Preclinical investigations with remacemide hydrochloride

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Abstract

There is now convincing neurochemical and neurophysiological evidence to suggest that increased excitatory neurotransmission is involved in epilepsy. Antagonists of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors are powerful anticonvulsants in many animal models of epilepsy. Early attempts to pharmacologically modulate glutamatergic transmission resulted in anticonvulsant compounds such as dizocilpine (MK801) and phencyclidine. Preclinical testing of these agents generally showed efficacy but also demonstrated severe side effects. A clinical application of pure glutamate antagonists has yet to be established.

Remacemide hydrochloride (RMD) is an anticonvulsant compound which exhibits efficacy in a wide range of seizure models. Both RMD and its active metabolite, desglycinyl-remacemide (DGR), are said to have inhibitory actions on voltage-gated sodium channels and NMDA glutamate receptors with DGR possessing greater potency at both sites. The following studies were designed to further investigate the pharmacology of RMD and DGR in various neurochemical and epilepsy models. Clinical trial results reported while this project was in progress demonstrated that the drug did not perform well as monotherapy. It was initially believed that the additional action on NMDA receptors would result in RMD possessing an improved clinical profile compared to standard Na^+ channel blockers such as carbamazepine (CBZ) and phenytoin (PHT). Further investigations as to why a drug with such a

promising preclinical portfolio performed so poorly in the clinical environment were therefore conducted.

Pharmacokinetic studies suggested that although some conversion of RMD to DGR does occur in the peripheral tissues the majority of DGR generation occurs at the blood/brain barrier, resulting in an accumulation of DGR in the brain. The pharmacokinetics of DGR were seen to outlast those of the parent compound both in serum and brain.

The effects of hepatic enzyme induction on the pharmacokinetics and pharmacodynamics of RMD were investigated. Administration of phenobarbital (PB), a known hepatic enzyme inducer, resulted in a significant increase in both the content and activity of cytochrome P450 enzymes in the livers of mice. This increase in hepatic enzyme activity was shown to significantly decrease the brain concentrations of RMD and DGR although the effects on DGR concentrations were more pronounced. The efficacy of RMD against maximal electroshock (MES)-induced seizures was seen to decrease in induced animals when compared to saline-treated controls.

In vitro investigations in the hippocampal slice permitted the effects of RMD and DGR to be studied separately. CBZ and ARR-15896, a putative NMDA antagonist, were employed for comparative purposes. RMD was found to have no effect on zero Mg^{2+} / 4-aminopyridine (4-AP) induced epileptiform burst firing in the rat hippocampal slice. In contrast, DGR, CBZ and ARR-15896 all significantly reduced epileptiform activity. These results suggested

that blockade of voltage-gated Na^+ channels and the NMDA receptor protect against paroxysmal discharges in this model. The relatively low potency of RMD at these pharmacological targets may explain its lack of efficacy in this regard.

A neurochemical study investigating the effects of RMD and DGR on sodium channel activity was conducted in rat brain synaptosomes. RMD and DGR were seen to reduce voltage-gated sodium channel activity in a concentration-related manner in agreement with previous electrophysiological investigations and confirmed the reported separation in potency at this site.

Several antiepileptic drugs (AEDs) have effects on γ-aminobutyric acid (GABA)/glutamate homeostasis. Previous studies have reported that DGR has effects on GABA-transaminase (GABA-T) and glutamic acid decarboxylase (GAD) following chronic administration to mice. The effects of RMD and DGR on the activity of glutamate dehydrogenase, one of the enzymes responsible for glutamate homeostasis, were analysed in rat brain mitochondria. RMD and DGR were shown to significantly reduce the formation of glutamate from α-ketoglutarate (α-KG) via an action on the enzyme glutamate dehydrogenase in rat brain mitchondria. It is possible that this action may contribute to the anticonvulsant properties of the compounds although further investigations are required.

There is growing evidence to suggest that monoamines have an important role in epilepsy. The effects of RMD and DGR on the reuptake processes of

serotonin (5-HT), norephinephrine (NE) and dopamine (DA) were investigated in rat brain synaptosomes. RMD was seen to significantly reduce the reuptake of DA at therapeutically relevant concentrations while DGR reduced the reuptake of 5-HT and NE at therapeutic concentrations. Further investigations are required to assess the mechanism and importance of the results obtained to RMD pharmacology in terms of epilepsy and possibly affective disorders.

In conclusion, observations from the above studies indicate that there are differences in the pharmacology of RMD and DGR. Pharmacokinetic studies demonstrated that DGR is present in the brain at higher concentrations than the parent compound. The increased susceptibility of DGR to hepatic enzyme induction and a subsequent change in the ratio of RMD to DGR in the brain may have contributed to the decrease in pharmacological effect observed in MES investigations. Alternatively, the concentrations of RMD and DGR may have simply decreased to sub-effective levels. Results from hippocampal slice studies suggest that blockade of sodium channels or inhibition of NMDA receptors is sufficient to decrease epileptiform bursting in the zero Mg^{2+}/4-AP model. It appears, therefore, that RMD, in contrast to DGR, does not exert a blockade at either of these two sites of action sufficient to decrease burst firing. Results obtained for both compounds with regards to monoamine uptake and glutamate dehydrogenase activity may influence the anticonvulsant activities of RMD *in vivo* although further investigations are required to assess their importance.

In conclusion, it is suggested that RMD monotherapy results in NMDA receptor blockade by DGR at concentrations lower than those required to block Na^+ channels. In induced patients, where DGR levels are lower, the block of Na^+ channels exerted by RMD results in an improved antiepileptic profile. As a result it is suggested that NMDA receptor blockade is not as important a mechanism of action for an antiepileptic compound to possess as first believed. This suggestion has possible implications for the future development of NMDA receptor antagonists as AEDs.

Contents

List of figures

List of tables

Declaration

I declare that all the work presented in this thesis was carried out by myself except where referenced and that it has not been submitted for any previous higher degree.

Sarah Santangeli

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Publications

Santangeli, S., McNeill, C., Sills, G.J., Brodie, M.J. (2000) Simultaneous determination of remacemide hydrochloride and desglycinyl-remacemide (ARR-12495XX) in brain tissue by high performance liquid chromatography. *J. Chromatog. B.*, **746,** 325-329

Santangeli, S., Thompson, G.G., Sills, G.J., Brodie, M.J. Effects of remacemide and selected anticonvulsants on sodium transport in rat brain synaptosomes. *Eur. J. Pharmacol.* (Submitted)

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Abstracts

Santangeli, S., Sills, G.J., Stone, T.W., Holmes, O., Brodie, M.J. (2000) Preclinical pharmacological studies with remacemide. *Epilepsia,* **41,** 157

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Abbreviations

A (mA)	amperes (milliamperes)
ACSF	artifical cerebrospinal fluid
ADP	adenosine diphosphate
AED	antiepileptic drug
AMPA	α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid
4-AP	4-aminopyridine
ATP	adenosine triphosphate
BGT	betaine γ-aminobutyric acid transporter
BSA	bovine serum albumin
BZD	benzodiazepine
cAMP	cyclic adenosine monophosphate
CBZ	carbamazepine
CD_{97}	dose causing convulsions in 97% of animals
Ci	Curie
CNS	central nervous system
CO	carbon monoxide
COMT	catechol-O-methyl transferase
CSF	cerebrospinal fluid
CYP450	cytochrome P450
DA	dopamine
DES	desipramine

DGR	desglycinyl-remacemide
ED_{50}	effective dose in 50% of animals
EDTA	ethylene diamine tetra-acetic acid
EEG	electroencephalogram
EGTA	ethylene glycol-bis[β-aminoethyl ester]-N, N, N', N'-tetra-acetic acid
ESM	ethosuximide
FBM	felbamate
FLU	fluoxetine
g (kg, mg, μg, ng)	gramme (kilogramme, milligramme, microgramme, nanogramme)
GABA	γ-aminobutyric acid
GAT	GABA-transporter
GABA-T	GABA-transaminase
GAD	glutamic acid decarboxylase
GBP	gabapentin
GDH	glutamate dehydrogenase
GEPR	genetically epilepsy-prone rat
GHB	γ-hydroxybutyrate
Gln-S	glutamate synthetase
HEPES	N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine (serotonin)
HVA	homovanillic acid

IC_{50}	concentration reducing response by 50%
ILAE	International League Against Epilepsy
i.p.	intraperitoneal
α-KG	α-ketoglutarate
l (ml, μl)	litre (millilitre, microlitre)
LEV	levetiracetam
LTG	lamotrigine
M (mM, μM, nM)	molar (millimolar, micromolar, nanomolar)
mACSF	modified ACSF
MAO	monoamine oxidase
MES	maximal electroshock
mol (mmol)	moles (millimoles)
N	normal
NAD	nicotinyl adenine dinucleotide
NE	norepinephrine
NIH	National Institutes of Health
NMD A	*N*-methyl-D-aspartate
NMDLA	*N*-methyl-D,L-aspartate
NOM	nomifensine
6-OHDA	6-hydroxydopamine
OXC	oxcarbazepine
PB	phenobarbital
PCP	phencyclidine
PEMA	phenylethylmalonamide

PHT	phenytoin
PRIM	primidone
PTZ	pentylenetetrazol
RMD	remacemide
SEM	standard error of the mean
SSA	succinic semialdehyde
SSADH	succinic semialdehyde dehydrogenase
TCA	trichloro-acetic acid
TGB	tiagabine
THE	tonic-hindlinb extension
TTX	tetrodotoxin
TPM	topiramte
VTD	veratridine
VGB	vigabatrin
VPA	sodium valproate

Chapter 1

Introduction

1 INTRODUCTION

1.1 Epilepsy

Epilepsy is one of the most common neurological disorders in man. With a prevalence of approximately 0.5 - 1%, it is estimated that 50 million people world wide may have the disorder (Brodie et al, 1997). Incidence rates of the disease are highest in childhood and the elderly, with a plateau observed between the ages of 15 - 65 (Brodie and Dichter, 1997). Epilepsy is not a single disease, but rather a collection of diverse syndromes, some of which are secondary to brain derangements, and some of which are primarily genetic (Chadwick, 1990).

1.1.1 *Definition*

The characteristic event of all epileptic disorders is a seizure. These episodes are associated with high frequency discharges by a group of neurons in the brain (Rang et al, 1995). This transitory disturbance in brain function develops suddenly, ceases spontaneously and may be caused by a number of different provocations (Barker et al, 2000). The particular symptoms produced by a seizure depend on the region of the brain that is affected. Thus, involvement of the motor-cortex causes convulsions, involvement of the hypothalamus causes peripheral autonomic discharges, and involvement of the reticular formation in the upper brainstem leads to loss of consciousness (Rang et al, 1995). Seizures which begin as a local abnormal discharge may then spread to other regions of the brain.

1.1.2 *Classification of seizure types*

According to the classification established by the International League against Epilepsy, seizures can be divided into two groups, partial and generalised (Table 1; Brodie and Schachter, 2001).

Partial seizures are those in which the discharge remains localised. When consciousness is not impaired the seizure is classified as a simple partial seizure. In episodes where consciousness is affected, the seizure is termed complex partial. Simple partial seizures can develop into complex partial seizures. A partial seizure can progress into a generalised motor seizure (Commission, 1981).

Generalised seizures are those which involve both brain hemispheres. Immediate loss of consciousness is characteristic of generalised seizures. The main categories are tonic-clonic seizures, absences and myoclonic seizures. A tonic-clonic seizure consists of an initial strong contraction of the whole musculature, causing a rigid spasm. Respiration stops and defaecation, micturition and salivation often occur. This tonic phase is followed by a series of violent, synchronous jerks which gradually diminish over 2 - 4 minutes. Absence seizures usually occur in children. This seizure type is less severe than tonic-clonic seizures although their frequency is often much higher. Patients abruptly cease what they are doing, and stare vacantly for a few seconds with little or no motor disturbance. Recovery is rapid with the patient often suffering no ill-effects (Rang et al, 1995).

International classification of epileptic seizure types

◊ **Partial seizures (beginning locally)**

- Simple seizures (without impaired consciousness)
  - with motor symptoms
  - with somatosensory or special sensory symptoms
  - with autonomic symptoms
  - with psychological symptoms

- Complex partial seizures (with impaired consciousness)
  - simple partial onset followed by impaired consciousness
  - impaired consciousness at onset

- Partial seizures evolving into secondary generalised seizures

◊ **Generalised seizures (convulsive or non-convulsive)**

- Absence seizures
  - typical
  - atypical

- Myoclonic seizures
- Clonic seizures
- Tonic seizures
- Tonic-clonic seizures
- Atonic seizures
- Unclassified seizures

Table 1: Simplified version of the International classification of epileptic seizures (Brodie and Schachter, 2001).

Myoclonic seizures are more prevalent among infants and children. This type of generalised seizure is characterised by brief jerks usually in the arms that last 1 - 5 seconds (Gram, 1990). Occasionally, myoclonic seizures affect the legs, causing falls. As with all generalised episodes, myoclonic seizures often occur within the first few hours after arising from sleep (Brodie and Schachter, 2001).

1.1.1 *Treatment of epilepsy*

Most patients with recurrent epileptic seizures require treatment. Exceptions are those patients with provoked seizures and those whose episodes are separated by years (Brodie and Dichter, 1996). The treatment of epilepsy involves drug therapy, or a surgical approach may be taken if an underlying structural lesion is identified and can be readily removed (Barker et al, 2000).

Although epilepsy has been a recognised disorder for approximately 4000 years, it was not until the 19th century that the first effective antiepileptic therapy was introduced (Patsalos and Sander, 1994). The anticonvulsant actions of bromide, which was introduced in 1857 were associated with severe side-effects, most notably sedation (Patsalos and Sander, 1994). Screening of other sedative compounds resulted in the discovery of phenobarbital (PB) which was first used as treatment for epilepsy in 1912. The search for less sedative compounds resulted in the introduction of phenytoin (PHT; Merritt and Putnam, 1938b). The subsequent discovery of carbamazepine (CBZ; 1967), primidone, (PRIM; 1952),

ethosuximide (ESM; 1958) and numerous benzodiazepines (BZDs), including diazepam, clobazam and clonazepam (Harden, 1994; Rang et al, 1995), were largely due to serendipitous drug discoveries and extensive drug screening programmes (Brodie and Dichter, 1996). The anticonvulsant properties of sodium valproate (VPA) were discovered in 1963 when the compound was being used as a solvent for other agents. The drug was approved for use against epilepsy in Europe in 1974 (Brodie and Dichter, 1997).

In the last ten years there has been a huge increase in the number of antiepileptic drugs (AEDs) available to treat epilepsy with vigabatrin (VGB), lamotrigine (LTG), felbamate (FBM), gabapentin, (GBP), oxcarbazepine (OXC), topiramate (TPM), zonisamide (ZNS), tiagabine (TGB) and levetiracetam (LEV) being licensed in at least one country in the 1990's.

The decision about treatment with AEDs should be made following extensive discussion with the patient with the benefits and side-effects of drug treatment made clear. Many patients will have to take medication for the rest of their lives, therefore it is important that a drug suited to the patient's lifestyle and seizure type is selected (Brodie and Dichter, 1997). Approximately 30% of patients with epilepsy are refractory to treatment with a single AED (Kwan and Brodie, 2000). Before long-term treatment with more than one drug is undertaken, all reasonable options for monotherapy should be exhausted (Brodie and Dichter, 1996). Studies have shown that if a patient does not respond to two first-line AEDs as monotherapy, it is unlikely that their condition will be controlled on any monotherapy regime (Brodie and Schachter, 2001). Only a relatively small

subgroup of patients will benefit from a combination of two AEDs, often at the expense of an increased burden in terms of side effects. In some cases, polytherapy may even result in a paradoxical increase in seizure frequency as a manifestation of toxicity (Perucca et al, 1998). There is, however, increasing evidence of the beneficial effects of some AED combinations (Stephen et al, 1998). Many clinicians will combine AEDs that have different mechanisms of action for patients with more than one seizure type, and drugs with similar mechanisms when there is a single type of seizure that proves unresponsive to monotherapy (Brodie and French, 2000).

1.2 Neurochemistry of epilepsy

1.2.1 *Neurotransmission*

One of the fundamental features of the nervous system is its ability to generate and conduct electrical impulses. These can take the form of generator potentials, synaptic potentials and action potentials - the latter being defined as a single electrical impulse passing down an axon (Barker et al, 2000). Nerve cells usually have a potential difference across their resting membrane, with the inside being negatively charged with respect to the outside. This potential originates from the fact that Na^+ ions exist mainly outside the cell, and K^+ ions mainly inside, but permeability to the latter is about 10-times greater than to Na^+ (Stone, 1995).

A small increase in Na^+ permeability or decrease in K^+ permeability will lead to a fall of membrane potential known as a depolarisation. Once this depolarisation reaches the threshold stimulus intensity, voltage-activated Na^+ channels in the neuronal membrane open, which allows Na^+ ions to flow down their

electrochemical gradient. The rapid influx of Na^+ ions cannot be compensated by K^+ efflux and causes a robust depolarisation. This depolarisation is responsible for the upstroke of the neuronal action potential (Barker et al, 2000).

This is an all or nothing phenomenon. Once the threshold stimulus intensity is reached, an action potential will be generated. The threshold is defined as the value at which the net inward current (largely determined by Na^+ ions) is just greater than the net outward current (largely carried by K^+ ions), and is typically around -55 mV (Rang et al, 1995).

The falling phase of the action potential then follows as the Na^+ channels become inactivated (section 1.2.2). This inactivation is also voltage dependent, in that it occurs in response to the initial depolarising stimulus, but has slower kinetics than the activation process and consequently occurs later (Barker et al, 2000).

During this falling phase a voltage-dependent K^+ current becomes important as its activation by depolarisation of the membrane has even slower kinetics than sodium channel inactivation. This voltage activated K^+ channel current leads to a brief period of membrane hyperpolarisation before it deactivates and the membrane potential is returned to the resting state (Rang et al, 1995).

Immediately after the spike of the action potential there is a refractory period when the neuron is either inexcitable or only activated to submaximal levels by suprathreshold stimuli. The refractory period has two important implications for action potential generation and conduction. First, action potentials can only be

conducted in one direction, away from the site of generation, and secondly, they can generated only up to certain limited frequencies (Barker et al, 2000).

When an action potential reaches the synaptic terminal it triggers the influx of Ca^{2+} across the cell membrane, from the extracellular fluid into the cytoplasm. This influx of Ca^{2+} causes the neurotransmitter containing vesicles to migrate towards the presynaptic membrane, where they fuse and then release their contents into the synaptic cleft between the presynaptic and postsynaptic cells. Each vesicle may contain several thousand molecules of transmitter and several hundred vesicles are often released with each action potential. Even in the absence of stimulation, single vesicles occasionally collide with the presynaptic membrane and release their contents. This basal release of neurotransmitter is important in the mechanism of action of some AEDs (Stone, 1995).

1.2.2 *Ion channels*

Voltage-gated ion channels control the flow of cations across surface and internal cell membranes (Barchi, 1998). Of these, the Na^+ channel is arguably the most important. As discussed above, voltage-dependent Na^+ channels are responsible for the upstroke of neuronal action potentials, and ultimately control the excitability of the nervous system (Porter and Rogawski, 1992). The neuronal Na^+ channel is a multi-subunit structure that forms a Na^+ selective, voltage-gated pore through the plasma membrane. The protein structure undergoes conformational alteration in response to changes in membrane potential, regulating conductance through the intrinsic pore (Ragsdale and Avoli, 1998).

In order to detect a change in membrane potential the receptor protein must possess a charged structure within the hydrophobic portion of the membrane. The highly conserved and highly charged 4th transmembrane region of each receptor subunit is now widely accepted as forming the voltage sensors (Anger et al, 2000).

At normal membrane potentials, most Na^+ channels exist in a closed, resting state. Upon depolarisation, the channel activates, facilitating ion influx. Thereafter, the Na^+ channel enters an inactivated state from which it is not readily re-activated. Repolarisation of the neuronal membrane rapidly converts the channel back to the resting state, from which it can respond to subsequent depolarisations (Catterall, 1992; Ragsdale and Avoli, 1998). The ability of neuronal Na^+ channels to progress through these various functional states within a few milliseconds is an essential characteristic for sustaining normal brain function and is also implicated in the production of epileptic discharges. The complexity of Na^+ channels provides enormous scope for genetic mutations leading to pathophysiological changes in channel function and several forms of generalised epilepsy have been linked to Na^+ channel mutations (Wallace et al, 1998; Anger et al, 2001). The neuronal Na^+ channel represents one of the most important targets for AED action (Upton, 1994; Macdonald and Kelly, 1995; Meldrum, 1996; White, 1999).

Voltage-dependent Ca^{2+} channels share key structural elements and sequence homology with their Na^+ channel counterparts (Barchi, 1998). These channels can be broadly classified into low or high threshold, according to the

membrane potential at which they are activated (Hofmann et al, 1994). The low-threshold T-type Ca^{2+} channel is expressed predominantly in thalamocortical relay neurones, where it is believed to be instrumental in the generation of generalised absence seizures (Coulter et al, 1989a). High-threshold Ca^{2+} channels are sub-classified by their pharmacological properties into L-, N-, P-, Q-, and R-types (Hofmann et al, 1994; Catterall, 1995; Dolphin, 1995). These channels are distributed throughout the nervous system on dendrites, cell bodies, and nerve terminals. The N-, P-, and Q-type channels, in particular, have been implicated in the control of neurotransmitter release at the synapse (Stefani et al, 1997). Several AEDs have been reported to block voltage-sensitive Ca^{2+} channels in a subtype-specific manner, an effect that may contribute to their antiepileptic actions (Stefani et al, 1997).

Neuronal K^+ channels are large protein complexes which form tetrameric structures. The association of 4 subunits in the neuronal membrane is required for the formation of a K^+-sensitive pore and, therefore, channel function (Pongs, 1999). At a neuronal level, K^+ channels are intimately involved in excitability. They are responsible for the action potential downstroke or, more specifically, repolarisation of the plasma membrane following Na^+ channel activation (Pongs, 1999). Direct activation of voltage-dependent K^+ channels hyperpolarises the neuronal membrane and limits action potential firing (Porter and Rogawski, 1992). Accordingly, K^+ channel activators have anticonvulsant effects in some experimental seizure models (Gandolfo et al, 1989; Rostock et al, 1996), whereas K^+ channel blockers potentiate seizures (Yamaguchi and Rogawski, 1992). Potentiation of voltage-sensitive K^+

channel currents may prove to be an important target for future AED development (Rundfeldt and Netzer, 2000).

1.2.3 *Neurotransmitters*

Normal CNS function is maintained by a balance between excitatory and inhibitory neurotransmission. Disruption of this balance may result in the generation of abnormal electrical activity (Schwartzkroin, 1993). There are countless neurotransmitter systems that contribute to the balance of excitability of the brain.

GABA is the major inhibitory neurotransmitter in the central nervous system (CNS). It exerts an inhibitory action in all forebrain structures, and it may play a role in certain neurological conditions, including epilepsy (Olsen and Avoli, 1997). Following its identification in brain (Awapara et al, 1950; Roberts and Frankel, 1950; Udenfriend, 1950), it was discovered that direct application of GABA to the canine motor cortex could arrest a local epileptic discharge (Hayashi and Nagai, 1956; Hayashi, 1959). Around the same time it was shown that certain convulsant hydrazides inhibited glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of GABA in the brain (Killam and Bain, 1957).

GABA is synthesised entirely from glutamate. The reaction is catalysed by GAD with pyridoxal phosphate acting as a cofactor (Meldrum, 1975). GAD, a soluble or cytoplasmic enzyme is found exclusively in GABAergic neurones. Antibodies to this enzyme can be used as a marker for GABAergic neurones in the CNS (Snead, 1983).

GABA acts on at least 3 types of receptor, $GABA_A$, $GABA_B$ and $GABA_C$. $GABA_A$ receptors, which are mainly post-synaptic, form a ligand-gated ion channel. Activation of this receptor subtype leads to an increase in Cl^- permeability and thus hyperpolarisation. These receptors are activated by GABA and muscimol and antagonised by bicuculline and picrotoxin. $GABA_B$ receptors are located both pre- and post-synaptically and exert an inhibition of adenylate cyclase via g-protein activation as their transduction mechanism. The net result of $GABA_B$ receptor activation can be a cAMP-mediated increase in K^+ conductance or a decrease in Ca^{2+} conductance, both of which cause hyperpolarisation of the neuronal membrane. GABA and baclofen are agonists at this receptor subtype, while phaclofen and 2-hydroxy-saclofen are antagonists. (Stone, 1995). The $GABA_C$ receptor subtype is pharmacologically distinct from $GABA_A$ and $GABA_B$. Located predominantly in the vertebrate retina, $GABA_C$ receptors form a transmitter-gated Cl^- channel consisting of a single protein subunit. These receptors are not blocked or modified by bicuclline or baclofen, respectively. Instead, $GABA_C$ receptors are selectively activated by *cis*-4-aminocrotonic acid and (1S,2R)-2-(aminomethyl)-1-carboxycyclopropane, which are analogues of GABA. Another GABA analogue, (1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid, acts as an antagonist at this receptor subtype (Chebib and Johnston, 1999).

Following release and receptor activation, GABA is removed from the synaptic cleft into nerve terminals and astrocytes by high affinity uptake carriers. Four subtypes have been identified by molecular cloning and designated GABA-transporter (GAT) -1, GAT-2, GAT-3, and betaine GAT (BGT)-1 (Borden et al,

1994). Following uptake, GABA is successively metabolised by two mitchondrial enzymes, GABA-transaminase (GABA-T) and succinic acid semialdehyde dehydrogenase (SSADH). GABA-T catalyses the metabolism of GABA to succinic acid semialdehyde and glutamate with α-KG serving as a cofactor. SSADH completes the metabolism to the inactive compound succinic acid in the presence of nicotinamide-adenine dinucleotide (NAD) and water (Meldrum, 1975).

Considerable evidence suggests that impaired GABA function can cause seizures. Altered GABAergic synaptic functions, especially those linked with $GABA_A$ receptors, may contribute to inherited or acquired epilepsies. Whether caused by a GABAergic mechanism or not, many patients may have their seizures controlled by drugs acting on the GABA system (Olsen and Avoli, 1997).

Glutamate is a key substance in the brain. It is involved in metabolism, pathology and neurotransmission and is also an essential source of energy (Kvamme, 1998). Glutamate is the main excitatory neurotransmitter in the mammalian brain and is released at a large proportion of synapses in the CNS. Its concentration in the brain is higher than any other amino acid (Greenamyre and Porter, 1994). The epileptogenic properties of glutamate, when applied directly to brain tissue or when administered systemically, are well documented (Bradford and Dodd, 1975; Stone and Javid, 1983).

There are a number of ways in which glutamate can be synthesised although glutamine and glucose-derived α-KG are considered to be the main precursors of

metabolic and neurotransmitter glutamate both *in vitro* and *in vivo* (Kvamme, 1998).

As with all excitatory neurotransmitters, release of glutamate via Ca^{2+}-dependent exocytosis causes depolarisation of the post-synaptic neurone (Rang et al, 1995). Glutamate receptors can be divided into two categories, ionotropic and metabotropic. NMDA, α-amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA) and kainate are the recognised types of ionotropic glutamate receptor. All three receptor subtypes form ion channels with NMDA receptors being permeable to Na^+ and Ca^{2+}, AMPA receptors permeable to Na^+, K^+ and Ca^{2+} and kainate receptors permeable to Na^+ and Ca^{2+}. The permeability of each channel to individual ions is dependent of the subunit composition of the receptor with the exception of the NMDA receptor which is always permeable to Ca^{2+} (Dingledine et al, 1999). Under normal circumstances, neurotransmitter glutamate excites cells via an action on the kainate and AMPA receptors (Stone, 1995).

The NMDA receptor belongs to the ion channel linked superfamily. Accordingly, the receptor components each have 4 membrane-spanning sequences of which six distinct subunits have been identified - NMDA1, NMDA2A-D and the recently discovered NMDA3A subunit. It is believed that NMDA receptors are made up of tetrameric and heteromeric subunit assemblies which have different physiological and pharmacological properties (Danysz and Parsons, 1998). Differences include CNS distribution, gating properties and magnesium sensitivity (Sucher et al, 1996; Parsons et al, 1998).

As seen in Figure 1, NMDA receptors have several attachment sites for agents that can modulate, or may indeed be required for, receptor activity. The ion channel the receptor forms is partially blocked by Mg^{2+} ions under normal physiological conditions. When the neurones are subjected to prolonged periods of depolarisation the Mg^{2+} block is reduced. Convulsions, which involve the excessive firing of neurones will recruit NMDA receptors. Their involvement helps to maintain and prolong the seizure event (Stone, 1995). The recruitment of NMDA receptors during periods of prolonged depolarisation has made the receptor an important target for AED action.

There is an abundance of evidence suggesting an important role for glutamate in epilepsy. As discussed above, glutamate itself has been shown to cause seizures when administered systemically (Bradford and Dodd, 1975). An increase in glutamate receptor density has also been reported in children suffering from generalised seizures (Represa et al, 1989). Finally, elevated plasma levels of glutamate have been found in patients with primary generalised or focal epilepsy (Janjua et al, 1982).

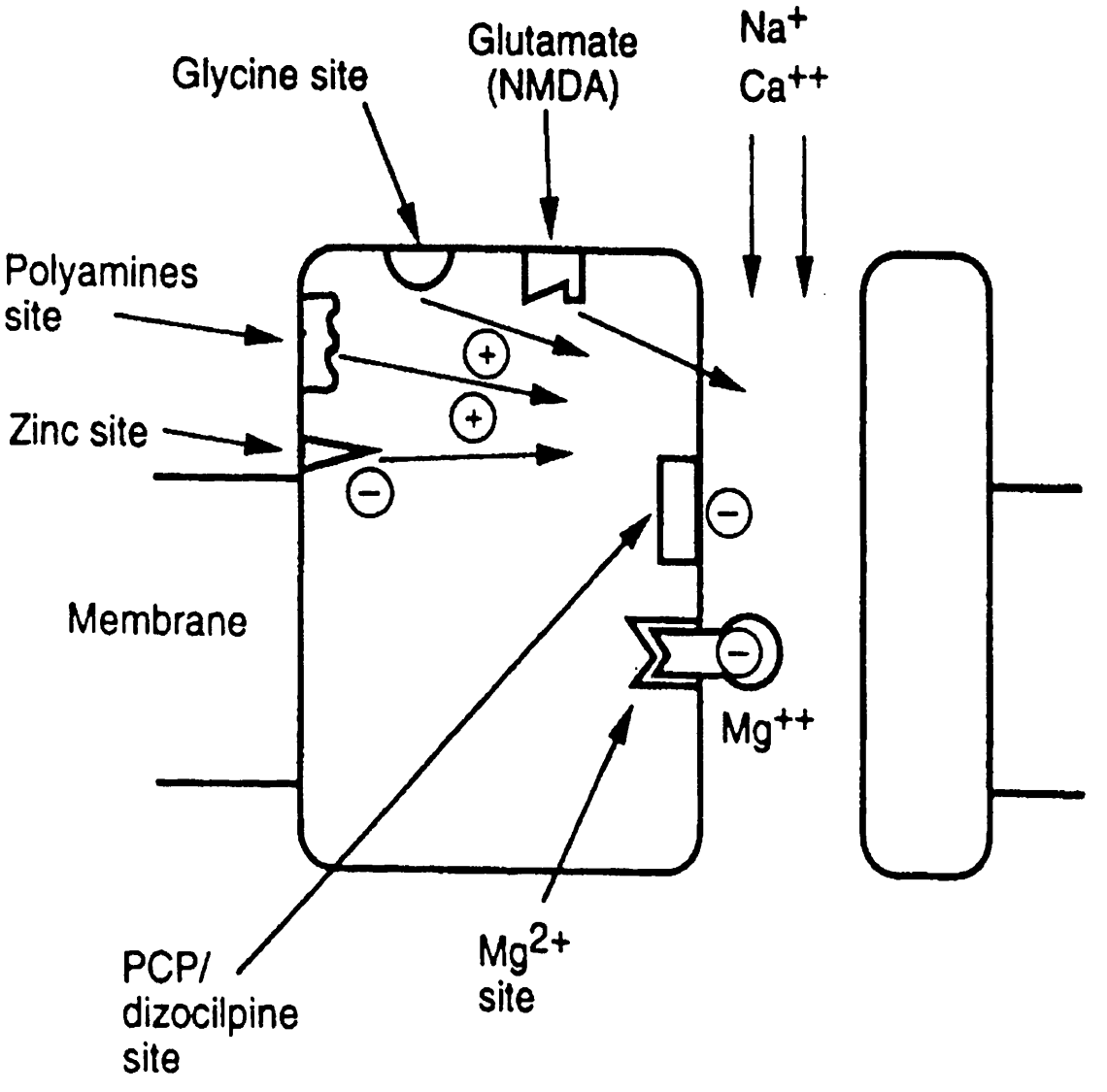


Figure 1: The NMDA receptor for glutamate. Receptor activation is facilitated by glycine and polyamine binding sites. The receptor can be blocked by zinc or by blockade of the channel by Mg^{2+}, phencyclidine (PCP) or dizocilpine (Stone, 1995).

As discussed above, there are countless neurotransmitter systems in the mammalian brain, each of which may contribute to intrinsic excitability. In addition to GABA and glutamate, the involvement of monoamines in the initiation or maintenance of epileptic phenomena has been extensively studied. Spontaneous and experimentally induced deficiencies in norepinephrine (NE), dopamine (DA) and/or 5-HT have been implicated in the onset and perpetuation of many seizure disorders (Racine and Coscina, 1979; Applegate et al, 1986; Zis et al, 1992; Bentue-Ferrer et al, 1994; Okada et al, 1997a; Applegate and Tecot, 1998). Conversely, many experimental procedures designed to increase monoaminergic activity have proven antiepileptic properties (Barry et al, 1989; Browning et al, 1989; Bengzon et al, 1992; Pelletier and Corcoran, 1993; Loscher and Hoenack, 1995; Dailey et al, 1997).

Electrophysiological studies have indicated that catecholamine and indoleamine systems can exert inhibitory neuronal control and modulate the release of other neurotransmitters (Jobe et al, 1972). Monoamines may, therefore, contribute to the regulation of neuronal excitability and hence seizure susceptibility (Kresch et al, 1989). The role of monoamines in epilepsy will be discussed in greater detail in chapter 8.

1.3 Animal models

In recent years, animal models involving induction of seizures by chemical or electrical stimuli have played an increasingly important role in epilepsy research. Although valuable in the screening of new AEDs, the majority of animal models serve only as tools for demonstrating single seizures than as true models of epilepsy (Loscher & Schmidt 1988). Despite the diversity of the clinical syndromes that they attempt to mirror, all models have significant limitations. Nevertheless, a great number of animal models remain in continual use for the investigation of epilepsy and the identification of novel AEDs and are likely to remain in use until they are superseded by more clinically relevant experimental paradigms.

1.3.1 *In vitro* models

Cell cultures of neurones and glial cells are now extensively employed in epilepsy research (Crain, 1972) and indeed many of the mechanisms of action of AEDs have been identified using cultured cells (Macdonald and Kelly, 1993). Primary cultures of such cells can be used for neurochemical (Gallo et al, 1982), neurophysiological (White et al, 1992), and neuropharmacological (Carboni and Wojcik, 1988) investigations. Such studies may incorporate whole cell recordings (McLean and Macdonald, 1986a; 1986b) or patch clamp techniques (Twyman et al, 1992).

The advantages of using primary cultured cells include a lack of interference of neighbouring cells, the ability to apply known concentrations of drug to the cell membrane, and ease of direct physiological recording. Disadvantages of such

preparations include a lack of correlation with whole animal studies and possible inter-assay heterogeneity (Stone, 1989).

The rodent hippocampal slice preparation has been utilised by investigators extensively since the early 1970's (Fisher, 1989). This *in vitro* preparation was initially used to investigate cerebral metabolism but was later adapted for physiological studies (Yamamoto and McIlwain, 1966). The isolation of the hippocampus has proved to be especially productive in terms of understanding the epilepsies. Hippocampal slices have the advantages of mechanical stability, absence of the blood-brain barrier for applied drugs, and absence of anaesthetics. Disadvantages include partial mechanical injury and hypoxia of the tissue. Despite this, hippocampal slices can exhibit an impressive range of neuronal behaviours, including synaptic plasticity and epileptiform bursting (Andersen et al, 1971; Schwartzkroin and Prince, 1978).

1.3.2 *Acute seizure models*

Acute models, which use either electrical or chemical stimulation to precipitate seizure activity, have been in widespread use for a number of years. The maximal electroshock (MES) test is arguably the best-studied and most useful seizure model available (Fisher, 1989). The fact that electrical stimulation of the brain results in seizure generation has been recognised for over a century (Fritsch and Hitzig, 1870; Albertoni, 1882). Quantification of these observations into a reliable laboratory technique facilitated the use of the MES test in anticonvulsant drug screening for the first time (Spiegel, 1937). In 1938, Merritt and Putnam described the identification of PHT following the

use of the MES test to evaluate the anticonvulsant properties of various phenyl derivatives. The test itself relies on administration of a supramaximal electrical stimulus, via ear-clip or corneal electrodes, to elicit tonic extension of the hind-limbs of both mice and rats. Anticonvulsant drug efficacy is determined by the ability of a drug to reduce the incidence of this 'all or nothing' event when compared to saline treated controls. The MES test may be a useful indicator for drugs with a likely clinical efficacy against generalised tonic-clonic seizures. One of the major drawbacks of this model is that it occasionally yields false negative results as a function of its supramaximal nature (Loscher and Schmidt, 1988).

Pentylenetetrazol (PTZ) is the most commonly employed chemoconvulsant in the laboratory evaluation of AED action (Loscher and Schmidt, 1988). The PTZ test involves the subcutaneous administration of a species-dependent dose of the convulsant (70 - 100 mg/kg) to induce sustained myoclonic seizures, generalised clonic seizures with loss of the righting reflex and, at higher doses, generalised tonic seizures within 3 - 5 minutes (Fisher, 1989). This test is sometimes referred to as the CD_{97} PTZ test, as it employs a convulsant dose (CD) sufficient to induce seizures in 97% of naive animals. The incidence of or latency to, individual seizure states is recorded and compared in drug evalution studies (Loscher et al, 1991a). The PTZ test has been proposed as an indicator for efficacy against absence seizures in man (Eadie, 1985). While this suggestion holds true for the efficacy of ethosuximide (ESM; Swinyard and Woodhead, 1989) and the inactivity of PHT (Krall et al, 1978) in this model, the rather loose correlation with the

clinical syndrome has been repeatedly questioned. Current opinion would now suggest that the PTZ seizure model has no direct parallel with the clinical disease (Loscher and Schmidt, 1988).

Gamma-hydroxybutyrate (GHB) is a naturally occurring metabolite of GABA found in the brain of several mammalian species, including man (Snead, 1996). When administered to a variety of animals, GHB can produce a response which is indistinguishable from a generalised absence seizure (Snead, 1988). The GHB treated animal shows an arrest of activity with a staring appearance, occasional myoclonic jerks, and, in the monkey, automatisms and pupillary dilatation. Behavioural characteristics and electroencephalogram (EEG) readings vary depending on the species. In rodents, a 4-6/second spike and wave pattern can be expected (Snead, 1978). It is not known how GHB produces an absence-like seizure, although it is believed that the underlying mechanism may involve a GHB-GABAergic interaction (Fisher 1989).

There are several compounds that have been proposed to exert their convulsant effects by antagonism of the $GABA_A$ receptor complex (Loscher and Schmidt, 1988). Those most commonly used include bicuculline and picrotoxin. Although these two agents differ in their precise mechanisms of convulsant action, with bicuculline competitively antagonising GABA binding and picrotoxin irreversibly interacting with the Cl^- ionophore of the $GABA_A$ receptor complex (Barolet et al, 1985), they tend to have similar sensitivities to AED action (Fisher, 1989). The seizures produced by these compounds are similar to those produced by PTZ and so do not offer any significant

advantages in this respect (Loscher and Schmidt, 1988). As with the PTZ model, the motor manifestations caused by systemic bicuculline and picrotoxin have no direct clinical counterpart (Loscher and Schmidt, 1988).

Strychnine is a chemoconvulsant compound which precipitates tonic extensor spasms following systemic administration (Fisher, 1989). An irreversible antagonism of glycine binding is reported to contribute to its convulsant action (Curtis et al, 1971). Glycine is an important inhibitory neurotransmitter in the brainstem and spinal cord and is also a co-agonist at the NMDA receptor via an action on a strychnine-insensitive site. In addition, strychnine interacts with the $GABA_A$ receptor (Braestrup and Nielsen, 1980). Like bicuculline and picrotoxin, this model is reported to have no clinical counterpart and furthermore, the tonic seizures produced by strychnine are not fully inhibited by doses of AEDs that are active in other chemoconvulsant models (Fisher, 1989).

The glutamate receptor agonists, NMDA and kainate are also employed in the production of experimental seizures in animals. Systemic kainic acid administration results in the acute production of seizures which may be manifested for several hours or days (Fisher, 1989). It is predominantly used as a model of partial seizures arising in the hippocampus. At higher doses, kainate has been employed as a model for the limbic cell damage which can occur following clinical status epilepticus (Sloviter, 1987). Systemic administration of NMDA to mice precipitates a chain of events characterised by initial behavioural abnormalities, followed by clonic and then tonic

seizures, and eventually death within 60 minutes (Fisher, 1989; Palmer et al, 1993). All aspects of the NMDA response have been employed for comparative drug evaluations. Like strychnine-induced seizures, NMDA responses are relatively resistant to conventional AED treatment. While the kainic acid model has a degree of relevance to a clinical epilepsy syndrome, no such correlation has been described for NMDA (Loscher and Schmidt, 1988). Similarly, following intravenous injection with the NMDA receptor agonist, NMDLA, mice develop convulsions within a few minutes followed by mortality. Investigations assessing the efficacy of drugs again both the convulsions and the mortality may be conducted (Czuczwar et al, 1986).

1.3.3 *Chronic seizure models*

The kindling phenomenon was developed on the basis that *'epilepsy induces epilepsy'* (Frey and Janz, 1985) and exploits the observation that repeated electrical stimuli to various parts of the brain results in increased local excitability (Fisher, 1989). The amygdala is reported to be the area of the brain most responsive to the kindling process, although other regions, including the hippocampus, are commonly used (Loscher and Schmidt, 1988). The process of kindling an animal is time-consuming, taking up to three weeks in most cases. Bi-polar electrodes are inserted into the brain area of interest and screw electrodes placed in the skull to record the EEG (Morimoto et al, 1997; Amano et al, 1998).

Following recovery, the kindling stimulus is applied on a once daily basis. After a few days, this initially sub-convulsive current begins to induce

progressively more complex and prolonged afterdischarges and modest seizure manifestations (Goddard, 1983).

Continued daily stimulation results in the generation of kindled seizures which progressively evolve through the classes as described by Racine (1972).

Stage 0- after discharge without motor manifestations

Stage 1- facial twitching

Stage 2- head nodding with simultaneous mastication

Stage 3- forelimb clonic convulsion

Stage 4- rearing on hind legs

Stage 5- full blown generalised convulsion with falling

Table 2: Racine scale of seizure severity (1972).

An animal is described as being "fully kindled" when the stimulus produces stage 5 seizures on five consecutive days (Morimoto et al, 1997; Paschoa et al, 1997; Amano et al, 1998). Once the enhanced sensitivity to the stimulus has developed, the animal retains the same high responsiveness to the low intensity stimulation for the rest of its life (Ferencz et al, 1997).

The various stages of the kindled seizure can be compared to the human disorder. The behaviour observed in stages 1 and 2 mimics that found in human complex partial seizures which most often originate from foci within the limbic system. Stage 3 seizures are said to mimic simple partial seizures

while the later two stages (4 & 5) represent secondary generalised motor seizures (McNamara, 1984).

One of the benefits of this model is its variety of end-points. The efficacy of drugs can be evaluated on the electrical discharges, the development of the kindled response (epileptogensis) and on the fully kindled (Fisher, 1989).

The genetically epilepsy-prone rat (GEPR) possesses an inborn hypersensitivity to a variety of seizure-inducing stimuli. The GEPR was developed from the Sprague-Dawley strain of which some 15-30% of the population exhibits spontaneous spike-wave discharges (Loscher and Schmidt, 1988). The incidence of these seizures was found to be markedly increased when pairs of these rats were used for breeding, therefore demonstrating the genetic nature of the phenomenon (Jobe and Laird, 1987). Although these rats were originally selected for their sensitivity to sound-induced seizures, they are also more prone to exhibit convulsions in response to hyperthermia, electroshock, PTZ and bicuculline. The GEPR has increased 5-HT and acetylcholine levels in the thalamus and striatum. It is, however, impossible to determine whether these alterations are responsible for the increased seizure susceptibility or merely a consequence of it. Audiogenic seizures similar to those in the GEPR may be produced in normal rats by infusing with NMDA into the inferior colliculus (Jobe and Laird, 1987).

The DBA/2 strain of mouse is another genetic model of reflex epilepsy. These animals exhibit severe audiogenic (sound-induced) seizures between the ages of 2

and 4 weeks (Seyfried and Glaser, 1985). At 8 weeks of age this audiogenic susceptibility is replaced by an increased sensitivity to seizures induced by MES or administration of excitatory amino acids (Engstrom and Woodbury, 1988). It is believed that the audiogenic seizure susceptibility is caused by a monoamine imbalance in the inferior colliculus area of the brain (Fisher, 1989). Seizure severity is again measured on the Racine (1972) scale. The majority of AEDs in clinical use are active in this model and while this may restrict its use in the search for novel therapies for drug-resistant epilepsy, it is unlikely that any potential anticonvulsant compound would fail in this model (Loscher and Schmidt, 1988).

The tottering mouse (tg/tg strain) suffers from spontaneous myoclonus and frequent partial and absence seizures from approximately 4 weeks of age (Kaplan et al, 1979; Noebels and Sidman, 1979). This strain has been especially useful for the investigation of neurotransmitters in epilepsy (Fisher, 1989). Tottering mice possess increased numbers of noradrenergic terminals and elevated levels of NE in the hippocampus, cerebellum and locus coeruleus (Levitt and Noebels, 1981). Lesions of noradrenergic terminals by the selective neurotoxin 6-hydroxydopamine (6-OHDA) have been shown to protect these animals against seizures (Noebels, 1984).

1.4 Antiepileptic drugs

As discussed in section 1.1.3, the majority of patients with recurrent epileptic seizures require treatment (Brodie and Dichter, 1996). These drugs afford symptomatic relief from seizures in up to 70% of the epileptic population (Kwan and Brodie, 2000). The following section describes the cellular mechanisms of action of both established and new AEDs. The majority of these effects have been determined by *in vitro* investigations in isolated cells and tissue preparations.

1.4.1 *Established antiepileptic drugs*

CARBAMAZEPINE

CBZ was first synthesised in 1953 in an attempt to produce a new antipsychotic drug (Brodie and Dichter, 1997). Following successful clinical studies, CBZ was introduced as a treatment for partial and generalised tonic-clonic seizures in 1967 (Editorial, 1989a).

CBZ is effective in a variety of seizure models including the MES seizure test in mice. It acts by preventing the repetitive firing of action potentials in depolarised neurones through a voltage- and use-dependent blockade of Na^+ channels (Dichter, 1993). This proposed mechanism of action has been confirmed by investigations in cultured mouse central neurones (McLean and Macdonald, 1986b), batrachotoxin-induced $^{22}Na^+$ flux measurements in rat brain synaptosomes and channel binding studies (Willow and Catterall, 1982). CBZ reduces the rate of recovery of Na^+ channels from the inactive state, thus preventing neuronal firing. The voltage-dependent nature of the block produced by CBZ means that it is removed by hyperpolarisation. Voltage-clamp studies

have shown that CBZ exerts a frequency-dependent block resulting in the maintenance of normal brain neurotransmission (Macdonald and Kelly, 1995). Clinically, the use of CBZ is complicated by the fact that it is a hepatic enzyme inducer, accelerating the breakdown of a number of other lipid soluble drugs (Brodie, 1992). CBZ also induces its own metabolism resulting in the need for careful monitoring following administration (Macphee et al, 1987).

PHENYTOIN

PHT was first synthesised in 1908 (Blitz, 1908), but pharmacological studies were not reported until 30 years later. Preliminary screening for a hypnotic action was conducted by Parke-Davis Laboratories (Gruhzit, 1939). However, Merritt and Putnam (1938a) reported efficacy of PHT against MES in cats and an anticonvulsant action in man (Merritt and Putnam, 1938b).

PHT is effective against partial and tonic-clonic seizures (Brodie and Dichter, 1996). Like CBZ, PHT appears to act via a voltage- and use-dependent block of Na^+ channels (Yaari et al, 1977; Selzer, 1978). Experimental evidence suggests that PHT binds to the fast inactivated state of the channel and reduces the frequency of sustained repetitive firing of action potentials without affecting their amplitude or duration (McLean and Macdonald, 1983). PHT reduces the rate of recovery of Na^+ channels from their inactivated state more efficiently than CBZ but requires a longer period of high frequency firing to exert a frequency-dependent block (Macdonald and Kelly, 1995). Evidence suggests that PHT may preferentially bind to different subtypes of Na^+ channels (Song et al, 1996). It has also been reported to block high voltage-activated Ca^{2+} channels (Schumacher et

al, 1998), attenuate post-ictal glutamate release (Rowley et al, 1995) and, paradoxically, reduce K^+ currents (Nobile and Vercellino, 1997). PHT is a hepatic enzyme inducer and is capable of accelerating the metabolism of a range of lipid soluble drugs including CBZ, VPA and ESM.

PHENOBARBITAL

The discovery of the antiepileptic properties of PB was a classic example of serendipity. Initially prescribed as a hypnotic, Alfred Hauptmann, a German physician, discovered the anticonvulsant properties of the drug in 1912. PB is used world wide and clinical trials have confirmed that it is as effective as CBZ and PHT in reducing partial and generalised tonic-clonic seizures (Mattson et al, 1985). The anticonvulsant effects of PB are believed to be mediated through potentiation of the actions of GABA by binding to a specific site on the $GABA_A$ receptor Cl^- channel complex (Brodie and Dichter, 1997). At the cellular level, PB increases post-synaptic potentials by increasing the mean Cl^- channel opening time and hence the duration of GABA-induced inhibitory post-synaptic bursts (Twyman et al, 1989). PB is a well-known hepatic enzyme inducer and can accelerate the metabolism of a long list of lipid-soluble drugs, including other AEDs (Patsalos and Duncan, 1993).

SODIUM VALPROATE

Sodium valproate (VPA) was discovered when it was used as a solvent for other compounds being screened for anticonvulsant effects (Meunier et al, 1963). VPA is effective over a wide range of seizure types and has been particularly valuable for the treatment of the idiopathic generalised epilepsies (Editorial, 1988).

The precise mechanisms by which VPA exerts its anticonvulsant effects remain to be confirmed. It has been shown to inhibit the sustained repetitive firing of mouse neurones in cell culture, an action associated with blockade of voltage-dependent Na^+ channels (McLean and Macdonald, 1986a). VPA may also have an inhibitory action on T-type Ca^{2+} channels, similar to that of ESM (see below). Such an action would explain the efficacy of the drug against generalised absence seizures (Kelly et al, 1990). There is also evidence to suggest that VPA elevates whole brain GABA levels and potentiates GABA responses, possibly via an action on the enzymes responsible for the synthesis and degradation of GABA (Loscher, 1999). VPA is an inhibitor of several hepatic metabolic processes and may decrease the metabolism of other AEDs such as PHT, CBZ and PB (Brodie and Dichter, 1996).

PRIMIDONE

Primidone (PRM) is a prodrug which is biotransformed *in vivo* to PB and another active metabolite, phenylethylmalonamide (PEMA; Brodie and Dichter, 1997). PEMA has a similar spectrum of anticonvulsant efficacy to PB with efficacy against both MES and PTZ seizures, but is 16 - 30 times less potent (Leal et al, 1979; Bourgeois et al, 1983). In contrast, PRM itself is nearly as potent as PB against MES seizures but has little activity in the PTZ test, suggesting that these drugs have different actions on Na^+ channels (Bourgeois et al, 1983). The clinical efficacy of PRM is similar to that of PB although it is less well tolerated (Mattson et al, 1985). There is little to recommend this drug over PB for patients in whom treatment with a barbiturate is contemplated (Brodie and Dichter, 1996).

ETHOSUXIMIDE

ESM, which was first introduced in 1958, is used for the treatment of uncomplicated generalised absence seizures (Brodie and Dichter, 1996). It was discovered during a search for less toxic alternatives to trimethadione (Brodie and Dichter, 1997). ESM acts by reducing low threshold, voltage-dependent T-type Ca^{2+} conductance in thalamic neurones (Coulter et al, 1989b). ESM alone does not influence hepatic metabolism although its concentrations are altered by enzyme inducers such as PHT and CBZ and by enzyme inhibitors such as (Pisani et al, 1990).

BENZODIAZEPINES

Benzodiazepines (BZDs), such as clobazam, diazepam and clonazepam, are used in the treatment of for absence, tonic-clonic, myoclonic and atonic seizures (Fisher and Blum, 1995). Like PB, BZDs act on the $GABA_A$ receptor Cl^- channel complex. They increase the frequency of channel opening, thereby potentiating the effects of GABA (Rang et al, 1995). The use of BZDs is complicated by their sedative effect and tolerance following prolonged administration (Brodie and Dichter, 1996). Diazepam and lorazepam can be used for the acute treatment of status epilepticus (Treiman et al, 1998).

1.4.2 *New antiepileptic drugs*

LAMOTRIGINE

Lamotrigine (LTG) is a novel AED that was developed as a result of an erroneous link between anticonvulsant and antifolate properties (Reynolds et al, 1966). The drug has a broad spectrum of activity and is effective against partial, absence, myoclonic and tonic-clonic seizures (Leach and Brodie, 1995).

LTG inhibits the sustained repetitive firing of Na^+-dependent action potentials in mouse neuroblasts, suggesting an action on Na^+ channels (Cheung et al, 1992). It has been suggested that LTG may selectively target Na^+ channels on neurones that synthesise glutamate and aspartate (Leach et al, 1986). LTG has also been shown to reduce whole cell Ca^{2+} currents in rat amygdalar neurones, possibly via an action on the N- and P-type channels that have been implicated in neurotransmitter release (Stefani et al, 1996, 1997; Wang et al, 1996). Like PHT, it is ineffective in the PTZ-induced seizure model, suggesting a similar mechanism of action (Rogawski and Porter, 1990). LTG is susceptible to the actions of enzyme-inducing and enzyme inhibiting drugs (Brodie and Schachter, 2001).

VIGABATRIN

Vigabatrin (VGB) was the first AED to be developed on the basis of a targeted mechanism of action. It is an irreversible inhibitor of the enzyme responsible for GABA degradation, GABA-T (Dichter and Brodie, 1996). Inhibition of this enzyme increases the amount of GABA in the brain (Petroff et al, 1995). VGB is used for the treatment of partial seizures in adults and is the drug of choice in children with infantile spasms (Chiron et al, 1991; Dulac et al, 1991). VGB has

demonstrated efficacy in a variety of seizure models including those induced by picrotoxin (Bernasconi et al, 1988) and amygdaloid kindling (Loscher et al, 1989). It is also effective in genetic models of reflex epilepsy (Schechter et al, 1977). VGB elevates GABA levels in plasma, cerebrospinal fluid and in the brains of experimental animals (Bohlen et al, 1979) as well as in the plasma and cerebrospinal fluid of man (Ben-Menachem et al, 1989). There is evidence to suggest that, in addition to its action on GABA-T, VGB may block the uptake of GABA into glial cells (Leach et al, 1996b).

In recent years, it has become apparent that long-term VGB treatment is associated with persistent visual field problems (Eke et al, 1997; Wilson et al, 1997; Harding et al, 1998). Recent studies (Sills et al, 2001) suggest that following acute administration in rats, VGB accumulates in the retina. It is unclear whether the VGB accumulation or the associated increase in GABA levels is responsible for visual field problems. VGB does not interfere with hepatic enzymes but does produce a reduction of PHT levels of around 20% by an unknown mechanism (Brodie and Schachter, 2001).

TOPIRAMATE

Topiramate (TPM) was licensed in the UK for use as add-on therapy in refractory epilepsy in 1995. It is effective against partial-onset and generalised seizures in man (Brodie and French, 2000; Stephen et al, 2000). TPM possesses multiple mechanisms of action, including inhibition of Na^+ and Ca^{2+} currents, blockade of AMPA/kainate subtypes of glutamate receptor and facilitation of GABA effects at the $GABA_A$ receptor. TPM also inhibits carbonic anhydrase although this modest

effect is unlikely to contribute to its anticonvulsant efficacy (Shank et al, 1994). In animal models, TPM is effective against seizures induced by MES (Shank et al, 1994) and amygdaloid kindling (Wauquier and Zhou, 1996) and also in genetic models of epilepsy (Nakamura et al, 1994). TPM has been found to be ineffective against chemically-induced seizures (Shank et al, 2000).

TPM does not appear to alter the metabolism of other drugs. It is a target for hepatic enzyme inducing agents such as PHT and CBZ (Bourgeois, 1996) with TPM plasma concentrations reduced by 48% and 40% (Sachdeo et al, 1996), respectively following co-administration.

TIAGABINE

TGB is a product of rational drug design which acts specifically on the GAT-1 GABA uptake transporter. By blocking its action, the post-release actions of GABA are prolonged (Krogsgaard-Larsen et al, 1987). TGB is effective for the treatment of partial seizures with or without secondary generalisation (Leach and Brodie, 1998).

In animal models, TGB is effective against audiogenic seizures in DBA/2 mice, the development (Dalby and Nielsen, 1997) and motor manifestations of amygdaloid kindled seizures (Pierce et al, 1991) and against the tonic-clonic components of PTZ-induced seizures. TGB is ineffective against MES-induced seizures in rodents (Nielsen et al, 1991). No other mechanisms of action have been proposed for TGB, and it does not affect Na^+ or Ca^{2+} channels (Schachter, 1998).

TGB is rapidly and extensively metabolised by the cytochrome P450 isoenzyme CYP3A. Due to its short half-life (5-9 hours), TGB is particularly susceptible to the actions of hepatic enzyme inducers such as CBZ and PHT (Brodie and Schachter, 2001).

GABAPENTIN

Gabapentin (GBP) is a structural analogue of GABA which can cross the blood/brain barrier via an amino acid transport system (Leach et al, 1997c). The drug was licensed in the UK in 1993 as adjunctive therapy for the treatment of refractory partial seizures, with or without generalisation (Wilson and Brodie, 1996). GBP is effective in a variety of animal seizure models, including the MES test (Rogawski and Porter, 1990). The precise mechanism of action of GBP remains unclear. Studies have shown that it does not directly interact with GABA receptors (Taylor et al, 1998) or transporters (Su et al, 1995; Macdonald and Greenfield, 1997) although there is some evidence to suggest that it may increase the synthesis (Taylor et al, 1992) and non-vesicular release of GABA (Gotz et al, 1993). In addition, GBP has been shown to increase GABA turnover in isolated areas of the brain (Loscher et al, 1991b). The results of these experiments have been validated by the work of Taylor and colleagues (1998) who reported an increase in the activity of GAD, the enzyme responsible for the conversion of glutamate to GABA, following GBP administration. GBP has also been reported to reduce the sustained repetitive firing of neurones in a similar manner to PHT (Wamil and McLean, 1994) and has been found to bind with high affinity to a Ca^{2+} channel subunit in the brain (Gee et al, 1996). The implication of this finding remains to be fully investigated, but the lack of effect of GBP on whole cell Ca^{2+}

currents in human dentate granule cells acutely isolated from patients with temporal lobe epilepsy (Schumacher et al, 1998) questions the pharmacological relevance of this finding. GBP is not metabolised, and does not induce or inhibit hepatic enzymes. Drug interactions are, therefore, not an issue with this agent (Brodie and Schachter, 2001).

OXCARBAZEPINE

Oxcarbazepine (OXC) is the 10-keto analogue of CBZ (White, 1999). It is essentially a pro-drug and is rapidly and completely reduced in the liver to its active metabolite, the monohydroxy derivative (Editorial, 1989b). OXC and CBZ have a similar profile and degree of efficacy against partial and generalised tonic-clonic seizures (Dam et al, 1989; Friis et al, 1993). Like CBZ, OXC is not effective against absence seizures or myoclonic jerks (Dichter and Brodie, 1996). OXC appears to exert its effects by blockade of voltage-dependent Na^+ channels in a similar manner to CBZ and PHT (McLean et al, 1994). It has also been shown to reduce presynaptic glutamate release possibly via an action on Ca^{2+} currents (Calabresi et al, 1995; Stefani et al, 1995, 1997). Unlike any other licensed AED, OXC may also increase K^+ channel conductance (McLean et al, 1994).

LEVETIRACETAM

LEV is an ethyl analogue of the no-otropic agent piracetam and is the most recently licensed AED (Genton and Van Vleymen, 2000). Results of clinical trials suggest that LEV is effective against partial seizures with or without secondary generalisation (Bialer et al, 1999), although extensive clinical use of the drug is

required to determine its full spectrum of clinical activity (Kwan et al, 2001). LEV would appear to have a unique mode of action which, at this time, is unclear. Preclinical investigations have demonstrated that LEV does not interact with Na^+, Ca^{2+} or K^+ channels or with GABA and glutamate neurotransmitter systems (Noyer et al, 1995; Sills et al, 1997). However, LEV has been found to reduce GABA turnover in the striatum of the rat by increasing GABA-T activity and reducing GAD activity (Loscher et al, 1996).

In acute animal seizure models, such as MES and PTZ induced seizures, LEV is ineffective at doses of up to 540 mg/kg (i.p.). However, this lack of anticonvulsant activity against acute seizures is contrasted by the protection afforded in amygdala and corneally kindled animals, DBA/2 mice and other genetic models of epilepsy (Loscher et al, 1996). The metabolism of LEV is independent of the cytochrome P450 system and so there are no pharmacokinetic interactions with other drugs (Patsalos, 2000)

FELBAMATE

Felbamate (FBM) was licensed in the USA for use as monotherapy and adjunctive therapy in refractory partial onset seizures in adults and children in July 1993. FBM has multiple mechanisms of action. It potentiates GABA responses at the $GABA_A$ receptor complex, blocks Na^+- and dihydropyridine-sensitive Ca^{2+}-channels and interacts with the strychnine-insensitive glycine modulatory site on the NMDA receptor (McCabe et al, 1993; Rho et al, 1994; White et al, 1995). FBM has been shown to be effective in a variety of animal seizure models including PTZ, NMDA and MES induced seizures in mice (Swinyard et al, 1986).

Over 100,000 patents received FBM in the first year following its launch. Shortly after, however, cases of aplastic anaemia and hepatotoxicity emerged and the use of the drug has been limited to patients with severe intractable epilepsy (Leppik and Wolff, 1995; Wilson and Brodie, 1996; Pellock and Brodie, 1997).

1.5 Remacemide

There is now convincing neurochemical and neurophysiological evidence to suggest that increased excitatory neurotransmission is involved in epilepsy (Meldrum, 1991; Loscher et al, 1993; Scheyer, 1998). As described in section 1.4, many of the currently available drugs act by increasing the effects of GABA, either by elevating GABA concentrations or by modifying its postsynaptic action (Rogawski and Porter, 1990). The other major group of drugs are those which block voltage-gated Na^+ channels. These drugs are classed as those which decrease excitatory transmission (Macdonald and Kelly, 1994).

It is known that glutamatergic synapses play a critical role in all epileptic phenomena and enhanced activation of post-synaptic glutamate receptors is proconvulsant (Chapman, 1998). Consequently, antagonists of NMDA receptors are powerful anticonvulsants in many animal models of epilepsy (Tauboll and Gjerstad, 1998). Early attempts to pharmacologically modulate glutamatergic transmission resulted in anticonvulsant compounds such as dizocilpine (MK801) and phencyclidine. Preclinical testing of these agents generally showed efficacy but also demonstrated severe side effects including

neuropathological changes (vacuolisation of neuronal cytoplasm in the neocortex) and significant behavioural malfunctions (McNamara et al, 1988; Olney, 1989; Willetts et al, 1990). A clinical application of pure glutamate antagonists has not yet been established (Chapman, 1998).

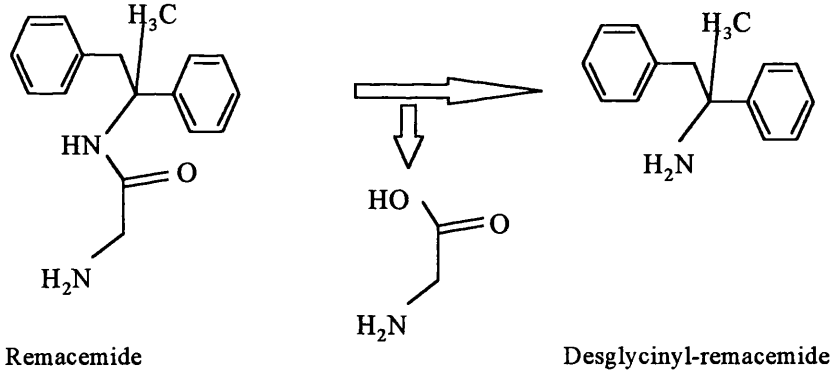
1.5.1 Background and pharmacology

Remacemide hydrochloride (RMD; (±) 2-amino-N-(1-methyl-1,2-diphenylethyl)-acetamide monohydrochloride) emerged from a drug screening programme conducted by Fisons' laboratories (now AstraZeneca) in conjunction with the National Institutes of Health AED development programme (Palmer et al, 1993). It resulted from a strategy to create compounds with a similar three-dimensional structure to phenytoin (Rogawski and Porter, 1990). RMD is a racemic mixture of two enantiomers that do not have clinically meaningful stereoselective pharmacological properties (Schachter and Tarsy, 2000). The drug undergoes desglycination to form desglycinyl-remacemide (DGR; formerly known as AR-R12495; figure 2). The hydrolase enzymes responsible for this conversion exist in almost all peripheral tissues (Heyn et al, 1994b), although the majority of the conversion is said to take place at the blood/brain barrier, resulting in an accumulation of DGR in the brain (Heyn et al, 1994a).

Conversion of RMD to DGR results in the production of glycine, an inhibitory neurotransmitter and co-agonist at the NMDA receptor (Stone, 1995). A number of other RMD metabolites have been identified in man, rodents and dogs including ARR-14464 and ARR-14330. These metabolites are also

active although the concentrations required for a pharmacological action are never likely to be reached following RMD administration (Palmer et al, 1992).

A unique dual mechanism of action has been proposed for RMD and DGR (Schachter and Tarsy, 2000). Both compounds inhibit the sustained repetitive firing of cultured spinal cord neurones (Wamil et al, 1996) and reduce [^{3}H]-batrachotoxin binding (Palmer et al, 1992), actions associated with a blockade of voltage-gated Na^+ channels. DGR was shown to be the more potent of the two compounds with an IC_{50} of 1.2 μM compared to an IC_{50} of 7.9 μM calculated for the parent drug (Wamil et al, 1996).



Remacemide Desglycinyl-remacemide

Figure 2: The biotransformation of remacemide to desglycinyl-remacemide

In addition to the proposed action on Na^+ channels, investigations have shown that both RMD and DGR inhibit the binding of [^{3}H]MK801 (Palmer et al,

1992), a channel blocking ligand at NMDA receptors (Huettner and Bean, 1988). As with the action on Na^+ channels, DGR appears to be the more active moiety at the NMDA receptor with an IC_{50} for the displacement of MK801 of 0.48 μM compared to 68 μM for RMD. There does appear, however, to be slight differences in the mechanism of block exerted by the two compounds (Subramaniam et al, 1996). The block produced by DGR was found to be strongly use- and voltage-dependent and could be occluded by Mg^{2+} (figure 1), indicating that it occurs by an open channel mechanism. More recent evidence also suggests an open channel block with DGR (Ahmed et al, 1999). Although RMD is also said to displace MK801, the block exerted was found to be only partially voltage-dependent, suggesting that at least a portion of its action occurs via an non-channel blocking mechanism. Previous studies have reported that RMD has slight affinity for both the glutamate and glycine binding sites on the NMDA receptor (Garske et al, 1991), whereas DGR displacement of [^{3}H]MK801 has been shown to be unaffected by glutamate or glycine (Ray et al, 1992). Despite RMD having a much higher IC_{50} than DGR in this model, the block exerted by RMD was found to be much more rapidly produced and prolonged under periods of depolarisation. Therefore, *in vivo*, the contribution of the parent drug to overall NMDA receptor blockade cannot be discounted (Subramaniam et al, 1996).

The neurochemical effects of DGR have also been investigated (Leach et al, 1997d). Repeated dosing of DGR to mice was found to increase the activity of GABA-T, the enzyme responsible for the breakdown of GABA to succinic semialdehyde (SSA) and glutamate. In addition, DGR was also found to

decrease the activity of GAD, the enzyme responsible for the synthesis of GABA from glutamate. Despite these apparent proconvulsant actions, DGR was seen to slightly reduce brain glutamate concentrations although the results obtained were not statistically significant (Leach et al, 1997d).

1.5.1 *Preclinical evaluation*

RMD and DGR have been tested in many animal models and a number of reviews have been published (Muir and Palmer, 1991; Clark et al, 1995; Davies, 1997; Schachter and Tarsy, 2000).

Both RMD and DGR have been found to be effective against MES-induced seizures in mice and rats with ED_{50} values of 21.5 and 17.1 mg/kg (i.p.) in mice, respectively (Palmer et al, 1992). Following administration of the approximate ED_{98}, RMD continued to protect against MES seizures in 20% of animals after 4 hours. DGR offered 40 - 50% protection after 4 hours (Palmer et al, 1992). The MES-seizure test is said to be indicative of a likely clinical efficacy against generalised tonic-clonic seizures (Fisher, 1989).

Both RMD and DGR prevent against the convulsions produced by NMDLA with ED_{50} values of 57.4 and 32.4 mg/kg, respectively. Protection against NMDLA-induced mortality was also afforded by both compounds, with ED_{50} values of 22.4 and 17.1 mg/kg respectively (Palmer et al, 1992).

Studies in the kindling model of epilepsy have found that RMD offers only 8% protection from established seizures in rats at 400 mg/kg. CBZ was seen

to protect 40% of animals at 200 mg/kg. RMD was previously found to be ineffective against the development of kindled seizures (Garske et al, 1991). DGR, however, was found to reduce seizure development (Palmer et al, 1992).

RMD is ineffective against seizures induced by PTZ, bicuculline, picrotoxin and strychnine (Garske et al, 1991). However, efficacy is observed for both compounds against seizures induced by kainate and 4-AP (Cramer et al, 1994) and against audiogenic seizures in the DBA/2 mouse model (Clark et al, 1995). In addition, both RMD and DGR have shown efficacy in the WAG/Rij strain of rat, a genetic model of absence epilepsy. This finding is unexpected as, traditionally, sodium channel blockers such as CBZ and PHT are without effect in this model (Van Luitelaar and Coenen, 1995).

In vitro investigations have found that RMD and DGR are almost equipotent in their limitation of sustained repetitive firing in CA1 cultured neurones from rats (Norris and King, 1997a). This is in contrast to the different profiles observed in cultured rat spinal cord neurones (Wamil et al, 1996). The reason for this difference is unclear but could reflect species variation, differential neuronal susceptibility, variations in recording conditions and drug exposure times (Norris and King, 1997b). In the rat hippocampal slice model, RMD was found to have no significant effects on evoked responses, recurrent inhibition, induction of long-term potentiation, penicillin-induced discharges or veratridine (VTD)-induced depolarisations (Garske et al, 1991). In

contrast, DGR was seen to decrease the amplitude and rate of rise of action potentials in the hippocampal slice (Norris and King, 1997b).

Consistent with its proposed mechanism of action, DGR has been found to significantly reduce spontaneous depolarisations and associated afterpotentials in cortical wedges prepared from DBA/2 mice subjected to Mg^{2+}-free conditions. NMDA- but not AMPA-induced depolarisations were also reduced in this model (Hu and Davies, 1995). DGR has also been shown to significantly reduce both VTD- and K^+-induced release of glutamate and aspartate from cortical slices prepared from mice (Srinivasan et al, 1995). The inhibition of K^+-stimulated glutamate release required much higher concentrations of DGR than those required to decrease VTD-induced glutamate release (Srinivasan et al, 1995). This finding is consistent with suggestions that DGR is a relatively weak NMDA antagonist and therefore inhibits the entry of Ca^{2+} into neurones (Muir and Palmer, 1991; Clark et al, 1995).

Neuronal impairment investigations have found that RMD is less toxic than DGR (Palmer et al, 1992). This is perhaps to be expected due to the greater efficacy of DGR at the NMDA receptor.

1.5.2 *Clinical findings*

RMD and DGR are weak bases with good water and lipid solubility (Clark et al, 1995). When taken orally, RMD is quickly and extensively absorbed from the gastrointestinal (GI) tract with approximately 74% of the drug bound to

plasma proteins in man. Due to its physicochemical properties, RMD is subjected to extensive metabolism. First pass metabolism, although high in rodents (>85%), is relatively low in humans (~10%; Clark et al, 1995). In man, RMD is said to reach peak serum concentrations within 1 - 2 hours of administration (Patsalos and Sander, 1994) with DGR levels reaching maximal 2-3 hours following dosing (Stables et al, 1995). DGR has a longer half-life than RMD, reportedly 12 - 24 hours compared to 3 - 4 hours respectively, (Stables et al, 1995). Steady-state concentrations of RMD and DGR are reached within 24 hours and 3 - 4 days following initiation of treatment, respectively (Clark et al, 1995).

In healthy volunteers, doses of up to 300 mg RMD are well tolerated. Above this dose, lightheadedness, headache, fatigue, and GI upset can be observed with increasing frequency (Muir and Palmer, 1991; Brodie et al, in prep.). Similar side effects have recently been reported in children following RMD treatment (Besag et al, 2001).

In an early double-blind, placebo-controlled crossover study of 28 patients with refractory epilepsy, RMD reduced seizure frequency by 33% with 4/28 of the patients being seizure-free during the RMD treatment section (Crawford et al, 1992). In another early monotherapy study, RMD (600 mg/day) was administered to patients undergoing acute withdrawal of their AEDs during evaluation for possible epilepsy surgery. Results indicated that seven of the nine patients suffered fewer partial seizures in the 24 following RMD treatment compared to the preceding 24 hours (Alarcon et al, 1992).

Two major trials assessing the efficacy (Richens et al, 2000) and safety (Chadwick et al, 2000) of RMD as add-on therapy in refractory epilepsy patients have been conducted in the UK. Richens and colleagues reported a significant reduction in seizure frequency during treatment with 600 mg/day RMD when compared with placebo. Analysis of the individual seizure types in the study showed no statistical differences. No patients were found to be seizure-free during the placebo arm of the treatment, however, 13% of patients were seizure-free during treatment with RMD (Richens et al, 2000). Of the 28 patients in the study, 24 were taking CBZ as one of their concomitant AEDs.

Only one major, international, multicentre trial of RMD monotherapy has been conducted. The SEReNE (Sequential Evaluation of RMD in Newly diagnosed Epilepsy) trial compared 600 mg/day RMD with 600 mg/day CBZ in newly diagnosed epilepsy patients (Brodie et al, in prep). A total of 570 patients were randomised to receive RMD (288) or CBZ (282). Patient demographics were well matched in terms of sex, age and race. The results suggest that CBZ was significantly superior at increasing the time to first, second, third and fourth seizures following randomisation of treatment (Brodie et al, in prep).

Pharmacokinetic interactions are important factors to consider when co-administering AEDs (Patsalos, 1998). As a result, the effects of co-administration of various AEDs on the pharmacokinetics of RMD have been investigated (Leach et al, 1996a; Leach et al, 1997a; Leach et al, 1997b;

Mawer et al, 1999). RMD itself undergoes cytochrome P450 (CYP450) dependent oxidation and induces the activity of various CYP450 isoforms in rats and mice (Palmer et al, 1992; Riley et al, 1995). The main isoforms believed to be affected are those which are also inducible by PB administration, namely those from the CYP2B and 3A families (Palmer et al, 1993). There appears to be no evidence of autoinduction (Leach et al, 1996a; Leach et al, 1997b). The addition of RMD to already established CBZ therapy results in a 22% increase in CBZ concentrations although levels of CBZ-10, 11 epoxide are unaffected (Leach et al, 1996a). In the same study, concentrations of RMD and DGR were reduced to 60 and 30% of control, indicating that DGR is more susceptible to the hepatic enzyme inducing properties of CBZ. A similar study found that co-administration of CBZ and RMD resulted in the need for CBZ dose withdrawal in 63% of patients (Mawer et al, 1999). Results with PHT co-administration were similar to those observed with CBZ (Leach et al, 1997b). Once again, RMD treatment was shown to increase the circulating concentrations of the co-administered drug. PHT is almost exclusively metabolised by the CYP2C9 and 2C19 isoforms (Levy et al, 1995). It is therefore possible that RMD or DGR inhibits the activity of one or both of these isoforms. PHT caused a decrease in circulating RMD and DGR levels. As with CBZ treatment, DGR appeared to be more susceptible to the enzyme inducing properties of PHT (Leach et al, 1997b). Co-administration with the hepatic enzyme inhibitor VPA has no affect on the pharmacokinetics of RMD, DGR or indeed VPA itself (Leach et al, 1997a).

In addition to the known effects against seizures, RMD exhibits neuroprotective properties in cerebral ischaemia (Bannan et al, 1994; Dyker and Lees, 1999; Halonen et al, 1999; Pisani et al, 2001). Investigations have also indicated that RMD treatment may be beneficial in patients suffering from either Parkinson's or Huntington's disease (Clarke and Feigin, 1999; Kieburtz et al, 1999; Alexi et al, 2000; Shoulson et al, 2000; Clarke et al, 2001; Schwid, 2001). It also appears to have efficacy as an analgesic, (Asghar et al, 2000).

In summary, clinical trials have found that RMD, when administered as add-on therapy is effective in reducing seizure frequency in patients who have been unsuccessfully treated with other AEDs (Richens et al, 2000). Monotherapy trials have demonstrated that CBZ is superior to RMD when comparing time to first, second, third and fourth seizures following randomisation (Brodie et al, in prep). CBZ and PHT, two known inducers of hepatic enzymes cause a decrease in the circulating concentrations of RMD and DGR (Leach et al, 1996a; Leach et al, 1997b). It appears from these studies that DGR is significantly more susceptible to the effects of this induction. RMD itself inhibits the metabolism of PHT and CBZ although cases of toxicity were predictable, rare and reversible following dosage reduction (Leach et al, 1997b; Mawer et al, 1999).

1.6 General aims

The aim of this project was to investigate the preclinical pharmacology of RMD and DGR. Particular emphasis was placed on the relative contribution of each moiety to the antiepileptic actions of the parent compound and the relative importance of individual mechanisms of action. In the development of RMD, it was assumed that additional inhibitory effects at the NMDA receptor might confer advantages for the drug over standard sodium channel blocking AEDs. This premise was subsequently challenged by clinical trial data, which emerged during the conduct of these studies, suggesting that RMD monotherapy was inferior to that with CBZ. In light of these observations, the preclinical pharmacological investigations described herein assumed greater significance. An attempt to formulate a hypothesis to explain why RMD, a drug with a promising preclinical profile and recognised clinical efficacy as add-on treatment, performed so poorly as monotherapy was subsequently undertaken.

Chapter 2

Materials and recurrent methods

2.1 MATERIALS

2.1.1 *Chemicals and reagents*

Adenosine diphosphate (ADP; sodium salt), 4-aminopyridine (4-AP), β-nicotinamide dinucleotide (β-NAD), β-NADH (reduced form; disodium salt), bovine serum albumin (BSA), calcium chloride ($CaCl_2$), DMI hydrochloride (10,11-dihydro-N-methyl-5H-dibenz[b,f]azepine-5-propanamine), dimethyl sulfoxide (DMSO), 7-ethoxycoumarin, EDTA (ethylenediaminetetra-acetic acid; free acid), EGTA (ethylene glycol-bis[β-aminoethyl ester]-N, N, N', N'-tetra-acetic acid), Ficoll, glycerol, gramicidin D, D-glucose, L-glutamic acid (monosodium salt), glycofurol, HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]), 5-hydroxytryptamine (serotonin), α-ketoglutarate (α-KG), magnesium sulphate ($MgSO_4$), NE, NMDA, pargyline (N-methyl-N-propargylbenzylamine), nomifensine (NMF; 1,2,3,4-tetrahydro-2-methyl-4-phenyl- 8-isoquinolinamine), pluronic F-127, potassium chloride (KCl), potassium EDTA (di-potassium salt), potassium phosphate (K_2HPO_4; monobasic), rotenone, SBFI-AM, sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), sodium dithionite ($Na_2S_2O_4$), TES (N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid), tetracaine, tris HCl, and veratridine (VTD), were all obtained from Sigma Chemical Company (Poole, UK).

Acetonitrile, ammonium chloride (NH_4Cl), chloroform, methanol, potassium dihydrogen orthophosphate ($KHPO_4$), potassium orthophosphate (KPO_4), sodium hydroxide (NaOH) and sucrose were bought from Fisher Scientific (Loughborough, UK). Hydrochloric acid (HCl), magesium chloride ($MgCl_2$),

perchloric acid ($HClO_4$) were purchased from Merck (UK). Ascorbic acid, trichloro-acetic acid (TCA) and Triton X-100 (iso-octylphenoxypoly-ethoxyethanol) were obtained from BDH (Poole, UK). Tetrodotoxin (TTX) was purchased from Tocris (UK). Dopamine (3-hydroxytyramine hydrochloride) was bought from Fluka Chemicals (UK). Ecoscint A scintillation fluid was purchased from National Diagnostics (Hull, UK). Coomassie Brilliant Blue G-250 protein dye was obtained from BIORAD (Hertfordshire, UK).

2.1.2 *Radioisotopes*

Dopamine (dihydroxyphenylethylamine hydrochloride, 3,4 -[ring-2, 5-6-^{3}H]), NE (norepinephrine hydrochloride, DL-[7-^{3}H(N)]), and 5-HT (hydroxytryptamine, creatinine sulfate, 5-[1,2-^{3}H(N)]) radioisotopes were purchased from New England Nuclear (Stevenage, UK).

2.1.3 *Pharmaceuticals*

RMD ((±)-2-amino-N-(1-methyl-1,2-diphenylethyl) acetamide hydrochloride), DGR ((±)-1-methyl-1,2-diphenylethylamine-monohydrochloride), AR-R15896 ((+)-α-phenyl-2-pyridine-ethanamine dihydrochloride) and the internal standard (2-(methyl-amino)-*N*-[1-phenyl-1-(phenylmethyl)propyl]-acetamide hydrochloride) for the HPLC assay were obtained from AstraZeneca, R&D Charnwood (Loughborough, UK). CBZ (5H-dibenz(b, f) azepine-5-carboxamide), PB (5 ethyl-5-phenyl-2, 4, 6-trioxohexahydropyrimidine) and PHT (5, 5-diphenyl-2, 4-imidazolidinedione) were purchased from Sigma Chemical

Company (Poole, UK). LTG (6-(2, 3-dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine) was obtained from GlaxoWelcome R&D (Stevenage, UK).

2.1.4 *Equipment*

Centrifugation: Blood samples were centrifuged on a Wifug haemicrofuge. All other samples were centrifuged in a Heraeus Sepatec 15R Biofuge (<1,000 x g) or in a Sorvall OTD-50 ultracentrifuge (> 1,000 x g).

Cytochrome P450 analysis: The enzyme content of samples was determined using a Cecil CE 594 Double Beam Spectrophotometer. Analysis of 7-hydroxycoumarin activity was measured using a Perkin Elmer LS-5 Luminescence Spectrometer.

Hippocampal slice studies: Recordings were made using a Neurolog system. Results were integrated on a Hitachi VC-6023 digital storage oscilloscope and displayed on a Reba Instruments PAR 1000 chart recorder.

Homogenisation: Whole brain samples were homogenised using a Polytron PT 1200 CL homogeniser. Cortex and liver samples were homogenised using a motorised teflon pestle and glass mortar.

HPLC Experiments: The chromatography equipment consisted of a Gilson 231 sample injector and Gilson 401 dilutor, a Shimadzu SPD-6A UV spectrophotometric detector (218 nm), a Shimadzu LC-10AT liquid

chromatograph and a Shimadzu C-R6A chromatopac recorder. The HPLC column was a Sphereclone C_6 (100 x 4.6 mm; 3 μm) which was used at room temperature. Solid-phase extraction columns were benzenesulfonic acid columns with pore size Å54 (100 mg; 1 ml columns).

Maximal electroshock: An Ugo Basile 7800 ECT unit was employed.

Monoamine uptake experiments: A Brandel harvester was employed for rapid filtration purposes. Liquid scintillation counting was carried out on a Canberra Packard TriCarb 2100TR liquid scintillation counter.

Protein assay and measurements of glutamate dehydrogenase activity: Analysis was performed using a Dynex MRX version 2.01 microplate reader. Results were integrated on a Viglen Contender ATX P5/166 MMX computer using Revelation (version 3.2) software.

Sodium uptake measurements: Analysis was performed using a Perkin Elmer LS-5 luminescence spectrometer.

2.2 Recurrent methods

2.2.1 *Animal use*

All the animals used in this project were purchased from Harlan Olac, Bicester, UK. Animals were housed in the Central Research Facility at the University of Glasgow where they were exposed to a controlled temperature and humidity

environment. All animals had access to food and water *ad libitum* and were subject to a 12 hour light/dark cycle.

2.2.2 *Determination of protein concentration*

The BIORAD method is a sensitive test of protein concentration which is particularly useful when samples contain low amounts of protein. The method utilises the colour change of a dye (Coomassie Brilliant Blue G-250) in response to protein. Standards were prepared, in duplicate, covering the range of 0.5 - 2 μg/ml using BSA. Unknown samples, again analysed in duplicate, were diluted into this range. BIORAD protein assay dye (100 μl) was added to standards and unknown samples. Following vortex-mixing, 200 μl of each standard and sample was transferred to a 96-well plate and read at 595 nm on the microplate reader. The results were corrected for dilution, averaged, and expressed in mg/ml.

Chapter 3

Pharmacokinetics of remacemide and desglycinyl-remacemide in brain tissue and serum

3.1 Introduction

Preclinical studies of AEDs often fail to consider the brain concentrations at which the candidate therapies exert their pharmacological effects (Santangeli et al, 2000). It is important to know the pharmacokinetics of compounds so that future dosing schedules can be developed without risk of toxicity. In addition, pharmacokinetic interactions, which are common with AED administration (Patsalos, 1998), can also involve active metabolites (Leach et al, 1996a) and so baseline levels of these compounds have to be known.

As discussed in section 1.5, the actions of RMD can be largely attributed to DGR, an active metabolite. Following acute RMD administration in man, DGR is present in much lower concentrations in plasma compared to the parent compound (Clark et al, 1995). These findings are consistent with reports that the majority of the conversion of RMD to DGR occurs at the blood/brain barrier, resulting in an accumulation of DGR in the brain (Heyn et al, 1994a).

3.2 Aims

The aim of the following experiments was to construct pharmacokinetic profiles of RMD and DGR in serum and brain tissue. An insight as to the extent of RMD conversion to the reportedly more active DGR would have applications for further concentration-effect studies and AED interaction investigations.

3.3 Methods

The following high-performance liquid chromatography (HPLC) assay was adapted from the previously published human plasma methodology (Flynn and O'Brien, 1992; Wilson et al, 1992).

3.3.1 *Standards and solutions*

Aqueous stock solutions of RMD (0.5 mg/ml), DGR (0.5 mg/ml) and internal standard (0.2 mg/ml) were prepared, aliquoted and stored at -70^{0}C until required. Two monobasic potassium phosphate buffers (0.5 M and 0.05 M) were prepared weekly with the pH adjusted to 2.5 and 3.3, respectively. Mobile phase (29:71 acetonitrile / 0.05 M potassium phosphate buffer) and working standard solutions of RMD (50 μg/ml) and DGR (50 μg/ml) were prepared daily as required. Prior to use, the mobile phase was passed through an aqueous filter (pore size 0.45 μm; Millipore, France) and degassed with helium. The flow rate of the mobile phase was 1 ml/min.

3.3.2 *Preparation of tissue*

For serum samples, truncal blood samples obtained from mice were centrifuged for one minute. Following collection, serum and brain samples were immediately placed in liquid nitrogen. The assay technique was validated with the use of naïve mouse brains (adult male ICR mice; 25-30 g) homogenised at high speed for 15 seconds in 15 volumes (v/w) of 1% perchloric acid. Blank homogenates were spiked with known concentrations of RMD and DGR. Internal standard (50 μl of 0.2 mg/ml stock) was then added to each sample. Homogenates were vortex mixed and then centrifuged

at 800 x g for 15 minutes at 4°C. One ml of the supernatant from each sample was then transferred into a tube containing 1.5 ml of 0.5 M potassium phosphate buffer and, thereafter, samples were gently mixed prior to extraction. Unknown brain samples were treated in the same manner, although no drug was added to the homogenate. It was at this point that serum samples entered the sample preparation method. In cases where less than 1 ml of serum was obtained, the volume was adjusted to 1 ml with 0.5 M potassium phosphate buffer and samples extracted without further intervention.

3.3.3 *Solid-phase extraction*

Solid phase extraction columns were conditioned immediately prior to use by 1ml methanol, 1ml elution fluid (60:40 acetonitrile / 0.05 M sodium bicarbonate) and 2 ml 0.05 M potassium phosphate buffer. Individual samples were added and washed with 1 ml 0.05 M potassium phosphate buffer. The columns were then rinsed with 1.5 ml methanol, 2 ml distilled water and allowed to dry. The analytes of interest were subsequently eluted from the columns into Pyrex borosilicate glass culture tubes (10 x 75 mm) with 3 x 0.25 ml elution fluid with the columns being allowed to dry between each application. Finally, the culture tubes were vortex mixed, samples transferred into glass autosampler vial inserts, and 75 µl injected into the chromatography system.

Limits of detection and quantification were determined by analysing samples of brain homogenate that had been spiked to a known concentration (0.5 -

100 ng/ml) with the minimum concentrations giving visible and measurable peaks noted.

3.4 Experimental protocol

Time study: Adult, male ICR mice were randomised into 15 groups of 6 animals. Each group was administered a single, acute injection of 40 mg/kg RMD (i.p.) and sacrificed at a different time point (5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 360 or 480 minutes) following injection, with brain and truncal blood samples obtained.

Concentration study: Adult, male ICR mice were randomised into 6 groups of 5 animals. One animal from each group was administered a single dose of RMD (5, 10, 20, 40 or 80 mg/kg; i.p.) and were again sacrificed at a variety of time points (15, 30, 60, 120, 240 or 480 minutes). Brain and truncal blood samples were obtained. This protocol was repeated on 6 occasions.

RMD and DGR concentrations in serum and brain were measured according to the methods described in section 3.3.

3.5 Data analysis and statistics

Statistical analysis was performed using MINITAB for Windows version 10.1 on a Viglen P2 400 microcomputer. Results are expressed as the mean of absolute concentrations (μg/ml, serum; μg/g, brain) ± the standard error of the mean (SEM).

3.6 Results

RMD was detectable in serum 5 minutes after intraperitoneal administration (40 mg/kg). Serum RMD concentrations appeared to peak at 15 minutes post-dosing (28.1 μg/ml; ± 2.3) with no drug detected after 90 minutes (figure 3).
DGR was not detected in serum until 10 minutes after i.p. administration of the parent compound (figure 3). Serum concentrations of DGR appeared to be maximal at 60 minutes post-dosing (13.3 μg/ml; ± 1.3) with no drug detected after 240 minutes.

Brain sample analysis suggested that RMD and DGR concentrations reached maximal levels at 20 minutes (10.3 μg/g; ± 0.2) and 60 minutes (17.9 μg/ml; ± 0.9) post-dosing, respectively (figure 4). RMD was not detectable in brain tissue 240 minutes following dosing. DGR was still measurable at 8 hours post-dosing.

RMD levels in serum decreased from 15 minutes following dosing (5 - 80 mg/kg RMD; figure 5). RMD was only detected in serum at 4 hours following 80 mg/kg administration. DGR levels in serum appeared to peak at 60 minutes post-dosing, independent of the dose of RMD administered (figure 6). Only following 40 and 80 mg/kg of RMD could DGR be measured more than 8 hours after administration. Half-lives of 62 and 250 minutes in serum were calculated for RMD and DGR, respectively, following an 80 mg/kg dose of RMD.

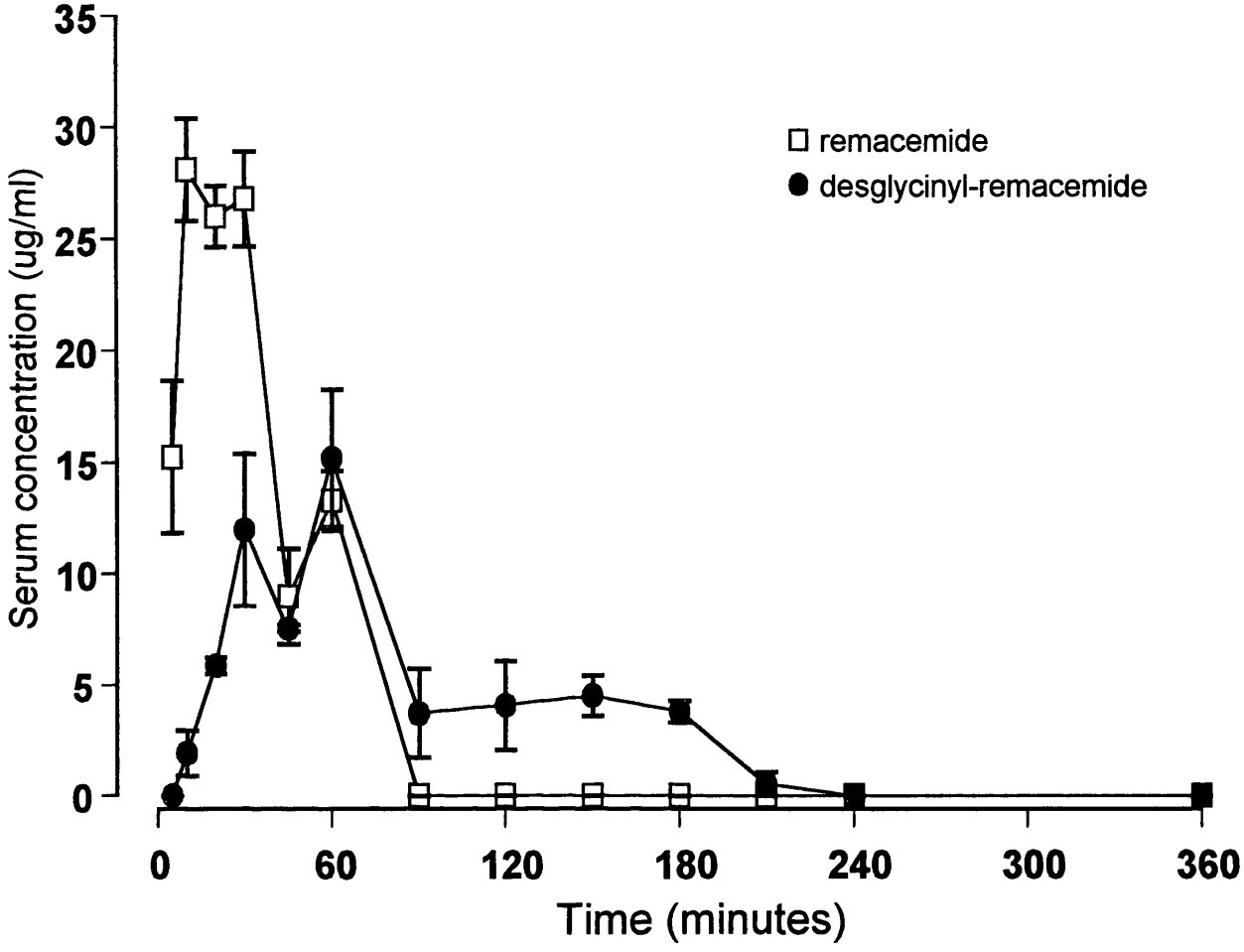


Figure 3: Pharmacokinetic profiles of remacemide and desglycinyl-remacemide in mouse serum 0 - 360 minutes after a single intraperitoneal injection of 40 mg/kg remacemide. Results (n = 6) are expressed as the mean of absolute serum concentrations (μg/ml) with error bars denoting the standard error of the mean.

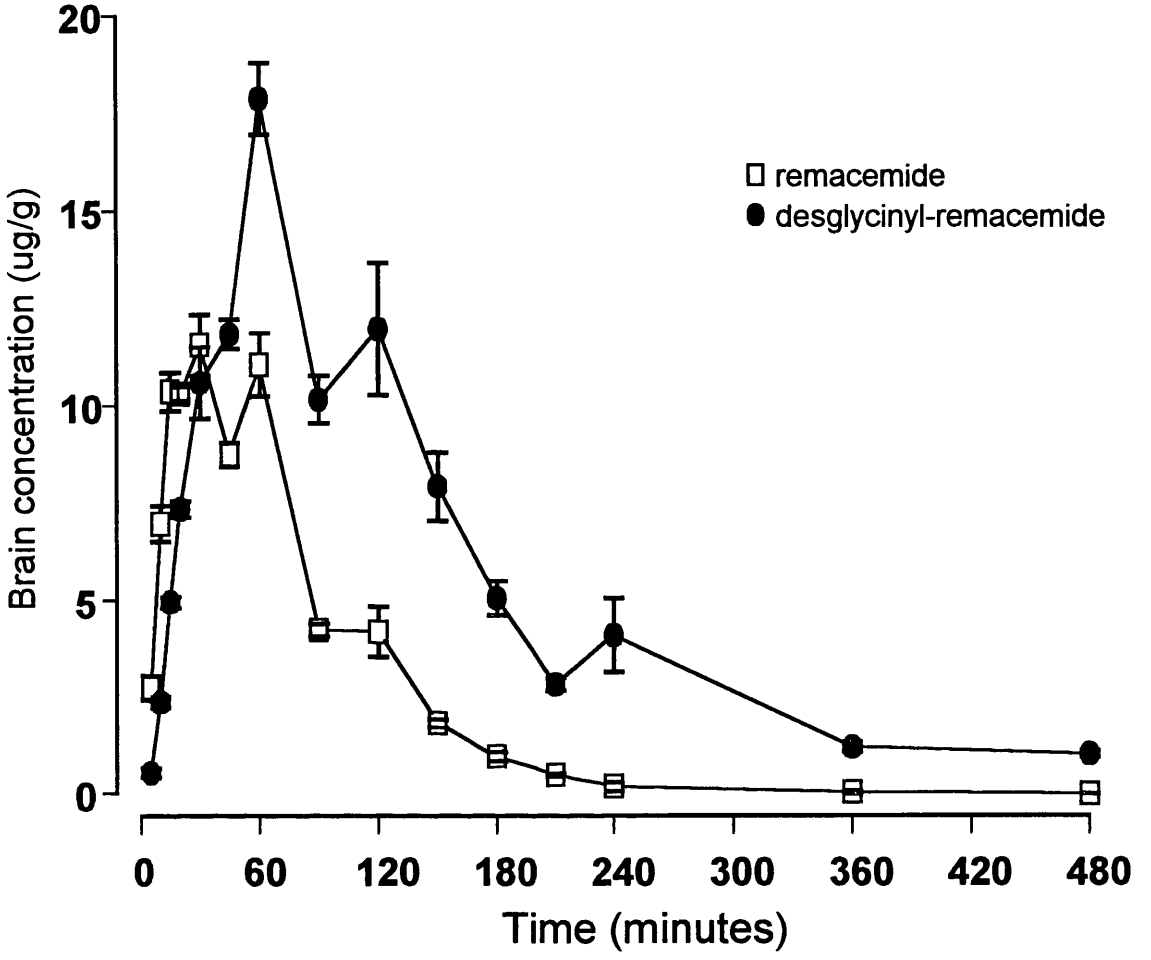


Figure 4: Pharmacokinetic profiles of remacemide and desglycinyl-remacemide in mouse brain 0 - 360 minutes after a single intraperitoneal injection of 40 mg/kg remacemide. Results (n = 6) are expressed as the mean of absolute brain concentrations (μg/g) with error bars denoting the standard error of the mean.

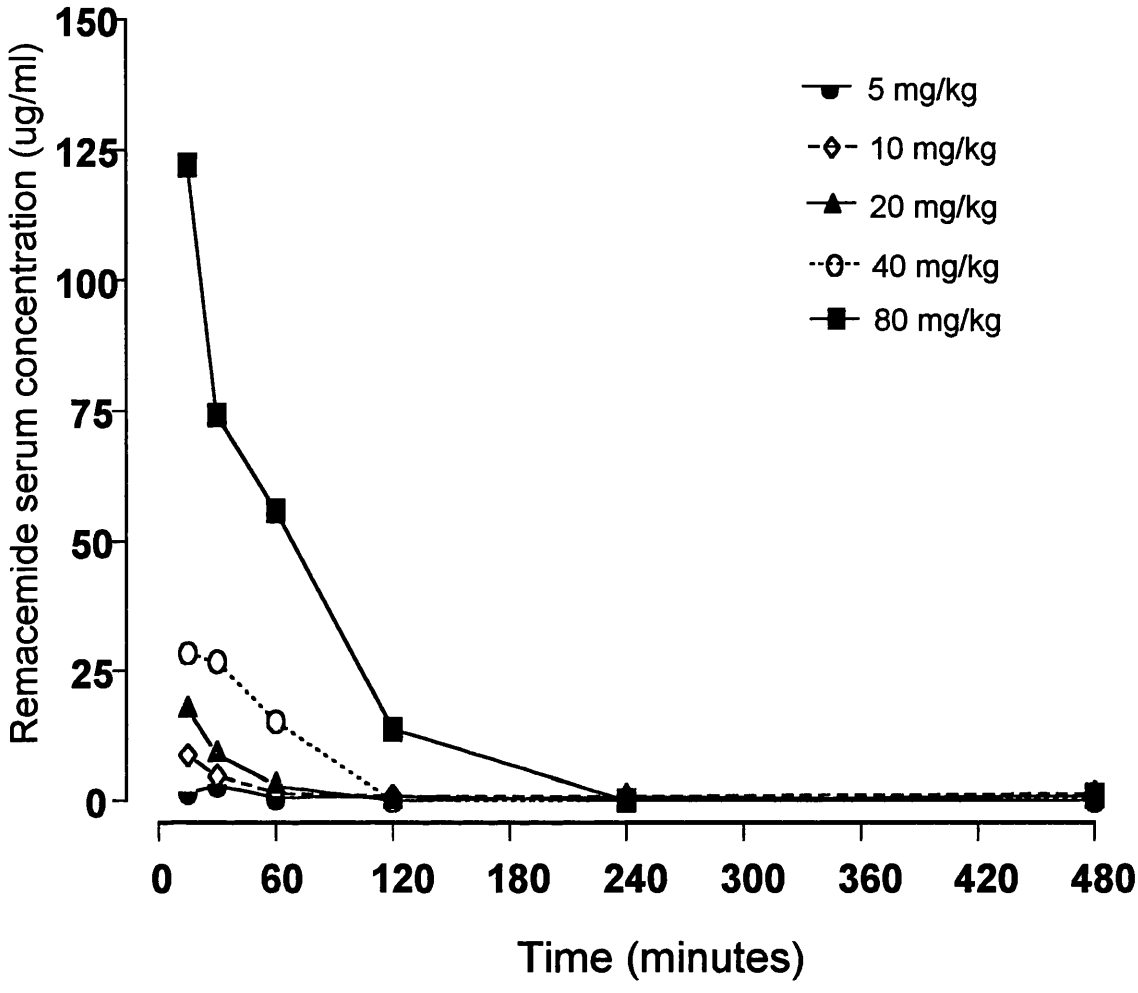


Figure 5: Pharmacokinetic profile of remacemide in mouse serum following a single intraperitoneal injection (5 - 80 mg/kg). Results (n = 6) are expressed as the mean of absolute serum concentrations (μg/ml). Error bars have been omitted for ease of comprehension.

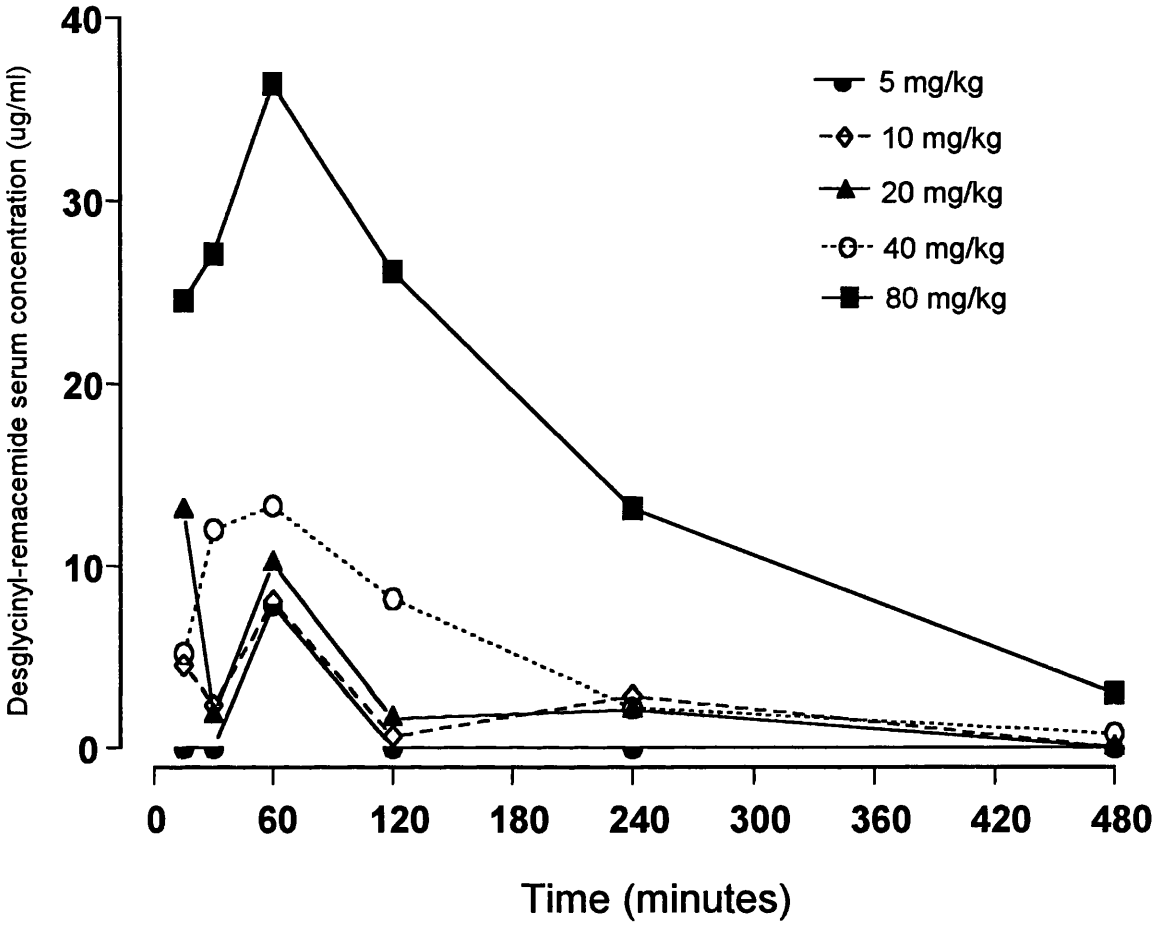


Figure 6: Pharmacokinetic profile of desglycinyl-remacemide in mouse serum following a single intraperitoneal injection of remacemide (5 - 80 mg/kg). Results (n = 6) are expressed as the mean of absolute serum concentrations (μg/ml). Error bars have been omitted for ease of comprehension.

In brain, RMD concentrations reached maximal at 30 minutes post-dosing, irrespective of the dose of RMD administered (figure 7). RMD was only detected in brain tissue after 4 hours following 80 mg/kg administration.

With the exception of the 10 mg/kg RMD group, DGR levels in the brain were maximal at 60 minutes post-dosing (figure 8). DGR levels following 40 and 80 mg/kg RMD were detectable 8 hours following administration.

3.7 Discussion

Previously published literature has shown that DGR is more active than RMD in a variety *in vitro* models (Clark et al, 1995; Davies, 1997). The above studies were conducted to determine to what degree RMD is converted to DGR in mice following acute administration of RMD. The results obtained would be useful for further concentration effect studies and would give an indication of the concentrations of RMD and DGR to use for further *in vitro* experiments.

Results from serum measurements of RMD and DGR suggest that conversion of RMD to DGR occurs in the peripheral tissues. The results also suggest that RMD is rapidly and extensively converted to DGR in the brain following acute administration to mice. The pharmacokinetics of DGR outlast those of the parent compound both in serum and brain.

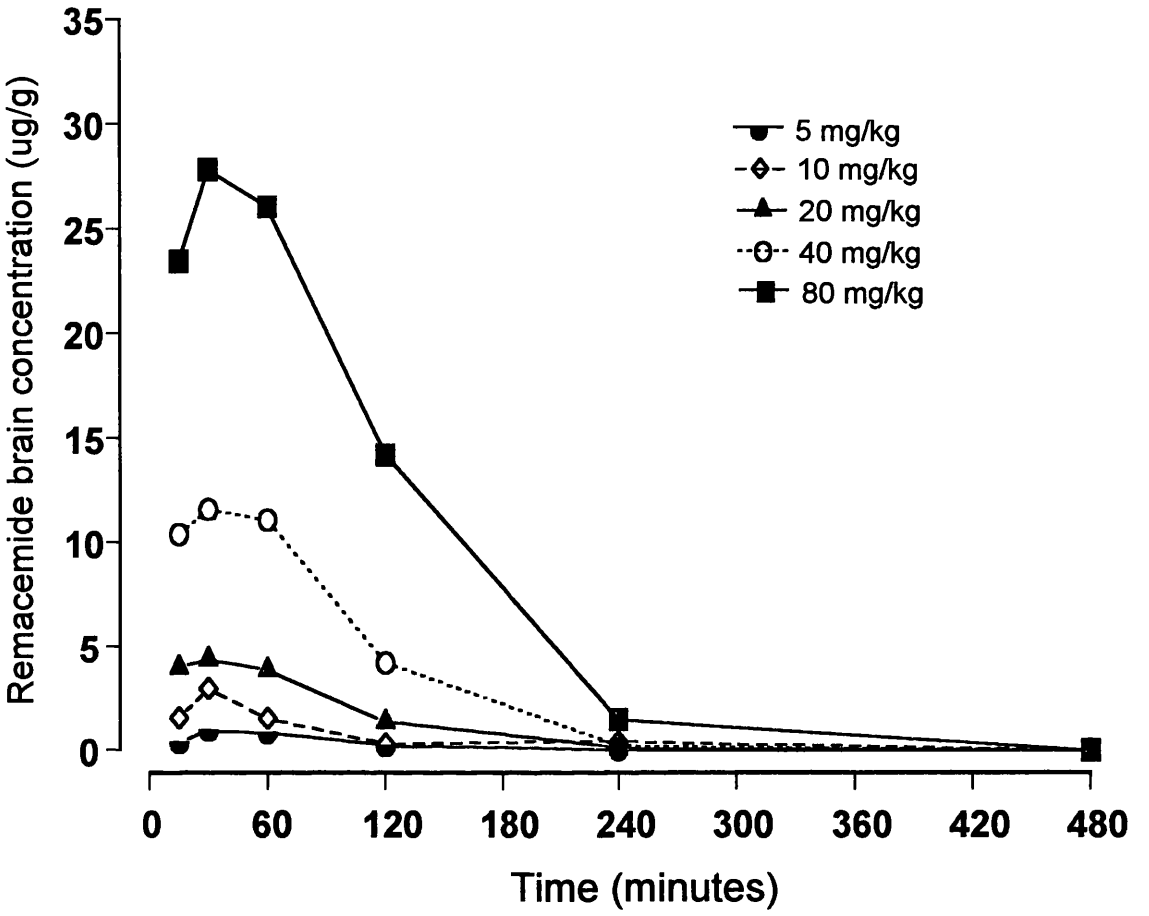


Figure 7: Pharmacokinetic profile of remacemide in mouse brain following a single intraperitoneal injection (5 - 80 mg/kg). Results (n = 6) are expressed as the mean of absolute brain concentrations (μg/g). Error bars have been omitted for ease of comprehension.

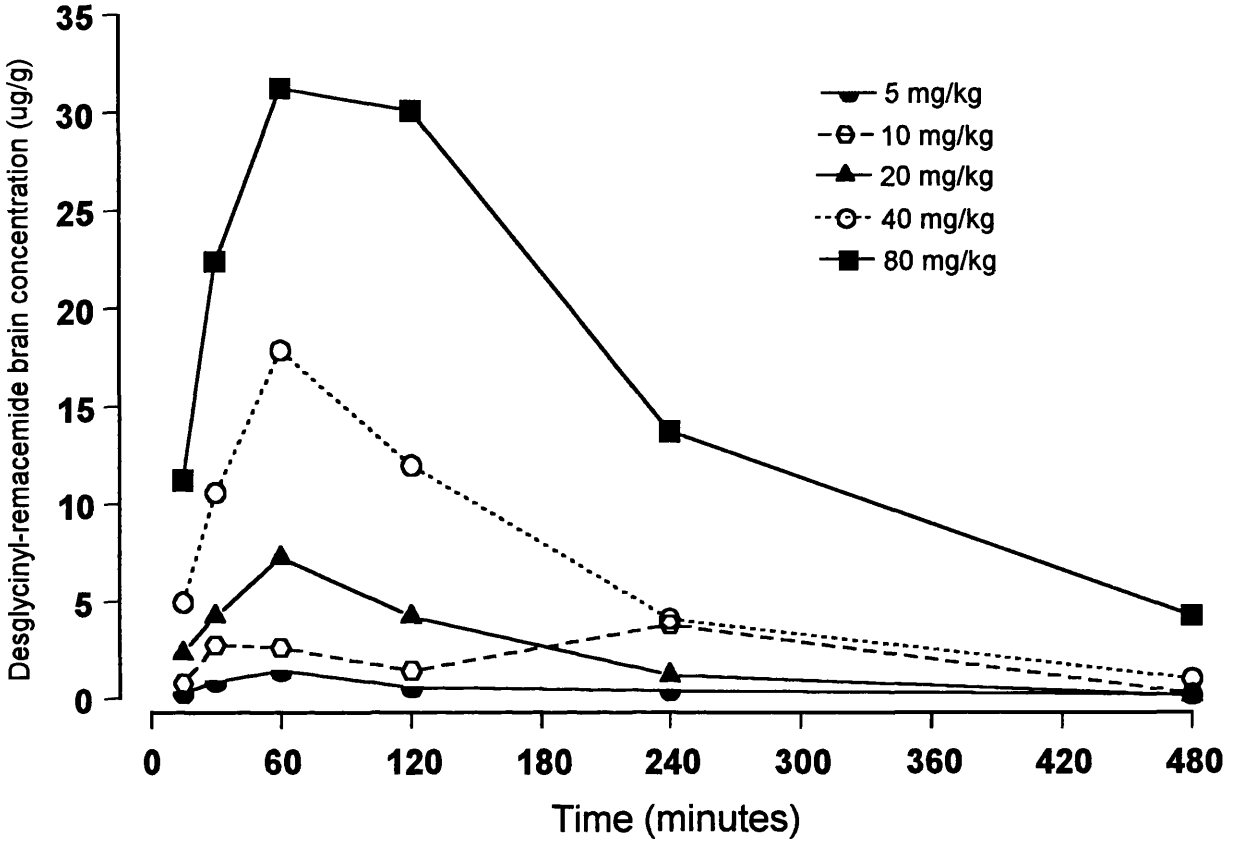


Figure 8: Pharmacokinetic profile of desglycinyl-remacemide in mouse brain following a single intraperitoneal injection of remacemide (5 - 80 mg/kg). Results (n = 6) are expressed as the mean of absolute brain concentrations (μg/g). Error bars have been omitted for ease of comprehension.

In man, RMD reaches peak serum concentrations within 1 - 2 hours following oral administration (Patsalos and Sander, 1994). DGR is said to reach maximal concentrations at 2 - 3 hours (Stables et al, 1995). Clinical studies have also found that CSF concentrations of DGR are much higher than those of RMD (Lindstrom et al, 1996). Our measurements of RMD and DGR concentrations in the brain concur with these findings. The terminal half-lives of RMD and DGR in man are reported to be approximately 3.8 and 12.9 hours, respectively (Palmer et al, 1993). Following multiple dosing, a greater degree of accumulation is observed with DGR than RMD, as would be expected from its half-life. In the investigations above, half-lives of 62 and 250 minutes were calculated for RMD and DGR, respectively, following an 80 mg/kg dose of RMD. Terminal half-lives of RMD and DGR in the rat are reported to be 42 and 150 minutes (Clark et al, 1995).

Results from the above study indicate that, when maximal, RMD levels in the brain were found to be 7.45% of those measured in the serum with DGR concentrations in the brain 25.6% of those in the serum. There are a number of reasons why the ratios of RMD to DGR may differ between brain and peripheral circulation. As previously discussed, the blood/brain barrier is said to be an important site for the conversion of RMD to DGR (Heyn et al, 1994a). It is possible that the conversion at this site results in the apparent accumulation of DGR in the brain. Alternatively DGR may simply pass into the brain via passive diffusion more easily than RMD by virtue of its smaller molecular size. If the actions of RMD and DGR differ in any way, as has been

previously suggested, it is possible that the ratio of RMD to DGR may be critical to the actions of the drug.

In conclusion the kinetics of DGR appear to outlast those of the parent compound both in serum and brain with a significantly greater half-life calculated. This is in agreement with previous studies (Clark et al, 1995). The findings presented in this chapter have consequences for later concentration-effect studies and AED interaction investigations.

Chapter 4

The effects of hepatic enzyme induction on the pharmacokinetics and pharmacodynamics of remacemide

4.1 Introduction

A drug interaction can be described as the modification of the effect of one drug by prior or concomitant administration of another (Patsalos, 1998). Drug interactions can be divided into two categories, pharmacokinetic and pharmacodynamic (Rang et al, 1995).

Pharmacokinetic interactions are those which can affect the way that drugs are absorbed, distributed, metabolised or excreted (Neal, 1992). The key mechanism of interaction of the AEDs relates to inhibition or induction of drug metabolism. The mono-oxygenases, known collectively as cytochrome P450, are by far the most important drug-metabolising enzymes.

Four of the established AEDs (CBZ, PHT, PB and PRIM) and three new AEDs (FBM, TPM and OXC) are recognised inducers of the cytochrome P450 system. Most pharmacokinetic drug interactions have been discovered as a result of an unexpected change in the clinical status of a patient upon addition to, or withdrawal from, existing medication (Brodie, 1992). The interaction potential of a drug can substantially affect its clinical use. Consequently, interaction studies are now an integral part of phase I and II development for all new AEDs (Patsalos, 1998).

Cytochrome P450 is the generic term for the superfamily of haem-containing mono-oxygenases that are responsible for the oxidative biotransformation of a wide range of chemically and biologically related exogenous and endogenous substrates (Porter and Coon, 1991). Located primarily in the endoplasmic

reticulum of the liver, the cytochrome P450 enzymes are the most powerful oxidising agents *in vivo* (Park et al, 1995). The general equation for P450-dependent oxidation is:

$$\mathbf{RH + NADPH + O_2 + H^+ \Rightarrow ROH + NADP^+ + H_2O}$$

The insertion of oxygen into the substrate (R) makes the compound more reactive, a process sometimes termed functionalisation (Gibson & Skett, 1986), and thereby more susceptible to further metabolism. There are many different cytochrome P450 subtypes, or isoenzymes, and more than 30 of these can be expressed in a particular tissue at any one time (Nebert et al, 1981). The capacity of the cytochrome P450 system can be altered by enzyme induction or inhibition. This can be selective or non-selective and provides a source of inter- and intra-individual variation in both rates and routes of drug metabolism (Park and Kitteringham, 1990).

Enzyme induction by drugs and other xenobiotic chemicals was initially discovered by virtue of the profound effects that induction can have on pharmacological responses. Thus its phenomenon was reported before the discovery and naming of the cytochrome P450 superfamily (Okey, 1990). Cytochrome P450 was first identified by Omura and Sato (1964). They named the pigment P450 because it exhibited a spectral peak at 450nm when the cytochrome was reduced and bound to carbon monoxide. Their spectral characterisation also provided the first means of quantitating the P450 content of tissue fractions.

A number of assays employing various model substrates have been developed as measurements of basal and PB-inducible mono-oxygenase activities (Greenlee and Poland, 1978). Sensitive fluorometric assays have been reported for the O-deethylation of 7-ethoxycoumarin (Ullrich and Weber, 1972; Jacobson et al, 1974) and 7-ethoxyresorufin (Burke and Mayer, 1974), and the O-demethylation of harmine (Burke and Upshall, 1976). The O-deethylation of 7-ethoxycoumarin to the highly fluorescent product, 7-hydroxycoumarin, is a particularly sensitive indicator of mono-oxygenase activity, with the apparent potential to serve as a screening assay for a wide range of inducers (Greenlee and Poland, 1978).

PB, chronically administered to rats, gradually decreases its own sedative effect by enhancing barbiturate metabolism and clearance (Remmer, 1962; Remmer and Merker, 1963). After induction with PB, the concentration of total P450 in rat liver microsomes, measured spectrally, typically increases 2- to 3-fold and is elevated in proportion to the increase in enzyme catalytic activity (Conney, 1967). Repeated injections of 80 mg/kg PB have been used to induce the hepatic enzymes of mice in a number of studies (Honkakoski and Lang, 1989; Kim et al, 1990; Corcos, 1992; Noguchi et al, 1994; Panesar et al, 1996; Charpentier et al, 1997; Carlile et al, 1999).

4.2 Aims

A pharmacokinetic study was conducted to assess the effects of hepatic enzyme induction with CBZ and PB on the pharmacokinetics of RMD and DGR. A further pharmacodynamic study investigated the effects of hepatic

enzyme induction on the efficacy of RMD against MES-induced seizures following acute administration in mice.

4.3 Methods

4.3.1 *Induction of cytochrome P450*

The cytochrome P450 hepatic enzyme system of male ICR mice was induced by administration of 100 mg/kg CBZ or 80 mg/kg PB (i.p.) once daily for 4 days.

4.3.2 *Maximal electroshock-seizure test*

This test was performed in accordance with the method described by Loscher and Schmidt (1988). Constant current electroshock stimuli were delivered to mice via auricular electrodes from an ECT unit. Stimuli consisted of 0.2 seconds of rectangular positive pulses (pulse width = 0.4 ms) at a frequency of 60 Hz. The stimulation current used to induce tonic hind-limb extension (THE) was 50 mA.

4.3.3 *Remacemide and desglycinyl-remacemide pharmacokinetics*

RMD and DGR concentrations were determined in brain tissue by the method described in section 3.3.

4.3.4 *Measurement of cytochrome P450 content*

The cytochrome P450 content of liver microsomes was determined by a modification of the method of Omura and Sato (1964). Microsomes were prepared from livers that had been perfused *in situ* with ice-cold saline. The

excised liver was homogenised with a motorised teflon pestle in 3 ml ice-cold 0.25 M sucrose. The homogenates were then centrifuged at 10,000 x g for 20 minutes at 4°C. The supernatant produced was centrifuged at 105,000 x g for one hour at 4°C.

The resulting pellets were resuspended in 3 ml 0.01 M Tris-HCl buffer which contained 151 mM KCl, 1 mM EDTA and 20% glycerol (pH 7.6). The prepared microsomes were then stored at -80°C until required.

On the day of the assay, the samples were removed from the freezer and allowed to defrost completely. Following centrifugation at 105,000 x g for one hour at 4°C, the pellets were resuspended in 0.1 M KH_2PO_4 (pH 7) to a protein concentration of 2 mg/ml as determined by the BIORAD method (section 2.2.2).

The resuspended microsomes were split between two cuvettes, each with 1 cm path lengths. Carbon monoxide (CO) was bubbled through one of the cells (designated the test cell). An excess of sodium dithionite was then added to both cuvettes and CO bubbled through the test cell once again. The test cell was then scanned against the reference cell (no CO) on a double beam spectrophotometer. The cytochrome P450 content of the microsomes was determined from the CO-induced difference spectrum of dithionite-reduced microsomes produced between 400 - 500 nm at 20°C. The extinction coefficient $\varepsilon = 91$ nm was used to account for the difference in cytochrome extinction between 450 and 490 nm. The figure indicating the peak difference

in absorbance observed between the test cell and control cell between 450 and 490 nm was therefore multiplied by 91 to obtain an accurate result.

4.3.5 *Measurement of cytochrome P450 activity*

The cytochrome P450 activity of liver microsomes was determined by measuring the 7-ethoxycoumarin O-deethylase activity of the preparation by the method described by Greenlee and Poland (1978). The O-deethylation of 7-ethoxycoumarin to the highly fluorescent product, 7-hydroxycoumarin, is a particularly sensitive indicator of mono-oxygenase activity.

Microsomes were prepared from livers perfused *in situ* with ice-cold saline. The excised livers were homogenised with a motorised teflon pestle in 3 ml 0.15 M KCl. The homogenate was centrifuged at 10,000 x g for 20 minutes at 4°C. The resulting supernatant was diluted with 0.15 M KCl to give a final protein concentration of 1 mg/ml as determined by the BIORAD method (section 2.2.2). At this stage the microsomes could be stored at -80° C until required.

Each sample was divided into three 100 µl aliquots and was measured in duplicate plus a blank. Test and blank samples were added to a buffer containing final concentrations of 0.5 µM NADH, 0.5 µM NADPH, 5.0 µM $MgCl_2$, 5 µM BSA and 65 µM KH_2PO_4 buffer (pH 7.2) to give a total volume of 1 ml. The reaction was initiated by the addition of 50 µl of the substrate (7-ethoxycoumarin; 0.5 µM final concentration). In the blank samples, 125 µl

15% (w/v) TCA was added prior to the addition of the substrate to prevent any reaction. All samples were then incubated at 37°C. After 10 minutes, 125 µl TCA (15%) and 2 ml chloroform were added, in sequence, to the test samples. Chloroform (2 ml) only was added to the blank samples. The product was extracted into the chloroform by shaking the tubes vigorously for 10 minutes. The chloroform extract was seen to separate into two phases. A 1 ml portion of the organic (lower) phase was removed and added to 1.5 ml 50% (v/v) 0.01 N NaOH/1 M NaCl. The sample was then vortex mixed. The alkaline phase of this solution (upper layer) was then removed and diluted 1:1 with 1 M NaCl. The concentration of 7-hydroxycoumarin in the samples was determined fluorometrically with excitation at 368 nm and emission at 456 nm.

The quantity of 7-hydroxycoumarin formed was calculated by comparing the net fluorescent units observed (sample minus blank) in the final extract with a standard solution of 7-hydroxycoumarin, after correcting for the dilution factors in the two extractions. Activity was corrected for percentage recovery of product and is expressed as the quantity of product formed (nmol/mg/min).

4.4 Experimental protocol

Pharmacokinetic study: Adult male ICR mice were randomised into three groups (n = 30) and administered either saline, 100 mg/kg CBZ or 80 mg/kg PB by intraperitoneal (i.p.) injection. Treatment continued once daily for 4 days. On day 5, 24 hours after the last chronic treatment, animals were given a single injection of RMD (40 mg/kg; i.p.). Six animals from each group were

sacrificed at 30, 60, 120, 240 and 360 minutes following RMD administration. Their brains were removed and analysed for RMD and DGR concentrations by the method described in section 3.3. Livers were also removed following perfusion *in situ* with ice cold saline and were analysed for cytochrome P450 content (section 4.3.3) and cytochrome P450 activity (section 4.3.4).

Pharmacodynamic study: Adult male ICR mice were randomised into two groups (n = 48) and administered either saline or 80 mg/kg PB i.p. once daily for 4 days. On day 5, 24 hours after the last chronic treatment, eight animals from each group were given a single injection of 0, 0.3, 1, 3, 10 or 30 mg/kg RMD (i.p.). One hour later, animals were subjected to the MES seizure test with the incidence of THE recorded. Following the seizure test, animals were sacrificed and had their brains removed. RMD and DGR concentrations in brain samples were analysed by the HPLC method described in section 3.3.

4.5 Data analysis and statistics

Statistical analysis was performed using MINITAB for Windows version 10.1 on a Viglen P2 400 microcomputer. Results for both cytochrome P450 content and activity are given in absolute values (nmol/mg and nmol/mg/min, [± SEM] respectively). Statistical differences from control ($p < 0.05$) were determined by one-way analysis of variance with a Dunnett correction for multiple comparisons. MES results were expressed as the percentage of animals per group exhibiting THE. ED_{50}values were calculated by simple regression analysis of the data. ED_{50} values are defined as the dose of RMD required to protect 50% of the animals in any particular group from THE.

Area under the curve analysis was performed using the Fig P scientific processor package.

4.6 Results

Pharmacokinetic study: Repeated administration of PB caused a decrease in the brain concentrations of RMD (figure 9) and DGR (figure 10). CBZ significantly decreased the concentrations of DGR only (figure 10). The results from saline and PB-treated animals are compared in figure 11. Area under the curve analysis demonstrated that DGR was significantly ($p < 0.05$) more susceptible than RMD to hepatic enzyme induction by PB (figure 12). Cytochrome P450 content analysis demonstrated that PB-treated animals had 66% more cytochrome P450 when compared to the saline treated controls (figure 13A). Enzyme activity was also increased in the PB-treated animals with an increase of 516% when compared to the control group (figure 13B). Data for animals treated with CBZ is unavailable due to contamination of the assay.

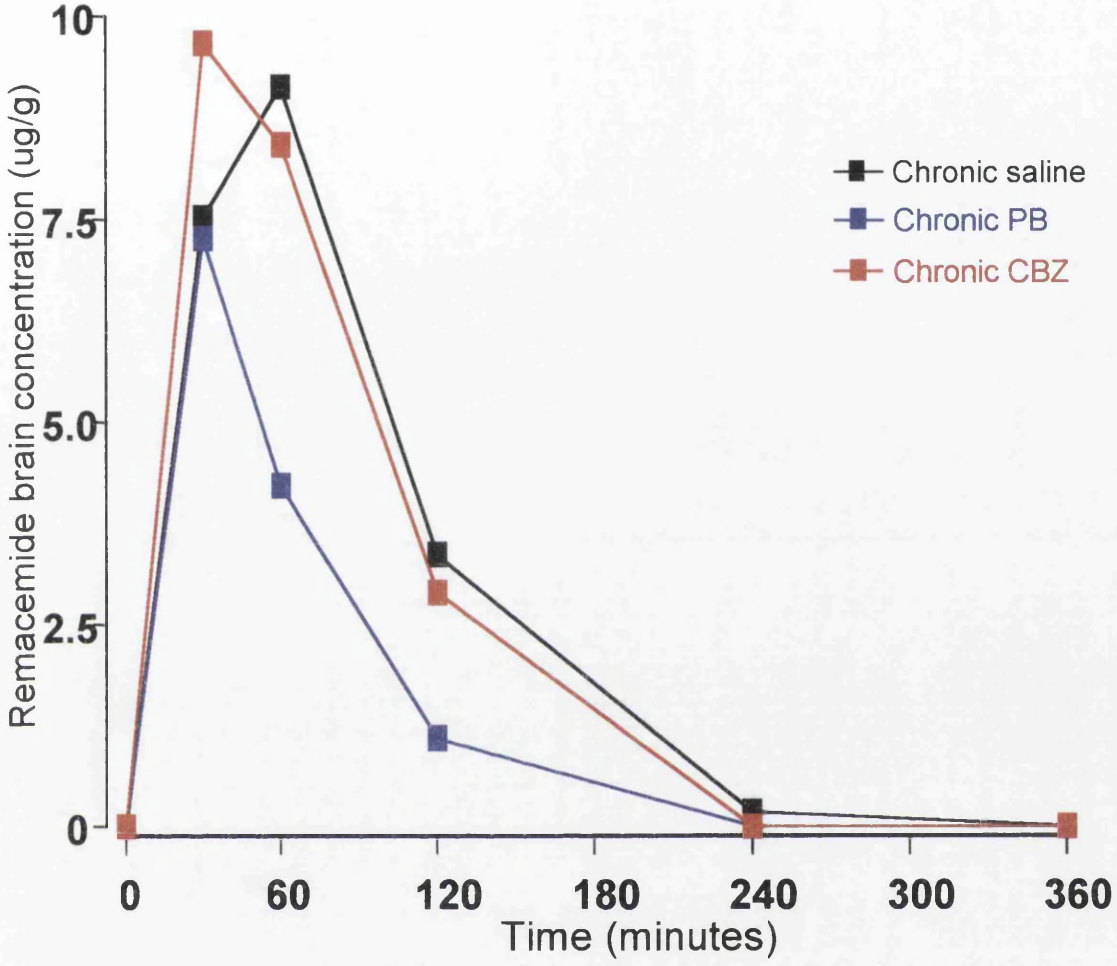


Figure 9: Pharmacokinetic profile of remacemide (40 mg/kg) in mouse brain 24 hours after chronic treatment with either saline, carbamazepine (CBZ; 100 mg/kg) or phenobarbital (PB; 80 mg/kg) once daily for 4 days. Results (n = 6) are expressed as the mean of absolute brain concentrations (μg/g). Error bars have been omitted for ease of comprehension.

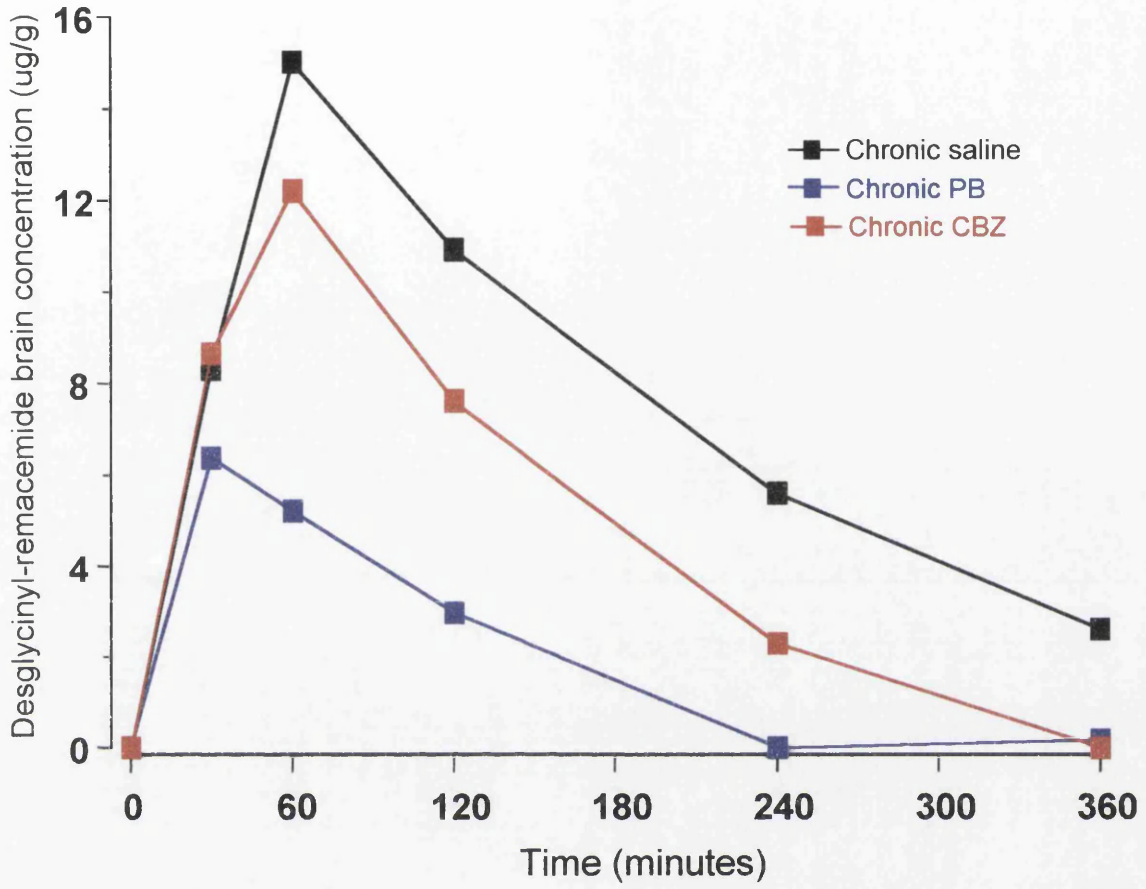


Figure 10: Pharmacokinetic profile of desglycinyl-remacemide in mouse brain after a single intraperitoneal injection of remacemide (40 mg/kg) administered 24 hours after chronic treatment with either saline, carbamazepine (CBZ; 100 mg/kg) or phenobarbital (PB; 80 mg/kg) once daily for 4 days. Results (n = 6) are expressed as the mean of absolute brain concentrations (μg/g). Error bars have been omitted for ease of comprehension.

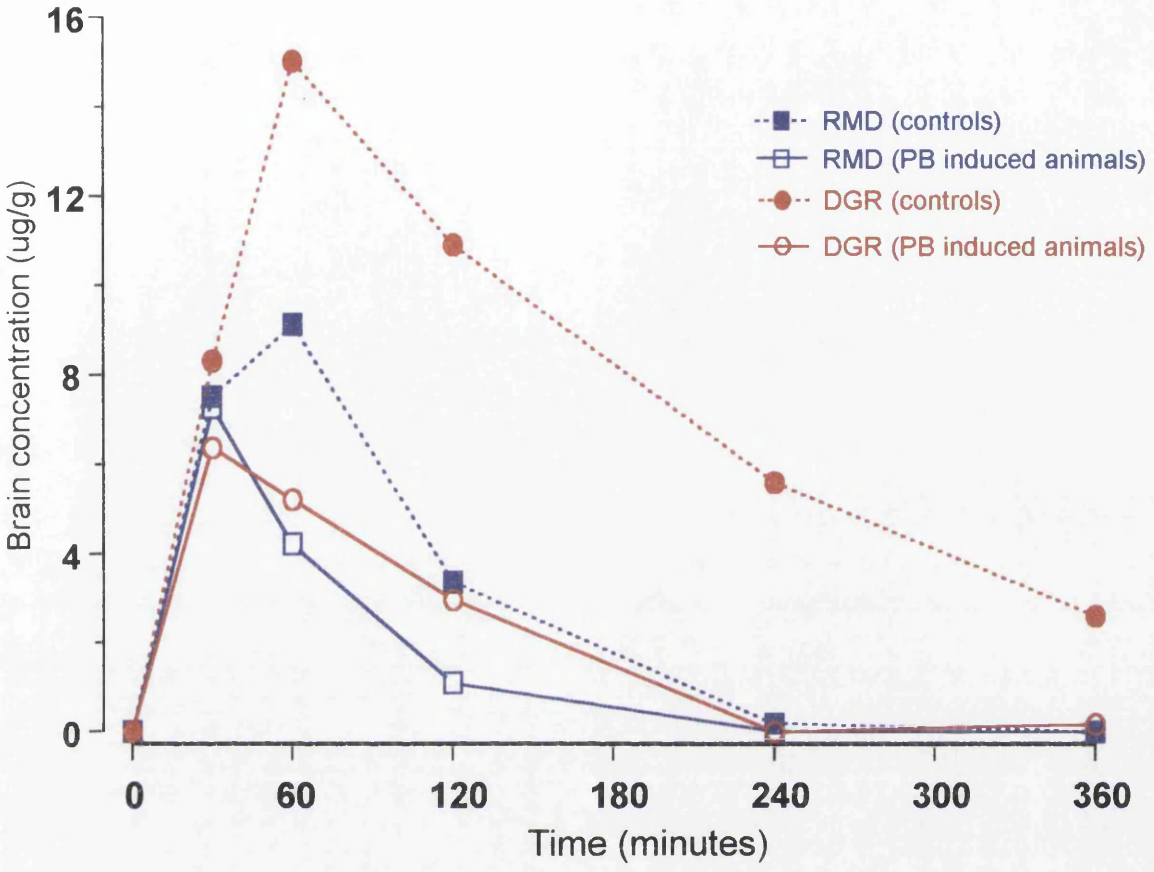


Figure 11: Pharmacokinetic profiles of remacemide (RMD) and desglycinyl-remacemide (DGR) in brain tissue of animals following a single injection of remacemide (40 mg/kg). Animals had been previously treated for 4 days with either saline or phenobarbital (PB; 80 mg/kg). Results (n = 5) are expressed in absolute brain concentrations (μg/g). Error bars have been omitted for ease of comprehension.

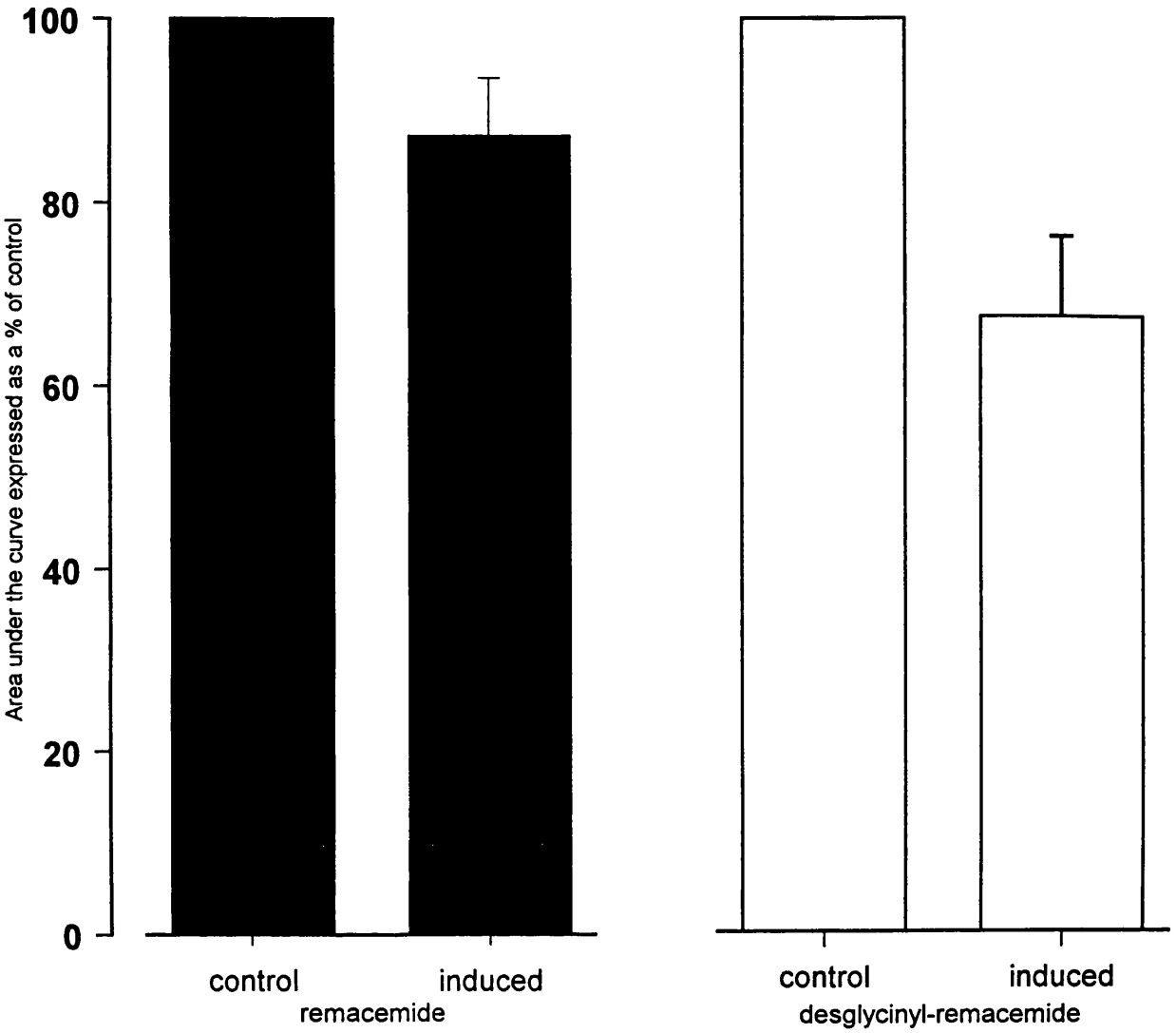


Figure 12A: Area under the curve analysis for brain concentrations of remacemide and desglycinyl-remacemide following a single intraperitoneal injection of remacemide (40 mg/kg) in mice chronically treated with saline (controls) or carbamazepine (induced). Results (n= 30) are expressed as the mean percentage of control (saline-treated) and error bars denote the standard error of the mean.

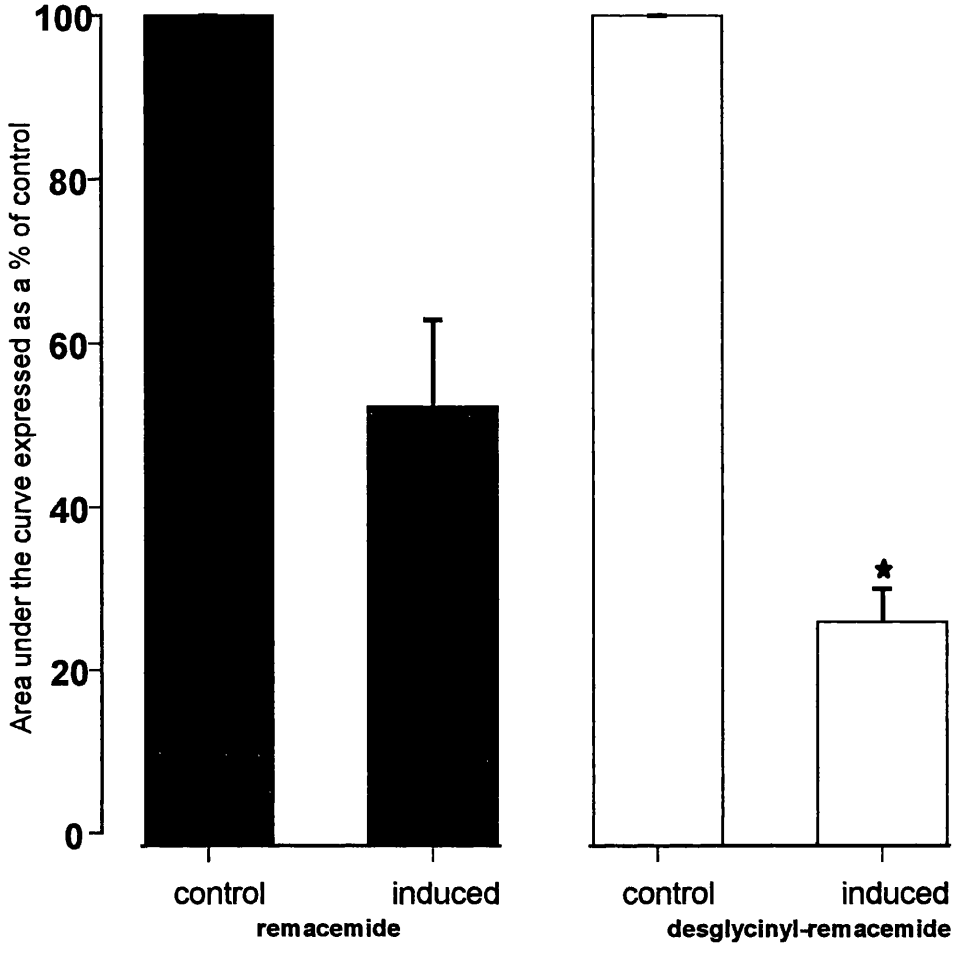


Figure 12B: Area under the curve analysis for brain concentrations of remacemide and desglycinyl-remacemide following a single intraperitoneal injection of remacemide (40 mg/kg) in mice chronically treated with saline (controls) or phenobarbital (induced). Results (n= 30) are expressed as the mean percentage of control (saline-treated) and error bars denote the standard error of the mean. Statistical differences (* $p < 0.05$) between remacemide and desglycinyl-remacemide levels in induced animals was determined by performing a Student's t-test.

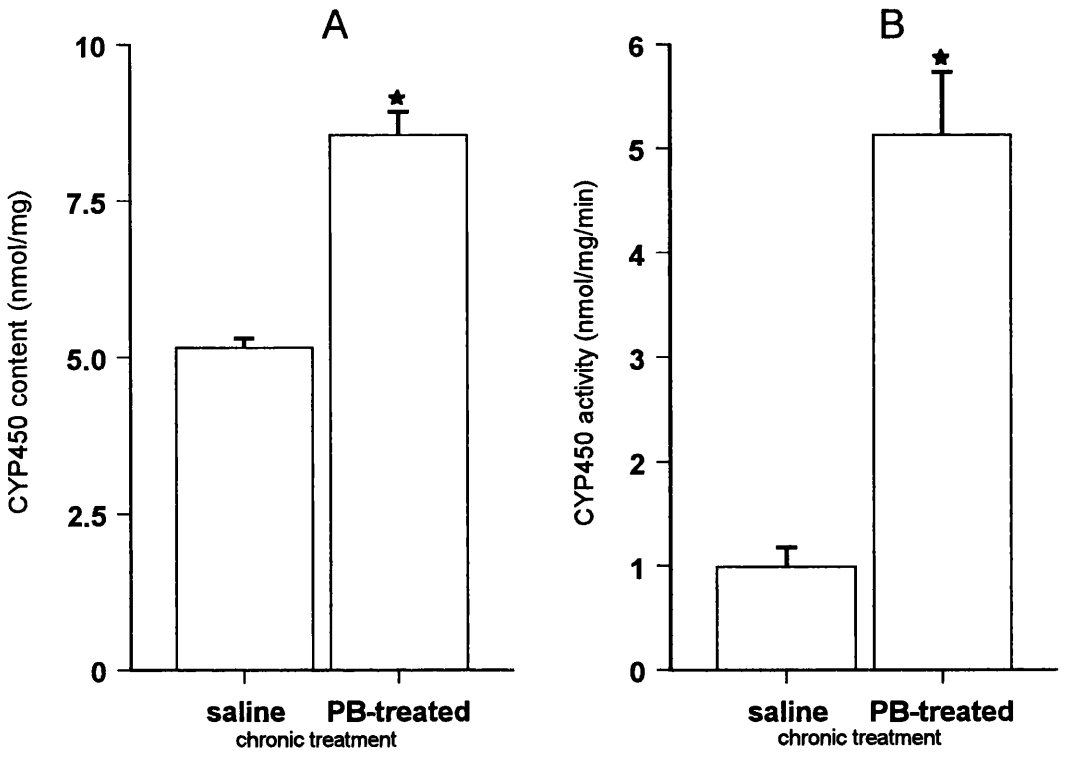


Figure 13: (A) Cytochrome P450 (CYP450) content of liver microsomes prepared from mice chronically treated with saline or phenobarbital (PB; 80 mg/kg) once daily for 4 days. The mean enzyme content of the microsomes (n = 30) is expressed in nmol/mg. Error bars denote the standard error of the mean. Statistical significance (*$p < 0.05$) from control was determined by a Student's t-test. (B) Cytochrome P450 (CYP450) activity of liver microsomes prepared from mice chronically treated with saline or phenobarbital (PB; 80 mg/kg) once daily for 4 days. Mean enzyme activity of the microsomes (n = 30) is expressed in nmol/mg/min. Error bars denote the standard error of the mean. Statistical differences from control were determined by a Student's t-test.

Pharmacodynamic study: Animals treated for 4 days with 80 mg/kg PB prior to RMD (figure 14) exhibited lower brain concentrations of RMD and DGR than animals that were previously treated with saline (figure 15). When expressed as a percentage of non-induced control results, DGR concentrations were found to be significantly ($p < 0.05$) lower than RMD concentrations following administration of 10 and 30 mg/kg RMD (figure 16). An ED_{50} value of 2.1 mg/kg RMD in the MES test was calculated for control animals. In PB treated animals the ED_{50} value was calculated 41 mg/kg (figure 17).

4.7 Discussion

Results from clinical trials (Chadwick et al, 2000; Richens et al, 2000) suggest that RMD is effective in reducing seizure frequency when administered as adjunctive therapy. There is also the suggestion that DGR is more susceptible to the effects of hepatic enzyme induction than the parent compound (Leach et al, 1996a; Leach et al, 1997b; Chadwick et al, 2000; Richens et al, 2000). The above study was designed to investigate the effects of hepatic enzyme induction on the relationship between RMD and DGR and also on efficacy against MES-induced seizures in mice.

Combination therapy is common in patients with active epilepsy and in patients with multiple seizure types. Due to the chronic nature of epilepsy and its treatment, the possibility of AED interactions is high. Pharmacokinetic interactions may affect the absorption, distribution, metabolism, or excretion of a drug. The effects of enzyme induction and

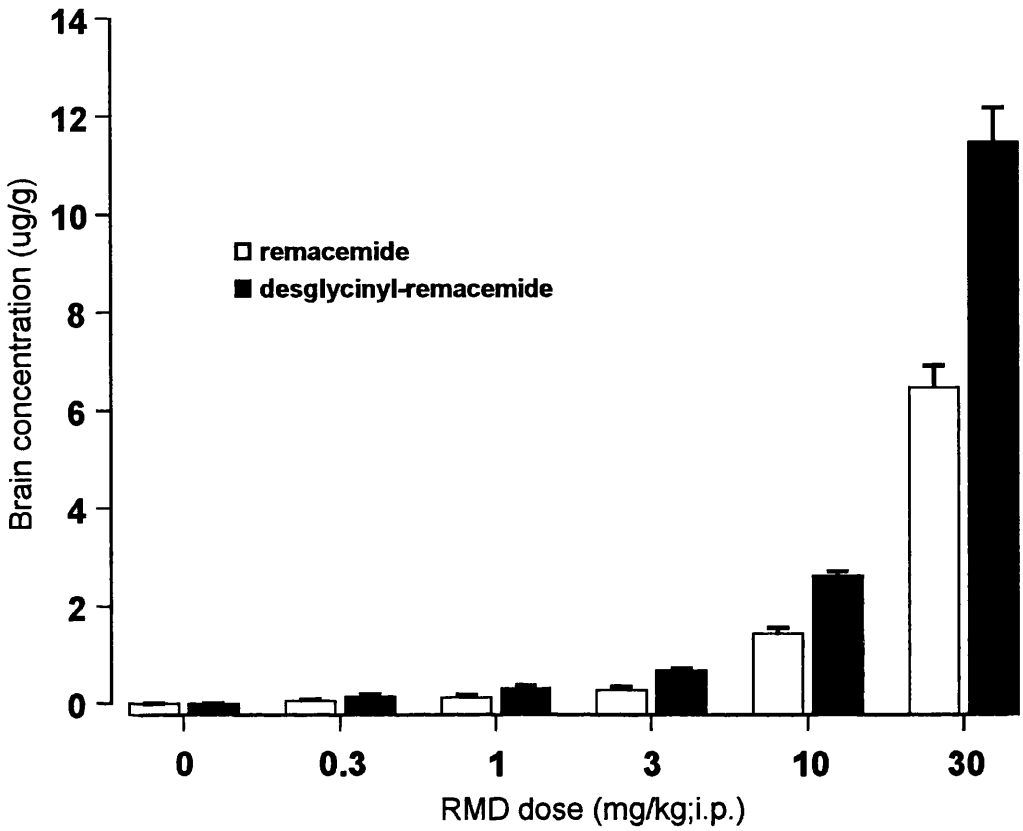


Figure 14: Pharmacokinetic profiles of remacemide (RMD) and desglycinyl-remacemide in mouse brain one hour following a single intraperitoneal injection of RMD (0 - 30 mg/kg). All animals were previously treated with saline (0.3 ml) once daily for 4 days. Results (n = 8) are expressed as the mean of the absolute brain concentrations (μg/g) with error bars representing the standard error of the mean.

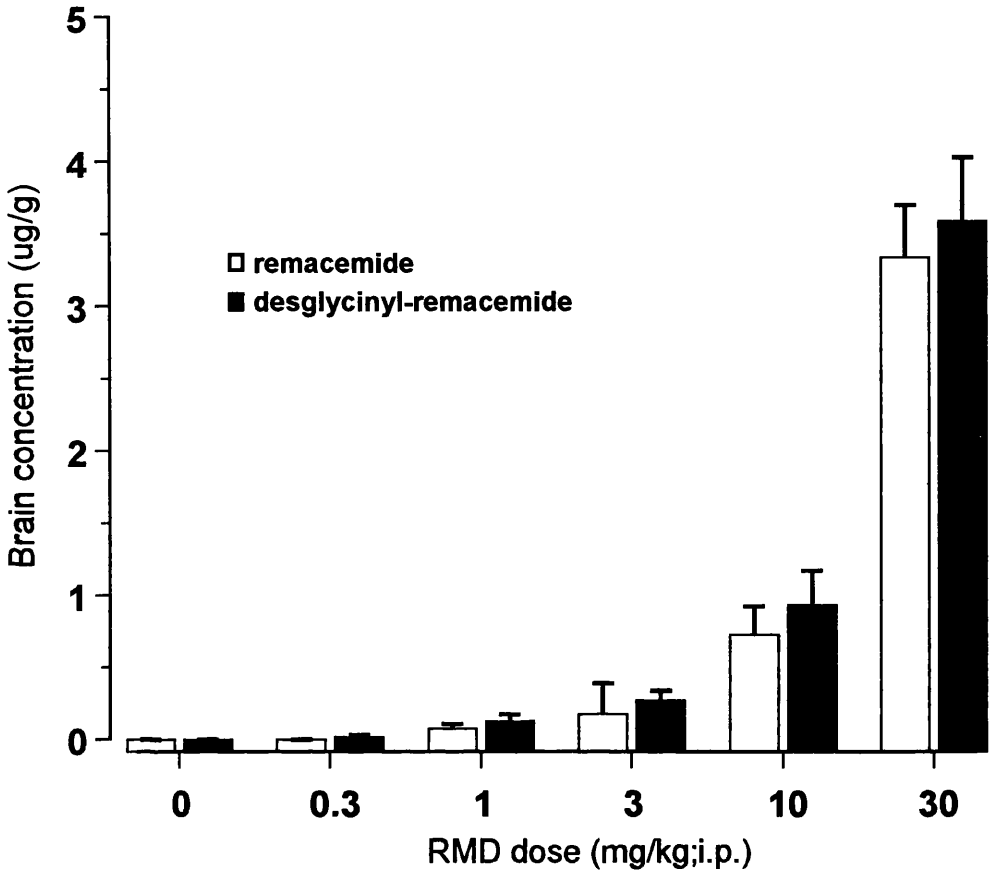


Figure 15: Pharmacokinetic profiles of remacemide (RMD) and desglycinyl-remacemide in mouse brain one hour following a single intraperitoneal injection of RMD (0 - 30 mg/kg). All animals were previously treated with phenobarbital (80 mg/kg) once daily for 4 days. Results (n = 8) are expressed as the mean of the absolute brain concentrations (μg/g) with error bars representing the standard error of the mean.

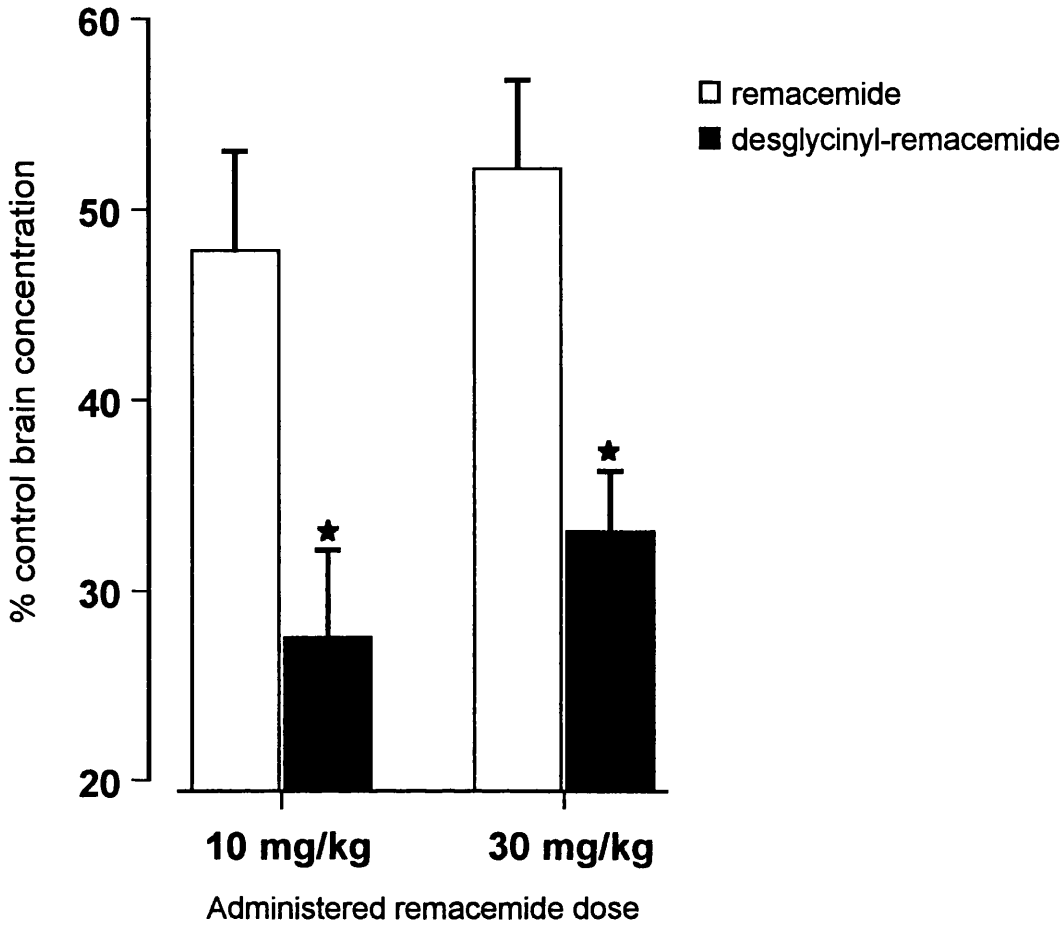


Figure 16: Brain concentrations of remacemide and desglycinyl-remacemide following a single intraperitoneal injection of remacemide. Animals had been previously treated with phenobarbital (80 mg/kg) for 4 days. Results (n = 8) are expressed as a percentage of control (saline treated) concentrations. Statistical significance (* $p < 0.05$) between remacemide and desglycinyl-remacemide results was determined by a Student's t-test.

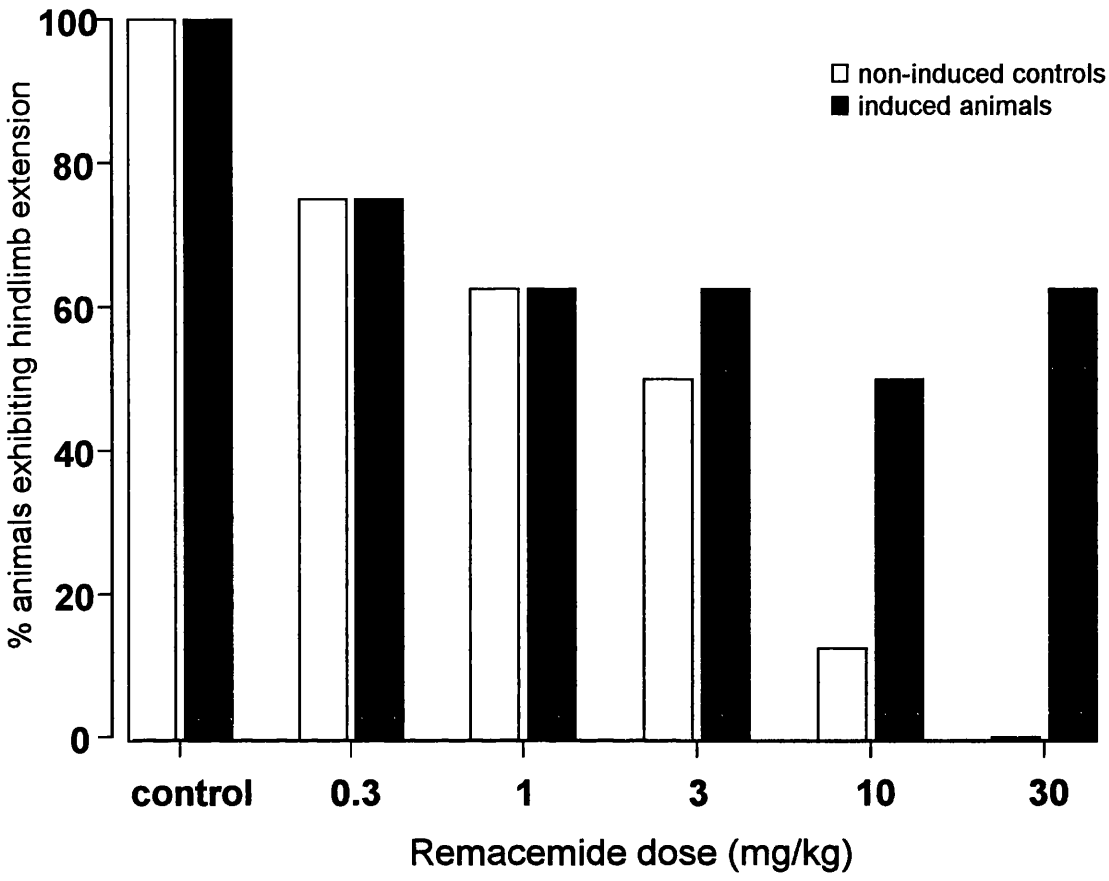


Figure 17: Effects of hepatic enzyme induction, caused by four days pretreatment with 80 mg/kg phenobarbital, on the efficacy of remacemide against maximal electroshock (MES)-induced seizures. Animals were subjected to the MES seizure test one hour following intraperitoneal administration of remacemide (0 - 30 mg/kg). Results (n = 8) are expressed as a percentage of animals exhibiting tonic hind-limb extension.

inhibition are the most common cause of pharmacokinetic problems (Brodie, 1992). The clinical result of hepatic enzyme induction is increased metabolism of the target drug, with a reduction in circulating concentrations which can result in attenuation of its pharmacological effect (Brodie, 1992).

In the above study, chronic administration of CBZ (100 mg/kg; i.p) and PB (80 mg/kg; i.p) once daily for 4 days was found to decrease brain concentrations of DGR with the effects of PB being more pronounced. CBZ was seen to have no significant effects of the pharmacokinetics of RMD. CYP450 content of liver microsomes prepared from PB-treated animals were shown to be significantly greater than those from saline-treated control animals. Similarly, a significant increase in CYP450 activity was observed in PB-treated animals when compared to controls. The effects of hepatic enzyme induction by PB on the brain concentrations of RMD and DGR were shown to be dependent on the dose of RMD administered. DGR was found to be significantly more susceptible to the effects of hepatic enzyme induction when results were expressed as a percentage of control. Chronic PB treatment caused the ED_{50} value for RMD in the MES seizure test to increase from 2.1 mg/kg for non-induced controls to 41 mg/kg RMD (i.p.) in PB-treated animals.

Previous clinical pharmacokinetic studies have found that RMD inhibits the metabolism of CBZ, and CBZ itself induces metabolism of RMD and DGR (Leach et al, 1996a). This mutual interaction was not significant enough to warrant an alteration of either treatment regimen. Closer analysis of the data

shows that DGR was more susceptible to the effects of hepatic enzyme induction than the parent compound. Another study (Mawer et al, 1999) reported that reduction of CBZ dosage was required when administered with RMD in order to avoid toxicity although this study employed greater doses of RMD and a longer treatment schedule to that mentioned above (Leach et al, 1996a). PHT, a well known inducer of CYP450 (Brodie, 1992) decreases the terminal half-lives of both RMD and DGR following co-administration with RMD (Leach et al, 1997b). RMD was also shown to cause a slight inhibition of PHT metabolism via an action on the CYP2C9 isoform. This study also reported that DGR was more susceptible to the effects of this induction than RMD (Leach et al, 1997b). VPA, a well known hepatic enzyme inhibitor (McKee and Brodie, 1994), was shown to have no effects on the kinetics of RMD or DGR following single or multiple dosing with RMD (Leach et al, 1997a).

Our results demonstrate that PB induces the metabolism of RMD and DGR to a greater degree than CBZ. The reasons for this are unclear. CBZ induces its own metabolism (Macphee et al, 1987) so it is possible that the circulating levels of CBZ were not sufficient to produce the same degree of induction as PB. PB also induces its own metabolism but not to the same extent as CBZ (Remmer, 1962; Remmer and Merker, 1963). The known variability of the extent of CBZ induction is perhaps also involved (Macphee et al, 1987). Alternatively, CBZ may not induce the appropriate isoforms of CYP450 required to alter RMD and DGR metabolism. PB is known to induce multiple forms of cytochrome P450 in rat liver, including CYP2A1, 2B1, 2B2, 2C6,

2C7, 2C11, 3A1, and 3A2 (Gonzalez, 1989; Waxman and Azaroff, 1992). Some of the induction responses are substantial with increases approximately 50- to 100-fold (2B1/2B2), whereas others are much smaller (2- to 4-fold with 2A1/2C6).

As discussed above, RMD and CBZ have a mutual interaction in terms of metabolism (Leach et al, 1996a). RMD inhibits CBZ metabolism by decreasing the activity of the CYP3A4 isoform. Clinically, CBZ, a known inducer of CYP3A4, increases the metabolism of RMD and DGR (Leach et al, 1996a), suggesting that both the drug and the parent compound are metabolised to some degree by this isoform. In the above study, CBZ only significantly decreased the concentrations of DGR suggesting that it is more susceptible to CYP3A4 metabolism than RMD. There is evidence to suggest that RMD and DGR are also metabolised by other isoforms of CYP450. As described above, PB induces a variety of CYP450 isoforms to various degrees but has no significant effect on CYP3A4 (Gonzalez, 1989; Waxman and Azaroff, 1992). The results described above indicate that PB does significantly induce the metabolism of both RMD and DGR. It is therefore possible that the 2B1 and 2B2 CYP450 isoforms, the principal isoforms induced by PB, are largely responsible for the metabolism of both RMD and DGR.

The increased metabolism of DGR compared to RMD reported here and in previous clinical investigations (Leach et al, 1996a; Leach et al, 1997b; Chadwick et al, 2000; Richens et al, 2000) suggests that either additional

isoforms of CYP450 may contribute to its clearance or that the compound, being of smaller molecular size, is simply more susceptible to drug metabolism. Alternatively, RMD, under induced conditions, may be converted more readily to one of its other inactive metabolites. The oxidation of RMD to the phenol metabolite AR-R14330 is catalysed by the 3A4 and 2D6 (AstraZeneca, unpublished results). Although neither of these isoforms are significantly induced by PB, an increase in the formation of this metabolite would explain the observations regarding CBZ and PHT co-administration (Leach et al, 1996a; Leach et al, 1997b; Mawer et al, 1999). The PB-induced increase in other isoforms of CYP450 may cause RMD to be converted to other metabolites rather than DGR, resulting in lower concentrations of the desglycinyl derivative.

P450 induction requires *de novo* protein (enzyme) synthesis. It is not merely an increase in activation of latent enzyme that is observed (Conney, 1967). Increased catalytic activity of P450-mediated mono-oxygenases is primarily the consequence of increased levels of P450 apoproteins. In turn, specific P450 protein contents are elevated, mainly as a result of increased gene transcription (Adesnik et al, 1981; Adesnik and Atchison, 1985; Whitlock, 1986; Nebert and Gonzalez, 1987). The results presented above demonstrate a disproportional increase in enzyme activity when compared to enzyme content. It is possible that sensitivity of the enzyme content assay is the reason for this observation. Another possibility is that the observed PB-induced change in whole CYP450 content is mainly due to an increase in CYP2B, the

isoform primarily responsible for the metabolism of 7-ethoxycoumarin to 7-hydroxycoumarin (Greenlee and Poland, 1978).

Efficacy in the MES model is described as an indicator of activity against primary and secondary generalised tonic-clonic seizures (Fisher, 1989). The ED_{50} levels calculated from non-induced animals in this study are significantly lower than those previously reported (Stagnitto et al, 1990; Garske et al, 1991; Palmer et al, 1991; Palmer et al, 1992; Clark et al, 1995; Davies, 1997). There are a number of possible reasons for this observation, including variation in pre-treatment times. Investigations that have performed the MES test 30 minutes following injection have significantly higher ED_{50} values than the above study in which animals were tested after one hour (Stagnitto et al, 1990; Palmer et al, 1992). DGR has been shown to be significantly more potent than RMD in the MES test and therefore it is possible that the lower levels of DGR at 30 minutes post-dosing account for the higher ED_{50} values in these studies. Variation in the type of electrodes used may also contribute to differing results. The majority of previous studies have used corneal electrodes, whereas auricular electrodes were employed in the above study (Stagnitto et al, 1990; Palmer et al, 1992).

The observed decrease in the efficacy of RMD against MES-induced seizures following PB treatment may be due to various factors. The most plausible explanation is the decrease in circulating drug concentrations. However, it is also possible that the increase in DGR metabolism when compared to RMD and the subsequent change in ratio may contribute to the lack of

pharmacological effect given that DGR is the more effective of the two compounds in the MES model (Clark et al, 1995; Davies, 1997).

With hindsight, an additional study investigating the effects of hepatic enzyme induction on the efficacy of DGR alone would have afforded a greater degree of understanding of the above results. Circulating plasma levels of RMD and DGR and a more detailed analysis of the specific isoforms of CYP450 that were induced by PB would also have been useful.

In conclusion, the above study demonstrates that administration of PB (80 mg/kg; i.p.) for 4 days, results in a significant increase in both the content and activity of cytochrome P450 enzymes in the livers of mice. This increase in hepatic enzyme activity was shown to significantly decrease the brain concentrations of RMD and DGR. The efficacy of RMD against MES-induced seizures when compared to saline-treated control animals was also decreased in induced animals. The increased susceptibility of DGR to enzyme induction and a subsequent change in the ratio of RMD to DGR in the brain is suggested to contribute to the observed decrease in pharmacological effect, although further investigations are required.

Chapter 5

Effects of remacemide and desglycinyl-remacemide on zero Mg^{2+}/4-AP-induced epileptiform activity in the rat hippocampal slice

5.1 Introduction

Many sources indicate that the hippocampus and associated structures are critical for complex partial seizures originating in the mesial temporal lobe in humans (de Lanerolle et al, 1989; Lothman et al, 1991; Stringer and Lothman, 1992). Electrophysiological recordings from the hippocampus of epileptic patients either *in situ* or following resective surgery exhibit marked epileptiform activity (Babb et al, 1987). Many features common to human epilepsy and experimental models of epilepsy have been shown to be hippocampal in origin, for example, mossy fibre sprouting and dentritic changes (Ross, 1998).

Despite being an *in vitro* model, the hippocampal slice retains all of its distinct neuronal pathways which can be selectively activated (Skrede and Westgaard, 1971). The afferent pathways into the hippocampus as well as the intrinsic connections and outputs are all glutamatergic with both ionotropic (NMDA, AMPA and kainic acid) and metabotropic subclasses of receptor present (Collingridge et al, 1982). Under normal physiological conditions, transmission within the hippocampus is carried by AMPA and kainic acid receptors with NMDA receptors only recruited during periods of prolonged depolarisation or when Mg^{2+} is removed from the surrounding medium (Collingridge et al, 1983).

The hippocampal slice model has a number of advantages over *in vivo* electrophysiological models. Ionic environments can be tightly controlled which permits easy manipulation, drugs can be applied without the restrictions

of the blood/brain barrier and systemic metabolism, and distinct intact pathways can be visualised and selectively activated (Skrede and Westgaard, 1971). Manipulation can be achieved by either changing the perfusion medium or by the direct application of compounds to the slice. Epileptiform activity is generated by raising the basal level of excitation by the use of excitatory compounds or procedures, compounds which reduce inhibition or by altering the ionic content of the perfusing medium (Voskul and Albus, 1985; Watts and Jefferies, 1993; Morris et al, 1996).

The extracellular concentration of K^+ is raised during seizures induced *in vivo* in cats (Moody et al, 1974; Fisher et al, 1976) and *in vitro* in hippocampal slices (Yaari et al, 1986). Interictal activity in the CA3 region of the hippocampus occurs when slices are bathed in a high K^+ medium (~ 12 mM; Rutecki et al, 1985; Korn et al, 1987; Traynelis and Dingledine, 1988). This activity propagates to the CA1 region where intense seizure-like activity results (Traynelis and Dingledine, 1988; Leschinger et al, 1993). Increased extracellular K^+ reduces K^+ efflux as a result of a modified concentration gradient (Dietzel et al, 1980). It is suggested that seizures develop when the CA1 region can no longer clear the excess K^+ from the interstitial space before the next interictal burst (Dichter et al, 1972; Traynelis and Dingledine, 1988).

Reduced levels of Mg^{2+} have been associated with the symptoms of clinical epilepsy (Durlach, 1967). Slice preparations of various CNS tissues generate epileptiform activity when perfused with a medium devoid of added Mg^{2+}

(Mody et al, 1987; Stanton et al, 1987; Gean and Shinnick-Gallagher, 1988; El-Beheiry and Puil, 1990). The absence of Mg^{2+} removes the voltage-dependent Mg^{2+} block from the NMDA subtype of glutamate receptor, again facilitating enhanced excitatory neurotransmission (Traub et al, 1995). Mg^{2+} and Ca^{2+}, as divalent cations, are involved in membrane charge screening which acts to stabilise membrane currents. The removal of Mg^{2+}, therefore, reduces membrane screening and facilitates the production of inward ionic currents and action potentials (Frankenhaeuser and Hodgkin, 1957; McLaughlin et al, 1971). Similarly, low or zero Ca^{2+} can also be used to cause epileptiform activity in hippocampal slices (Jefferies and Haas, 1982; Konnerth et al, 1986).

4-aminopyridine (4-AP) is known to induce epileptiform activity *in vivo* (Szenet and Pongracz, 1979) and *in vitro* (Chesnut and Swann, 1988; Watts and Jefferies, 1993; Arvanov et al, 1995; Traub et al, 1995). The principal action of 4-AP is the blockade of neuronal K^+ currents (Storm, 1988) although an enhanced release of neurotransmitters can also be stimulated (Thesleff, 1980). This release is thought to be secondary to the elongation of action potential duration allowing a greater influx of Ca^{2+} into the presynaptic neurone (Molgo et al, 1977; Flores-Herandez et al, 1994).

Other methods of inducing epileptiform activity in the hippocampal slice include perfusion of GABA receptor antagonists, such as picrotoxin (Lee and Halbitz, 1989) and bicuculline (Herron et al, 1985), and other convulsive agents such as kainic acid (Westbrook and Lothman, 1983), tetanus toxin,

(Whittington and Jefferies, 1994), penicillin (Wong and Prince, 1979), PTZ (Mirski et al, 1994) and pilocarpine (Nagao et al, 1996).

5.2 Aims

An *in vitro* conversion study was conducted to determine whether RMD is metabolised to DGR in hippocampal slices. Subsequent investigations were designed to compare and contrast the effects of RMD and DGR on the generalised epileptiform activity produced by omission of Mg^{2+} and the addition of 4-AP to the bathing medium of rat hippocampal slices. CBZ, a prototypic sodium channel blocking anticonvulsant, and ARR-15896, a putative NMDA receptor blocker were employed for comparative purposes.

5.3 Methods

5.3.1 *Preparation of tissue*

Hippocampal slices were prepared from adult, male Wistar rats (200 - 250 g) according the the method of Ross and co-workers (1998). Animals were anaesthetised with 1.3 g/kg urethane and sacrificed by cervical dislocation. After decapitation the brain was carefully removed and placed in oxygenated, ice-cold ACSF (NaCl 115 mM, $CaCl_2$ 2.5 mM, KCl, 2 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.2 mM, D-glucose 10 mM). The cerebellum and pons/medulla were removed and the two cerebral hemispheres separated using a scalpel blade. The hippocampi were dissected from each hemisphere using microspatulas.

Transverse hippocampal slices (450 μm) were prepared using a McIlwain tissue chopper. The slices were kept in an interface chamber containing

ACSF, gassed with 95% O_2 / 5% CO_2 and allowed to recover for at least one hour prior to experimentation. Following the recovery period, individual slices were transferred to a 1 ml submersion chamber which was continually perfused with ACSF at a rate of 3-4 ml/minute. The temperature of the chamber was held at 34^{O}C.

5.3.2 *Measurement of remacemide and desglycinyl-remacemide*

RMD and DGR concentrations in hippocampal slices and in ACSF from the *in vitro* conversion study were determined by the method described in section 3.3.

5.3.3 *Stimulation and recording*

A bipolar stimulation electrode was placed in the CA1 region of the hippocampal slice (figure 18) to allow orthodromic stimulation of the mossy fibres. The response to this stimulation (0.2 Hz) was recorded via a glass capillary electrode, filled with 0.9% NaCl, whose tip was positioned in the pyramidal cell layer of the CA1 region (figure 18). The brief period of stimulation confirmed slice viability and correct positioning of the electrode indicated by the ability to record a population spike in the CA1 stratum pyramidals.

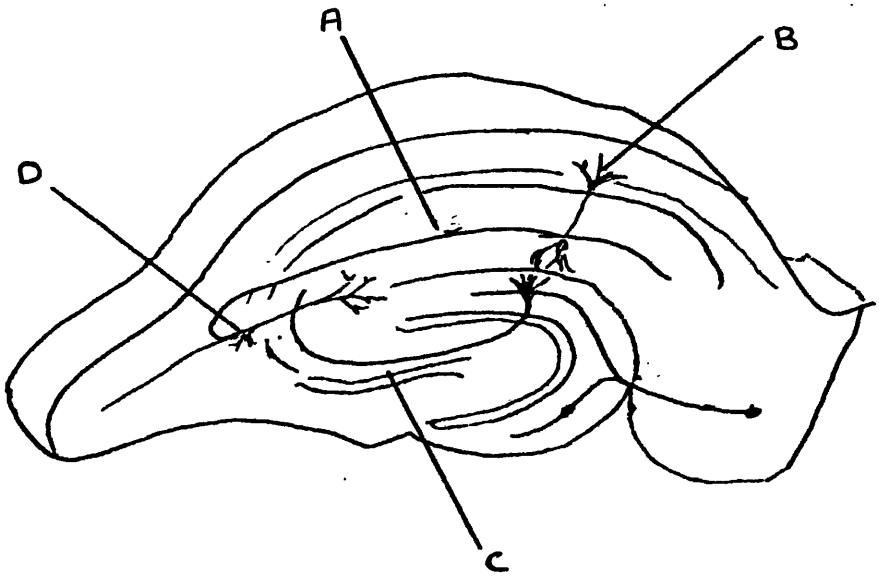


Figure 18: Diagram of a hippocampal slice. (A) - Stimulating electrode CA1; (B) - Recording electrode in CA1; (C) – Dentate gyrus. (D) – Schaffer collaterals

5.3.4 *Generation of epileptiform activity*

For initiation of epileptiform bursting, stimulation was stopped and the slices perfused with a modified ACSF (mACSF) which contained no Mg^{2+} and 50 μM 4-AP (zero Mg^{2+}/4-AP). Bursts were observed on a digital storage oscilloscope and recorded on a chart recorder. Burst frequency was determined by counting the number of spikes displayed on the chart recorder per minute. Experiments were performed when the burst frequency was constant and greater than 20 bursts per minute.

5.3.5 *Drug preparation*

RMD, DGR, CBZ and ARR-15896 (all 0 -100 μM) were dissolved in mACSF. CBZ was first dissolved in 2 ml glycofurol before being diluted with mACSF to give a final concentration of 2% glycofurol. Pilot studies indicated that 2% glycofurol had no effect on epileptiform activity.

5.4 Experimental protocols

5.4.1 *In vitro conversion study*

Concentration study- Slices were randomised into 7 groups (n = 5) with individual slices placed in the wells of 96 well plates with 250 μl of oxygenated ACSF containing 0, 62.5, 125, 250, 500, 750 or 1000 μM RMD. An additional well contained 1000 μM RMD with no tissue present. Plates were incubated for 10 minutes at 37°C after which time the ACSF and tissue were removed from each well and stored at -20°C until required for analysis of RMD and DGR concentrations.

Time study: Slices were randomised into 8 groups (n = 5) and placed individually in the wells of a 96 well plate. All slices were incubated with 250 μl oxygenated ACSF containing 500 μM RMD at 37°C for 1, 2, 5, 7.5, 10, 15, 20 or 30 minutes. Following incubation, tissue and ACSF were removed from each well and stored at -20°C until required for analysis of RMD and DGR concentrations.

7.4.2 *Electrophysiological studies*

The effects of RMD, DGR, CBZ and ARR-15896 (all 0 - 100 μM) were investigated in one hippocampal slice preparation from each of five different rats and individual slices were employed for the study of one drug alone. All drugs were perfused for 25 minutes and slices washed with mACSF alone for 35 minutes between drug concentrations.

5.5 Data analysis and statistics

Statistical analysis was performed using MINITAB for Windows version 10.1 on a Viglen P2 400 microcomputer. *In vitro* conversion study results are expressed as the mean of absolute values (μM) ± the standard error of the mean (SEM). In the electrophysiological studies, the effects of individual drug concentrations were expressed as the percentage of control frequency and group results expressed as mean percentage (± SEM). Control frequency was determined as the mean burst frequency for the 5 minute period immediately preceding drug perfusion. Drug effects were assessed by recording the mean burst frequency in the final 5 minutes of perfusion. Statistical differences from control were determined by Student's paired t-test with Bonferroni correction for multiple comparisons.

5.6 Results

5.6.1 *In vitro conversion study*

Concentration study: DGR was detected in ACSF samples when the incubated concentration of RMD was greater than 250 μM (figure 19). The concentration of

DGR measured when the maximum RMD concentration was incubated (1000 μM) was found to be 1 μM (± 0.05). No DGR was measured in the tissue samples.

Time study: DGR was measured in all ACSF samples (figure 20) where 500 μM RMD was present. Again no DGR was found in the tissue samples. The concentration of RMD in the tissue samples was shown to increase with time (figure 21).

5.6.2 *Electrophysiological studies*

Mean burst frequency in the slices used for experimentation was found to be 37 bursts per minute (± 18). RMD was without effect on zero Mg^{2+} / 4-AP induced epileptiform burst firing at all concentrations (0.1 - 100μM) investigated (figure 22). In contrast, DGR, CBZ and ARR-15896 (0.1 - 100μM) significantly ($p < 0.05$) reduced mean burst frequency (± S.E.M.) to 42.5% (± 4.4; figure 23), 19.5% (± 12.2; figure 24) and 35.9% (± 3.5; figure 25) of control, respectively.

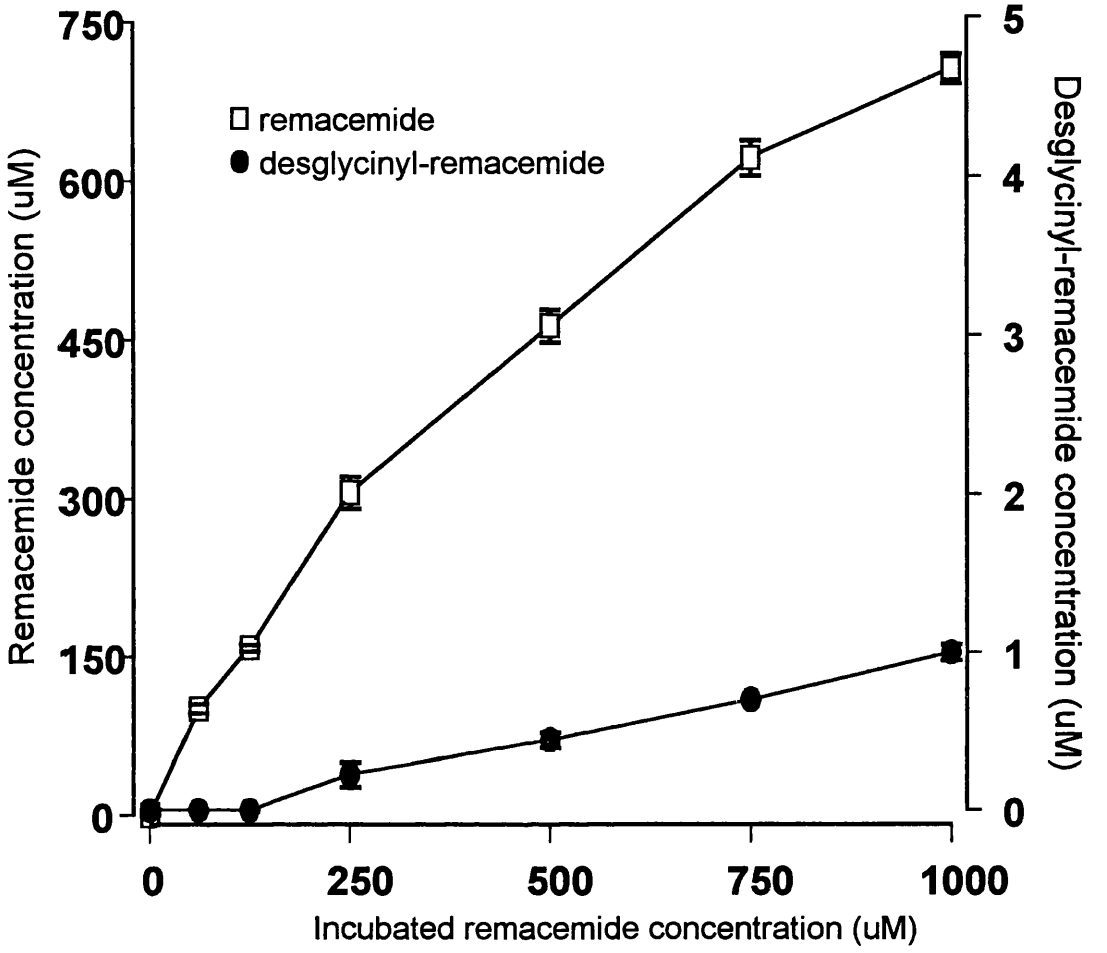


Figure 19: Bathing medium concentrations of remacemide (□) and desglycinyl-remacemide (•) following the incubation of rat hippocampal slices with remacemide (0 - 1000 μM) for 10 minutes at 37°C. Results (n = 5) are expressed as the mean absolute drug level (μM) with the error bars denoting the standard error of the mean.

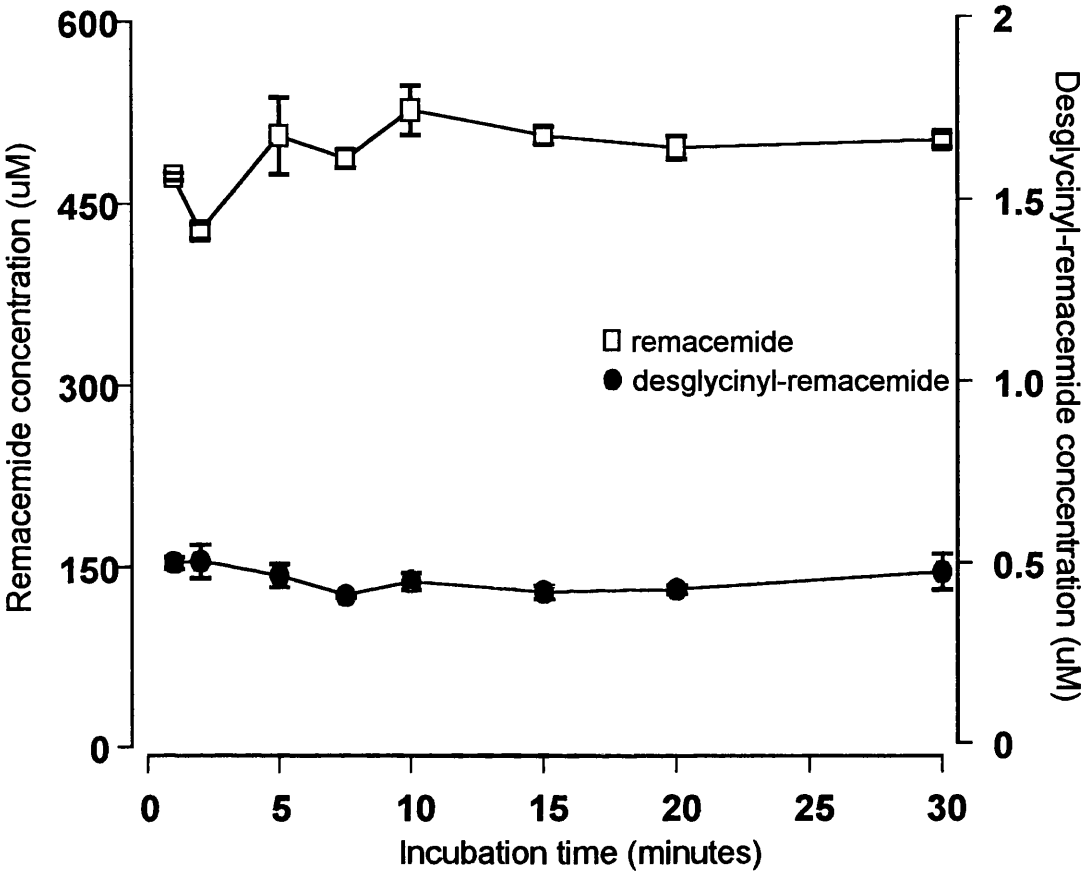


Figure 20: Bathing medium concentrations of remacemide (□) and desglycinyl-remacemide (•) following the incubation of rat hippocampal slices with 500 μM remacemide for 0 - 30 minutes at 37°C. Results (n = 5) are expressed the mean absolute drug level (μM) with the error bars denoting the standard error of the mean.

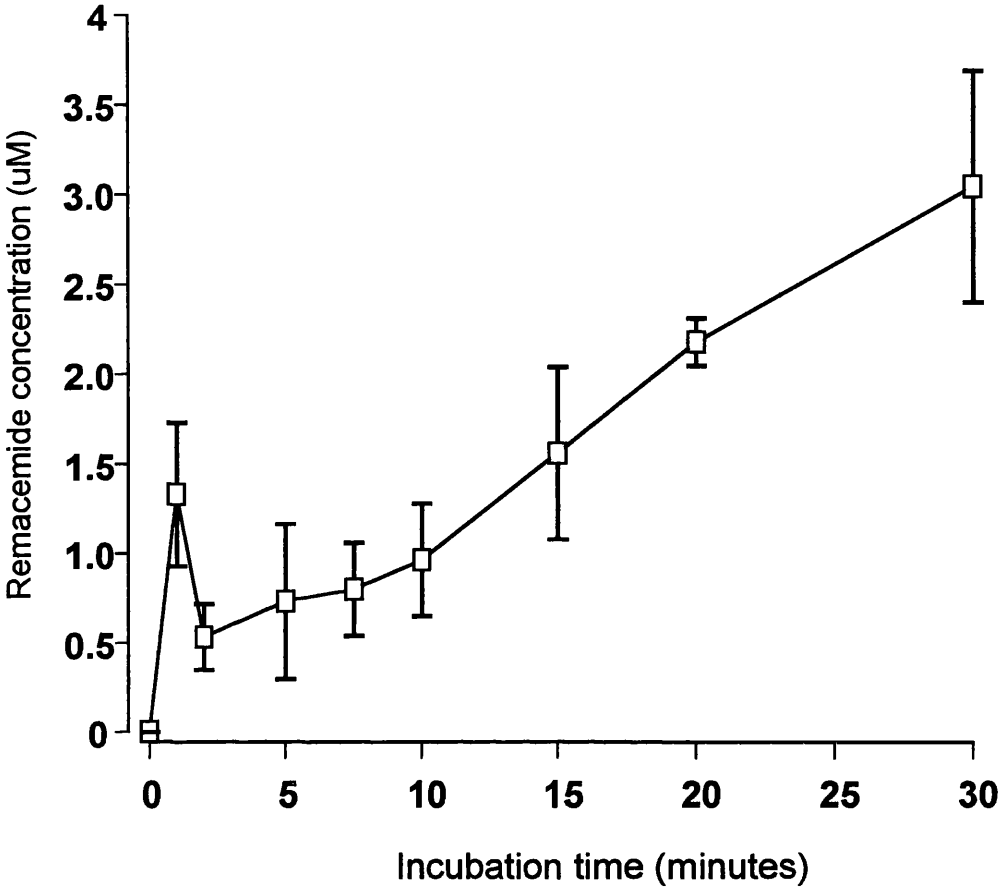


Figure 21: Tissue concentrations of remacemide in hippocampal slices (n = 5) incubated with 500 μM remacemide at 37^{0}C for 0 - 30 minutes. Remacemide concentrations are expressed as the mean concentration (μM) with the error bars representing the standard error of the mean.

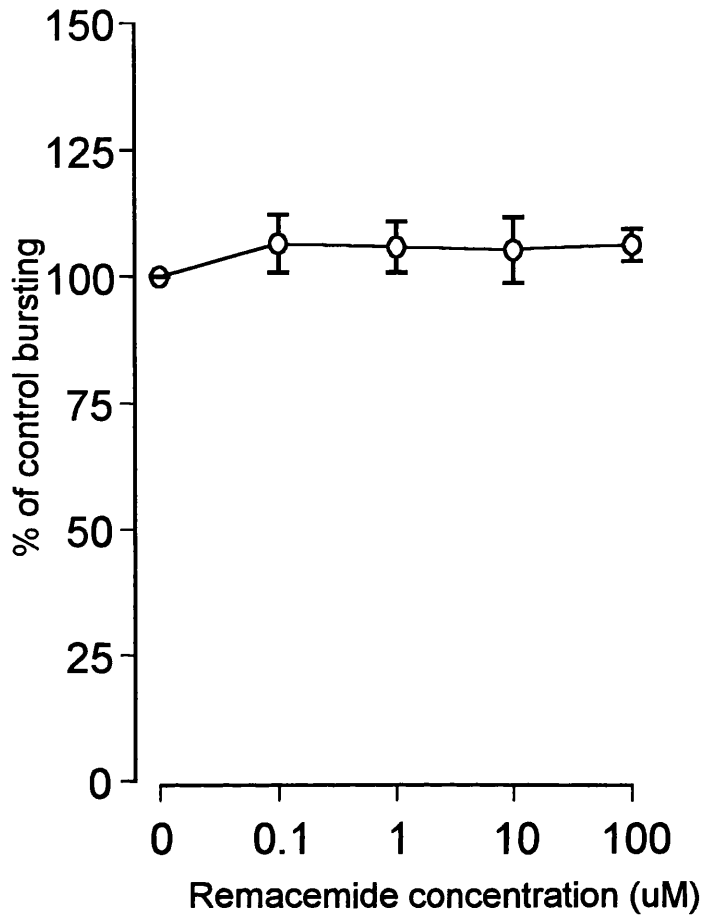


Figure 22: The effects of remacemide (0 - 100 μM) on zero Mg^{2+}/4-AP induced epileptiform bursting in the rat hippocampal slice following a 25 minute perfusion. Results (n = 5) are expressed as the mean percentage of control bursting. Error bars denote the standard error of the mean.

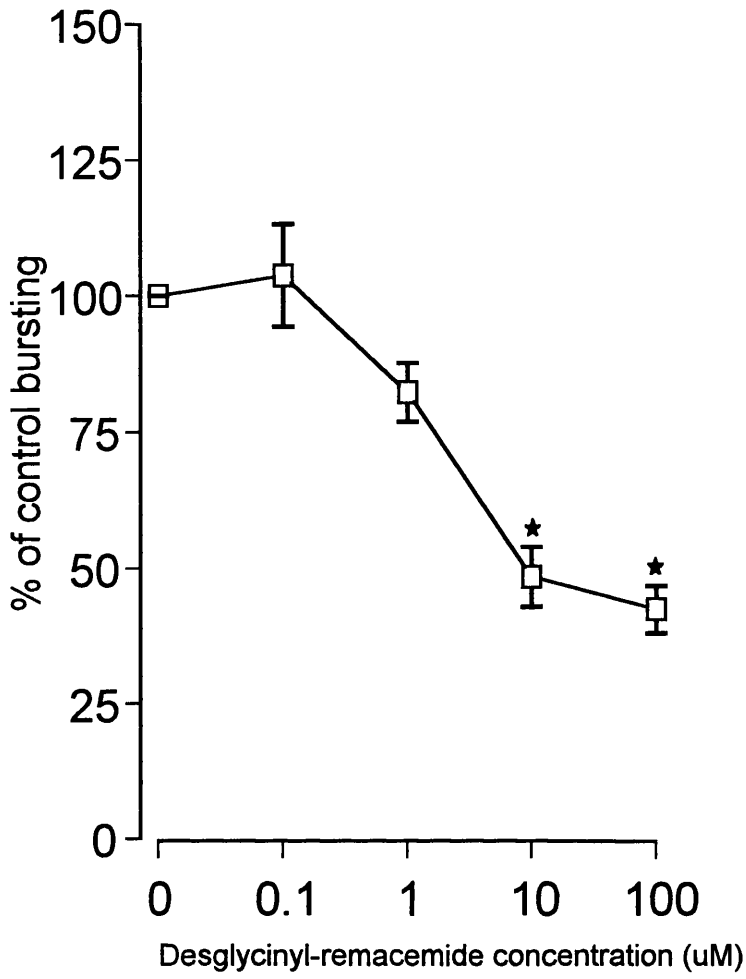


Figure 23: The effects of desglycinyl-remacemide (0 - 100 μM) on zero Mg^{2+}/4-AP induced epileptiform bursting in the rat hippocampal slice following a 25 minute perfusion. Results (n = 5) are expressed as the mean percentage of control bursting. Error bars denote the standard error of the mean. Statistical differences (* $p < 0.05$) from control were determined by Student's paired t-test with Bonferroni correction for multiple comparisons.

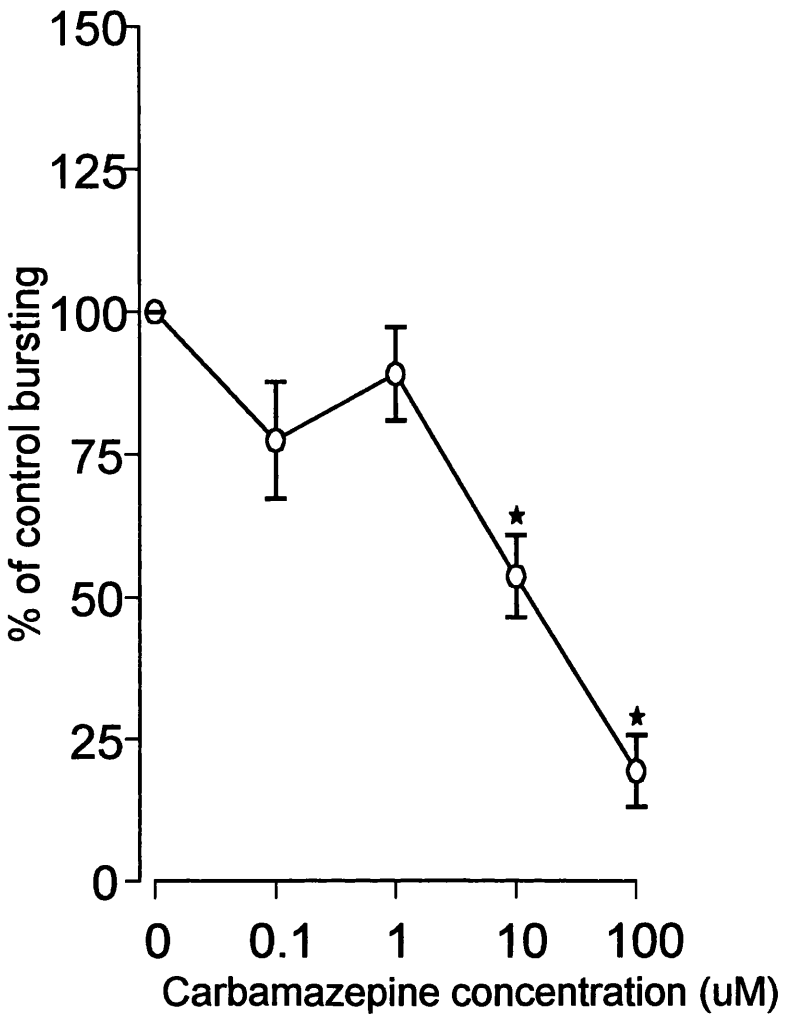


Figure 24: The effects of carbamazepine (0 - 100 μM) on zero Mg^{2+}/4-AP induced epileptiform bursting in the rat hippocampal slice following a 25 minute perfusion. Results (n = 5) are expressed as the mean percentage of control bursting. Error bars denote the standard error of the mean. Statistical differences (* $p < 0.05$) from control were determined by Student's paired t-test with Bonferroni correction for multiple comparisons.

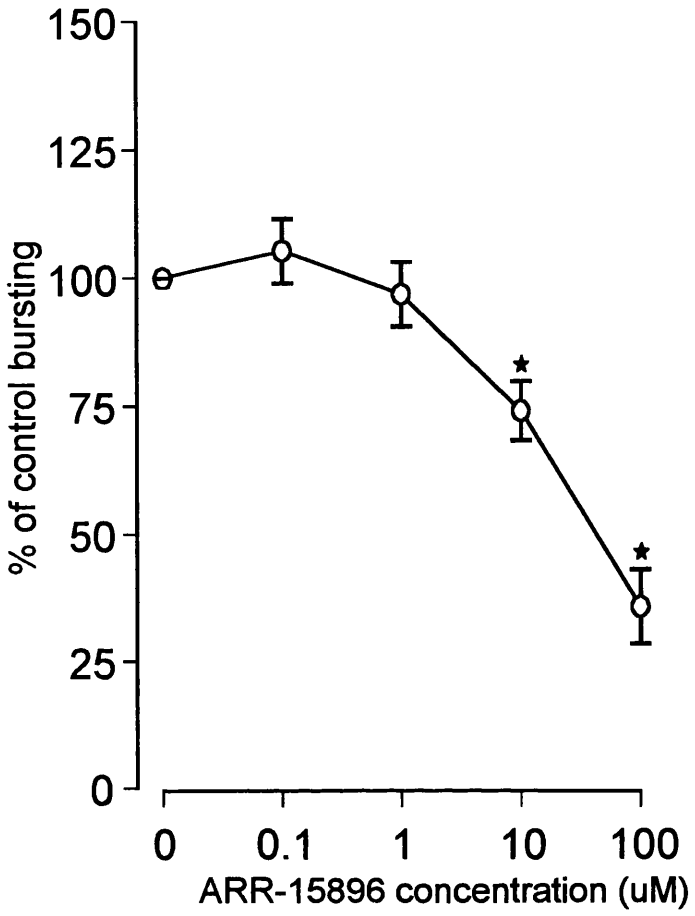


Figure 25: The effects of ARR-15896 (0 - 100 μM) on zero Mg^{2+}/4-AP induced epileptiform bursting in the rat hippocampal slice following a 25 minute perfusion. Results (n = 5) are expressed as the mean percentage of control bursting. Error bars denote the standard error of the mean. Statistical differences (* $p < 0.05$) from control were determined by Student's paired t-test with Bonferroni correction for multiple comparisons.

5.7 Discussion

The above investigations were designed to investigate the conversion of RMD to DGR *in vitro* and compare and contrast the effects of both compounds on the generalised epileptiform activity produced by omission of Mg^{2+} and the addition 4-AP to the bathing medium of rat hippocampal slices. CBZ and ARR 15896 were employed for comparative purposes.

The hippocampal slice is a useful *in vitro* tool for the investigation of AED action (Yonekawa et al, 1995). Perfusion with 4-AP alone is generally sufficient to induce epileptiform burst firing in this model, as is removal of Mg^{2+} from the bathing medium (Mody et al, 1987; Chesnut and Swann, 1988; Watts and Jefferies, 1993; Traub et al, 1994; Arvanov et al, 1995; Traub et al, 1995). However, in this case a dual stimulus was chosen to provoke the robust, frequent and reproducible discharges required for pharmacological study. The primary induction of burst firing in the zero Mg^{2+} / 4-AP model is a consequence of multiple cellular mechanisms.

Three mechanisms have been proposed to underlie burst generation caused by the omission of Mg^{2+} from the perfusate. As previously described, divalent cations, such as Mg^{2+} and Ca^{2+}, are involved in the process of stabilising membrane currents. The removal of Mg^{2+} facilitates the production of inward ionic currents and therefore action potentials (Frankenhaeuser and Hodgkin, 1957; McLaughlin et al, 1971). In addition, Mg^{2+} and Ca^{2+} are mutual antagonists, with Mg^{2+} blocking Ca^{2+} entry into pre- and post-synaptic membranes (Katz and Miledi, 1969; Czeh and Somjen, 1989). By removing

Mg^{2+}, neurotransmitter release is facilitated, causing enhanced excitability of the postsynaptic neurone (Mody et al, 1987). Finally, the absence of Mg^{2+} removes the voltage-dependent Mg^{2+} block from the NMDA subtype of glutamate receptor, again facilitating enhanced excitatory neurotransmission (Traub et al, 1995). Blockade of voltage-gated K^+ channels by 4-AP further increases the hyperexcitability by preventing repolarisation of the neuronal membrane (Traub et al, 1994). The resultant epileptiform burst firing is, however, a manifestation of generalised hyperexcitability involving non-synaptic events and non-specific synaptic and neuronal activation (Kohling et al, 2001). Accordingly, drugs with a variety of mechanisms of action are effective in this model (Yonekawa et al, 1995).

The results from the conversion study indicate that small amounts of DGR are measurable in the bathing medium of hippocampal slices following incubation with 250 μM RMD and above. The concentration of DGR increased with the incubated RMD concentration. DGR concentrations following incubation with 500 μM RMD were time-independent suggesting that the presence of DGR in the samples is due to contamination of the parent drug and not conversion of RMD to DGR although small levels of conversion by hydrolase enzymes in the hippocampal slice cannot be discounted. The concentrations of DGR detected, even at the highest concentration of RMD incubated, are not thought to be of a sufficient concentration to influence any results observed in the hippocampal slice model. This is in contrast to the low concentrations of DGR required to block NMDA currents (Subramaniam et al, 1996) and for MK801 displacement (Palmer et al, 1992). However, the model utilised in this

study is an extremely general model of excitation and therefore higher concentrations of any compound will be required to exert an effect on the various mechanisms of excitation. From the results shown in figure 23, it is also clear that 1 μM DGR has no effect in this model.

Electrophysiological studies revealed that DGR, CBZ and ARR-15896 all decrease zero Mg^{2+} / 4-AP induced epileptiform activity in a concentration dependent manner. RMD was found to have no effect in this model at any of the concentrations studied.

CBZ and ARR-15896 reduced zero Mg^{2+} / 4-AP induced epileptiform activity in the rat hippocampal slice in a concentration-dependent manner, suggesting that blockade of voltage-gated Na^{+} channels and the NMDA receptor are effective mechanisms for the prevention of burst firing in this model. This finding is substantiated by the efficacy of DGR. In contrast, RMD was without effect on zero Mg^{2+} / 4-AP induced epileptiform burst firing. To confirm that the RMD findings were due to lack of efficacy and not slice insensitivity, tetracaine (100 μM) was perfused at the conclusion of each RMD study. In all cases, spiking was rapidly and completely abolished (data not shown). The contrasting effects of RMD and DGR in this study may be related to the relative potencies of the two compounds at the voltage-gated Na^{+} channel and the NMDA receptor.

This proposal has implications for investigations of the anticonvulsant efficacy of RMD in animals and man. RMD and DGR have a common anticonvulsant

profile in experimental animals characterised by efficacy against seizures induced by maximal electroshock and the systemic administration of NMDA, 4-AP and kainate (Garske et al, 1991; Palmer et al, 1993; Cramer et al, 1994). The ED_{50} values in these models are similar (Palmer et al, 1997), giving the impression that RMD and DGR are roughly equi-potent. However, one must consider the rapid conversion of RMD to DGR in both animals and man before making such comparisons. One hour after systemic administration of the parent compound, the metabolite accounts for more than 50% of circulating drug concentrations in experimental animals (Section 3.6). In human studies, under steady state conditions, DGR represents approximately 30% of the total drug load (Schacher and Tarsy, 2000). These findings, together with the recognised separation in potency and longer half-life of the metabolite (Schachter and Tarsy, 2000), suggest that, following systemic administration of the parent compound, DGR is the principal pharmacological moiety and NMDA receptor blockade the primary mechanism of action.

Although in general terms RMD and DGR have a common pharmacological profile, the desglycinyl derivative is reported to be more potent, at both voltage-gated Na^+ channels and the NMDA receptor (Subramaniam et al, 1996; Wamil et al, 1996). In cultured mouse spinal cord neurones, RMD and DGR prevent sustained repetitive firing, a marker for Na^+ channel activity, with IC_{50} values of 7.9 μM and 3.3 μM, respectively (Wamil et al, 1996). Displacement of [^{3}H]-batrachotoxin binding to neuronal Na^+ channels has also been reported, with IC_{50} values of 15.6 μM for RMD and 7.9 μM for DGR (Palmer et al, 1992). At the NMDA receptor, RMD and DGR displace [^{3}H]-

MK801 binding with half maximal inhibitory concentrations of 68 μM and 0.48 μM, respectively (Palmer et al, 1992). In addition, NMDA currents are reduced by 50% at concentrations of 75 μM RMD and 0.7 μM DGR (Subramaniam et al, 1996). These studies reveal an important two-fold separation in potency at the Na^+ channel and up to a 100-fold difference at the NMDA receptor which may explain the contrasting effects of RMD and DGR on zero Mg^{2+} / 4-AP induced epileptiform activity.

However, previous investigations conducted *in vitro* have indicated that there may be possible differences in the NMDA receptor blocking actions of RMD and DGR (Subramaniam et al, 1996). The NMDA receptor block produced by DGR was shown to be strongly use- and voltage-dependent and could be occluded by Mg^{2+}, indicating an open channel block mechanism. In contrast, the partial voltage dependence of RMD suggests that at least a portion of its action on NMDA receptors occurs via a non-channel blocking/allosteric mechanism (Subramaniam et al, 1996). Previous evaluation of RMD in the hippocampal slice demonstrated that it has no effects on evoked CA1 discharges, recurrent inhibition, induction of long term potentiation, penicillin-induced discharges or the latency of depolarisation produced by VTD (Palmer et al, 1991).

Previously reports of CBZ effects in the hippocampal slice model are conflicting. CBZ has been reported to significantly decrease 4-AP induced bursting in the hippocampus of both young and old rats at concentrations similar to those used in the above study (Fueta and Avoli, 1992). The drug

was also found to have efficacy in both the low calcium and low Mg^{2+} models (Dost and Rundfeldt, 2000). In contrast, it has also been reported that CBZ has no effect on burst frequency in the 4-AP model at a concentration of 51 μM. A decrease in burst amplitude however, was observed (Yonekawa et al, 1995).

As with all models, the hippocampal slice preparation has its disadvantages. As discussed in section 1.3.1, the trauma of dissection may alter the metabolism of the slice or cause partial mechanical injury and hypoxia of the tissue. In addition, ionic environments do not exactly mimic normal extracellular conditions *in vivo* and variability between different areas in the one region of the slice, perhaps caused by damage, may affect responses (Bak et al, 1980; Grinvald and Segal, 1984; Reid et al, 1988). Although the hippocampus can be considered a two-dimensional structure, longitudinal and contralateral pathways do exist. These pathways are severed during the preparation process thus altering both excitatory and inhibitory inputs. Despite this, the hippocampal slice preparation is widely regarded as an important *in vitro* preparation (Fisher, 1989) and can exhibit an impressive range of neuronal behaviours. This model can help differentiate drugs with similar *in vivo* spectrums of anticonvulsant activity (Yonekawa et al, 1995).

In conclusion, it would appear that small levels of DGR are detectable in ACSF samples following hippocampal slice incubation with RMD. It is suggested that this is a result of contamination of the RMD supply. The concentrations of DGR measured, even at the highest concentrations of

RMD, were not sufficient to affect further studies. RMD was without effect on zero Mg^{2+} / 4-AP induced epileptiform burst firing in the rat hippocampal slice. In contrast, DGR, CBZ and ARR-15896 all significantly reduced epileptiform activity. These results suggest that blockade of voltage-gated Na^{+} channels and the NMDA receptor protect against paroxysmal discharges in this model. The relatively low potency of RMD at these pharmacological targets may explain its lack of efficacy in this regard. In addition, the different mechanism of NMDA receptor blockade produced by RMD and DGR may have contributed to the results. These findings, together with those of pharmacokinetic investigations (Schachter and Tarsy, 2000), suggest that the anticonvulsant effects of RMD in both animals and man may be mediated by its desglycinyl metabolite.

Chapter 6

Effects of remacemide and desglycinyl-remacemide on sodium influx in rat brain synaptosomes

6.1 Introduction

Blockade of voltage-gated sodium channels is a recognised mechanism of action of several commonly used AEDs, including CBZ, PHT and LTG (Rogawski and Porter, 1990; White, 1999). A plethora of electrophysiological investigations suggest that these agents produce a characteristic voltage- and use-dependent blockade of sodium channels, reducing high frequency neuronal firing with little or no effect on the amplitude or duration of single action potentials (Schwartz and Grigat, 1989; Ragsdale et al, 1991; Lang et al, 1993; Kuo and Bean, 1994; Kuo et al, 1997). Binding studies (Willow and Catterall, 1982) and numerous neurochemical investigations (Willow et al, 1984; Leach et al, 1986a; Waldmeier et al, 1995; Deffois et al, 1996; Lingamaneni and Hemmings, 1999) substantiate these observations.

The sodium channel effects of RMD and DGR are considerably less characterised. There is evidence to support inhibition of sustained repetitive firing of sodium-dependent action potentials (Wamil et al, 1996; Norris and King, 1997b) and an interaction with [^{3}H]-batrachotoxin binding (Palmer et al, 1992). However, specific neurochemical investigations of sodium channel blockade with RMD and DGR are limited (Srinivasan et al, 1995) and the results potentially confounding (Davies, 1997).

6.2 Aims

The following study was designed to characterise the sodium channel blocking properties of RMD and DGR using a simple neurochemical technique in rat brain synaptosomes. CBZ, ARR-15896, LTG and PHT were included for comparative

purposes. ARR-15896 is a neuroprotective compound with structural similarities to RMD and DGR (Palmer et al, 1999). It is an NMDA receptor ion channel antagonist with a pharmacological profile similar to that of MK801 (Greene et al, 1996).

6.3 Methods

The following methodology was adapted from the methods of Urenjak and coworkers (1991) and Deri and Adam-Vizi (1993).

6.3.1 *Preparation of synaptosomes*

The cortex of an adult, male Wistar rat (200-250 g) was homogenised with a motorised teflon pestle in 15 ml ice-cold homogenisation buffer (0.32 M sucrose, 5 mM Na-2-([2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]-amino)ethanesulphonate, 0.5 mM EDTA, 16 μM BSA; pH 7.4). The homogenate was then centrifuged at 1,000 x g for 10 minutes at 4°C. Thereafter, the resulting supernatant was centrifuged at 28,000 x g for 20 minutes at 4°C to produce a crude mitochondrial/synaptosomal pellet. Following resuspension in 4 ml homogenisation buffer, the pellet was layered on a discontinuous Ficoll gradient (2.5 ml 12% Ficoll, 1.5 ml 9% Ficoll, 2.5 ml 6% Ficoll, 2 ml homogenate). This gradient was then centrifuged at 90,000 x g for 60 minutes at 4°C.

The two synaptosomal layers found within the 9% Ficoll region were removed and diluted to 18 ml with homogenisation buffer (minus EDTA). This suspension was then centrifuged for 20 minutes at 20,000 x g at 4°C. The

resulting pellet was resuspended in 9.96 ml standard incubation medium (3mM KCl, 2mM $MgCl_2$, 25mM HEPES, 10mM glucose and 140 mM sucrose; pH 7.4) and placed on ice.

6.3.2 *Sodium-sensitive dye uptake*

Before being added to the synaptosomes, 20 µl 2mM SBFI-AM was mixed with 20 µl 2% pluronic F-127. Previous studies have shown that the presence of the detergent facilitates the influx of the dye into the synaptosomes. The 40 µl solution of dye and detergent was added to 9.96 ml of synaptosomal suspension and continually shaken in an enclosed incubator for 70 minutes at 37^0C. During this time the dye was taken up by the synaptosomes and hydrolysed to SBFI. The influx of the dye was monitored by removing a 2 ml aliquot of the suspension and observing the increase in fluorescence at an excitation of 340 nm and an emission of 510 nm on a Perkin Elmer LS-5 Luminescence Spectrometer.

To account for the possible leakage of dye from the synaptosomes, a ratio was taken between the excitation at 340 nm, the Na^+-dependent intensity and at 380 nm, the Na^+-independent intensity as suggested by Harootunian and coworkers (1989) and Borin and Siffert (1990).

Following incubation, the synaptosomal suspension was centrifuged at 12,000 x g for 10 minutes at 4°C. In order to remove any dye that was not taken up, the synaptosomes were then resuspended in standard incubation medium and a further spin of 5 minutes at 12,000 x g was performed at 4°C. Thereafter,

the pellet was again resuspended in standard incubation medium to a protein concentration of 6 mg/ml as determined by the BIORAD method (section 2.2.2) and stored on ice.

6.3.3 *Construction of standard curve*

In order to relate fluorescence to internal sodium concentration, a standard curve was prepared. This was carried out in the presence of gramicidin D, an ionophore which makes plasma membranes permeable to monovalent cations (Pressman et al, 1967; Podleski and Changeux, 1969, Keen and White, 1971). In the presence of gramicidin D (2 μM), it was assumed that Na^+ concentrations between the external medium and synaptosome cytoplasm were in equilibrium. Synaptosomes were prepared in a sodium free environment, as described above. The samples were then exposed to known concentrations of sodium (12 - 200 mM) in the presence of gramicidin D for 5 minutes (at 37^0C) after which time the 340/380 nm fluorescence intensity ratio (at an emission of 510 nm) was noted. A standard curve was constructed by plotting the 340/380 nm ratios as a function of Na^+ concentration (figure 26).

6.3.4 *Determination of resting sodium*

To determine drug effects on the resting internal sodium concentration of the synaptosomes, 100 μl of the synaptosomes was added to 1.85 ml standard incubation medium containing the appropriate concentration of drug and supplemented with 140 mM NaCl and 2 mM $CaCl_2$ (at 37^0C). The 340/380 fluorescence intensity ratio was noted at 0 and 5 minutes. The internal sodium concentration at 5 minutes was deemed to be the resting concentration for

that preparation and also served as the control level for the veratridine (VTD)- stimulated influx investigations.

6.3.5 *Veratridine-induced sodium influx*

VTD is known to open voltage-dependent Na^+ channels, which in turn causes a Na^+ influx and, consequently, depolarisation (Blaustein and Goldring, 1975). Once the resting Na^+ concentration for the preparation was established (see above), 50 µl 8 mM VTD was added to the cell giving a final concentration of 200 µM (final volume 2 ml). Following a 5 minute incubation at 37^0C, the 340/380 fluorescence intensity ratio was noted.

6.3.6 *Assay validation*

To ensure that the methodology was specific for measuring changes in internal sodium concentrations, the assay was validated by using the sodium channel blocker, tetrodotoxin (TTX). Synaptosomes were prepared and loaded with dye as described above. The effects of TTX (0-10 µM) on resting sodium concentrations and veratridine-stimulated Na^+ influx were then investigated.

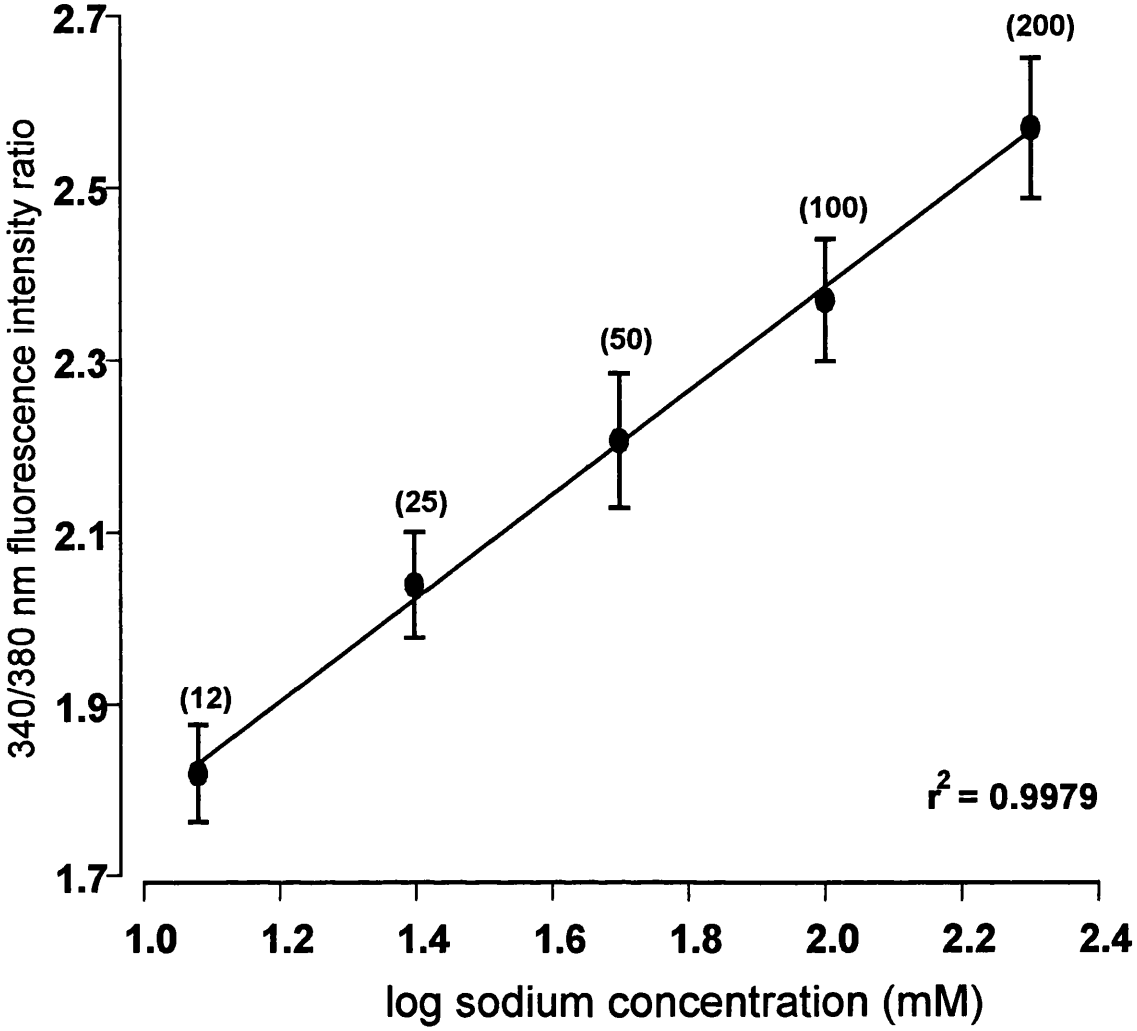


Figure 26: Relationship between absolute sodium concentration (mM; in parentheses) and 340/380nm fluorescence ratio in rat cortical synaptosomes in the presence of the sodium ionophore gramicidin D (n = 5). Linearity ($r^2 = 0.9979$) was determined by simple regression analysis.

6.4 Experimental protocol

The effects of RMD, DGR, CBZ, ARR-15896, PHT (all 0 - 1000 μM) and LTG (0 - 100 μM) were investigated in this model. Each drug concentration was tested on 5 occasions.

6.5 Data analysis and statistics

Results for VTD-stimulated sodium uptake are expressed as a percentage of control ± SEM. Resting sodium concentrations are given in absolute values (mM; ± SEM). Linear regression analysis was employed in the determination of IC_{50} values. Results were compared to control by one-way analysis of variance with Dunnett correction for multiple comparisons.

6.6 Results

TTX decreased VTD-stimulated sodium uptake to 6.9% (± 6.2) of control at 10 μM (figure 27).

RMD (figure 28A) and DGR (figure 29A) significantly ($p < 0.05$) reduced VTD-stimulated sodium influx (± SEM) to 30.6% (± 4.7) and 13.2% (± 6.2) of control at 1000 μM, with IC_{50}values of 160.6 μM and 85.1 μM, respectively. CBZ (figure 30A), ARR15896 (figure 31A), LTG (figure 32A) and PHT (figure 33A) reduced VTD-stimulated sodium influx (± SEM) to 20.1% (± 2.2), 47.4% (± 13.1), 27.9% (± 10.0) and 79.8% (± 6.6) of control, respectively at the maximum concentrations studied. IC_{50} values for CBZ and LTG were 325.9 μM and 23.0 μM, respectively.

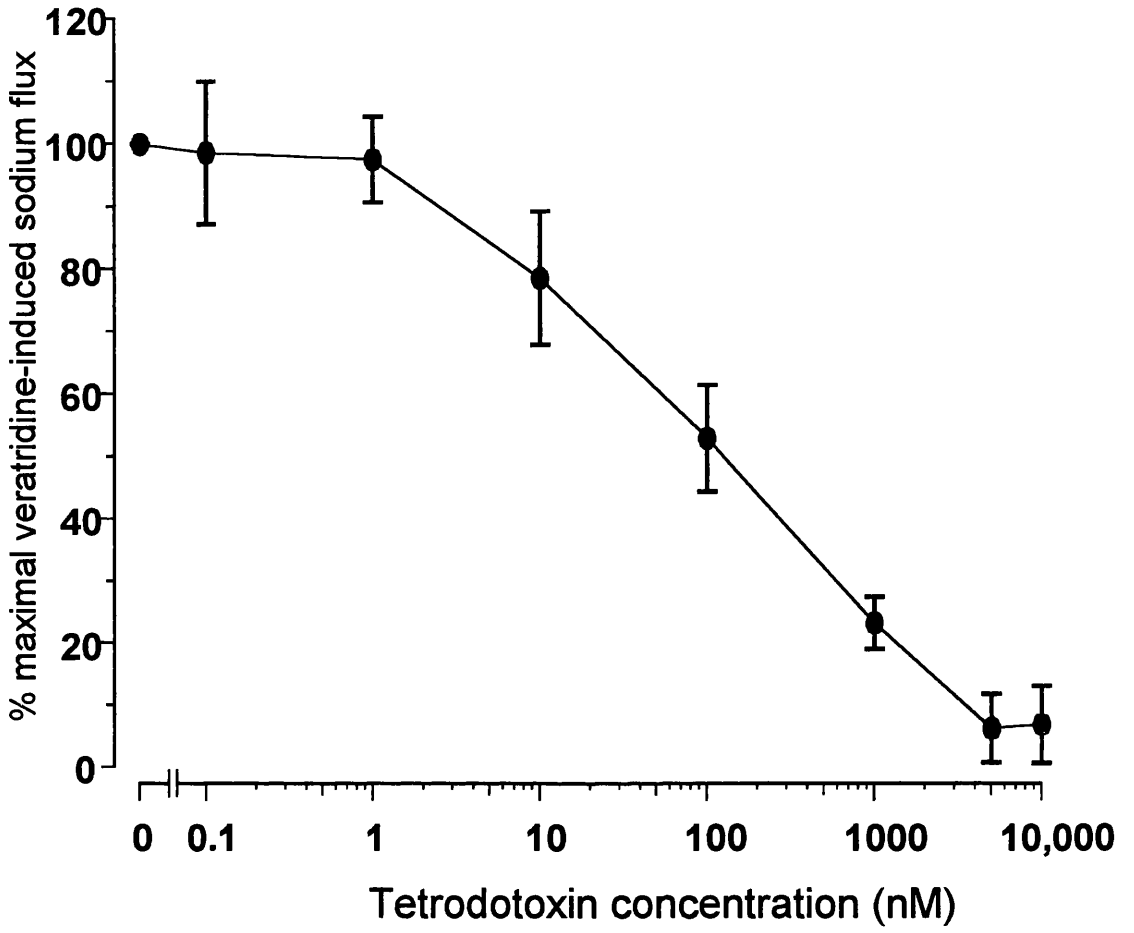


Figure 27: Effect of tetrodotoxin (0 - 10 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean.

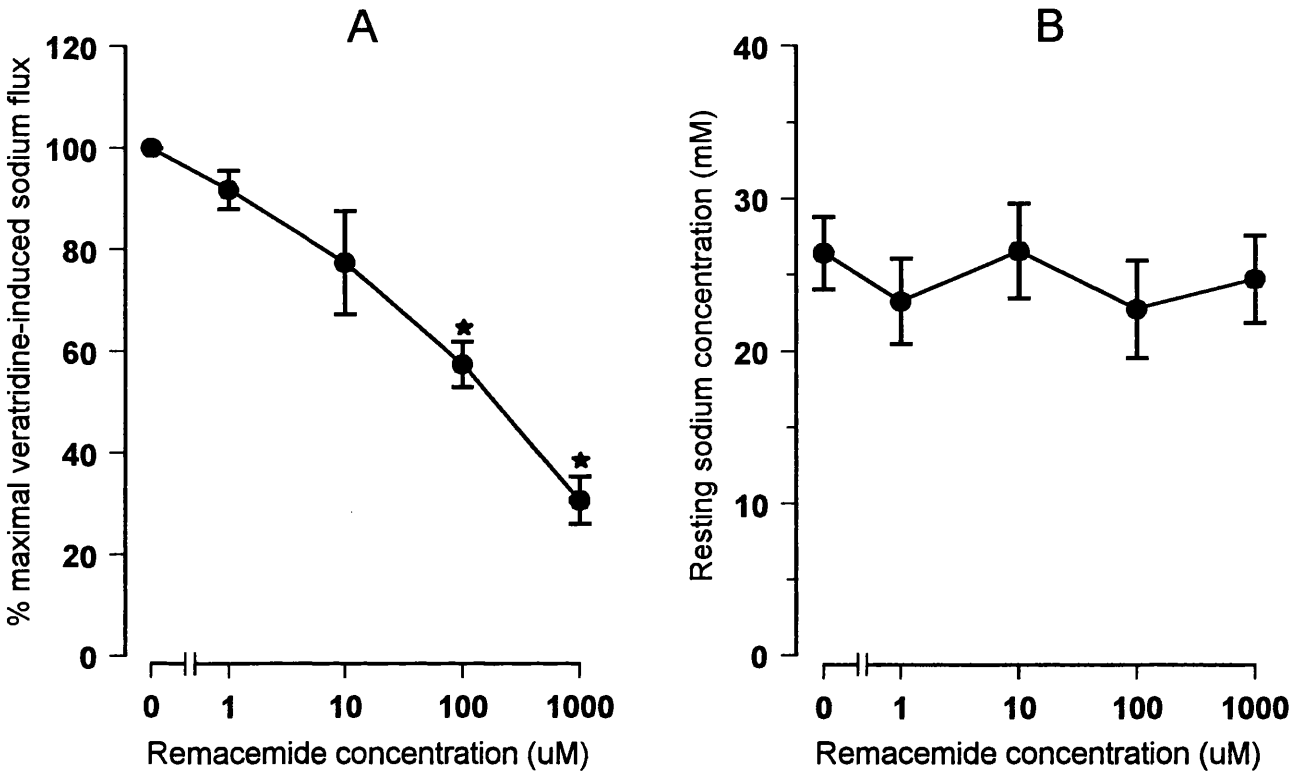


Figure 28: (A) Effect of remacemide (0 - 1000 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. Statistical significance (* $p < 0.05$) was determined by one-way analysis of variance with Dunnett correction for multiple comparisons. (B) Effect of remacemide (0 - 1000 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM).

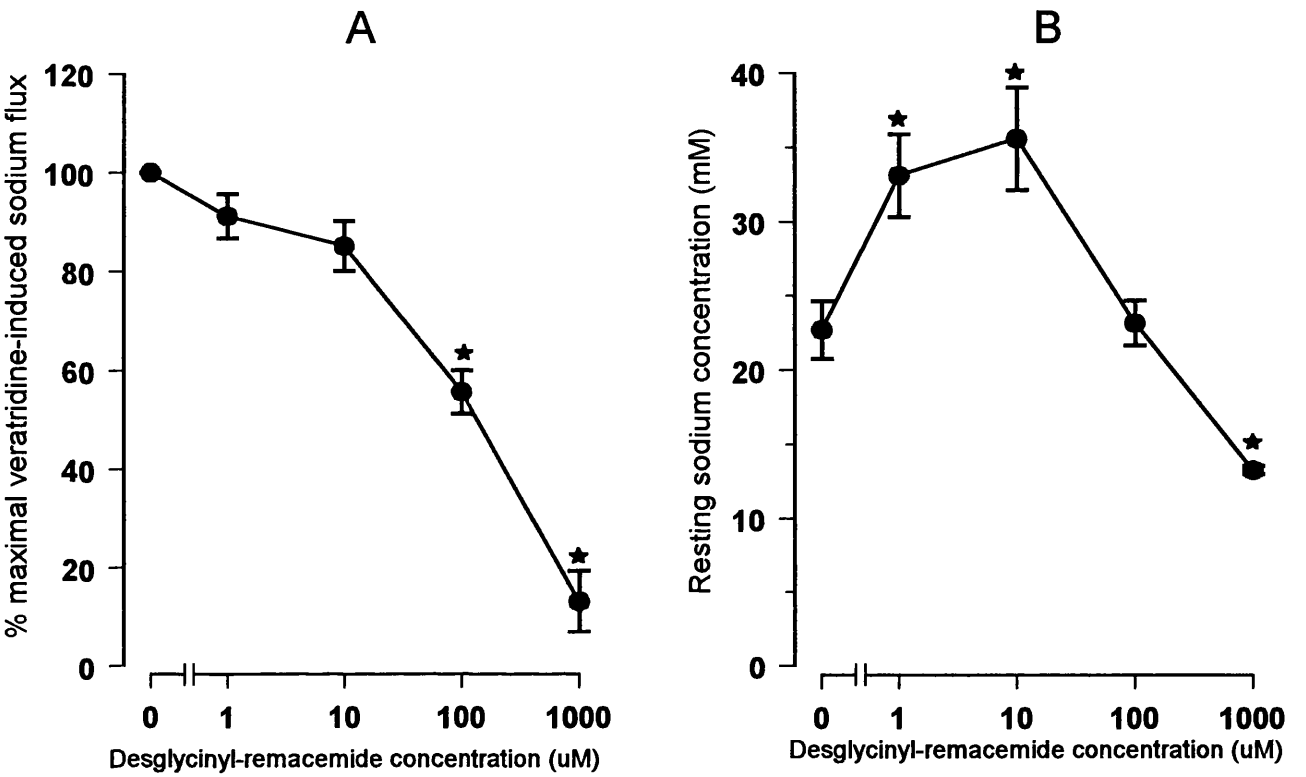


Figure 29: (A) Effect of desglycinyl-remacemide (0 - 1000 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. (B) Effect of desglycinyl-remacemide (0 - 1000 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM). Statistical significance ($p < 0.05$) in (A) and (B) was determined by one-way analysis of variance with Dunnett correction for multiple comparisons.

Although ARR-15896 reduced VTD-stimulated sodium influx to less than 50% of control, the data was not conducive to calculating IC_{50} levels.

The mean (± S.E.M.) resting sodium concentration of the synaptosomes was 22.5 mM (± 2.0; n = 25). DGR (1 and 10 μM) significantly ($p < 0.05$) increased the resting sodium concentration (figure 29B). In contrast, a significant ($p < 0.05$) decrease in resting sodium levels was observed with 1000 μM DGR and also with 1000 μM CBZ (figure 30B). RMD (figure 28B), ARR15896 (figure 31B), LTG (figure 32B) and PHT (figure 33B) had no effect. TTX had no effect on resting sodium levels (data not shown).

6.7 Discussion

As discussed in previous sections, considerable electrophysiological and neurochemical evidence suggests that blockade of voltage-gated sodium channels is a common and important mechanism of AED action (Rogawski and Porter, 1990; White, 1999).

This investigation was designed to confirm and characterise the sodium channel effects of RMD and DGR using a simple neurochemical assay in rat brain synaptosomes.

Assay validation with the prototypic sodium channel blocker TTX confirmed the specificity of the technique and its suitability for the study of voltage-gated sodium channels and their pharmacological amelioration. Subsequent studies with RMD and DGR suggested that both agents blocked voltage-

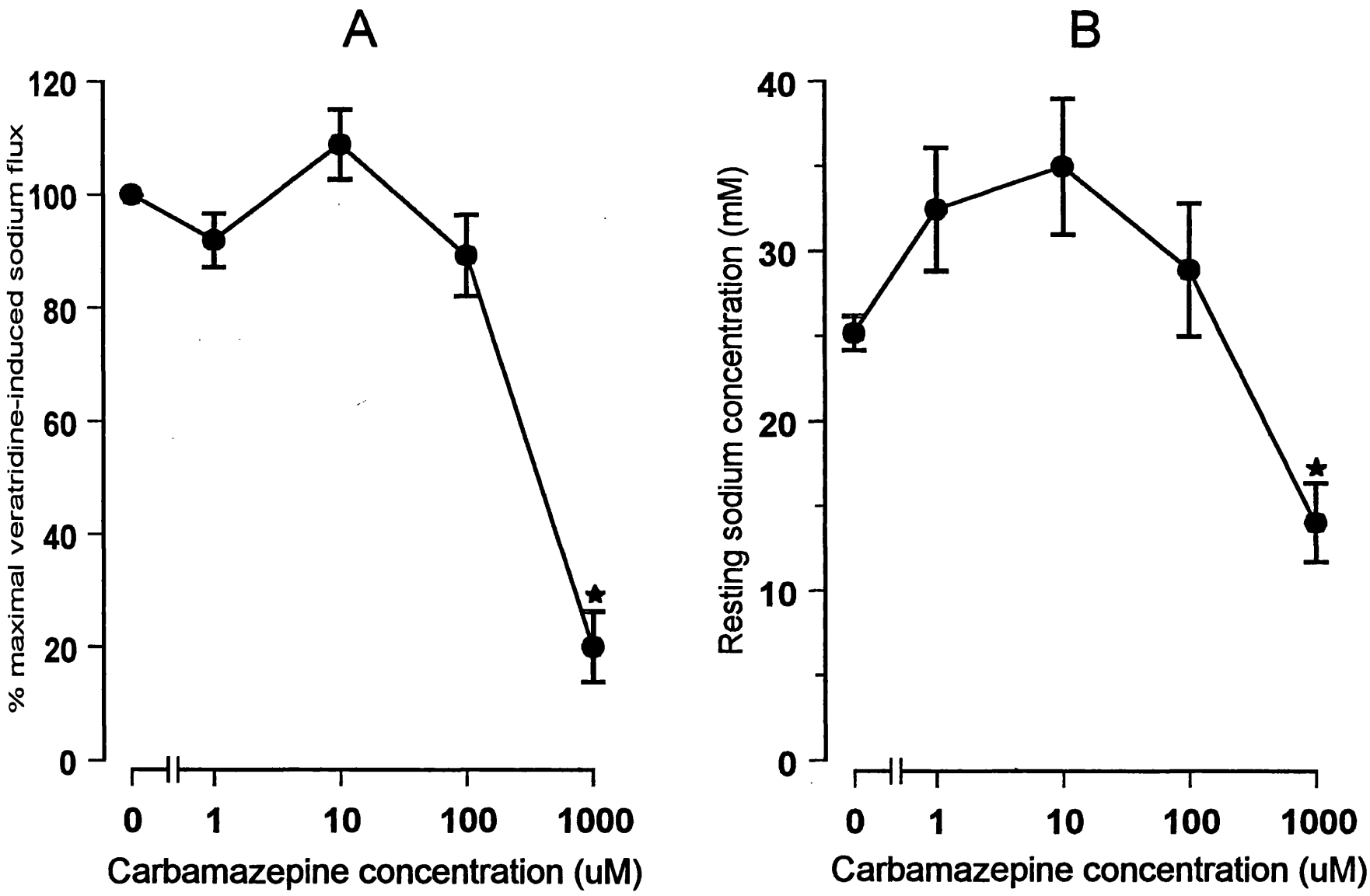


Figure 30: (A) Effect of carbamazepine (0 - 1000 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. (B) Effect of carbamazepine (0 - 1000 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM). Statistical significance (* $p < 0.05$) in (A) and (B) was determined by one-way analysis of variance with Dunnett correction for multiple comparisons.

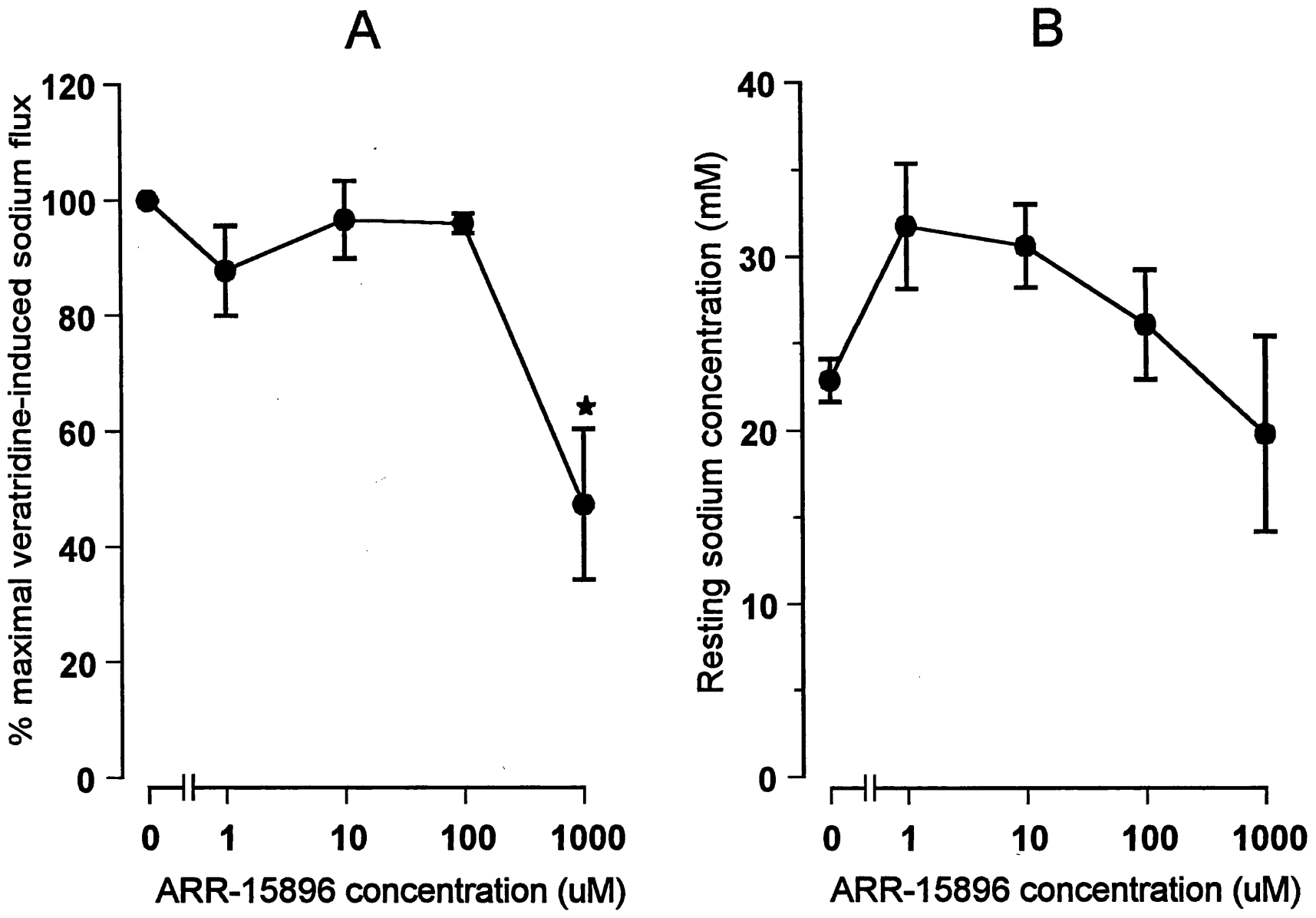


Figure 31: (A) Effect of ARR-15896 (0 - 1000 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. Statistical significance (* $p < 0.05$) was determined by one-way analysis of variance with Dunnett correction for multiple comparisons. (B) Effect of ARR-15896 (0 - 1000 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM).

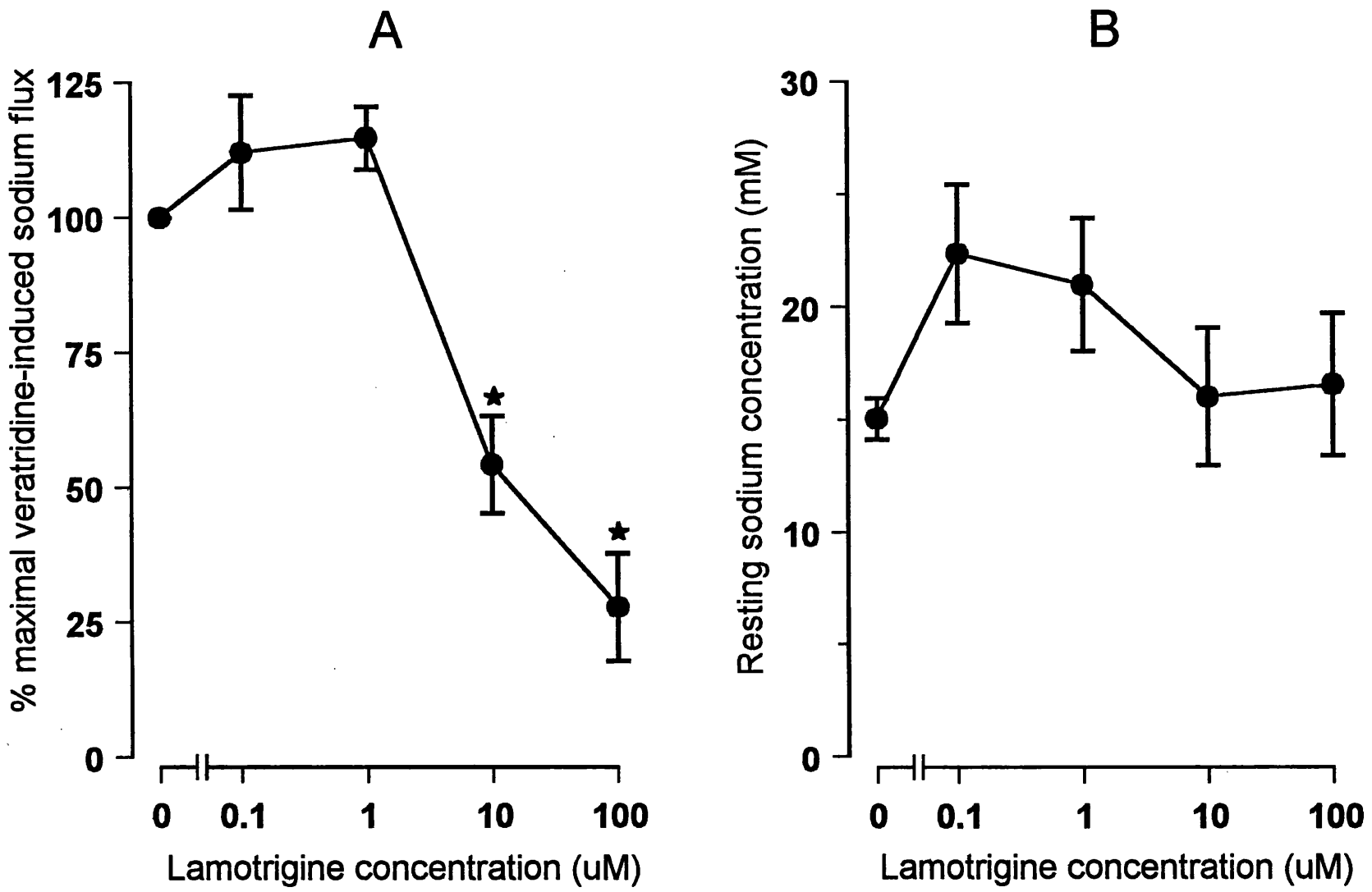


Figure 32: (A) Effect of lamotrigine (0 - 100 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. (B) Effect of lamotrigine (0 - 100 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM). Statistical significance (* $p < 0.05$) in (A) and (B) was determined by one-way analysis of variance with Dunnett correction for multiple comparisons.

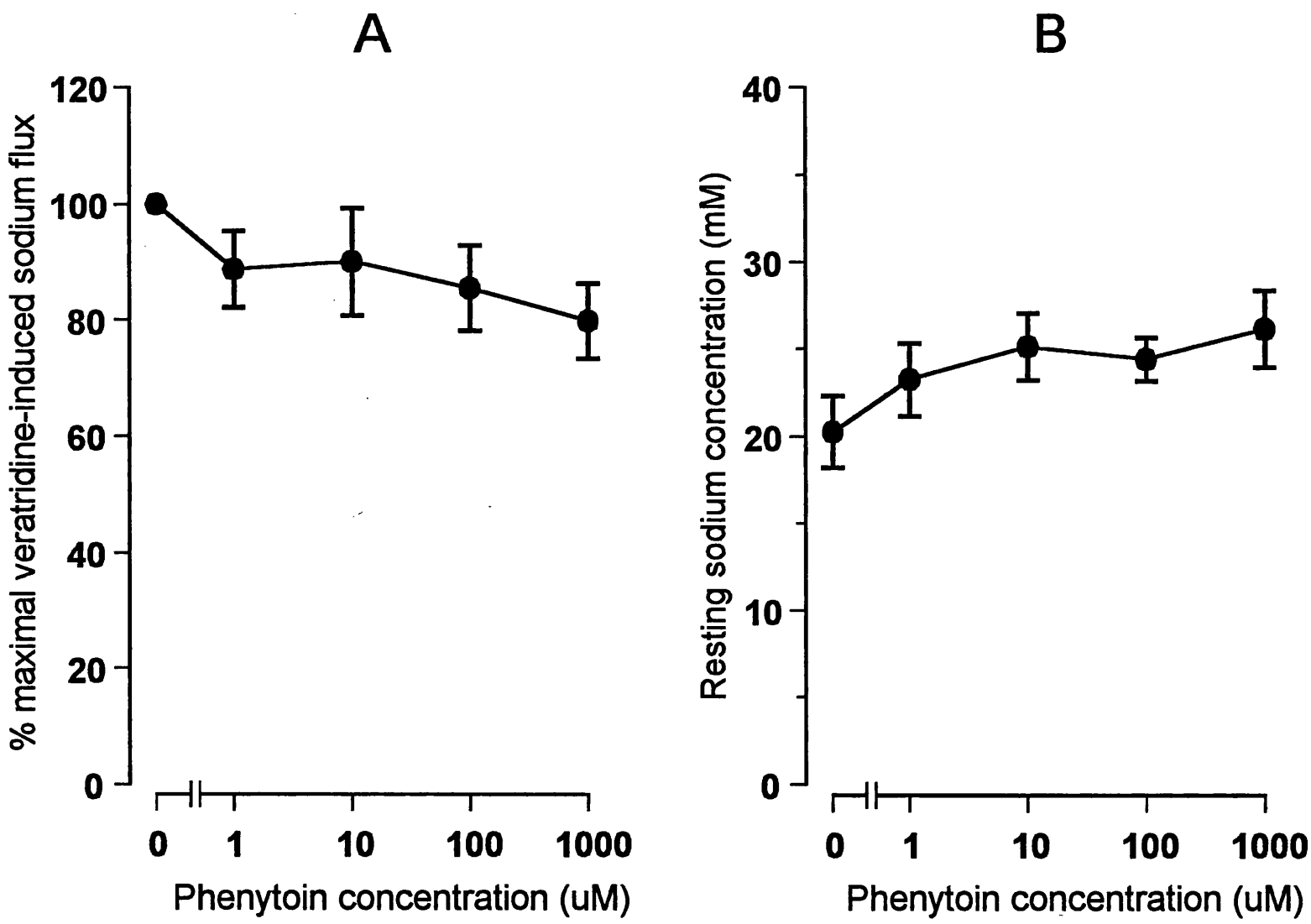


Figure 33: (A) Effect of phenytoin (0 - 1000 μM) on veratridine-induced sodium influx in rat cortical synaptosomes. Results (n = 5) are expressed as the mean percentage of the maximal response to veratridine. Error bars denote the standard error of the mean. (B) Effect of phenytoin (0 - 1000 μM) on resting internal sodium concentration in rat cortical synaptosomes. Results (n = 5) are expressed as absolute sodium concentrations (mM).

gated sodium channels in a concentration dependent manner, confirming previous electrophysiological findings in this regard. The reference compounds, CBZ, ARR-15896, PHT and LTG, similarly reduced VTD-stimulated sodium influx, in accordance with their reported mechanisms of action. ARR-15896 was included due to its action on the NMDA subtype of glutamate receptor. In terms of relative potency, however, a wide variability was observed. LTG was the most potent agent (IC_{50}= 23 μM), followed by DGR (IC_{50}= 85 μM), RMD (IC_{50} = 161 μM), CBZ (IC_{50} = 326 μM) and PHT which, even at supra-therapeutic concentrations, reduced sodium influx by only 20%, compared to control. An IC_{50} value for ARR-15896 could not be calculated.

Previous studies suggest that both RMD and DGR displace [^{3}H]-batrachotoxin binding to voltage-gated sodium channels with IC_{50} values of 15.6 μM and 7.9 μM, respectively (Palmer et al, 1992). In cultured mouse spinal cord neurones, RMD and DGR reduce sustained repetitive firing, an electrophysiological marker for voltage-gated sodium channel activity, with IC_{50} values of 7.9 μM and 1.2 μM, respectively (Wamil et al, 1996). A similar study, recording sustained repetitive firing of CA1 neurones in the intact hippocampal slice preparation reported half maximal inhibitory concentrations of 66 μM and 60 μM for RMD and DGR, respectively (Norris and King, 1997a). Finally, a neurochemical investigation, employing VTD-induced glutamate and aspartate release as surrogate markers of sodium channel activity, reported an IC_{50} of 12.5 μM for DGR (Srinivasan et al, 1995).

Although these findings largely support the two-fold separation in potency between RMD and DGR in the current investigation, when IC_{50} values are compared, questions of pharmacological sensitivity arise.

However, similarly disparate results are reported from a surfeit of studies with other sodium channel blocking AEDs. CBZ reduces sustained repetitive firing in cultured mouse central neurones with an IC_{50} of 4 μM (McLean and Macdonald, 1986b) and prevents batrachotoxin-induced $^{22}Na^{+}$ flux into rat brain synaptosomes at 22 μM (Willow et al, 1984). In addition, it displaces [^{3}H]-batrachotoxin binding with an IC_{50} of 131 μM (Willow and Catterall, 1982) and prevents VTD-induced glutamate release from rat brain synaptosomes at 200 μM (Lingamaneni and Hemmings, 1999). A similar range of potencies have been reported from multiple and diverse studies with PHT (4 - 200 μM; Willow and Catterall, 1982; Willow et al, 1984; Willow et al, 1985; Lang et al, 1993; Deffois et al, 1996; Lingamaneni and Hemmings, 1999). ARR-15896 has no reported effects on sodium channels and was included so that the effects of NMDA receptor blockade could be assessed in this model. Given the high concentrations at which they occurred, it is thought that the effects observed with ARR-15896 in this study were down to synaptosomal toxicity and not a pharmacological effect.

In order to understand wide variations in potency when comparing the findings of pharmacological studies of sodium channel blockade, it is important to consider methodological influences and to appreciate that

sodium channels are considerably more sensitive to AED-mediated amelioration under depolarising conditions (Rogawski and Porter, 1990). This reflects the significantly greater affinity of these agents for the inactivated state of the channel and is fundamental in their ability to reduce high frequency neuronal firing with little or no effect on the amplitude or duration of single action potentials (Kuo, 1998). As such, electrophysiological investigations of the voltage- and use-dependent blockade of sodium channels by AEDs are consistently more sensitive than neurochemical studies which are usually conducted under physiological conditions and accordingly restricted to reporting tonic inhibitory effects of AEDs on sodium channel function. Moreover, the response to any given compound is generally greater in studies employing acutely isolated or cultured neurones than brain slices. Similarly, receptor binding studies often reveal impressive drug potencies while those employing surrogate markers of sodium channel activity, such as neurotransmitter release, are less sensitive. These variabilities in pharmacological response may reflect differences in drug penetration, non-specific binding and, in the case of brain slice preparations, glial cell interaction.

The results of this study, and countless previous investigations, suggest that, at therapeutic concentrations, the sodium channel effects of AEDs are restricted to a characteristic voltage- and use-dependent blockade which reflects affinity for the inactivated state of the channel and underlies the ability of these drugs to prevent high frequency neuronal burst firing. Tonic

inhibition of sodium channels, with the potential to influence action potential generation, is only observed at supra-therapeutic concentrations.

There is no evidence to suggest that AEDs influence sodium homeostasis at resting membrane potential. In this study, however, DGR (1 and 10 μM) significantly increased resting sodium concentration. A contrasting decrease was observed with both DGR and CBZ at 1000 μM, although this finding can be discounted on the basis of drug toxicity. The effect of DGR on resting internal sodium concentration is unlikely to be related to voltage-gated sodium channel blockade, given the wide separation in effective concentrations. It is possible that DGR has a weak pharmacological action on one of the many transport systems, such as Na^+/K^+ ATP-ase and the Na^+/H^+ antiport, which maintain the sodium ion gradient across the cell membrane. This hypothesis requires further detailed investigation before appropriate conclusions can be made.

In conclusion, the novel anticonvulsant compound RMD, and its active metabolite DGR, reduce voltage-gated sodium channel activity in a concentration-related manner. Previous electrophysiological investigations have reported voltage- and use-dependent blockade of sodium channels with these agents (Wamil et al, 1996; Norris and King, 1997a). This neurochemical study supports these findings and suggests that tonic inhibition of sodium channel function is only observed at supra-therapeutic concentrations. Given the relative potencies and steady state concentrations of both agents following RMD administration in man, it is likely that the desglycinyl metabolite is the

principal pharmacological moiety and NMDA receptor blockade the primary mechanism of action (Davies, 1997). Nevertheless, inhibition of voltage-gated sodium channels by RMD undoubtedly contributes to its anticonvulsant effect.

Chapter 7

Effects of remacemide and desglycinyl-remacemide on glutamate dehydrogenase activity in rat brain mitochondria

7.1 Introduction

Glutamate dehydrogenase (GDH) is one of the enzymes involved in glutamate homeostasis. It is responsible for the conversion of glutamate to α-KG by oxidative deamination and also for the conversion of α-KG to glutamate by reductive amination (figure 34; Kuo et al, 1994). α-KG, which is derived from glucose in both neurones and astrocytes, is a cofactor in the conversion of GABA to glutamate and succinic semialdehyde and an important precursor for metabolic and neurotransmitter glutamate (Shank and Aprison, 1981).

The activity of GDH in brain homogenates is relatively high compared to other enzyme systems (Salganicoff and De Robertis, 1965; Cooper et al, 1985), which indicates that the enzyme is capable of rapid catalytic turnover. However, several studies in cultured astrocytes (Yudkoff et al, 1986; Farinelli and Nicklas, 1992), neuronal fragments (Erecinska and Nelson, 1990; Yudkoff et al, 1991), and whole brain *in vivo* (Cooper et al, 1979), have shown that the reductive amination of α-KG and oxidative deamination of glutamate are slow processes. This suggests that the activity of GDH may be under the control of an inhibitory factor or factors. The rapid increase in extracellular glutamate concentrations that are measured following ischaemic episodes suggests that these factors may be destroyed when the integrity of the nervous tissue is lost (Kuo et al, 1994).

Increased levels of GDH have been measured in seizure foci (Sherwin and Gelder, 1986) indicating a possible involvement of the enzyme in seizure processes.

Previous neurochemical studies (Leach et al, 1997d) have demonstrated that DGR exerts an effect on the GAD and GABA-T enzymes that are involved in GABA/glutamate homeostasis following chronic administration. The following study was designed to investigate the effects of RMD and DGR on the activity of GDH for both the reductive amination of α-KG and the oxidative deamination of glutamate.

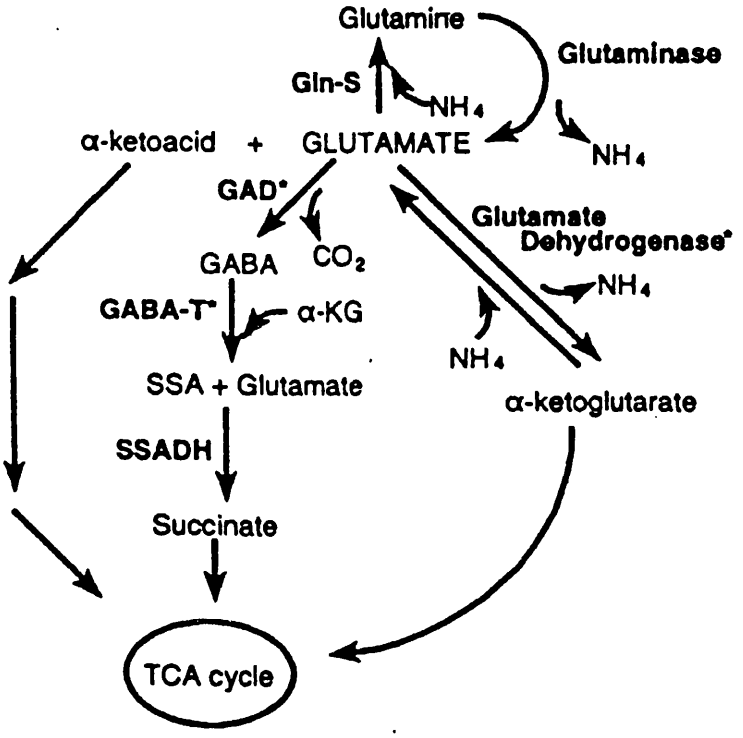


Figure 34: A simplified diagram demonstrating the various routes of glutamate and GABA formation (Goldlust et al, 1995). Abbreviations: GAD, glutamate decarboxylase. Gln-S, glutamine synthetase; SSA, succinic acid semialdehyde; SSADH, succinic acid semialdehyde dehydrogenase; α-KG, α-ketoglutarate.

7.2 Aims

The following experiments were conducted to investigate the effects of RMD and DGR on the actions of GDH in isolated rat brain mitochondria. CBZ and ARR-15896 were employed for comparative purposes.

7.3 Methods

The following method was adapted from the published methodologies of Kaur and Kanungo (1970) and Erecinska and Nelson (1990).

7.3.1 *Preparation of tissue*

Mitochondria were prepared from adult, male Wistar rats (200-250 g). Animals were sacrificed by cervical dislocation with the forebrain immediately removed and placed in an ice-cold isolation medium consisting of 0.32 M sucrose, 1 mM K^+ EDTA and 10 mM Tris HCl (pH 7.4). The tissue was then chopped into small pieces using a scalpel blade. Blood and other debris were removed by adding 10 ml of isolation medium and decanting the supernatant following a 3 minute spin at 1,000 x g at 4°C. This process was carried out twice prior to the tissue being homogenised with a motorised teflon pestle in 30 ml of isolation medium.

The homogenate was centrifuged at 1,300 x g for 3 minutes at 4°C. The resulting supernatant was centrifuged at 17,000 x g for 10 minutes at 4°C to produce a crude mitchondrial/synaptosomal pellet. The pellet was resuspended in 2 ml isolation medium and diluted to 15 ml with a 12%

Ficoll/sucrose medium (12% (w/w) Ficoll, 0.32 M sucrose, 50 μM K^+ EDTA; pH 7.4). This suspension was given 5 hand held turns with a teflon pestle. The suspension was divided into three centrifuge tubes with 2 ml of a 7.5% Ficoll buffer (7.5% (w/w) Ficoll, 0.32 M sucrose, 50 μM K^+ EDTA; pH 7.4) placed on top of the suspension. Finally, 2 ml of isolation medium was layered on top. This gradient was centrifuged at 99, 000 x g for 30 minutes at 4°C. The pellet formed as a result of this process contained the free mitochondria. They were resuspended to a protein concentration of 2 mg/ml in a final incubation medium consisting of 0.32 M sucrose, 10 mM Tris-HCl and 0.5 mM EDTA (pH 7.4) and placed on ice until required.

7.3.2 *Measurement of enzyme activity*

Analysis of glutamate dehydrogenase activity was carried out using a microplate reader and was integrated using Revelation (version 3.2) software. Absorbance readings at 340 nm were obtained every 10 seconds for 3 minutes.

Drugs were dissolved in the final incubation medium described above. Mitchondria were incubated for three hours with the drug at room temperature. Control preparations were incubated with blank final incubation medium. Triton X-100 (2%) was also added to all samples to permeabilise the mitchondria. Enzyme activity in the direction of glutamate $\Rightarrow$ α-KG (oxidative deamination) was measured in the following buffer - 150 mM KCl, 20 mM Tris HCl, 2 mM EGTA, 1 mM NAD^+, 50 mM glutamate, 0.01 mM

rotenone, 1.5 mM ADP (pH 7.6). Following incubation, 100 µl of the mitochondria was placed in a well of a 96-well plate with 900 µl of the assay buffer resulting in a final protein concentration of 200 µg/ml. The plate was immediately analysed for changes in absorbance at 340 nm over a 5 minute period.

Glutamate dehydrogenase activity in the direction of α-KG ⇒ glutamate (reductive amination) was measured in the following buffer- 150 mM KCl, 20 mM Tris HCl, 0.16 mM NADH, 2 mM EGTA, 50 mM NH_4Cl, 1 mM 2-oxoglutarate (α-KG), 0.01 mM rotenone, 1.5 mM ADP (pH 7.6). Again, 100 µl of the drug/medium incubated mitochondria was added to 900 µl of the assay buffer in a 96-well plate. The plate was immediately analysed for changes in absorbance at 340 nm over a 5 minute period.

7.4 Experimental protocol

Mitochondria, prepared from rat brain as described in section 7.3.1, were incubated for three hours with either RMD, DGR, CBZ or ARR-15896 (0 - 1000 µM). Following the three hour incubation, glutamate dehydrogenase activity was assessed by the methods described in section 7.3.2. Each experiment was repeated on 5 separate occasions.

7.5 Data analysis and statistics

Changes in absorbance were calculated from an immediate first reading compared to one taken after 5 minutes. The results were compared to those

obtained from non-drug containing control samples. Results for both directions of the reaction (reductive amination and oxidative deamination) are expressed as a mean percentage of control ± SEM. Results were compared to control by one-way analysis of variance with Dunnett correction for multiple comparisons.

7.6 Results

RMD, DGR and CBZ significantly ($p < 0.05$) decreased the conversion of α-KG to glutamate (reductive amination) to 60.5% (± 6.6; figure 35), 79.3% (± 3.5; figure 36) and 82.3% (± 5.9; figure 37) of control, respectively. ARR-15896 was without effect at any of the concentrations studied (figure 38).

RMD and DGR significantly ($p < 0.05$) decreased the conversion of glutamate to α-KG (oxidative deamination) to 80.2% (± 3.0; figure 39) and 73.0% (± 7.2; figure 40) of control, respectively. CBZ and ARR-15896 significantly ($p < 0.05$) increased the rate of α-KG formation to 126.33% (± 9.85; figure 41) and 149.1% (± 9.0; figure 42) of control respectively.

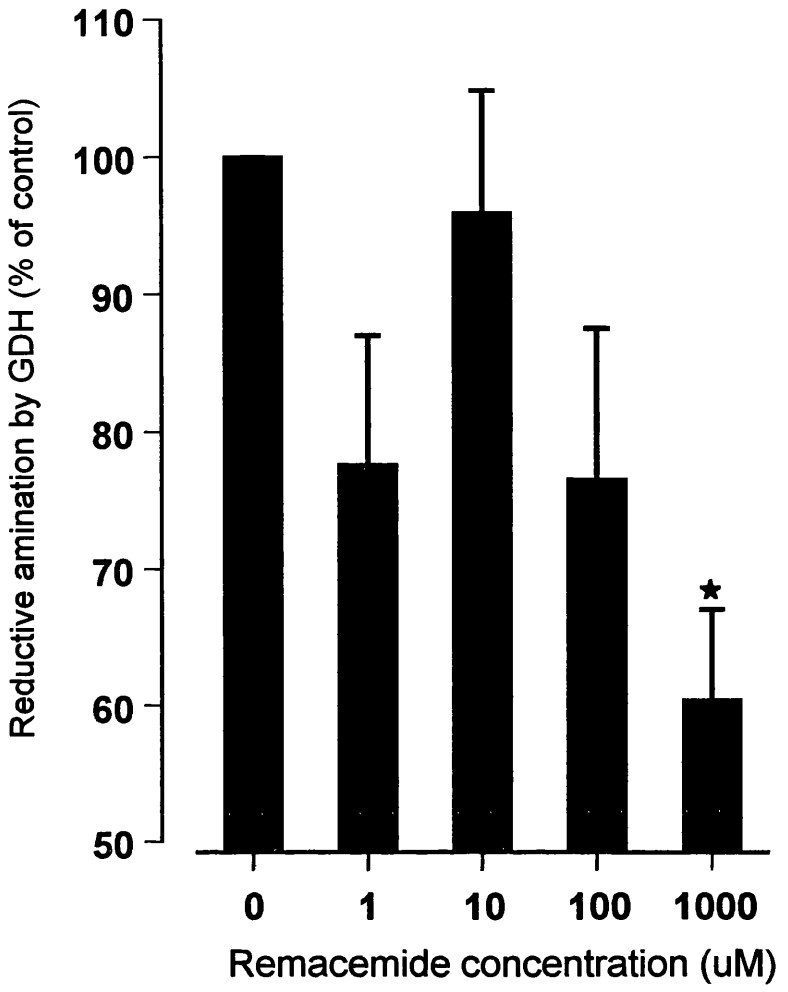


Figure 35: Effects of remacemide (0 - 1000 μM) on the reductive amination of α-ketoglutarate by glutamate dehydrogenase following a 3 hour incubation. Results (n = 5) are expressed as a mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance ($*p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

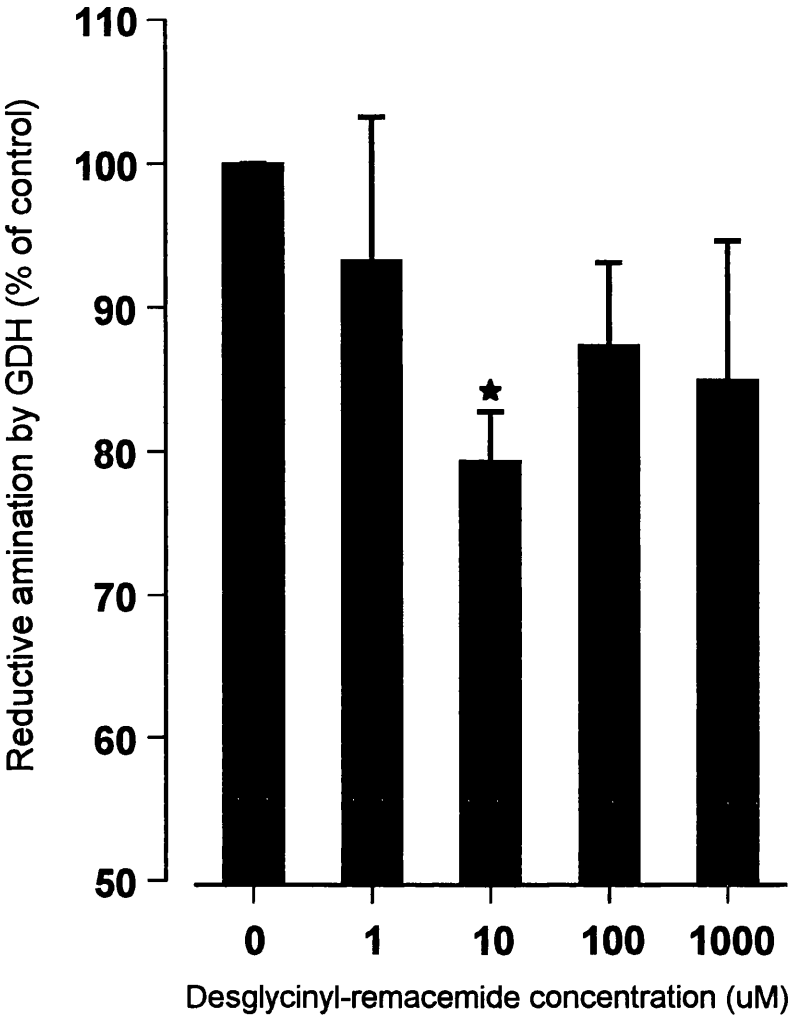


Figure 36: Effects of desglycinyl-remacemide (0 - 1000 μM) on the reductive amination of α-ketoglutarate by glutamate dehydrogenase following a 3 hour incubation. Results (n = 5) are expressed as a mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance (*$p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

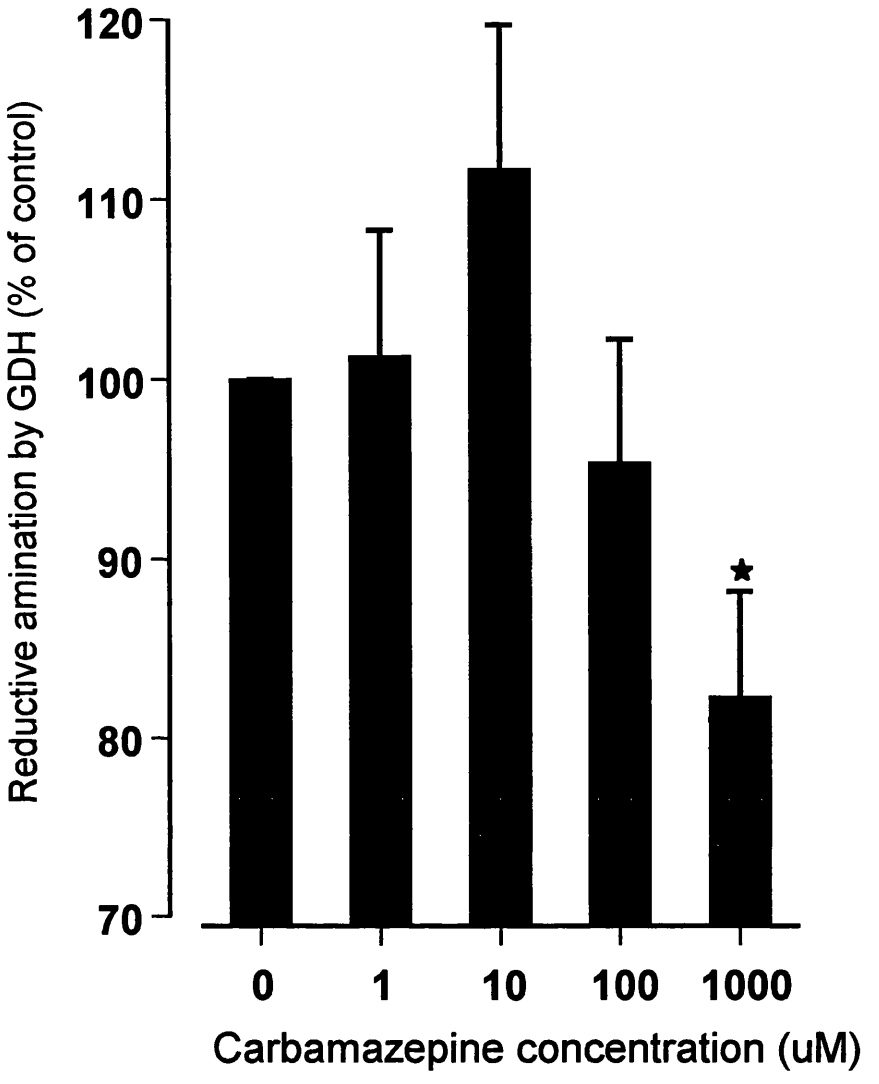


Figure 37: Effects of carbamazepine (0 - 1000 μM) on the reductive amination of α-ketoglutarate by glutamate dehydrogenase following a 3 hour incubation. Results (n = 5) are expressed as a mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance ($*p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

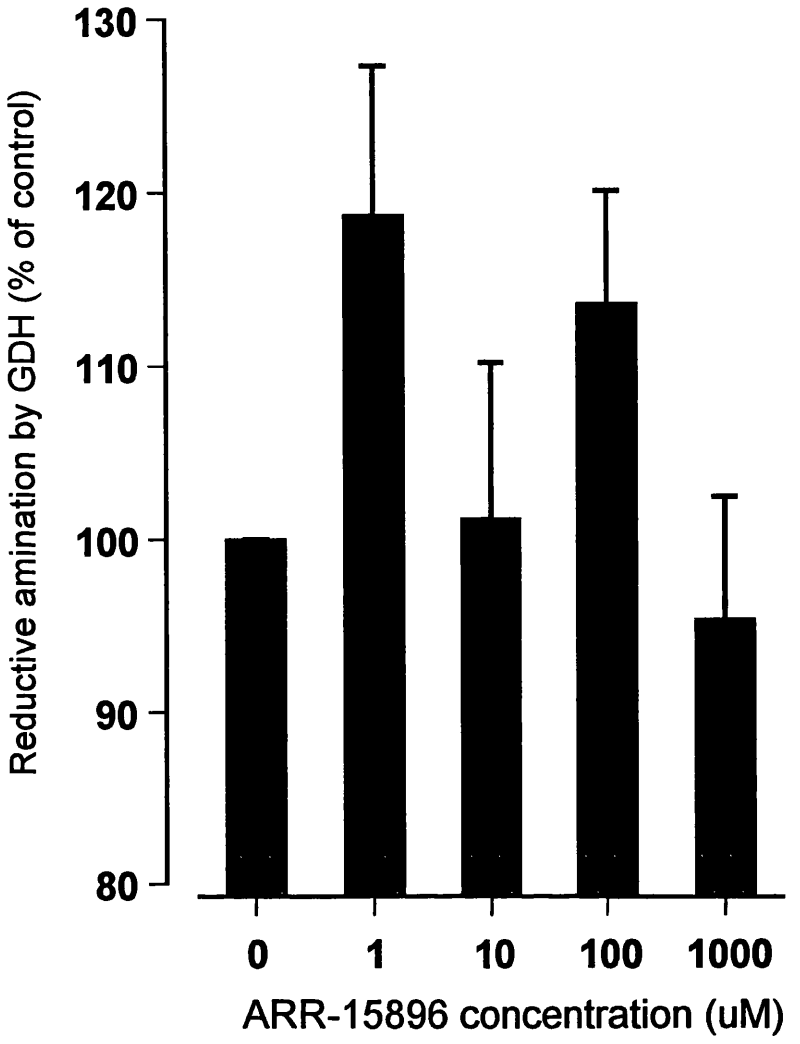


Figure 38: Effects of ARR-15896 (0 - 1000 μM) on the reductive amination of α-ketoglutarate by glutamate dehydrogenase following a 3 hour incubation. Results (n = 5) are expressed as a mean percentage of control activity with error bars representing the standard error of the mean.

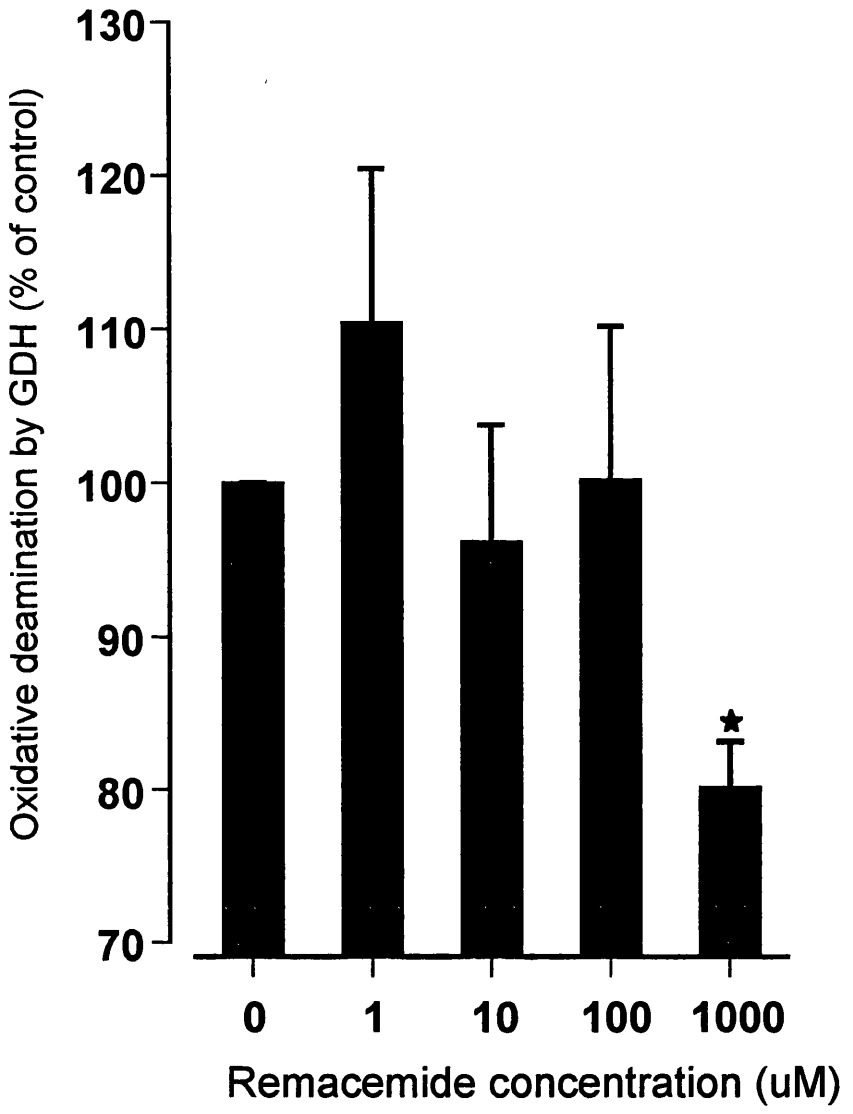


Figure 39: Effects of remacemide (0 - 1000 μM) on the oxidative deamination of glutamate by dehydrogenase activity following a 3 hour incubation. Results (n = 5) are expressed as the mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance (*$p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

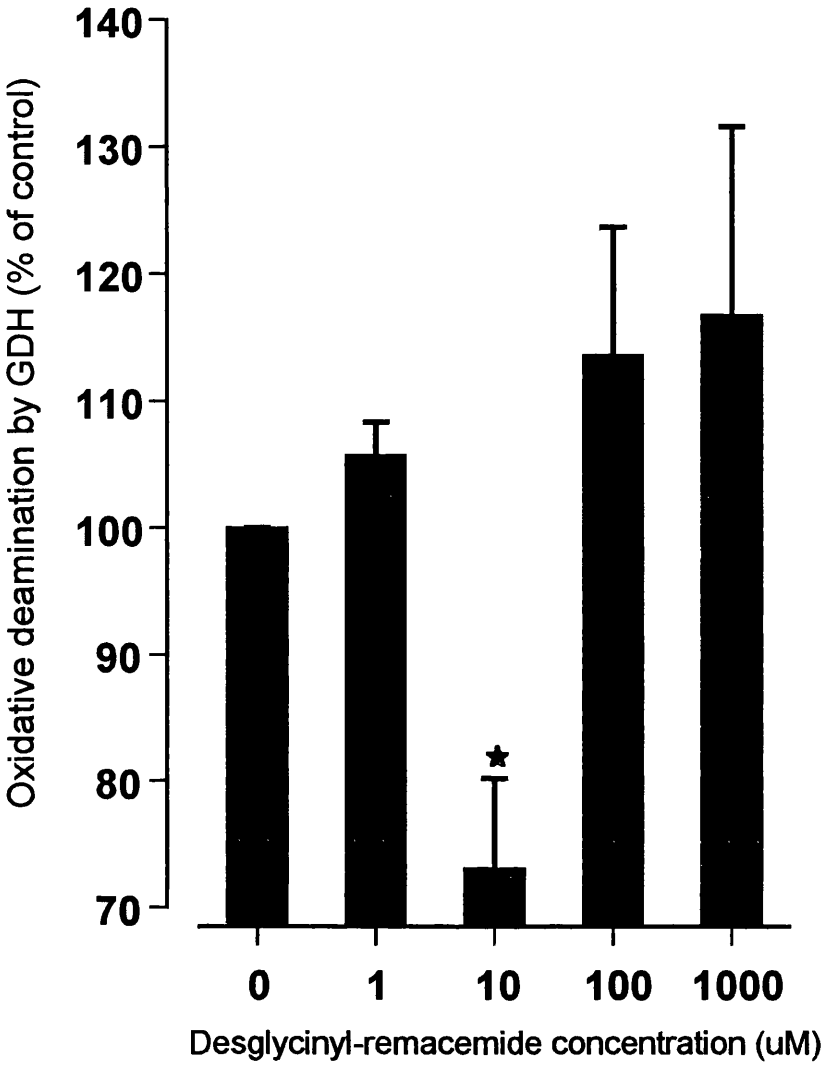


Figure 40: Effects of desglycinyl-remacemide (1 - 1000 μM) on the oxidative deamination of glutamate by dehydrogenase activity following a 3 hour incubation. Results (n = 5) are expressed as the mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance (*$p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

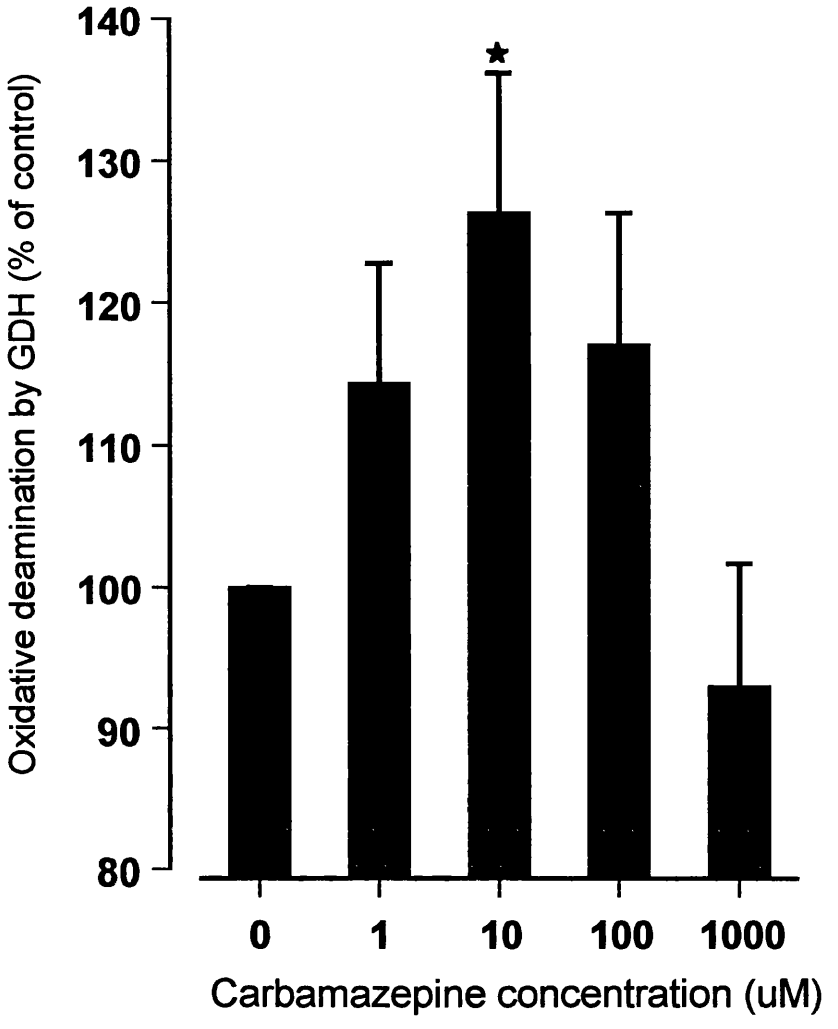


Figure 41: Effects of carbamazepine (1 - 1000 μM) on the oxidative deamination of glutamate by dehydrogenase activity following a 3 hour incubation. Results (n = 5) are expressed as the mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance (*$p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

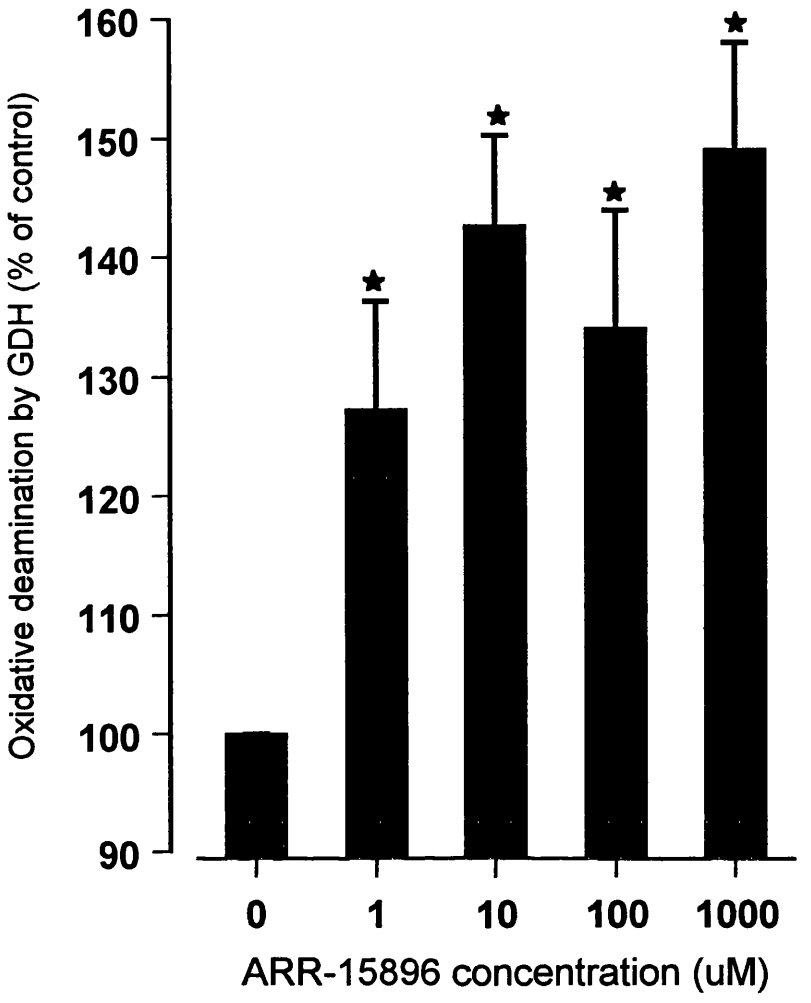


Figure 42: Effects of ARR-15896 (1 - 1000 μM) on the oxidative deamination of glutamate by dehydrogenase activity following a 3 hour incubation. Results (n = 5) are expressed as the mean percentage of control activity with error bars representing the standard error of the mean. Statistical significance (*$p < 0.05$) was determined by one way analysis of variance with Dunnett correction for multiple comparisons.

7.7 Discussion

The role of glutamate in epilepsy is well documented (Greenamyre and Porter, 1994). Compounds which mimic or increase glutamate levels have been shown to have seizure producing properties (Fisher, 1989) and human brain dialysis studies have shown that the extracellular glutamate concentration in the epileptic focus is markedly increased during seizures (Carlson et al, 1992; During and Spencer, 1993; Wilson et al, 1996). The homeostasis of glutamate, and indeed GABA, is therefore critical to the maintenance of normal brain activity.

The above study was designed to investigate the effects of RMD and DGR on the activity of GDH, the enzyme responsible for the formation of glutamate from α-KG (reductive amination) and also for the conversion of glutamate to α-KG (oxidative deamination).

RMD, DGR and CBZ significantly decreased the formation of glutamate from α-KG in the above study while ARR-15896 had no effect. RMD and CBZ were shown to have no effect at therapeutically relevant concentrations which are said to be between 1 and 10 μM for both compounds (McLean and Macdonald, 1986; Schachter and Tarsy, 2000). The formation of α-KG from glutamate was significantly reduced by RMD and DGR. However RMD had no effect at therapeutically relevant concentrations. CBZ and ARR-15896 significantly increased the conversion of α-KG to glutamate. Interestingly, DGR significantly decreased both actions of the GDH enzyme at 10 μM. In both cases the results

obtained appeared to be against the general trend observed with other DGR concentrations. There is no clear reason for these findings and further experiments would be require to assess their integrity.

Previous studies have found that DGR significantly increases the activity of GABA-T following repeated administration (Leach et al, 1997d). The same study found that DGR had no effect on GABA or glutamine concentrations, however, a trend towards reduced brain glutamate levels was observed although this finding was not statistically significant. Further investigations (Fraser et al, 1999) revealed that DGR has no effect on the enzyme glutamine synthetase which indicates that the decrease in glutamate levels observed in the original study was not the result of an increase in the conversion of glutamate to glutamine. DGR has also been reported to decrease the activity of GAD, the enzyme responsible for the conversion of glutamate to GABA (Leach et al, 1997d; figure 34).

Limited work has been carried out to assess the effects of AEDs on the activity of GDH. One study (Hitchcock and Teixeira, 1982) found that chronic PHT decreased GDH levels although no measurements of activity were performed. The same study also stated that VPA increased GDH and GABA levels. More recently, GBP was found to increase the oxidative deamination of glutamate to 342% of control (Goldlust et al, 1995).

In brain, the role of GDH has yet to be fully elucidated (Plaitakis et al, 2000). There is a general consensus that formation of glutamate in cultured astocytes occurs mainly via the actions of glutaminase (figure 34) rather than reductive

amination by GDH (Yudkoff et al, 1991; McKenna et al, 1996; Westergaard et al, 1996). However, the GDH reaction may account for some fraction of glutamate synthesised in nerve tissue under conditions of hyperammonemia (Kanamori and Ross, 1995) which can be caused by VPA treatment (Marescaux et al, 1983). This could account for the results obtained in previous studies with VPA (Hitchcock and Teixeira, 1982).

RMD and DGR were shown to have no effects on the activity of GDH at therapeutically relevant concentrations which are estimated to be between 1 and 10 μM for both RMD and DGR (Schachter and Tarsy, 2000). This is in agreement with previous studies that suggested that acute DGR administration was without effects on other enzymes involved in GABA/glutamate homeostasis (Leach et al, 1997d). CBZ was shown to increase the conversion of glutamate to α-KG at therapeutic concentrations. These actions may contribute to the anticonvulsant properties of CBZ although further investigations are required. ARR-15896 significantly increased the conversion of glutamate to α-KG, which again may contribute to its anticonvulsant action. It may also have implications for its efficacy against stroke-induced ischemia.

An *in vivo* study measuring the effects of anticonvulsants on GDH activity would have been a useful follow-up study given more time. Chronic administration of compounds would perhaps validate the above results and give further insight into the possible implications of any observations.

In conclusion, the activity of the enzyme GDH was measured in rat brain mitochondria with the effects of RMD, DGR, CBZ and ARR-15896 being investigated. RMD, DGR and CBZ all decreased the formation of glutamate from α-KG although none of the effects occurred at therapeutically relevant concentrations. ARR-15896 was found to have no effect. CBZ and ARR-15896 increased the conversion of glutamate to α-KG which may contribute to their anticonvulsant effects by decreasing glutamate concentrations. The results may also be important for the use of ARR-15896 against stroke-induced ischaemia. Although the role of GDH in glutamate homeostasis remains to be fully elucidated it is possible that in disease states, such as epilepsy, glutamate regulation via the GDH system plays an important role. Further investigations are required to confirm the above findings *in vivo* and to assess the clinical importance of the results.

Chapter 8

The effects of remacemide and desglycinyl-remacemide on monoamine uptake in rat brain synaptosomes

8.1 Introduction

There is growing evidence to suggest that monoamines have an important role in epilepsy (Starr, 1996). The initial indication of the involvement of monoamines in the process of epileptogenesis was provided by studies employing reserpine, which, by depletion of neuronal monoamine levels, facilitates the onset of seizures in DBA/2 mice (Chen et al, 1954; Lehmann, 1967; Chapman and Meldrum, 1989). Selective destruction of monoaminergic nerve terminals with 6-OHDA, or strategically placed electrolytic lesions in both genetic epilepsy models and non-epileptic mammals (Jobe et al, 1999), provides further evidence for the importance of monoamines in seizure suppression.

Synaptic inactivation of monoamine neurotransmitters occurs via a combination of both neuronal and extraneuronal re-uptake and intracellular enzymatic degradation (Rang et al, 1995). Re-uptake of monoamines from the synaptic cleft is mediated via transporter proteins, belonging to the Na^+- and Cl^--dependent amine transporter family that exist in both neuronal and glial cell membranes (Fleckenstein et al, 2000). In addition, within the nerve terminals, vesicular monoamine transporters operate to sequester monoamines into vesicles prior to release as neurotransmitters (Fleckenstein et al, 2000). Drugs may act on these transporters to alter their activity and consequently influence synaptic transmission (Haughey et al, 1999). Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) represent the degradative enzymes responsible for the inactivation of monoamines following re-uptake (Rang et al, 1995). Inhibition of these enzymes therefore increases synaptic

monoamine concentrations. MAO inhibitors, such as clorgyline, increase monoamine levels in the synaptic cleft (Lehmann, 1967), with a resultant decrease in convulsion intensity in animal seizure models (Finberg et al, 1993; Mishra et al, 1993).

Fluoxetine (FLU), an antidepressant that acts selectively by 5-HT reuptake inhibition, produces dose dependent anticonvulsant effects in the GEPR (Dailey et al, 1992). These anticonvulsant effects can be correlated with increased extracellular 5-HT concentrations (Yan et al, 1994). Results obtained from experimental models of epilepsy (Alexander and Kopeloff, 1970; Kellogg, 1971; Racine and Coscina, 1979) support the involvement of serotonergic systems in inhibition of seizure activity. The absence of a steady state 5-HT level in animals susceptible to audiogenic seizures (Naffah-Mazzacoratti et al, 1996) and the ability of endogenous 5-HT to suppress picrotoxin induced epileptiform activity in Sprague-Dawley rats (Lu and Gean, 1998) provides further evidence.

There is, similarly, growing evidence to suggest that NE exerts an inhibitory influence in the CNS, therefore enhanced NE transmission may limit the spread of seizure discharges (Lehmann, 1967; Jobe et al, 1972; McIntyre et al, 1979; Applegate et al, 1986; Shouse et al 1996). Restoration of NE levels via a foetal locus coeruleus graft (NE containing neurones), reverses this effect (Kokaia et al, 1994), therefore indicating a NE involvement in seizure suppression (McIntyre et al, 1979; Applegate et al, 1986; McIntyre and Edson, 1989; Bengzon et al, 1992). In addition, tricyclic antidepressants, such

as desipramine (DES), inhibit NE reuptake and increase both synaptic NE concentration and the seizure threshold (Wood et al, 1977). Confirmation of these effects in epileptic patients was achieved by electrical activation of the locus coeruleus through cerebellar stimulation, resulting in elevated NE levels in the CSF and an improvement in seizure tolerance (Wood et al, 1977; Bengzon et al, 1992).

Previous research has revealed reduced levels of homovanillic acid (HVA), a DA metabolite, in patients with temporal lobe epilepsy (Kresch et al, 1989), an effect that can be reversed by CBZ (Okada et al, 1997b). This suggests a role for DA in seizure susceptibility. Administration of levodopa, a precursor for both NE and DA, diminishes the occurrence of tonic extensor convulsions in audiogenic seizure susceptible DBA/2 mice, while increasing brain NE and DA concentrations (Dailey and Jobe, 1984). However, inhibition of the conversion of levodopa to NE, via DA β hydroxylase suppression, does not alter this anticonvulsant effect therefore indicating the importance of DA (Dailey and Jobe, 1984).

There is limited data to suggest that some drugs that block monoamine reuptake are effective anticonvulsants in man (Ojemann et al, 1983). Whether an action on monoamine reuptake is important in terms of RMD and currently used AEDs remains to be investigated.

8.2 Aims

The aim of these experiments was to determine whether inhibition of monoamine re-uptake might contribute to the antiepileptic actions of RMD and DGR. CBZ, PHT, LTG and ARR-15896 were included for comparative purposes.

8.3 Methods

8.3.1 *Preparation of synaptosomes*

Adult male Sprague- Dawley rats (200 – 250 g) were sacrificed by cervical dislocation and the brain removed into ice-cold 0.9% saline. Brain regions were isolated according to the method of Glowinski and Iversen (1966). The cerebral cortex was employed for studies of 5-HT and NE uptake and the striatum for DA investigations. The tissue was weighed and homogenised in 40 volumes of ice-cold 0.32 M sucrose solution using a motorised glass-teflon pestle. The homogenate was centrifuged at 1,000 x g for 10 minutes at 4°C to remove erythrocytes and cellular debris. The resulting supernatant was centrifuged for a further 20 minutes at 12,000 x g at 4°C. The final supernatant was discarded and the pellet resuspended in 35 ml Krebs solution (NaCl 125 mM, KCl 3 mM, $NaHCO_3$ 22 mM, $CaCl_2.2H_2O$ 1.2 mM, $MgSO_4.7H_2O$ 1.2 mM, NaH_2PO_4 1 mM, D-glucose 10 mM, ascorbic acid 1 mM) containing pargyline (100 μM). Pargyline was included to prevent MAO-mediated neurotransmitter degradation.

8.3.2 *Monoamine uptake assay*

This technique was devised from modifications of the methods of Matsunaga and co-workers (1998) and Fleckenstein and colleagues (1999). A 400 µl aliquot of synaptosome suspension was incubated at 37^0C for 10 minutes in the presence of 50 nM radiolabelled monoamine (specific activities: NE, 2.5 Ci / mmol; DA, 1.22Ci / mmol; 5-HT, 2.53 Ci / mmol), and appropriate drug concentrations, in a final assay volume of 1 ml. Monoamine transport was determined in duplicate with a blank assay included for each sample. Blank assays contained an excess (200 µM) of unlabelled monoamine. After 10 minutes, the reaction was terminated by rapid filtration through pre-wet Whatman GF/B glass-fibre filter paper followed by three washes with ice cold Krebs solution in a Brandel harvester. Filter papers were air dried and placed in Pony vials containing 4 ml of Ecoscint A. Samples were analysed in comparison to standards of known radioactive content, corrected for background and blank sample counts, and expressed in relation to protein concentration and reaction time. Protein concentrations were determined by the BIORAD method as described in section 2.2.2.

8.4 Experimental protocol

Pilot studies were performed with the recognised monoamine uptake inhibitors FLU (0 - 1000 nM), DES (0 - 100 µM) and nomifensine (NOM; 0 -10 µM) to validate the methodologies for 5-HT, NE and DA uptake, respectively. Thereafter, the effects of RMD, DGR, CBZ, ARR-15896, LTG and PHT (all 0 - 1000 µM) on 5-HT, NE and DA uptake were investigated.

8.5 Data analysis and statistics

Results were calculated as a percentage of the individual control values for each uptake assay. Group results (n = 6) were then expressed as the mean percentage ± SEM. Statistical differences from control were determined by one-way analysis of variance with a Dunnett correction for multiple comparisons.

8.6 Results

FLU, DES and NOM significantly ($p < 0.05$) reduced the reuptake of 5-HT, NE and DA, respectively, into the rat brain synaptosomes, thereby validating the methodologies (figure 43-45).

RMD significantly ($p < 0.05$) reduced the reuptake of 5-HT, NE and DA to 37.8 % (± 5.4), 29.8 % (± 5.9) and 10.8% (± 3.2) of control, respectively (figure 46). DGR significantly ($p < 0.05$) reduced 5-HT, NE and DA reuptake to 8.6% (± 1.5), 23.4% (± 3.6) and 16.67% (± 6.7) of control, respectively (figure 47). CBZ significantly ($p < 0.05$) reduced the reuptake of 5-HT, NE and DA to 27.6% (± 3.1), 54.2% (± 5.6) and 30.4% (± 6.8) of control, respectively (figure 48). ARR-15896 significantly ($p < 0.05$) reduced 5-HT, NE and DA reuptake to 16.2% (± 6.9), 27.5% (± 3.1) and 14.0% (± 2.4) of control, respectively (figure 49). LTG significantly ($p < 0.05$) reduced 5HT and NE reuptake (± SEM) to 39.0% (± 4.1) and 49.4% (± 6.7) of control, respectively. LTG was without effect on DA reuptake at all the concentrations studied (figure 50). PHT significantly ($p < 0.05$) reduced DA

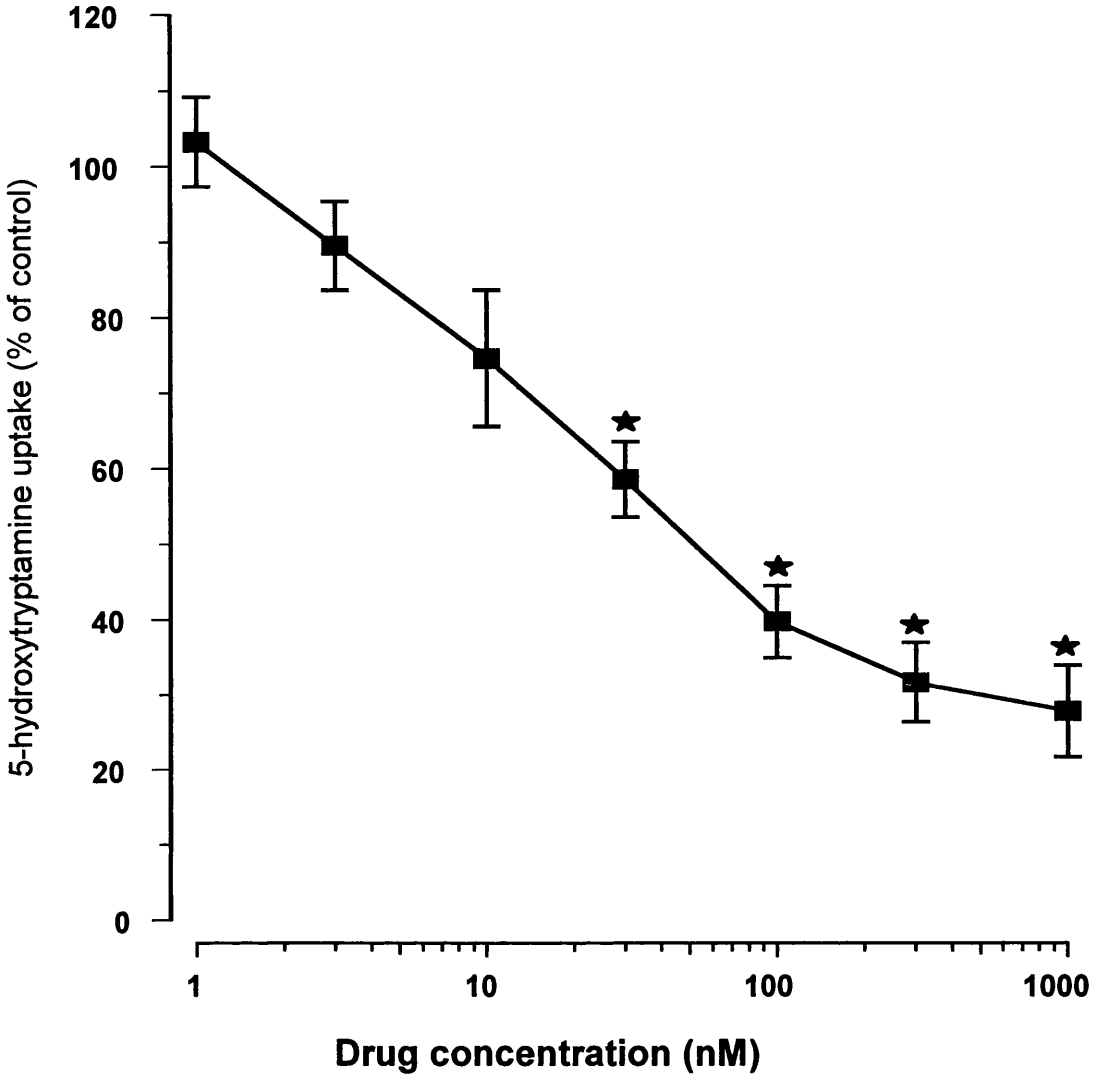


Figure 43: The effects of fluoxetine (1 - 1000 nM) on 5-hydoxytryptamine uptake in rat cortical synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values and error bars denote the standard error of the mean (± SEM). Statistical significance (*$p < 0.05$) from control was determined by one-way analysis of variance with a Dunnett correction for multiple comparisons.

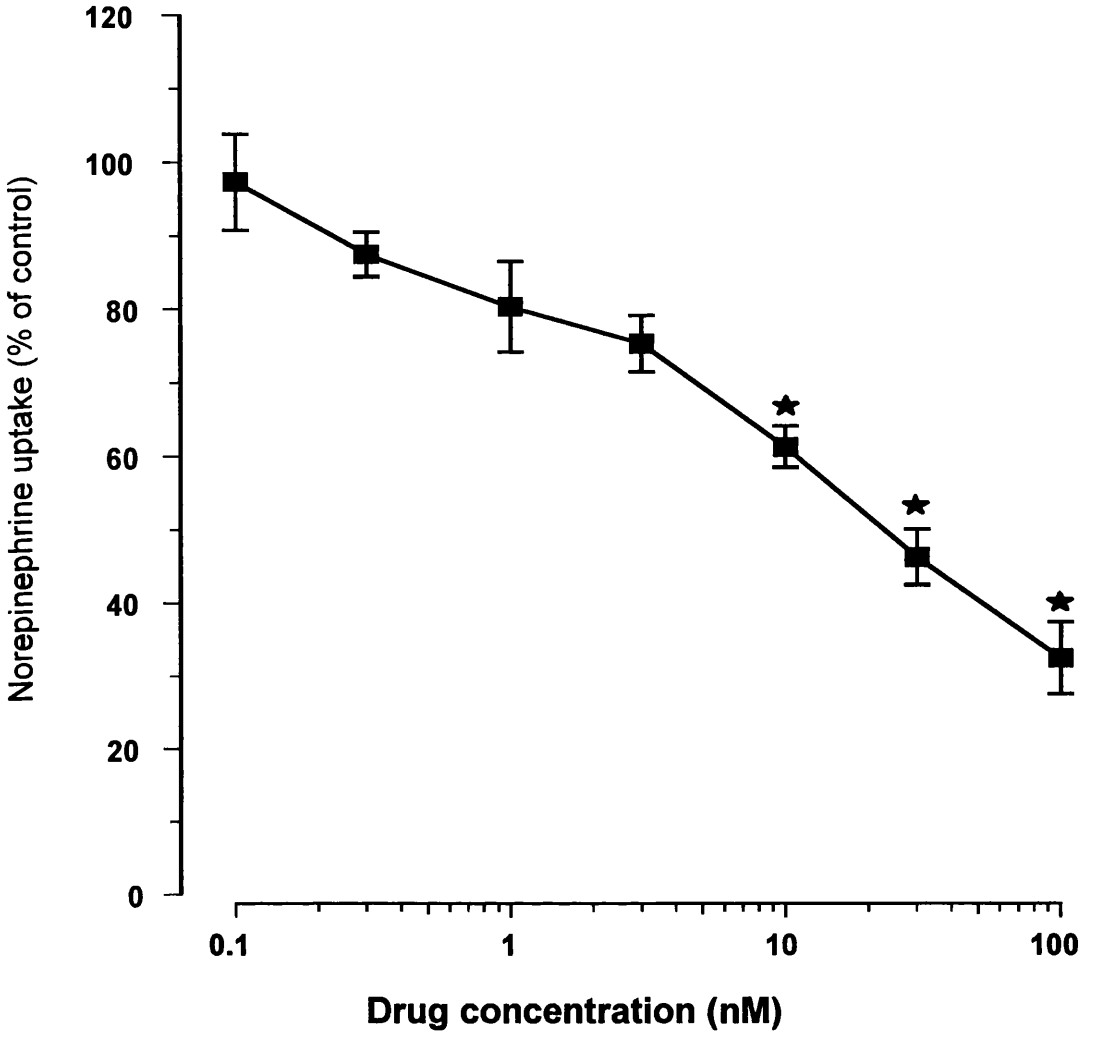


Figure 44: The effects of desipramine (0.1 - 100 μM) on norepinephrine uptake in rat cortical synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values and error bars denote the standard error of the mean (± SEM). Statistical significance (*$p < 0.05$) from control was determined by one-way analysis of variance with a Dunnett correction for multiple comparisons.

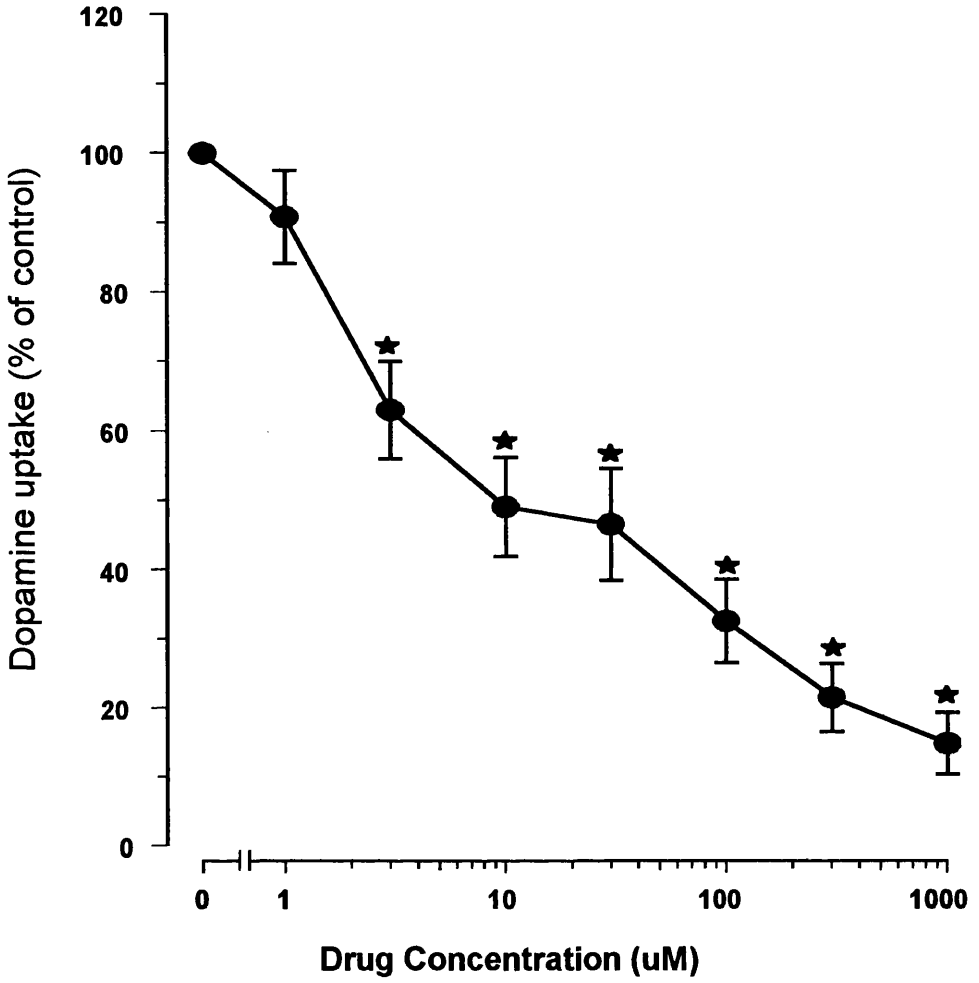


Figure 45: The effects of nomifensine (0 - 1000 μM) on dopamine uptake in rat striatal synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values and error bars denote the standard error of the mean (± SEM). Statistical significance (*$p < 0.05$) from control was determined by one- way analysis of variance with a Dunnett correction for multiple comparisons.

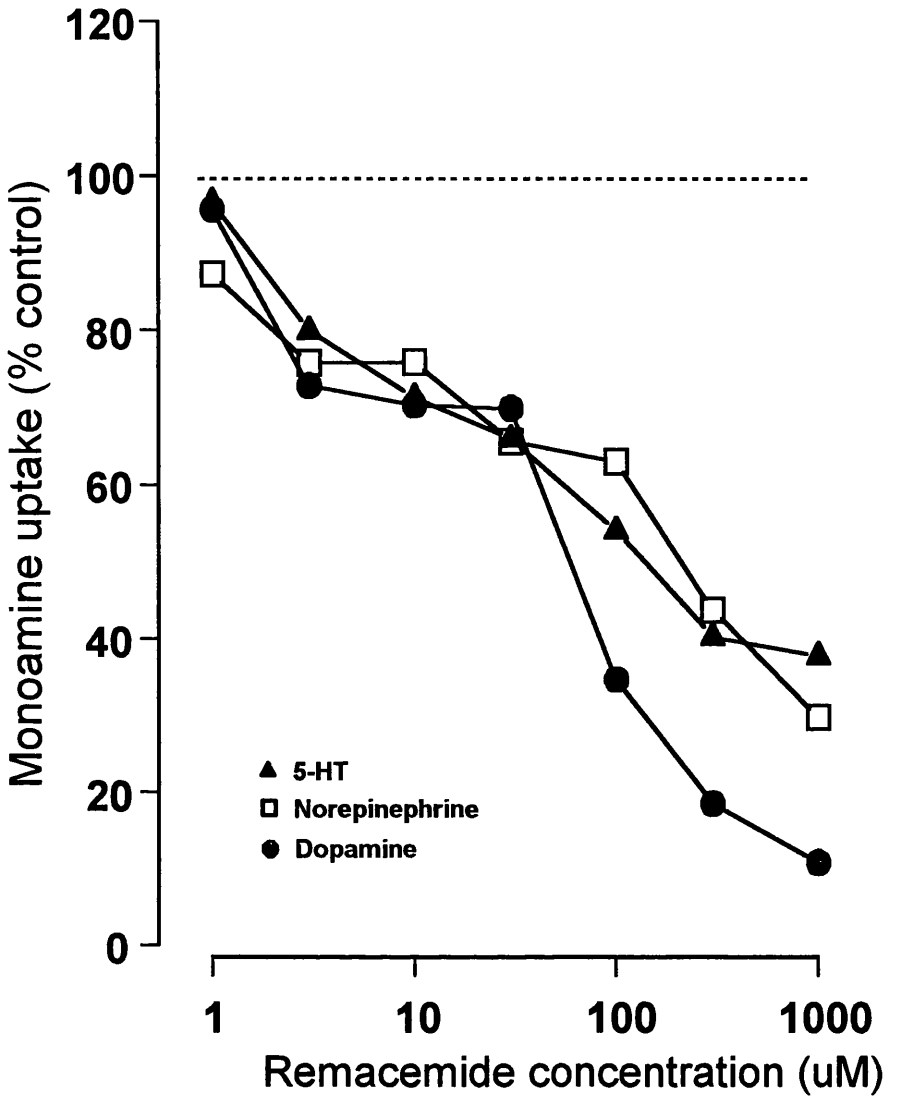


Figure 46: The effects of remacemide on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

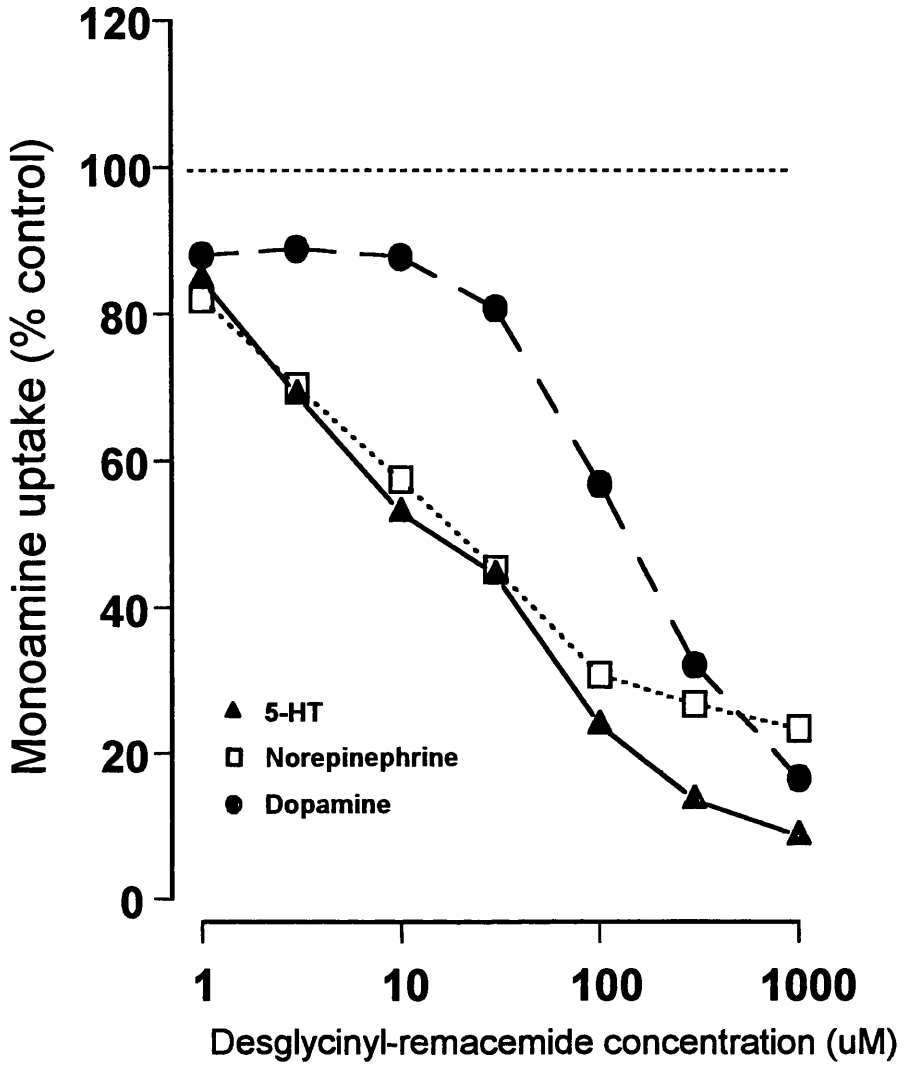


Figure 47: The effects of desglycinyl-remacemide on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

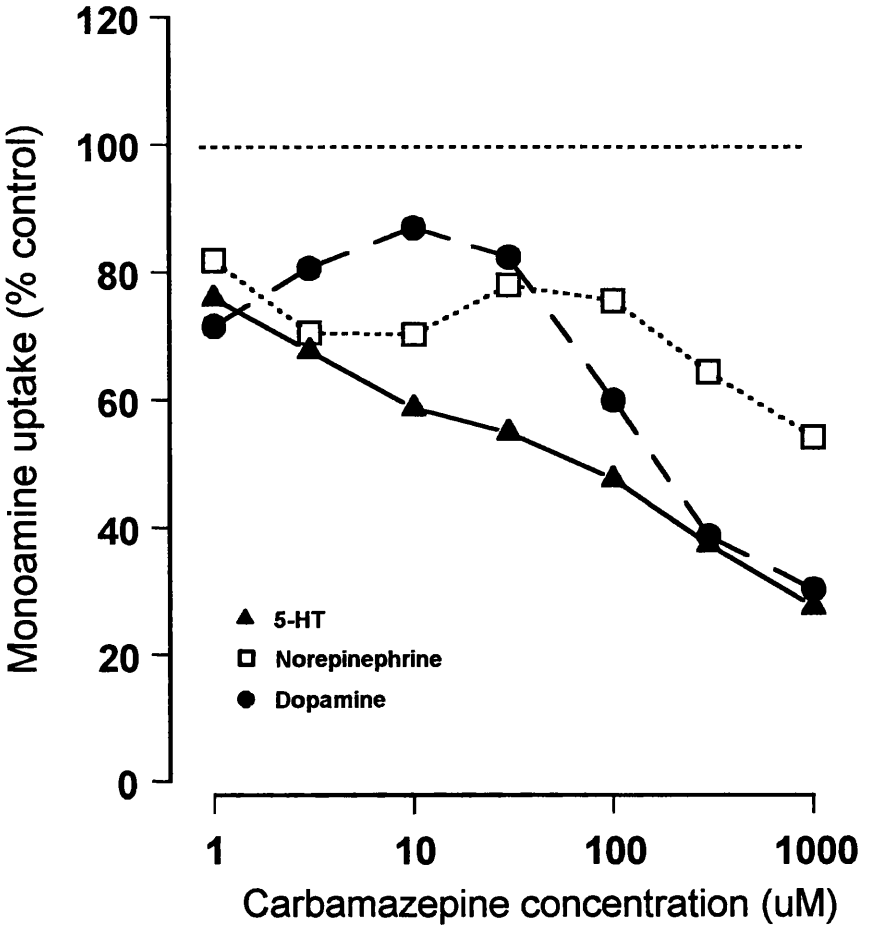


Figure 48: The effects of carbamazepine on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

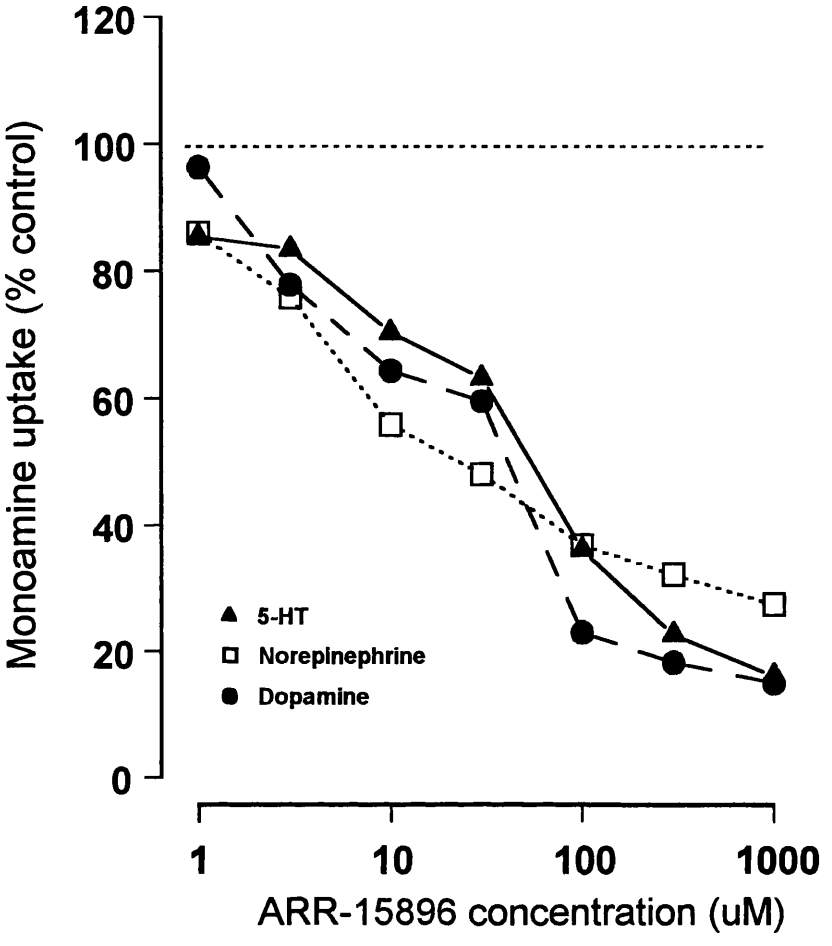


Figure 49: The effects of ARR-15896 on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

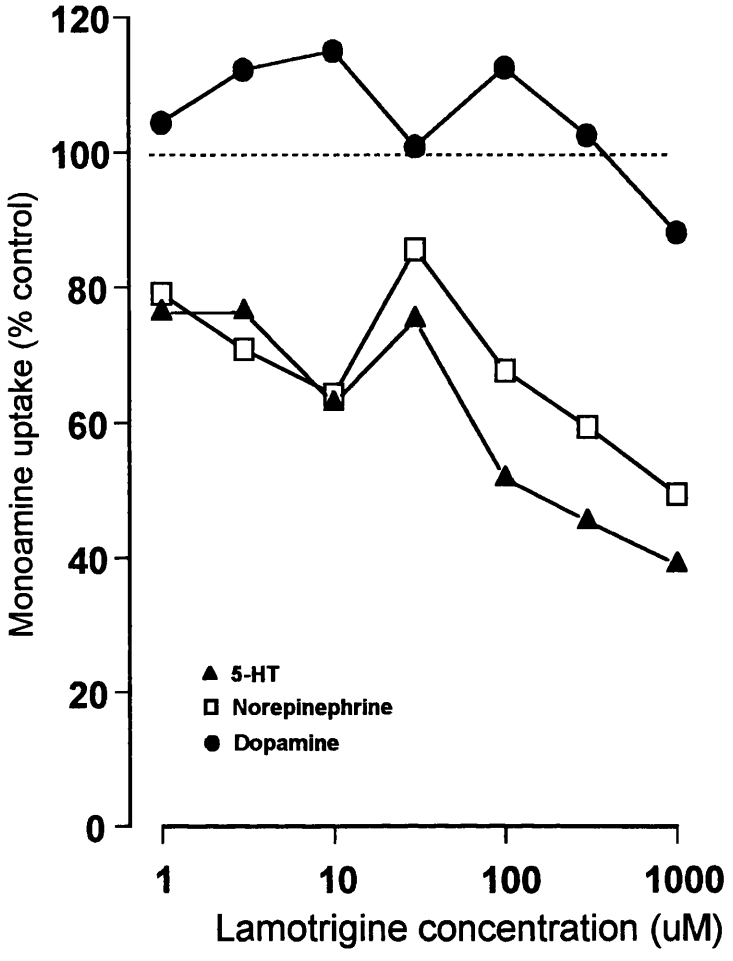


Figure 50: The effects of lamotrigine on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

reuptake to 35.6% (± 8.0) of control (figure 51) but had no significant effect on 5-HT or NE reuptake processes.

		(Percentage inhibition of control uptake)		
DRUG	CONCENTRATION	5-HT	NE	DA
RMD	1000 uM	37.8	29.8	10.8
DGR	1000 uM	8.6	23.4	16.6
CBZ	1000 uM	27.6	54.2	30.4
ARR-15896	1000 uM	16.2	27.5	14.0
LTG	1000 uM	39.0	49.4	NE
PHT	1000 uM	NE	NE	35.6

Table 3 Effects of anticonvulsants on monoamine uptake

8.7 Discussion

The aim of the above investigations was to assess the effects of RMD and DGR on monoamine uptake in rat brain synaptosomes. CBZ, ARR-15896, LTG and PHT were employed for comparative purposes.

There is increasing evidence to suggest monoamines may contribute to the regulation of neuronal excitability and hence seizure susceptibility (Kresch et al, 1989). Experimentally induced decrements in monoamine levels have, in general, been demonstrated to precipitate seizures (McIntyre et al, 1979; Applegate et al, 1986; Browning et al, 1989; Shouse et al, 1996; Jobe et al, 1999), whereas increasing monoamine levels are usually associated with a

reduction in convulsion intensity (Lehmann, 1967; Finberg et al, 1993; Mishra et al, 1993). Therefore, potentially AEDs may enhance monoaminergic transmission as part of their anticonvulsant mechanisms of action.

RMD significantly reduced the reuptake of all the three monoamines investigated although none of these reductions were statistically significant ($p < 0.05$) from control at therapeutically relevant concentrations which are reported to be between 1 and 10 μM for both RMD and DGR (Schachter and Tarsy, 2000). DGR also significantly reduced all three reuptake processes but only those of 5-HT and NE were significantly ($p < 0.05$) affected at therapeutically relevant concentrations. CBZ significantly reduced all three monoamine uptake processes although only the reduction in 5-HT reuptake was significant ($p < 0.05$) at therapeutically relevant concentrations which again are reportedly between 1 and 10 μM (McLean and Macdonald, 1986). The experimental NMDA receptor antagonist, ARR-15896 reduced the reuptake of all three monoamines. Statistically significant ($p < 0.05$) reduction of all the reuptake processes was achieved at 30 μM ARR-15896, the concentration required to block NMDA-induced depolarisation in hippocampal slices (Palmer et al, 1999). LTG significantly reduced the reuptake of 5-HT and NE and PHT the reuptake of DA, although no statistically significant ($p < 0.05$) effect was seen at therapeutically relevant concentrations.

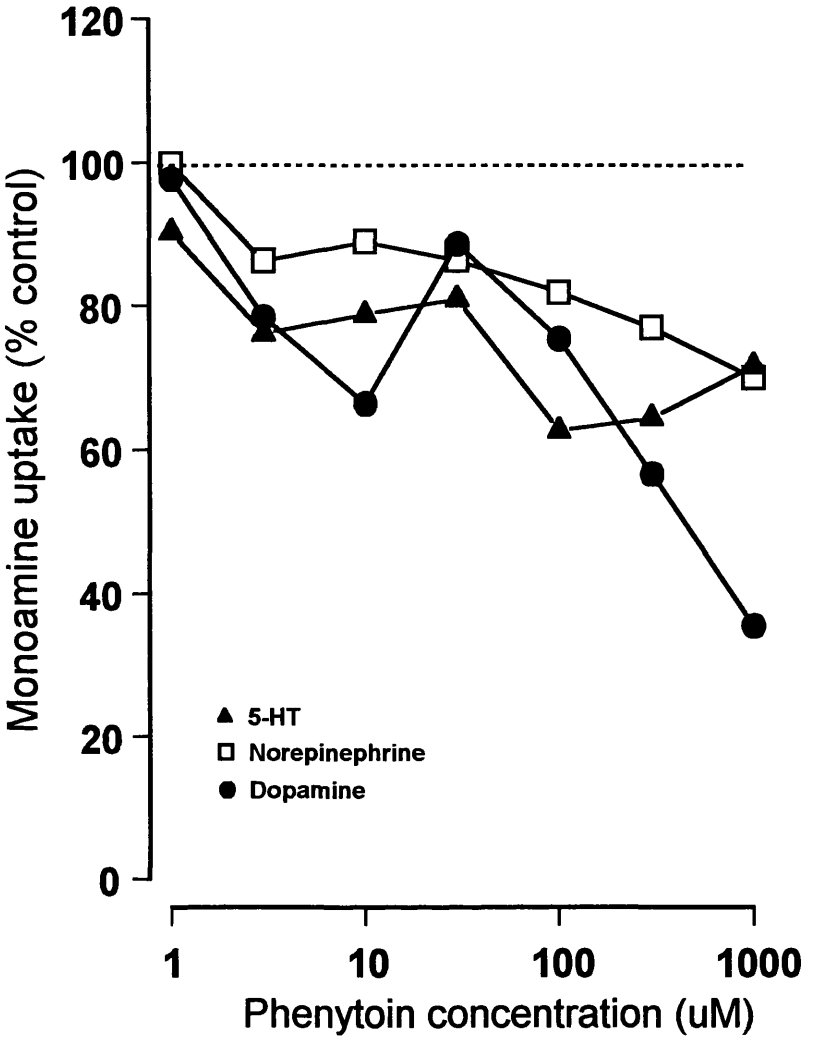


Figure 51: The effects of phenytoin on 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) reuptake in rat cortical (5-HT, NE) and striatal (DA) synaptosomes. Results (n = 9) are expressed as the mean percentage of individual control values. Error bars and indicators of significance have been omitted for ease of comprehension.

CBZ produces dose dependent anticonvulsant effects in both GEPRs (Yan et al, 1992) and outbred Sprague-Dawley rats (Dailey et al, 1997), with a corresponding increase in synaptic 5-HT (Yan et al, 1992; Dailey et al, 1996; Graumlich et al, 1999; Butler et al, 2000). TTX, despite reducing basal 5-HT release, did not alter the increase in synaptic 5-HT induced by CBZ, which implies the elevated 5-HT levels are independent of the nerve action potential (Dailey et al, 1997). Evidence suggests that the increased extracellular 5-HT is due to reuptake inhibition (Butler et al, 2000).

Depletion of 5-HT levels in GEPRs by prior administration of p-chloroamphetamine diminishes the anticonvulsant effect of CBZ (Yan et al, 1992; Dailey et al, 1996; Lu & Gean, 1998). PHT, which has a similar clinical profile to CBZ, also produces dose related anticonvulsant effects in GEPRs and Sprague-Dawley rats but with no corresponding increase in extracellular 5-HT (Dailey et al, 1996). The evidence suggests that several AEDs, notably CBZ, enhance monoaminergic neurotransmission, possibly by reuptake inhibition, and that these effects contribute to the antiepileptic actions of the drugs.

Previous work has suggested that LTG inhibits the reuptake of 5-HT, NE and DA *in vitro* (Southam et al, 1998). The findings presented above do suggest that LTG has an inhibitory effect on the reuptake of 5-HT and NE, however, a lack of action of therapeutically relevant concentrations questions the importance of such findings.

In contrast to the above findings, suppression of NE uptake by PHT has been reported in several studies with the concentrations required well within the therapeutic range (Hadfield, 1972; Weinberger et al, 1976). However, other studies have indicated that PHT only exerts an effect on NE transmission at concentrations above the therapeutic range and even then not necessarily via an action on the reuptake process (Azzaro et al, 1973). Although the results reported above were not statistically significant, there was a trend towards inhibition of NE reuptake observed with PHT (figure 51). Due to the concentrations of PHT required to produce that effect it is suggested that synaptosomal toxicity and not a pharmacological effect is being observed.

Biochemical evidence for a DA system dysfunction in the brain of epileptic patients is not straightforward (Starr, 1996). Both increases and decreases in the concentrations of DA and its major metabolite, HVA, have been detected in the CSF of epilepsy patients. Traditionally, DA has been regarded as anticonvulsant, although experimental work has occasionally produced conflicting results, suggesting that there might be other factors involved (Starr, 1996). Some clinical evidence exists suggesting that DA has an anticonvulsant action in man (Starr, 1996). The prototypic non-selective D_1/D_2 receptor agonist apomorphine was first proposed to be an antiepileptic over a century ago, but only recently has its anticonvulsant profile in man been established (Starr, 1996). The use of apomorphine for epilepsy is restricted by the high incidence of side effects and short-lived action. Side effects include nausea and vomiting, which could be minimised by administration of a peripherally acting DA antagonist such as domperidone.

Early investigations (Hadfield, 1972) demonstrated a PHT mediated inhibition of DA uptake in the DA rich caudate putamen. However, statistical significance was not indicated, and so the clinical relevance of these results cannot be assessed. In contrast, recent studies using *in vivo* microdialysis (Okada et al, 1997c) have reported no change in DA uptake in the presence of PHT at therapeutic concentrations. With respect to CBZ, significantly increased levels of extracellular DA have been reported in the same model (Okada, 1997a). The results were attributed to CBZ acting as an adenosine A_2 receptor agonist. Adenosine is said to have a role as a homeostatic modulator for the release of various neurotransmitters. Therefore agonists at A_2 receptors are reported to potently increase the seizure threshold and it was suggested that this is one of the mechanisms by which CBZ operates (Okada et al, 1997b). As this study was solely concerned with the contribution of uptake blockade, an action on adenosine receptors cannot be validated or disputed.

Results from 5-HT reuptake experiments indicate that CBZ, DGR and ARR-15896 significantly effect the reuptake process at pharmacologically relevant concentrations. All three agents are chemically related to the amine compounds that are known to inhibit monoamine uptake, for example imipramine and amitriptyline (Stone, 1995). It is possible that CBZ, DGR and ARR-15896 block the biogenic amine transporters responsible for the removal of monoamines from the synaptic cleft by virtue of their structure. A similar hypothesis can be suggested for the results obtained from the NE reuptake

analysis given that DGR and ARR-15896 were again the only compounds seen to have an affect at pharmacologically significant concentrations.

The contrasting effects of RMD and DGR presented above confirms that the two compounds have pharmacological differences. Whether the actions of DGR on 5-HT and NE reuptake are relevant to the antiepileptic properties of RMD requires further investigation.

Interestingly, the NMDA antagonist, ARR-15896 potently inhibited the reuptake of all three monoamines investigated most probably via an action on the amine transporter systems responsible for reuptake of monoamines. These findings suggest a previously undiscovered mechanism of action for this agent. Further studies are required to evaluate the importance of these findings to the anti-ischaemic actions of the compound.

The theoretical link between epilepsy and effective disorders is confirmed pharmacologically, because agents that elevate noradrenergic and/or serotonergic transmission, for example tricyclic antidepressants, are both anticonvulsant and antidepressant, whereas reserpine both induces depression and facilitates seizures (Yan et al, 1993; Moshe, 2000). AEDs such as CBZ and VPA are becoming increasingly prescribed prophylactically for mood stabilisation (Okuma, 1994; Post et al, 1996; Dunn et al, 1998; Jobe et al, 1999), and recently the beneficial effects of LTG in depression and bipolar disorders have been reported (Bowden, 1998; Post et al, 1998; Southam et al, 1998). The above findings suggest that RMD and ARR-15896 could have a

potential use against such disorders. Further investigations are required to confirm the actions of RMD, and therefore DGR, and ARR-15896 in this respect.

With hindsight, a greater accuracy of results could perhaps have been afforded with the use of pure, rather than crude, synaptosomes.

In conclusion, the above study investigated the effects of RMD and DGR on the reuptake processes of 5-HT, NE and DA. CBZ, ARR-15896, LTG and PHT were included for comparative purposes. RMD was found to have no significant effects at clinically relevant concentrations. CBZ, DGR and ARR-15896 were found to significantly reduce the reuptake of 5-HT perhaps via a simple blockade of the biogenic amine transporters responsible for the reuptake process. DGR and ARR-15896 were also found to inhibit NE reuptake, possibly by the same mechanism. Further investigations are required to assess the mechanism and importance of the results obtained to RMD pharmacology in terms of epilepsy and possibly affective disorders.

Chapter 9

General discussion and conclusions

RMD is a novel anticonvulsant that has been previously shown to be effective against MES-, kainate-, NMDA- and 4-AP induced seizures (Stagnitto et al, 1990; Garske et al, 1991; Palmer et al, 1992; Palmer et al, 1993; Cramer et al, 1994). RMD and its active metabolite, DGR, have been shown to inhibit the sustained repetitive firing of cultured neurones, suggested an inhibitory action on voltage-gated Na^+ channels with IC_{50} values of 7.9 and 1.2 μM reported, respectively (Wamil et al, 1996). Both RMD and its active metabolite, DGR, have an inhibitory effect on NMDA receptors with a low affinity for the receptor ion channel complex although DGR is more potent at this site with an IC_{50} value for [^{3}H]MK801 displacement of 0.48 μM compared to 68 μM observed for RMD (Palmer et al , 1992). Inhibition of NMDA-evoked currents in rat hippocampal neurones was achieved with RMD and DGR at IC_{50} values of 67 and 0.7 μM, respectively (Subramaniam et al, 1996). The same studies suggested that RMD has affinity for an alternative allosteric site on the NMDA receptor although the consequences of this are not clear (Subramaniam et al, 1996).

The aim of this project was to investigate the preclinical pharmacology of RMD and DGR. Particular emphasis was placed on the relative contribution of each moiety to the antiepileptic actions of the parent compound and the relative importance of individual mechanisms of action. In the development of RMD, it was assumed that its additional inhibitory effects at the NMDA receptor might confer advantages for the drug over standard Na^+ channel blocking AEDs.

During the conduct of this project, results emerged from clinical trials suggesting that while effective as add-on therapy (Richens et al, 2000), RMD was significantly inferior to the Na^+ channel blocker, CBZ in monotherapy trials (Brodie et al, in prep). In light of these observations, the preclinical pharmacological investigations described above assumed greater significance. An attempt was made to formulate a hypothesis to explain why RMD, a drug with a promising preclinical profile and recognised clinical efficacy as add-on treatment, performed so poorly as monotherapy.

The results presented above indicate that there are vital differences in the pharmacology of RMD and DGR. Investigations presented herein (section 3.6) have demonstrated that DGR is the predominant moiety, in terms of pharmacokinetics, following administration of the parent compound. Various preclinical investigations, including those presented here, have shown that DGR is the more active compound pharmacologically (Stagnitto et al, 1990; Garske et al, 1991; Palmer et al, 1991; Palmer et al, 1992; Clark et al, 1995; Davies, 1997). Given that DGR exerts an effect on NMDA receptors at concentrations lower than those required to block Na^+ channels, it is possible that the efficacy observed in the majority of animal models is the result of a reduction of NMDA receptor activity.

NMDA antagonists are effective anticonvulants in a wide variety of seizure models (Anderson et al, 1986; Avoli et al, 1987; Mody and Lambert, 1987; Aram et al, 1989). However, they are known to produce a variety of adverse neurobehavioural effects *in vivo* (Rogawski, 1992). The challenge therefore

has been to develop NMDA antagonists that not only have adequate bioavailability and appropriate pharmacokinetic properties, but which are also sufficiently free of side effects at anticonvulsant doses. There are several modulatory sites on the NMDA receptor (figure 1) which have been identified by a wide range of selective antagonists. Studies have suggested that there may be differences in the toxicity profiles of drugs acting selectively at these sites (Rogawski, 1992).

Investigations have been carried out to assess the therapeutic indices of a variety of selective NMDA antagonists (Ferkany et al, 1989; Rogawski et al, 1989; Saywell et al, 1990; Thurkauf et al, 1990; Rogawski et al, 1991). The therapeutic index (TI) of each compound was determined as the ratio of TD_{50}/ED_{50}, where TD_{50} is the dose producing impaired motor performance in 50% of animals, and the ED_{50} is the dose protecting 50% of the animals against THE in the MES-model. A TI of 1 indicates an equal potency for anticonvulsant activity and motor toxicity. High affinity uncompetitive NMDA antagonists such as MK801 and PCP were found to have TIs of < 1. However, low affinity uncompetitive antagonists, such as DGR, were found to have an average TI of 4. CBZ, which has no reported effects on NMDA receptors was found to have a TI of 6 (Rogawski, 1992). Although high affinity uncompetitive NMDA antagonists appear to have no future in epilepsy therapy (Sveinbjornsdottir et al, 1993), low affinity uncompetitive antagonists have shown promise. Preclinically, RMD and DGR displayed anticonvulsant activity with a much better therapeutic ratio than the high affinity antagonists (Rogawski et al, 1991; Rogawski, 1996).

As add-on therapy in clinical trials, RMD was shown to be effective at decreasing seizure frequency when compared to the first drug alone (Richens et al, 2000). It was noted that the vast majority of patients were taking CBZ, a known hepatic enzyme inducer as a concominant AED. Various clinical studies have demonstrated that RMD, and in particular DGR, are susceptible to the effects of hepatic enzyme induction (Leach et al, 1996a; Leach et al, 1997a; Chadwick et al, 2000; Richens et al, 2000). The results presented above demonstrate that DGR is also more susceptible than RMD to the enzyme inducing effects of PB in mice.

Despite reported efficacy as adjunctive therapy, a recently conducted systemic review and meta-analysis of earlier placebo-controlled add-on trials indicates that RMD has a poor response rate as add-on therapy (Marson et al, 2001). The analysis indicated that doses of 300 - 600 mg RMD per day were no different from placebo. Regression analysis in the same study suggests that approximately 10% of patients would however see a significant improvement in seizure control on 800 - 1200 mg of RMD per day. Clinical studies have indicated that 1200 mg RMD daily, administered as adjunctive therapy to patients taking hepatic enzyme inducers, increases the incidence of NMDA associated side effects (Chadwick et al, 2001). If 1200 mg RMD was taken daily by patients who do not have enzyme induction, the incidence of adverse events would surely increase to unacceptable levels.

The clinical trial results described above are in complete contrast to what would be expected from preclinical data. As described, low affinity NMDA

antagonists with acceptable pharmacokinetic profiles should be ideal candidates for antiepileptic therapy. A low affinity NMDA antagonist with an additional effect at voltage-gated sodium channels, on paper appears to be an ideal candidate for clinical efficacy. The fact that RMD does not significantly decrease seizure frequency raises a number of questions. Firstly the relevance of animal models in the development of AEDs. All preclinical information suggested that both RMD and DGR are anticonvulsant in a wide range of *in vitro* and *in vivo* models although in clinical practice neither compound appeared to have a significant effect on seizures. Perhaps the models that are utilised are more detached from the human condition as first thought. In addition, DGR levels in brain reported in the above studies appear to be much higher than those estimated for humans (AstraZeneca, unpublished results). It is possible that the kinetic differences between animal and man contribute to the lack of outcome correlation. Secondly, the findings question the entire concept of rational drug design. On paper, RMD was an ideal candidate for AED development. It is possible that the DGR was just too potent at the NMDA site. A number of successful AEDs, for example VPA, GBP, VGB, FBM and LEV, have a weak affinity for their respective sites of action although they are successful in the clinical setting.

In conclusion, the results presented herein suggest that there are differences in the pharmacology of RMD and DGR which may possibly affect the clinical profile of the parent drug. The studies confirm the reported differences in the potencies of the two moieties at the known sites of action. Monotherapy trials and meta-analysis of add-on trials indicate that RMD does not improve on the

efficacy afforded by CBZ, a traditional Na^+ channel blocker. These results suggest that NMDA receptor blockade is not as important a property for an antiepileptic compound as first believed. The lack of correlation between preclinical and clinical data questions the relevance of animal models to human epilepsy. This suggestion has possible implications for the future development of NMDA antagonists as AEDs.

References

Adesnik, M., Atchison, M. (1985) Genes for cytochrome P450 and their regulation. *CRC Crit. Rev. Biochem.,* **19,** 247-305.

Adesnik, M., Bar-nun, S., Maschio, F., Zunich, M., Lippman, A., Bard, E. (1981) Mechanism of induction of cytochrome P450 by phenobarbital. *J. Biol. Chem.,* **256,** 10340-10345.

Ahmed, M.S., Mather, A., Enna, S.J. (1999) Binding of [^{3}H]desglycinyl remacemide to rat brain membranes: association with the benzomorphan attachment site of the N-methyl-D-aspartic acid receptor channel. *Brain Res.,* **827,** 46-50.

Alarcon, G., Binnie, C.D., Elwes, R.D.C., Polkey, C.E. (1992) Remacemide monotherapy in patients undergoing acute antiepileptic drug withdrawal. *Seizure,* **suppl. A,** 7.

Albertoni, P. (1882) Untersuchung uberdie Wirkuing einier Arzneimittel auf die Erregbarkeit des Grosshirns nebst Beitragen zur Therapie der Epilepsie. *Arch. Exp. Path. Pharmacol.,* **15,** 248-288.

Albright, P.S., Burnham, W.M., Okazaki, M. (1979) Effect of atropine sulphate on AM kindling in the rat. *Exp. Neurol.,* **66,** 409-412.

Alexander, G.J., Kopeloff, L.M. (1970) Metrazol seizures in rats: effects of p-chlorophenylalanine. *Brain Res.,* **22,** 231-235.

Alexi, T., Borlongan, C.V., Faull, R.L.M., et al. (2000) Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. *Prog. Neurobiol.,* **60,** 409-470.

Ali, L., Grapengiesser, E., Gylfe, E., Hellman, B., Lund, P.E. (1989) Free and bound sodium in pancreatic β-cells exposed to glucose and tolbutamide. *Biochem. Biophys. Res. Commun.,* **164,** 212-218.

Amano, K., Hamada, K., Yagi, K., Seino, M. (1998) Antiepileptic effects of topiramate on amygdalaoid kindling in rats. *Epilepsy Res.,* **31,** 123-128.

Anderson, P., Bliss, T.V.P., Skrede, K.K. (1971) Lammellar organisation of hippocampal excitatory pathways. *Exp. Brain Res.,* **239,** 222-238.

Anger, T., Madge, D.J., Mulla, M., Riddall, D. (2001) Medicinal chemistry of neuronal voltage-gated sodium channel blockers. *J. Medicinal Chem.,* **44,** 115-137.

Applegate, C.D., Burchfiel, J.L., Konkol, R.J. (1986) Kindling antagonism: effects of norepinephrine depletion on kindled seizure suppression after concurrent, alternate stimulation in rats. *Exp. Neurol.,* **94,** 379-390.

Applegate, C.D., Tecot, L.H. (1998) Global increases in seizure susceptibility in mice lacking 5-HT_{2C} receptors: a behavioural analysis. *Exp. Neurol.,* **154,** 522-530.

Arnold, P.S., Racine, R.J., Wise, R.A. (1973) Effect of atropine, reserpine, 6-hydroxydopamine, and handling on seizure development in the rat. *Exp. Neurol.*, **40,** 457-470.

Arvanov, V.L., Holmes, K.H., Keele, N.B., Shinnick-Gallagher, P. (1995) The functional role of metabotropic glutamate receptors in epileptiform activity induced by 4-aminopyridine in the rat amygdala slice. *Brain Res.*, **669,** 140-144.

Asghar, A.U.R., Hasan, S.S., King, A.E. (2000) Actions of the anticonvulsant remacemide metabolite ARR-R12495AA on afferent-evoked spinal synaptic transmission in vitro and on models of acute and chronic inflammation in the rat. *J. Pharmacol. Exp. Ther.*, **294,** 876-883.

Awapara, J., Landua, A.J., Fuerst, R., Seale, B. (1950) Free γ-aminobutyric acid in the brain. *J. Biol. Chem.*, **187,** 35-39.

Azzaro, A.J., Gutrecht, J.A., Smith, D.J. (1973) Effect of diphenylhydantoin on the uptake and catabolism of L-[^{3}H]norepinephrine in vitro in rat cerebral cortex tissue. *Biochem. Pharmacol.*, **22,** 2719-2729.

Babb, T., Wilson, C.L., Isokaws-Akesson, M. (1987) Firing patterns of human limbic neurones during stereoencephalography (SEEG) and clinical temporal lobe seizures. *Electroencephalogy Clin Neurophysiol.*, **66,** 467-482.

Bak, I.J., Misgeld, U., Weiler, M., Mordan, E. (1980) The preservation of nerve cells in rat neostriatal slices maintained in vitro: a microdialysis study. *Brain Res.*, **197,** 341-353.

Bannan, P.E., Graham, D.I., Lees, K.R., McCulloch, J. (1994) Neuroprotective effect of remacemide hydrochloride in focal cerebral ischemia in the cat. *Brain Res.*, **664,** 271-275.

Barchi, L. (1998) Ion channel mutations affecting muscle and brain. *Curr. Opin. Neurol.*, **11,** 461-468.

Barker, R.A., Barasi, S., Neal, M.J. (2000) Neuroscience at a glance. Blackwell Science.

Barolet, A.W., Li, A., Liske, S., Morris, M.E. (1985) Antagonist actions of bicuculline methiodide and picrotoxin on extrasynaptic GABA receptors. *Can. J. Physiol. Pharmacol.*, **63,** 1465-1470.

Barry, D.I., Wancher, B., Kragh, J., Bolwig, T.G., Kokaia, M., Brundin, P. (1989) Grafts of fetal locus coerulus neurones in rat amygdala-piriform cortex suppress seizure development in hippocampal kindling. *Exp. Neurol.*, **106,** 125-132.

Beghi, E., Di Mascio, R., Tognoni, G. (1986) Drug treatment of epilepsy. Outlines, criticism and perspectives. *Drugs,* **31,** 249-265.

Bengzon, J.M., Brundin, P., Kalen, P., Kokaia, P., Lindvall, O. (1992) Host regulation of noradrenaline release from grafts of seizure suppressant locus coeruleus neurones. *Exp. Neurol.,* **111,** 49-55.

Ben-Menachem, E., Persson, L.I., Schechter, P.J., et al. (1989) The effect of different vigabatrin treatment regimes on CSF biochemistry and seizure control in epileptic patients. *Br. J. Clin. Pharmacol.,* **27,** 79-85.

Bentue-Ferrer, P., Belissant, E., Decombe, R., Allain, H. (1994) Temporal profile of aminergic neurotransmitter release in striatal dialysates in rats with post-ischemic seizures. *Exp. Brain Res.,* **437,** 437-443.

Bernasconi, R., Klien, M., Martin, P., et al. (1988) γ-vinyl GABA: Comparison of neurochemical and anticonvulsant effects in mice. *J. Neural. Trans.,* **72,** 213-233.

Besag, F.M.C., Newton, R.E., Blakey, G.E., Dean, A.D.P. (2001) Safety, tolerability, and pharmacokinetics of remacemide in children. *Pediatr. Neurol.,* **24,** 352-356.

Bialer, M., Johannessen, S.I., Kupferberg, H.J., Levy, R.H., Loiseau, P., Perucca, E. (1999) Progress report on new antiepileptic drugs: a summary of the fourth Eilat conference. *Epilepsy Res.,* **34,** 1-41.

Bianchi, C., Beani, L. (1985) Acetylcholine. In: *Antiepileptic drugs. Handbook of Experimental Pharmacology, Vol 74.* Frey H-H., Janz, D. (Eds) pp139-147. Berlin: Springer Verlag.

Blitz, H. (1908) Uber die konstitution der einwirkungsproukte von substituierten harnstoffen auf benzil und uber einige neue methoden zur darstellung der 5, 5-diphenylhydantoine. *Ber. Dtsch. Chem. Ges.,* **41,** 1379.

Bohlen, P., Hout, S., Palfreyman, M.G. (1979) The relationship between GABA concentrations in the brain and cerebrospinal fluid. *Brain Res.,* **167,** 297-305.

Borden, L.A., Murali Dhar, T.G., Smith, K.E., Weinshank, R.L., Branchek, T.A., Gluchowski, C. (1994) Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. *Eur. J. Pharmacol.,* **269,** 219-224.

Borin, M., Siffert, W. (1990) Stimulation of thrombin increases cytosolic free Na^+ concentration in human platelets. *J. Biol. Chem.,* **265,** 19543-19550.

Bourgeois, B.F.D. (1996) Drug interaction profile of topiramate. *Epilepsia,* **37,** S14-S17.

Bourgeois, B.F.D., Dobson, W.E., Ferrendelli, J.A. (1983) Primdone, phenobarbital and PEMA: I. Seizure protection, neurotoxicity and therapeutic index of individual compounds in mice. *Neurology,* **33,** 283-290.

Bowden, C.L. (1998) New concepts in mood stabilisation: evidence for the effectiveness of valproate and lamotrigine. *Neuropsychopharmacol.*, **19,** 194-199.

Bradford, H.F., Dodd, P.R. (1975) Convulsions and activation of epileptic foci induced by monosodium glutamate and related compounds. *Biochem. Pharmacol.*, **26,** 253-254.

Braestrup, C., Neilsen, M. (1980) Strychnine as a potent inhibitor of the brain GABA/benzodiazepine complex. *Brain Res. Bull.*, **5 (suppl. 2),** 681-684.

Brodie, M.J. (1992) Drug interactions and epilepsy. *Epilepsia,* **33 (suppl. 1),** S13-S22.

Brodie, M.J., Dichter, M.A. (1996) Antiepileptic drugs. *New Eng. J. Med.*, **334,** 168-175.

Brodie, M.J., Dichter, M.A. (1997) Established antiepileptic drugs. *Seizure,* **6,** 159-174.

Brodie, M.J., French, J.A. (2000) Management of epilepsy in adolescents and adults. *Lancet,* **356,** 323-329.

Brodie, M.J., Schachter, S.C. (2001) Epilepsy. Second edition. Health Press, Oxford.

Brodie, M.J., Shorvon, S.D., Canger, R., et al. (1997) Commission on European affairs: Appropriate standards of epilepsy care across Europe. *Epilepsia,* **38,** 1245-1250.

Brodie, M.J., Wroe, S.J., Dean, A.D.P., Holdich, T.A.H., Whitehead, J., Stevens, J.W. Efficacy and safety of remacemide versus carbamazepine in newly diagnosed epilepsy: comparison by sequential analysis. *New Engl. J. Med.,* in preparation.

Brophy, P.D., Borowski, T.B., Kokkindis, L. (1993) Effects of scopolamine on the rewarding and seizure-induced porperties of AM stimulation. *Pharmacol. Biochem. Behav.,* **44,** 425-428.

Browning, R.A., Lanker, M.L., Faingold, C.L. (1989) Injections of noradrenergists into the inferior colliculus: effects on audiogenic seizures in genetically epilepsy-prone rats. *Epilepsy Res.,* **4,** 119-125.

Burke, M.D., Mayer, R.T. (1974) Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholantrene. *Drug Metab. Dispos.,* **2,** 583-588.

Burke, M.D., Upshall, D.G. (1976) Species and phenobarbital-induced differences in the kinetic constants of liver microsomal harmine O-demethylation. *Xenobiotica,* **6,** 321-328.

Butler, E., Thompson, G.G., Sills, G.J., Brodie, M.J. (2000) Effects of antiepileptic drugs on 5-hydroxytryptamine uptake into rat cortical synaptosomes. *Epilepsia,* **41,** 157.

Calabresi, P., De Murtas, M., Stefani, A., et al. (1995) Action of GP 47779, the active metabolite of oxcarbazepine, on the corticostriatal system. I. Modulation of corticostriatal synaptic transmission. *Epilepsia,* **36,** 990-996.

Carboni, E., Wojcik, W.J. (1988) Dihydropyridine binding sites regulate calcium influx through specific voltage-sensitive calcium channels in cerebellar granule cells. *J. Neurochem.,* **50,** 1279-1286.

Carlile, D.J., Hakooz, N., Houston, J.B. (1999) Kinetics of drug metabolism clearance in rat liver slices: IV. Comparison of ethoxycoumarin clearance by liver slices, isolated hepatocytes, and hepatic microsomes from rats pretreated with known modifiers of cytochrome P450 activity. *Drug Met. Disp.,* **27,** 526-532.

Carlson, H., Ronne Engstrom, E., Understedt, U., Hillered, L. (1992) Seizure related elevations of extracullular amino acids in human focal epilepsy. *Neurosci. Lett.,* **140,** 30-32.

Catterall, W.A. (1992) Cellular and molecular biology of voltage-gated sodium channels. *Physiol. Rev,.* **72,** 15-48.

Catterall, W.A. (1995) Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.,* **64,** 493-531.

Cavalheiro, E.A. (1994) Spontaneous recurrent seizures in rats: amino acids and monoamine determination in the hippocampus. *Epilepsia,* **35,** 1-11.

Chadwick, D.W. (1990) Diagnosis of epilepsy. *Lancet,* **336,** 291-295.

Chadwick, D.W., Smith, D., Crawford, P., Harrison, B. (2000) Remacemide hydrochloride: a placebo-controlled, one month, double-blind assessment of its safety, tolerability and pharmacokinetics as adjunctive therapy in patients with epilepsy. *Seizure,* **9,** 544-550.

Chapman, A.G. (1998) Glutamate receptors in epilepsy. In: *Progress in brain research.* Ottersen, O.P., Langmoen, I.A., Gjerstad, L. (Eds), pp 371-381. Elsevier Science.

Chapman, A.G., Meldrum, B.S. (1989) Epilepsy-Prone Mice: Genetically Determined Sound-Induced Seizures. In: *Neurotransmitters and Epilepsy.* Jobe, P.C., Laird, H.E. (Eds) pp 9-40. New Jersey: Humana Press Inc.

Charpentier, K.P., Von Moltke, L.L., Poku, J.W., Harmatz, J.S., Shader, R.I., Greenblatt, D.J. (1997) Alprazolam hydroxylation by mouse liver microsomes in vitro: The effect of age and phenobarbital induction. *Biopharm. Drug Disp.,* **18,** 139-149.

Chebib, M., Johnston, G.A.R. (1999) The 'ABC' of GABA receptors: A brief review. *Clin. Exp. Pharmacol Physiol.,* **26,** 937-940.

Chen, G., Ensor, C.R., Bohner, B. (1954) A facilitation action of reserpine on the central nervous system. *Proc. Soc. Exp. Biol. Med.*, **86,** 507-510.

Chesnut, T.J., Swann, J.W. (1988) Epileptiform activity induced by 4-aminopyridine in immature hippocampus. *Epilepsy Res.,* **2,** 187-195.

Cheung, H., Kamp, D., Harris, E. (1992) An in vitro investigation of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Res.,* **13,** 107-112.

Chiron, C., Dulac, O., Beaumont, D., Palacios, L. Pajot, N., Mumford, J. (1991) Therapeutic trial of vigabatrin in refractory infantile spasms. *J. Child Neurol.,* **suppl. 2,** S52-S59.

Clark, B., Hutchison, J.B., Jamieson, V., Jones, T., Palmer, G.C., Scheyer, R.D. (1995) Potential antiepileptic drugs: remacemide hydrochloride. In: *Antiepileptic Drugs, Fourth Edition.* Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds), pp 1035-1044. Raven Press, New York.

Clarke, C.E., Cooper, J.A., Holdich, T.A.H. (2001) A randomised, double-blind, placebo-controlled, ascending-dose tolerability and safety study of remacemide as adjuvant therapy in Parkinson's disease with response fluctuations. *Clin. Neuropharmacol.*, **24,** 133-138.

Clarke, H., Feigin, A. (1999) Disease modifying agents for Huntington's disease: What is on the horizon? *CNS Drugs,* **12,** 383-389.

Collingridge, G.L., Kehl, S.J., McLennan, H. (1982) The action of an *N*-methyl-D-aspartate antagonist on synaptic processes in the rat hippocampus. *J. Physiol.*, **327,** 27.

Collingridge, G.L., Kehl, S.J., McLennan, H. (1983) Excitatory amino acids in synaptic transmission in the schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol.*, **334,** 33-46.

Commission on classification and terminology of the international league against epilepsy. (1981) Proposal for revised classification of epilepsy and epileptic syndromes. *Epilepsia,* **30,** 389-399.

Conney, A.H. (1967) Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.*, **19,** 317-366.

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogensis by polycyclic aromatic hydrocarbons: GHA Clowes Memorial Lecture. *Cancer Res.*, **42,** 4875-4917.

Cooper, A.J.L., Mcdonald, J.M., Gelbard, A.S., Gledhill, R.F., Duffy, T.E. (1979) The metabolic fate of ^{13}N-labeled ammonia in rat brain. *J. Biol. Chem.*, **254,** 4982-4992.

Cooper, A.J.L., Mora, S.N., Cruz, N.F., Gelbard, A.S. (1985) Cerebral ammonia metabolism in hyperammonemic rats. *J. Neurochem.*, **44,** 1716-1723.

Corcoran, M.E. (1988) Characteristics of accelerated kindling after depletion of noradrenaline in adult rats. *Neuropharmacol.*, **27,** 1081-1084.

Corcos, L. (1992) Phenobarbital and dexamethasone induce expression of cytochrome P450 genes from subfamilies IIB, IIC, and IIIA in mouse liver. *Drug Met. Disp.*, **20,** 797-801.

Coulter, D.A., Huguenard, J.R., Prince, D.A. (1989a) Calcium currents in rat thalamocortical relay neurones: kinetic properties of the transient low-threshold current. *J. Physiol.*, **414,** 587-604.

Coulter, D.A., Huguenard, J.R., Prince, D.A. (1989b) Characterisation of ethosuximde reduction of low-threshold calcium current in thalamic neurons. *Ann. Neurol.*, **25,** 582-593.

Coyne, W.E., Cusic, J.W. (1968) Anticonvulsant semicarbazides. *J. Med. Chem.*, **11,** 1158-1160.

Crain, S.M. (1972) Tissue culture models of epileptiform activity. In: *Experimental models of epilepsy - a manual for the laboratory worker.* Purpura, D.P., Penry, J.K., Tower, D., Woodbury, D.M., Walter, R.D. (Eds) pp 291-316. New York: Raven Press.

Cramer, C.L., Stagnitto, M.L., Knowles, M.A., Palmer, G.C. (1994) Kainic acid and 4-aminopyridine seizure models in mice: evaluation of efficacy of antiepileptic agents and calcium antagonists. *Life Sci.*, **54,** 271-275.

Crawford, P., Richens, A., Mawer, G., et al. (1992) A double-blind placebo controlled cross-over study of remacemide hydrochloride as adjunctive therapy in patients with refractory epilepsy. *Seizure,* **1,** 7-13.

Curtis, D.R., Duggan, A.W., Johnston, G.A.R. (1971) The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. *Exp. Brain Res.*, **12,** 547-565.

Czeh, G., Somjen, G. (1989) Changes in extracellular calcium and magnesium and synaptic transmission in isolated mouse spinal cord. *Brain Res.*, **486,** 274-285.

Czuczwar, S.J., Frey, H.H., Loscher, W. (1986) N-methyl-D,L-aspartic acid-induced convulsions in mice and their blockade by antiepileptic drugs and other agents. In: *Neurotransmitters, seizures and epilepsy, III.* Nistico,G. (Ed), pp 235-246, Raven Press, New York.

Dailey, J.W., Jobe, P.C. (1984) Effect of Increments in the Concentration of Dopamine in the Central Nervous System on Audiogenic Seizures in DBA/2J Mice. *Neuropharmacol.*, **23**, 1019-1024.

Dailey, J.W., Yan, Q.-S., Adams-Curtis, L.E., et al. (1996) Minireview - Neurochemical correlates of anti-epileptic drugs in the genetically epilepsy prone rat (GEPR). *Life Sci.*, **58,** 259-266.

Dailey, J.W., Yan, Q.S., Mishra, P.K., Burger, R.L., Jobe, P.C. (1992) Effects of fluoxetine on convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. *J. Pharmacol. Exp. Ther.*, **260,** 533-540.

Dailey, J.W., Reith, M.E., Yan, Q.S., Li, M.Y., Jobe, P.C. (1997) Carbamazepine increases extracelluar serotonin: lack of antagonism by tetrodotoxin or zero calcium. *Eur. J. Pharmacol.*, **328,** 153-162.

Dalby, N.O., Nielsen, E.B. (1997) Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin, and vigabatrin. *Epilepsy Res.*, **28,** 63-72.

Dam, M., Ekberg, R., Loyning, Y., Waltimo, O., Jakobsen, K. (1989) A double-blind study comparing oxcarbazepine and carbamazepine in patients newly diagnosed, previously untreated epilepsy. *Epilepsy Res.*, **2,** 70-76.

DanyszW., Parsons, C.G. (1998) Glycine and NMDA receptors: Physiological significance and possible therapeutic applications. *Pharmacological Revs.*, **50,** 597-664.

Davies, J.A. (1997) Remacemide hydrochloride: a novel anticonvulsant. *Gen. Pharmacol.*, **4,** 499-502.

Davis, M.A., Winthrop, S.O., Thomas, R.A., Herr, F., Charest, M.P., Gaudry, R. (1964) Anticonvulsants. I. Dibenzo (a,d)cycloheptadiene-5-carboxamide and related compounds. *J. Med. Chem.*, **6,** 88-94.

Deffois, A., Fage, D., Carter, C. (1996) Inhibition of synaptosomal veratridine-induced sodium influx by antidepressants and neuroleptics used in chronic pain. *Neurosci. Lett.*, **220,** 117-120.

Deri, Z., Adam-Vizi, V. (1993) Detection of intracellular free Na^+ concentration by a fluorescent indicator, Na^+-binding benzofuran isophthalate: the effect of veratridine, ouabain and α-latrotoxin. *J. Neurochem.*, **61,** 818-825.

Dichter, M.A. (1993) Old and new mechanisms of antiepileptic drug actions. *Epilepsy Res.*, **10 (suppl.),** 9-17.

Dichter, M.A., Brodie, M.J. (1996) New antiepileptic drugs. *New Eng. J. Med.*, **334,** 1583-1590.

Dichter, M.A., Herman, C.J., Selzer, M. (1972) Silent cells during interictal discharges and seizures in hippocampal penicillin foci. Evidence for the role of extracellular K^+ in the transition from the interictal state to seizures. *Brain Res.*, **48,** 173-183.

Dietzel, I., Heninemann, U., Hofmeier, G., Lux, H.D. (1980) Transient changes to the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. *Exp. Brain Res.*, **40,** 432-439.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F. (1999) The glutamate receptor ion channels. *Pharmacol. Revs.*, **51,** 7-61.

Dolphin, A.C. (1995) Voltage-dependent calcium channels and their modulation by neurotransmitters and G proteins. *Exp. Physiol.*, **80,** 1-36.

Dost, R., Rundfeldt, C. (2000) The anticonvulsant retagabine potently suppresses epileptiform discharges in the low Ca^{2+} and low Mg^{2+} model in the hippocampal slice preparation. *Epilepsy Res.*, **38,** 53-66.

Dulac, O., Chiron, C., Luna, D, et al. (1991) Vigabatrin in childhood epilepsy. *J. Child Neurol.*, **suppl. 2,** S30-S37.

Dunn, R.T., Frye, M.S., Kimbrell, T.A., Denicoff, K.D., Leverich, G.S., Post, R.M. (1998) The Efficacy and Use of Anticonvulsants in Mood Disorders. *Clin. Neuropharmacol.*, **21,** 215-235.

During, M.J., Spencer, D.D. (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet,* **341,** 1607-1610.

Durlach, J. (1967) A proposal of a case of the epileptic form of latent tetany due to a magnesium deficiency. *Revue Neurologique,* **117,** 189-196.

Dyker, A.G., Lees, K.R. (1999) Remacemide hydrochloride: a double-blind, placebo-controlled, safety and tolerability study in patients with acute aschemic stroke. *Stroke,* **30,** 1796-1801.

Eadie, M.J. (1985) Clinical use of antiepileptic drugs. In: *Antiepileptic drugs. Handbook of Experimental Pharmacology, Vol 74.* Frey H-H., Janz, D. (Eds) pp765-790. Berlin: Springer Verlag.

Editorial (1988) Sodium valproate. *Lancet.* **ii,** 1229-1231.

Editorial. (1989a) Carbamazepine update. *Lancet,* **ii,** 595-597.

Editorial. (1989b) Oxcarbazepine. *Lancet,* **ii,** 196-198.

Eke, T., Talbot, J.F., Lawden, M.C. (1997) Severe persistent visual field constriction associated with vigabatrin. *Br. Med. J.,* **314,** 180-181.

El-Beheiry, H., Puil, E. (1990) Effects of hypomagnesia on transmitter actions in neocortical slices. *Br. J. Pharmacol.,* **101,** 1006-1010.

Engstrom, F.L., Woodbury, D.M. (1988) Seizure susceptibility in DBA and C57 mice: the effects of various convulsants. *Epilepsia,* **29,** 389-395.

Erecinska, M., Nelson, D. (1990) Activation of glutamate dehydrogenase by leucine and its nonmetabolizable analogue in rat brain synaptosomes. *J. Neurochem.,* **54,** 1335-1343.

Farinelli, S.E., Nicklas, W.J. (1992) Glutamate metabolism in rat cortical astrocyte cultures. *J. Neurochem.,* **58,** 1905-1915.

Ferencz, I., Kokaia, M., Keep, M., et al. (1997) Effects of cholinergic denervation on seizure development and neurotrophin messenger RNA regulation in rapid hippocampal kindling. *Neurosci.,* **80,** 389-399.

Finberg, J.P.M., Pacak, K., Kopin, I.J., Goldstein, D.S. (1993) Chronic inhibition of monoamine oxidase type A increases noradrenaline release in rat frontal cortex. *Naun. Sch. Arch. Pharmacol.*, **347,** 500-505.

Fisher, R.S. (1989) Animal models of the epilepsies. *Brain Res. Revs.,* **14,** 245-278.

Fisher, R., Blum, D. (1995) Clobazam, oxcarbazepine, tiagabine, topiramate, and other new antiepileptic drugs. *Epilepsia,* **36 (suppl. 2),** S105-S114.

Fisher, R.S., Pedley, T.A., Moody, W.J., Prince, D.A. (1976) The role of extracellular potassium in hippocampal epilepsy. *Arch. Neurol.,* **33,** 76-83.

Fleckenstein, A.E., Gibb, J.W., Hanson, G.R. (2000) Differential effects of stimulants on monoaminergic transporters: Pharmacological consequences and implications for neurotoxicity. *Eur. J. Pharmacol.*, **406,** 1-13.

Fleckenstien, A.E., Haughey, H.M., Metzger, R.R., et al. (1999) Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function. *Eur. J. Pharmacol.*,**382,** 45-49.

Flores-Herandez, J., Galarraga, E., Pineda, J.C., Bargas, J. (1994) Patterns of excitatory and inhibitory synaptic transmission in the rat neostriatum as revealed by 4-AP. *J. Neurophysiol.*, **72,** 2246-2256.

Flynn, J.W., O'Brien, J.E. (1992) High-performance liquid chromatographic method for the simultaneous determination of remacemide (a novel anticonvulsant and cerebroprotectant) and an active metabolite in human plasma. *J. Chromatog. B.*, **583,** 91-97.

Fozzard, H.A., Hank, D.A. (1996) Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. *Physiol. Rev.*, **76,** 887-926.

Frankenhaeuser, B., Hodgkin, A.L. (1957) The action of calcium on the electrical properties of squid axon. *J. Physiol.*, **137,** 218-244.

Fraser, C.M., Sills, G.J., Forrest, G., Thompson, G., Brodie, M.J. (1999) Effects of anti-epileptic drugs on glutamine synthetase activity in mouse brain. *Br. J. Pharmacol.*, **126,** 1634-1638.

Frey, H-H., Janz, D. (Eds) (1985) *Antiepileptic drugs. Handbook of Experimental Pharmacology, Vol 74.* Berlin: Springer Verlag.

Friis, M.L., Kritensen, O., Boas, J., et al. (1993) Therapeutic experiences with 947 epileptic out-patients in oxcarbazepine treatment. *Acta. Neurol. Scand.,* **87,** 224-227.

Fritsch, G., Hitzig, E. (1870) Ueber die electrische Erregbarkeit des Grosshirns. *Arch. Anat. Physiol. Wissensch. Med.,* **37,** 300-332.

Fueta, Y., Avoli, M. (1992) Effects of antiepileptic drugs on 4-aminopyridine-induced epileptiform activity in young and adult rat hippocampus. *Epilepsy Res.,* **12,** 207-215.

Gallo, V., Ciotti, M.T., Coletti, A., Aloisi, F., Levi, G. (1982) Selective release of glutamate from cerebellar granule cells differentiating in culture. *Proc. Nat. Acad. Sci. USA,* **79,** 7919-7923.

Gandolfo, G., Romettino, S., Gottesman, C., Van Luijtelaer, G., Coenen, A., Bidard, J.N., et al. (1989) K^+ channel openers decrease seizures in genetically epileptic rats. *Eur. J. Pharmacol.,* **167,** 181-183.

Garske, E.G., Palmer, G.C., Napier, J.J., et al. (1991) Preclinical profile of the anticonvulsant remacemide and its enantiomers in the rat. *Epilepsy Res.,* **9,** 161-174.

Gean, P.W., Shinnick-Gallagher, P. (1988) Characterisation of the epileptiform activity induced by magnesium-free solution of the rat amygdala slices: an intracellular study. *Exp. Neurol.*, **101,** 248-255.

Gee, N.S., Brown, J.P., Dissanayake, V.U.K., Offord, J., Thurlow, R., Woodruff, G.N (1996) The novel anticonvulsant, gabapentin (Neurontin), binds to the α2δ subunit of a calcium channel. *J. Biol. Chem.*, **271,** 5768-5776.

Genton, P., Van Vleymen, B. (2000) Piracetam and levertiracetam: close structural similarities but different pharmacological and clinical profiles. *Epileptic Disord.*, **2,** 99-105.

Gibson, G., Skett, P. (1986) Introduction to drug metabolism. Chapman & Hall, London.

Glowinski, J., Iverson, L.L. (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J.Neurochem.*, **13,** 655-669.

Goddard, G.V. (1983) The kindling model of epilepsy. *Trends Neurosci.*, **6,** 275-279.

Goldlust, A., Su, T.Z., Welty, D.F., Taylor, C.P., Oxender, D.L. (1995) Effects of anticonvulsant drug gabapentin on the enzymes in metabolic pathways of glutamate and GABA. *Epilepsy Res.,* **22,** 1-11.

Gonzalez, F.J. (1989) The molecular biology of cytochrome P450s. *Pharmacol. Rev.,* **40,** 243-288.

Gotz, E., Feuerstein, T.J., Meyer, D.K. (1993) Effects of gabapentin on release of γ-aminobutyric acid from slices of rat striatum. *Drug Res.,* **43,** 636-638.

Gram, L. (1990) Epileptic seizures and syndromes. *Lancet,* **336,** 161-163.

Graumlich, J.F., McLaughlin, R.G., Birkhahn, D., et al. (1999) Carbamazepine pharmacokinetics-pharmacodynamics in genetically epilepsy-prone rats. *Eur. J. Pharmacol.*, **369,** 305-311.

Greenamyre, J.T., Porter, R.H.P. (1994) Anatomy and physiology of glutamate in the CNS. *Neurology,* **44 (suppl. 8),** S7-S13.

Greene, J.G., Porter, R.H.P., Greenamyre, J.T. (1996) ARL-15896, a novel NMDA receptor ion channel antagonist: neuroprotection against mitochondrial metabolic toxicity and regional pharmacology. *Exp. Neurol.,* **137,** 66-72.

Greenlee, W.F., Poland, A. (1978) An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Pharmacol. Exp. Ther.*, **205,** 596-605.

Grinvald, A., Segal, M. (1984) Optical monitoring of electrical activity: detection of spatiotemporal patterns of activity in hippocampal slices by voltage-sensitive probes. In: *Brain Slices* Dingledine, R. (Ed). pp 227-261. Plenum Press, New York.

Gruhzit, O.M. (1939) Sodium diphenyl hydantoinate. Pharmacological and histopathological studies. *Arch. Pathol.*, **28,** 761-762.

Hadfiled, M.G. (1972) Uptake and Binding of Catecholamines. *Arch. Neurology*, **26,** 78-84.

Halonen, T., Nissinen, J., Pitkanen, A. (1999) Neuroprotective effect of remacemide hydrochloride in a perforant pathway stimulation model of status epilepticus in the rat. *Epilepsy Res.*, **34,** 251-269.

Harden, C.L. (1994) New antiepileptic drugs. *Neurol.* **44,** 787-795.

Harding, G.F.A., Mackenzie, R., Klistorner, A. (1998) Severe persistent visual filed constriction associated with vigabatrin. *Br. Med. J.*, **316,** 232-233.

Harootunian, A.T., Kao, J.P.Y., Eckert, B.K., Tsien, R.T. (1989) Fluorescence ratio imaging of cytosolic free Na^+ in individual fibroblasts and lymphocytes. *J. Biol. Chem.*, **264,** 19458-194467.

Haughey, H.M., Brown, J.M., Fleckenstein, A.E., Hanson, G.R. (1999) Oxygen Radicals differentially affect Na^+/Cl^--dependent transporters. *Eur. J. Pharmacol.*, **379,** 107-110.

Hayashi, T. (1959) The inhibitory action of β-hydroxy-γ-aminobutyric acid upon the seizure following stimulation of the motor cortex of the dog. *J. Physiol.*, **145,** 570-578.

Hayashi, T., Nagai, K. (1956) Action of ω-amino acids on the motor cortex of higher animals, especially γ-amino-β-hydroxybutyric acid as the real inhibitory principle in the brain. *Abstr. Commun. Int. Physiol. Congr.*, **20,** 410 (abs).

Herron, C.E., Williamson, R., Collingridge, G.L. (1985) A selective NMDA antagonist depresses epileptiform activity in rat hippocampal slices. *Neurosci. Lett.*, **61,** 255-260.

Heyn, H., McCarthy, D.J., Curry, S.H., Eisman, M.S., Anders, M.W. (1994a) Brain uptake and biotransformation of remacemide hydrochloride, a novel anticonvulsant. *Drug Met. Disp.*, **22,** 443-446.

Heyn, H., Gietl, Y., Anders, M.W. (1994b) Tissue distribution and stereoselectivity of remacemide-glycine hydrolase. *Drug Met. Disp.*, **22,** 973-974.

Hitchcock, E., Teixeira, M. (1982) Anticonvulsant activation of pain-suppressive systems. *App. Neurophys.*, **45,** 582-593.

Hofmann, F., Biel, M., Flockerzi, V. (1994) Molecular basis of Ca^{2+} channel diversity. *Annu. Rev. Neurosci.*, **17,** 399-418.

Honkakoski, P., Lang, M.A. (1989) Mouse liver phenobarbital-inducible P450 system: purification, characterisation, and differential inducibility of four cytochrome P450 isozymes from the D2 mouse. *Arch. Biochem. Biophys.*, **273,** 42-57.

Hu, R.Q., Davies, J.A. (1995) The effect of desglycinyl metabolite of remacemide on cortical wedges prepared from DBA/2 mice. *Eur. J. Pharmacol.*, **287,** 251-256.

Huettner, J.E., Bean, B.P. (1988) Block of NMDA-activated current by the anticonvulsant MK801: selective binding to open channels. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 1307-1311.

Jacobson, M., Levin, W., Poppers, P.J., Wood, A.W., Conney, A.H. (1978) Comparison of the O-dealkylation of 7-ethoxycoumarin and the hydroxylation of benzo(a)pyrene in human placenta. *Clin. Pharmacol. Ther.*, **16,** 701-710.

Janjua, N.A., Metrakos, J.D., Van Gelder, N.M. (1982) Plasma amino acids in epilepsy. In: *Genetic basis of the epilepsies.* Anderson, V.E., Hauser, W.A., Penry, J.K., Sing, C.F. (Eds). pp 181-197. New York: Raven Press.

Jefferys, J.G.R., Haas, H.L. (1982) Synchonised bursting of CA1 hippocampal pyramidal cells in the absence of synaptic transmission. *Nature,* **300,** 448-450.

Jobe, P.C., Dailey, J.W., Wernicke, J.F. (1999) A noradrenergic and serotonergic hypothesis of the linkage between epilepsy and affective disorders. *Crit. Rev. Neurobiol.*, **13,** 317-356.

Jobe, P.C., Laird, H.E. (Eds) (1987) Neurotransmitters and epilepsy. Clifton N.J. Humana Press.

Jobe, P.C., Picchioni, A.L., Chin, L. (1972) Role of brain norepinephrine in audiogenic seizure in the rat. *J. Pharmacol. Exp. Ther.*, **184,** 1-9.

Jones, G.L., Amato, R.J., Wimbish, G.H., Peyton, G.A. (1981) Comparison of anticonvulsant potencies of cyheptamide, carbamazepine and phenytoin. *J. Pharm. Sci.,* **70,** 618-620.

Julien, R.M., Hollister, R.P. (1975) Carbamzepine: mechanism of action. *Adv. Neurol.*, **11,** 164-176.

Kallen, R.G., Cohen, S.A., Barchi, R.L. (1993) Structure, function and expression of voltage-dependent sodium channels. *Mol. Neurobiol.*, **7,** 383-428.

Kanamori, K., Ross, B.D. (1995) In vivo activity of glutaminase in the brain of hyperammonaemic rats measured by ^{15}N nuclear magnetic resonance. *Biochem. J.*, **305,** 329-336.

Kaplan, B.J., Seyfried, T.N., Glaser, G.H. (1979) Spontaneous poly-spike discharges in an epileptic mutant mouse (tottering). *Exp. Neurol.*, **66,** 577-586.

Katz, B., Miledi, R. (1969) Tetrodotoxin-resistant electric activity in presynaptic terminals. *J. Physiol.*, **203,** 459-487.

Kaur, G., Kanungo, M.S. (1970) Alterations in glutamate dehydrogenase of the brain of rats of various ages. *Can. J. Biochem.*, **48,** 203-206.

Keen, P., White, T.D. (1971) The permeability of pinched-off nerve endings to sodium, potassium, and chloride and the effects of gramicidin. *J. Neurochem.*, **18,** 1097-1103.

Kellogg, C. (1971) Serotonin metabolism in the brains of mice sensitive or resistant to audiogenic seizures. *J. Neurobiol.*, **2,** 209-219.

Kelly, K.M., Gross, M.A., Macdonald, R.L. (1990) Valproic acid selectively reduces the low-threshold (T) calcium current in rat nodose neurons. *Neurosci. Lett.*, **116,** 233-238.

Kieburtz, K., Nutt, J., Schwid, S., et al. (1999) The impact of remacemide hydrochloride on levodopa concentrations in Parkinson's disease. *Clin. Neuropharmacol.*, **22,** 220-225.

Killam, K.F., Bain, J.A. (1957) Convulsant hydrazides I: in vitro and in vivo inhibition of vitamin B6 enzymes by convulsant hydrazides. *J. Pharmacol. Exp. Ther.*, **119,** 255-262.

Kim, H.J., Choi, E.S., Wade, A.E. (1990) Effect of dietary fat on the induction of hepatic microsomal cytochome P450 isozymes by phenobarbital. *Biochem. Pharmacol.*, **39,** 1423-1430.

Koella, W.P., Levin, P., Baltzer, V. (1976) The pharmacology of carbamazepine and some other antiepileptic drugs. In: *Epileptic seizures - behaviour - pain.* Birkmayer W. (Ed) pp 23-48. Huber Bern.

Köhling, R., Gladwell, S.J., Bracci, E., Vreugdenhil, M., Jefferys, J.G.R. (2001) Prolonged epileptiform bursting induced by 0-Mg^{2+} in rat hippocampal slices depends on gap junctional coupling. *Neurosci.*, **105,** 579-587.

Kokaia, M., Cenci, M.A., Elmer, E., et al. (1994) Seizure development and noradrenaline release in kindling epilepsy after noradrenergic reinnervation of the subcortically deafferented hippocampus by superior cervical ganglion or fetal locus coeruleus grafts. *Exp. Neurology*, **130,** 351-361.

Konnerth, A., Heinemann, U., Yaari, Y. (1986) Non-synaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizure activity in low extracellular calcium. *J. Neurophysiol.*, **56,** 409-423.

Korn, S.J., Giacchino, J.L., Chamberlin, N.L., Dingledine, R. (1987) Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. *J. Neurophysiol.*, **57,** 325-340.

Krall, R.L., Penry, J.K., White, B.G., Kupferberg, H.J., Swinyard, E.A. (1978) Anticonvulsant drug screening. *Epilepsia,* **19,** 409-428.

Kresch, M.J., Shaywitz, B.A., Shaywitz, S.E., Anderson, G.M., Leckman, J.L., Cohen, D.J. (1989) Neurotransmitters in Human Epilepsy. In: *Neurotransmitters and Epilepsy.* Jobe, P.C. & Laird, H.E. (Eds). pp 321-338. New Jersey: Humana Press Inc.

Krogsgaard-Larsen, P., Falch, E., Larsson, O.M., Schousboe, A. (1987) GABA uptake inhibitors: relevance to antiepileptic drug research. *Epilepsy Res.*, **1,** 77-93.

Kubova, H., Mares, P. (1993) Anticonvulsant action of oxcarbazepine, hydroxycarbamazepine, and carbamazepine against metrazol-induced motor seizures in developing rats. *Epilepsia,* **34,** 188-192.

Kuo, C.C. (1998) Imipramine inhibition of transient K+ current: an external open channel blocker preventing fast inactivation. *Biophy. J.,* **75,** 2845-2857.

Kuo, C.C., Bean, B.P. (1994) Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. *Mol. Pharmacol.,* **46,** 716-725.

Kuo, C.C., Chen, R.S., Lu, L., Chen, R.C. (1997) Carbamazepine inhibition of neuronal sodium currents: quantitative distinction from phenytoin and possible therapeutic implications. *Mol. Pharmacol.*, **51,** 1077-1083.

Kuo, N., Michalick, M., Erecinska, M. (1994) Inhibition of glutamate dehydrogenase in brain mitochondria and synaptosomes by magnesium: a possible cause for its low *in vivo* activity. *J. Neurochem.*, **63,** 751-757.

Kvamme, E. (1998) Synthesis of glutamate and its regulation. In: *Progress in Brain Research.* Ottersen, O.P., Langmoen, I.A., Gjerstad, L. (Eds). Ch.6, pp 73-85. Elsevier Science

Kwan, P., Brodie, M.J. (2000) Early identification of refractory epilepsy. *N. Eng. J. Med.*, **342,** 314-319.

Kwan, P., Sills, G.J., Brodie, M.J. (2001) The mechanisms of action of commonly used antiepileptic drugs. *Pharmacol. Ther.*, **90,** 21-34.

de Lanerolle, N.C., Kim, J.H., Robbins, R.J., Spencer, D.D. (1989) Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. *Brain Res.*, **495,** 387-95.

Lang, D.G., Wang, C.M., Cooper, B.R. (1993) Lamotrigine, phenytoin and carbamazepine interactions on the sodium current present in N4TG1 mouse neuroblastoma cells. *J. Pharmacol. Exp. Ther.*, **266,** 829-835.

LaSalle, G.L.G. (1981) Amygdaloid kindling in the rat: regional differences and general properties. In: *Kindling II.* Wada, J.A. (Ed) pp 31-47. New York: Raven.

Leach, J.P., Blacklaw, J., Jamieson, V., Jones, T., Richens, A., Brodie, M.J. (1996a) Mutual interaction between remacemide hydrochloride and carbamazepine: two drugs with active metabolites. *Epilepsia,* **37,** 1100-1106.

Leach, J.P., Brodie, M.J. (1995) Lamotrigine: clinical use. In: *Antiepileptic drugs (4th edition).* Levy, R., Mattson, R.H., Meldrum, B.S., Penry, J.K. (Eds) pp 889-895. Raven Press, New York.

Leach, J.P., Brodie, M.J. (1998) Tiagabine. *Lancet,* **351,** 203-207.

Leach, J.P., Girvan, J., Jamieson, V., Jones, T., Richens, A., Brodie, M.J. (1997a) Lack of phamacokinetic interaction between remacemide hydrochloride and sodium valproate in epileptic patients. *Seizure,* **6,** 179-184.

Leach, J.P., Girvan, J., Jamieson, V., Jones, T., Richens, A., Brodie, M.J. (1997b) Mutual interaction between remacemide hydrochloride and phenytoin. *Epilepsy Res.,* **26,** 381-388.

Leach, J.P., Sills, G.J., Butler, E., Forrest, G., Thompson, G.G., Brodie, M.J. (1997c) Neurochemical actions of gabapentin in mouse brain. *Epilepsy Res.,* **27,** 175-180.

Leach, J.P., Sills, G.J., Butler, E., Forrest, G., Thompson, G.G., Brodie, M.J. (1997d) Neurochemical actions of the desglycinyl metabolite of remacemide hydrochloride (ARL 12495AA) in mouse brain. *Br. J. Pharmacol.,* **121,** 923-926.

Leach, J.P., Sills, G.J., Majid, A., et al. (1996b) Effects of vigabatrin on GABA uptake into rat cortical astrocytes in primary culture. *Seizure,* **5,** 229-234.

Leach, M.J., Marden, C.M., Miller, A.A. (1996) Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia,* **27,** 490-497.

Leal, K.W., Rapport, R.L., Wilensky, A.J., Friel, P.N. (1979) Single dose pharmacokinetics and anticonvulant efficacy of primidone in mice. *Ann. Neurol.,* **5,** 470-474.

Lee, W. -L., Hablitz, J.J. (1989) Involvement of non-NMDA receptors in picrotoxin-induced epileptiform activity in the hippocampus. *Neurosci. Lett.,* **107,** 129-134.

Lehmann, A. (1967) Audiogenic seizures data in mice supporting new theories of biogenic amines mechanisms in the central nervous system. *Life Sci.*, **6,** 1423-1431.

Leppik, I.E., Wolff, D.L. (1995) The place of felbamate in the treatment of epilepsy. *CNS Drugs,* **4,** 294-301.

Leschinger, A., Stabel, J., Igelmund, P., Heinemann, U. (1993) Pharmacological and electrographic properties of epileptiform activity induced by elevated K^+ and lowered Ca^{2+} and Mg^{2+} concentrations in rat hippocampus. *Exp. Brain Res.,* **96,** 230-240.

Levitt, P., Noebels, J. (1981) Mutant mouse tottering: selective increase of locus ceruleus axons in a defined single-locus mutation. *Proc. Natl. Acad. Sci. U.S.A.*, **78,** 4630-4643.

Levy, R. (1995) Cytochrome P450 isozymes and antiepileptic drug interactions. *Epilepsia,* **36 (suppl. 5),** 8-13.

Lindstrom, P., Ben-menachem, E., Soderfeldt, B. (1996) CSF concentrations of remacemide and its deglycinyl metabolite in a double blind placebo controlled study of 3 doses (300, 600, 1200 mg) remacemide hydrochloride as add-on therapy in patients with refractory epilepsy. *Epilepsia,* **37,** 166.

Lingamaneni, R., Hemmings, H.C. (1999) Effect of anticonvulsants on veratridine- and KCl-evoked glutamate release from rat cortical synaptosomes. *Neurosci. Lett.*, **276,** 127-130.

Liporace, J.D., Sperling, M.R., Dichter, M.A. (1994) Absence seizures and carbamazepine in adults. *Epilepsia,* **35,** 1026-1028.

Lothman E.W., Bertram III E.H. & Stringer J.L. (1991) Functional anatomy of hippocampal seizures. *Prog. Neurobiol.*, **37,** 1-82.

Loscher, W. (1999) Valproate: A reappraisal of its pharmacodynamic properties and mechanisms of action. *Prog. Neurobiol.*, **58,** 31-59.

Loscher, W., Hoenack D. (1995) Anticonvulsant and antiepileptic effect of L-deprenyl (selegilene) in the kindling model of epilepsy. *J. Pharmacol. Exp. Ther.*, **274,** 307-314.

Loscher, W., Hoenack, D., Bloms-Funke, P. (1996) The novel antiepileptic drug levertiracetam (ucb L059) induces alterations in GABA metabolism and turnover in discrete areas of rat brain and reduces neuronal activity in substantia nigra pars reticulata. *Brain Res.*, **753,** 208-216.

Loscher, W., Hoenack D., Fassbender, C.P., Nolting, B. (1991a) The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. *Epilepsy Res.*, **8,** 171-189.

Loscher, W., Hoenack, D., Taylor, C.P. (1991b) Gabapentin increases amino-oxyacetic acid-induced GABA accumulation in several rgions of rat brain. *Neurosci. Lett.*, **128,** 150-154.

Loscher, W., Jackel, R., Muller, F. (1989) Anticonvulsant and proconvulsant effects of inhibitors of GABA degradation in the amygdala-kindling model. *Eur. J. Pharmacol.*, **163,** 1-14.

Loscher, W., Rundfeldt, C., Hoenack, D (1993) Low doses of NMDA receptor antagonists synergistically increase the anticonvulsant effect of the AMPA

receptor antagonist NBQX in the kindling model of epilepsy. *Eur. J. Neurosci.*, **5,** 1545-1550.

Loscher, W., Schmidt, D. (1988) Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.*, **2,** 145-181.

Lu, K.-T., Gean, P.-W. (1998) Endogenous serotonin inhibits epileptiform activity in rat hippocampal CA1 neurons via 5-hydroxytryptamine$_{1A}$ receptor activation. *Neurosci*, **86,** 729-737.

Macdonald, R.L., Greenfield, L.J. Jr., (1997) Mechanisms of action of new antiepileptic drugs. *Curr. Opin. Neurol.*, **10,** 121-128.

Macdonald, R.L., Kelly, K.M. (1993) Antiepileptic drug mechanisms of action. *Epilepsia,* **34 (suppl. 5),** S1-S8.

Macdonald, R.L., Kelly, K.M. (1994) Mechanisms of action of currently prescribed and newly developed antiepileptic drugs. *Epilepsia,* **35 (suppl. 4),** S41-S50.

Macdonald, R.L., Kelly, K.M. (1995) Antiepileptic drug mechanisms of action. *Epilepsia,* **36 (suppl. 2),** S2-S12.

Macphee, G.J.A., Butler, E., Brodie, M.J. (1987) Intradose and circadian variation in circulating carbamzepine and its epoxide in epileptic patients: a consequence of autoinduction of metabolism. *Epilepsia,* **28,** 286-294.

Marescaux, C., Warter, J.M., Laroye, M., et al. (1983) Sodium valproate: a hyperammonemic drug - study in epileptics and normal subjects. *J. Neurol. Sci.,* **58,** 195-209.

Matsunaga, K., Sato, T., Shuoto, H., Tsuruta, Y., Suemaru, K., Gomita, Y., Oishi, R. (1998) Inhibition of neuronal dopamine uptake by some antiallergic drugs. *Eur. J. Pharmacol.*, **350**, 165-169.

Mattson, R.H., Cramer, J.A., Collins, J.F., et al. (1985) Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondary generalised tonic-clonic seizures. *New Eng. J. Med.,* **313,** 145-151.

Mawer, G.E., Jamieson, V., Lucas, S.B., Wild, J.M. (1999) Adjustment of carbamazepine dose to offset the effects of the interaction with remacemide hydrochloride in a double-blind, multicentre, add-on drug trial (CR2237) in refractory epilepsy. *Epilepsia,* **40,** 190-196.

McCabe, R.T., Wasterlain, C.G., Kucharczyk, N., Sofia, R.D., Vogel, J.R. (1993) Evidence for anticonvulsant and neuroprotectant action of felbamate mediated by strychnin-insensitive glycine receptors. *J. Pharmacol. Exp. Ther.,* **264,** 1248-1252.

McIntyre, D.C., Edson, N. (1989) Kindling-Based Status Epilepticus: Effects of Norepinephrine Depletion with 6-Hydroxydopamine. *Exp. Neurology*, **104,** 10-14.

McIntyre, D.C., Saari, M., Pappas, B.A. (1979) Potentiation of Amygdala Kindling in Adult or Infant Rats by Injections of 6-Hydroxydopamine. *Exp. Neurology*, **63,** 527-544.

McKee, P.J.W., Brodie, M.J. (1994) Pharmacokinetic interactions with antiepileptic drugs. In: *New anticonvulsants: advances in the treatment of epilepsy.* Trimble, M. (Ed) pp 1-33. John Wiley, UK.

McKenna, M.C., Sonnewald, U., Huang, X., Stevenson, J., Kielke, H.R. (1996) Exogenous concentration regulates the metabolic fate of glutamate in astrocytes. *J. Neurochem.*, **66,** 386-393.

McLaughlin, S.G.A., Szabo, G., Eisenman, G. (1971) Divalent ions and the surface potential of charged phospholipid membranes. *J. Gen. Physiol.*, **58,** 667-687.

McLean, M.J., Macdonald, R.L. (1983) Multiple actions of phenytoin on mouse spinal cord neurones in cell culture. *J. Pharmacol. Exp. Ther.*, **227,** 779-789.

McLean, M.J., Macdonald, R.L. (1986a) Sodium valproate, but not ethosuximde, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurones in cell culture. *J. Pharmacol. Exp. Ther.*, **237,** 1001-1011.

McLean, M.J., Macdonald, R.L. (1986b) Carbamazepine and 10, 11-epoxy carbamazepine produce use- and voltage-dependent limitation of rapidly firing action potentials of mouse central neurones in cell culture. *J. Pharmacol. Exp. Ther.*, **238,** 727-738.

McLean, M.J., Schmutz, M., Wamil, A.W., Olpe, H.R., Portet, C., Felmann, K.F. (1994) Oxcarbazepine: mechanisms of action. *Epilepsia,* **35 (suppl. 3),** S5-S9.

McNamara, J.O. (1984) Kindling: Animal model of complex partial epilepsy. *Annals. Neurol.*, **16,** 572-576.

McNamara, J.O., Russell, R.D., Rigsbee, L., Bonhaus, D.W. (1988) Anticonvulsant and antiepileptogenic actions of MK801 in the kindling ang electroshock models. *Neuropharmacol.*, **27,** 563-568.

Meldrum, B.S. (1975) Epilepsy and γ-aminobutyric acid-mediated inhibition. *Int. Rev. Neurobiol.*, **17,** 1-36.

Meldrum, B.S. (1991) Excitotoxicity and epileptic brain damage. *Epilepsy Res.*, **10,** 55-61.

Meldrum, B.S. (1996) Update on the mechanism of action of antiepileptic drugs. *Epilepsia,* **37 (suppl. 6),** S4-S11.

Merritt, H.H., Putnam, T.J. (1938a) A new series of anticonvulsant drugs tested by experiments on animals. *Arch. Neurol. Psychiat.,* **39,** 1003-1015.

Merritt, H.H., Putnam, T.J. (1938b) Sodium diphenyl hydantoinate in treatment of convulsive disorders. *JAMA.,* **111,** 1068-1073.

Meunier, H., Carraz, G., Meunier, Y., Eymard, P., Aimard, M. (1963) Proprietes pharmacodynamiques de l'acide n-propylacetique. 1er memoire: proprietes antiepileptiques. *Therapie,* **18,** 435-438.

Minta, A., Tsien, R.Y. (1989) Fluorescent indicators for cytosolic sodium. *J. Biol. Chem.,* **264,** 19449-19457.

Mirski, M.A.Z., Rossell, L.A., McPherson, R.W., Traystman, R.J. (1994) Dexmedetomidine decreases seizure threshold in a rat model of experimental generalised epilepsy. *Anaestheology,* **81,** 1422-1428.

Mishra, P.K., Kahle, E.H., Bettendorf, A.F., Dailey, J.W., Jobe, P.C. (1993) Anticonvulsant effects of intracerebroventricularly administered norepinephrine are potentiated in the presence of monoamine oxidase inhibition in severe seizure genetically epilepsy-prone rats (GEPR-9s). *Life Sci.*, **52,** 1435-1441.

Mody, I., Lambert, J.D.C., Heinemann, U. (1987) Low extracellular magnsium induces epileptiform activity and spreading depression in rat hippocampal slices. *J. Neurophysiol.*, **59,** 1033-1053.

Molgo, I., Lemeignan, M., Lechat, P. (1977) Effects of 4-aminopyridine at the frog neuromuscular junction. *J. Pharmacol. Exp. Ther.*, **203,** 653-663.

Moody, W.J., Futamachi, K.J., Prince, D.A. (1974) Extracellular potassium activity during epileptogenesis. *Exp. Neurol.*, **42,** 248-260.

Morimoto, K., Sato, H., Yamamoto, Y., Watanabe, T., Suwaki, H. (1997) Antiepileptic effects of tiagabine, a selective GABA uptake inhibitor, in the rat kindling model of temporal lobe epilepsy. *Epilepsia,* **38,** 966-974.

Morris, M.E., Obrocea, G.V., Avoli, M. (1996) Extracellular K^+ accumulations and synchronous GABA-mediated potentials evoked by 4-aminopyridine in the adult hippocampus. *Exp. Brain Res.*, **109,** 71-82.

Moshe, S.L. (2000). Mechanisms of action of anticonvulsant agents. *Neurology*, **55 (suppl. 1),** S32-S40.

Muir, K.T., Palmer, G.C. (1991) Remacemide. *Epilepsy Res.*, **suppl 3,** 147-152.

Naffah-Mazzacoratti, M.G., Amado, D., Cukiert, A., et al. (1996) Monoamines and their metabolites in cerebrospinal fluid and temporal cortex of epileptic patients. *Epilepsy Res.*, **25,** 133-137.

Nagao, T., Alonso, A., Aoli, M. (1996) Epileptiform activity induced by pilocarpine in the rat hippocampal-entorhinal slice preparation. *Neurosci.*, **72,** 399-408.

Nakamura, J., Tamura, S., Kanda, T., et al. (1994) Inhibition by topiramate of seizures in spontaneously epileptic rats and DBA/2 mice. *Eur. J. Pharmacol.*, **254,** 83-89.

Neal, M.J. (1992) Medical pharmacology at a glance. Second Edition. Blackwell Science.

Nebert, D.W., Eisen, H.J., Negishi, M., Lang, M.A., Hjelmeland, L.M., Okey, A.B. (1981) Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P450) activites. *Ann. Rev. Pharmacol. Toxicol.*, **21,** 431-462.

Nebert, D.W., Gelboin, H.V. (1968) Substrate inducible microsomal aryl hydroxylase in mammalian cell culture. *J. Biol. Chem.*, **243,** 6242-6249.

Nebert, D.W., Gonzalez, F.J. (1987) P450 genes: Structure, evolution, and regulation. *Ann. Rev. Biochem.*, **56,** 945-993.

Nebert, D.W., Nelson, D.R., Coon, M.J., et al. (1993) The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature. *DNA Cell Biol.*, **10,** 1-14.

Nielsen, E.B., Suzdak, P.D., Andersen, K.E., Knutsen, L.J.S., Sonnewald, U., Braestrup, C. (1991) Characterisation of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. *Eur. J. Pharmacol.*, **196,** 257-266.

Nobile, M., Vercellino, P. (1997) Inhibition of delayed rectifier K^+ channels by phenytoin in rat neuroblastoma cells. *Br. J. Pharmacol.*, **120,** 647-652.

Noebels, J.L. (1984) A single gene error of noradrenergic axon growth synchronises central neurones. *Nature,* **310,** 409-411.

Noebels J.L., Sidman, R.J. (1979) Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science,* **204,** 1334-1336.

Noguchi, M., Fujitsuka, T., Honda, K., Kawai, Y. (1994) Effects of phenobarbital on drug metabolizing enzyme activities and other biochemical parameters in rats with DL-ethionine-induced liver injury. *J. Toxicolog. Sci.*, **19,** 203-212.

Norris, S.K., King, A.E. (1997a) Electrophysiological effects of the anticonvulsant remacemide hydrochloride and its metabolite ARL 12495AA on rat CA1 hippocampal neurones in vitro. *Neuropharmacol.* **36,** 951-959.

Norris S.K., King A.E. (1997b) The stereo-isomers of the anticonvulsant ARL 12495AA limit sustained repetitive firing and modify action potential properties of rat hippocampal neurones in vitro. *J. Pharmacol. Exp. Ther.*, **281,** 1191-1197.

Noyer, M., Gillard, M., Matagne, A., Henichart, J.P., Wulfert, E. (1995) The novel antiepileptic drug levertiracetam (ucb L059) appears to act via a specific binding site in CNS membranes. *Eur. J. Pharmacol.*, **286,** 137-146.

Ojemann, L.M., Friel, P.N., Trejo, W.J., Dudley, D.L. (1983) Effect of doxepin on seizure frequency in depressed epileptic patients. *Neurol.*, **22,** 1151-1159.

Okada, M., Kiryu, K., Kawata, Y., Mizuno, K., Wada, K., Tasaki, H., Kaneko, S. (1997a) Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. *Eur. J. Pharmacol.*, **321,** 181-188.

Okada, M., Hirano, T., Mizuno, K., Chiba, T., Kawata,Y., Kiryu, K., Wada, K., Tasaki, H., Kaneko, S. (1997b) Biphasic effects of carbamazepine on the dopaminergic system in rat striatum and hippocampus. *Epilpesy Res.*, **28,** 143-153.

Okada, M., Kawata, Y., Kiryu, K., et al. (1997c) Effects of toxic and non-toxic concentration of phenytoin on monoamine levels in rat brain. *Epilepsy Res.*, **28,** 155-163.

Okada, M., Hirano, T., Mizuno, K., Kawata, Y., Wada, K., Murakami, T., Tasaki, H., Kaneko, S. (1998) Effects of carbamazepine on hippocampal serotonergic system. *Epilepsy Res.*, **31**, 187-198.

Okey, A.B. (1990) Enzyme induction in the cytochrome P450 system. *Pharmac. Ther.*, **45,** 241-298.

Okuma, T. (1994). Use of Antiepileptic Drugs in Schizophrenia. *CNS Drugs*, **1,** 269-280.

Olney, J.W. (1989) Excitotoxicity and NMDA receptors. *Drug Dev. Res.,* **17,** 299-319.

Olsen, R.W., Avoli, M. (1997) GABA and epileptogenesis. *Epilepsia,* **38,** 399-407.

Omura, T., Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.,* **239,** 2370-2378.

Palmer, G.C., Clark, B., Hutchison, J.B. (1993) Antiepileptic and neuroprotective potential of remacemide hydrochloride. *Drugs of the Future,* **18,** 1021-1042.

Palmer, G.C., Miller, J.A., Cregan, E.F., Gendron, P., Peeling, J. (1997) Low-affinity NMDA antagonists. The neuroprotective potential of ARL 15896AR, *Ann. NY. Acad. Sci.,* **825,** 220-231.

Palmer, G.C., Murray, R.J., Cramer, C.L., et al. (1999) [S]-AR-R 15896AR- a novel anticonvulsant: acute safety, pharmacokinetic and pharmacodynamic properties. *J. Pharmacol. Exp. Ther.,* **288,** 121-132.

Palmer, G.C., Murray, R.J., Wilson, T.C.M., et al. (1992) Biological profile ofthe metabolites and potential metabolites of the anticonvulsant remacemide. *Epilepsy Res.,* **12,** 9-20.

Palmer, G.C., Stagnitto, M.L., Ordy, J.M., et al. (1991) Preclinical profile of stereoisomers of the anticonvulsant remacemide in mice. *Epilepsy Res.*, **8,** 36-48.

Panesar, S.K., Bandiera, S.M., Abbott, F.S. (1996) Comparative effects of carbamazepine and carbamazepine-10,11-epoxide on hepatic cytochromes P450 in the rat. *Drug, Met. Disp.*, **24,** 619-627.

Park, B.K., Kitteringham, N.R. (1990) Assessment of enzyme induction and enzyme inhibition in humans: toxicological implications. *Xenobiotica,* **20,** 1171-1185.

Park, B.K., Pirmohamed, M., Kitteringham, N.R. (1995) The role of cytochrome P450 enzymes in hepatic and extrahepatic human drug toxicity. *Pharmac. Ther.* **68,** 385-424.

Parsons, C.G., Danysz, W., Quack, G. (1998) Glutamate in CNS disorders as a target for drug development. *Drug News Perspect.*, **15,** 45-57.

Paschoa, O.E.D., Kruk, M.R., Hamstra, R., Voskuyl, R.A., Danhof, M. (1997) Seizure patterns in kindling and cortical stimulation models of experimental epilepsy. *Epilepsia,* **770,** 221-227.

Patsalos, P.N. (1998) Pharmacokinetic and pharmacodynamic interactions: principles and interpretative pitfalls. *Epileptologia,* **6 (suppl. 2),** 9-19.

Patsalos, P.N. (2000) Antiepileptic drug pharmacogenetics. *Ther. Drug Monitor.*, **22,** 127-130.

Patsalos, P.N., Duncan, J.S. (1993) Antiepileptic drugs. A review of clinically significant drug interactions. *Drug Safety,* **9,** 156-184.

Patsalos, P.N., Duncan, J.S., (1994) New antiepileptic drugs. A review of their current status and clinical potential. *CNS Drugs,* **2,** 40-77.

Patsalos, P.N., Sander, J.W.A.S. (1994) Newer antiepileptic drugs. Towards an improved risk-benefit ratio. *Drug Safety,* **11,** 37-67.

Pelletier, M.R., Corcoran, M.E. (1993) Infusions of a2 noradrenergic agonists and antagonists into the amygdala: effects on kindling. *Brain Res.,* **632,** 29-35.

Pellock, J.M., Brodie, M.J. (1997) Felbamate: 1997 update. *Epilepsia,* **38,** 1261-1264.

Perucca, E. (1997) Pharmacological advances of antiepileptic drug monotherapy. *Epilepsia,* **38,** S6-S8.

Perucca, E., Gram, L., Avanzini, G., Dulac, O. (1998) Antiepileptic drugs as a cause of worsening seizures. *Epilepsia,* **39,** 5-17.

Petroff, O.A., Rothman, D.L., Behar, K.L., Mattson, R.H. (1995) Initial observations on effect of vigabatrin on in vivo 1H spectroscopic measurements of gamma-aminobutyric acid, glutamate, and glutamine in human brain. *Epilepsia,* **36,** 457-464.

Pierce, N.W., Suzdak, P.D., Gustavson, L.E., Mengel, H.B., McKelvey, J.F., Mant, T. (1991) Tiagabine. In: *New antiepileptic drugs.* Pisani, F., Perucca, E., Avanzini, G., Richens, A. (Eds) pp 157-160. Amsterdam: Elsevier.

Pisani, F., Pedale, S., Macaione, V., Torre, V., Oteri, G., Avanzini, G., Ientile, R. (2001) Neuroprotective effects of lamotrigine and remacemide on excitotoxicity induced by glutamate agonists in isolated chick retina. *Exp. Neurol.,* **170,** 162-170.

Pisani, F., Perucca, E., Di Perri, R. (1990) Clinically relevant antiepileptic drug interactions. *J. Int. Med. Res.,* **18,** 1-15.

Plaitakis, A., Metaxari, M., Shashidharan, P. (2000) Nerve tissue-specific (GLUD2) and housekeeping (GLUD1) human glutamate dehydrogenases are regulated by distinct allosteric mechanisms: Implications for biologic function. *J. Neurochem.,* **75,** 1862-1869.

Podleski, T., Changeux, J.P. (1969) Effects associated with permeability changes caused by gramicidin A in electroplax membrane. *Nature,* **221,** 541-545.

Pongs, O. (1999) Voltage-gated potassium channels: from hyperexcitability to excitement. *FEBS Lett.*, **452,** 31-35.

Porter, R.J., Rogawski, M.A. (1992) New antiepileptic drugs: from serendipity to rational discovery. *Epilepsia,* **33 (suppl. 1),** S1-S6.

Porter, T.D., Coon, M.J. (1991) Cytochrome P450. *J. Biol. Chem.* **266,** 13469-13472.

Post, R.M., Frye, M.A., Denicoff, K.D., Gabriele, S.L., Kimbrell, T.A., Dunn, R.T. (1998) Beyond Lithium in the Treatment of Bipolar Illness. *Neuropsychopharmacol.*, **19,** 206-219.

Post, R.M., Ketter, T.A., Denicoff, K., et al. (1996) The place of anticonvulsant therapy in bipolar illness. *Psychopharmacol.*, **128,** 115-129.

Pressman, B.C., Harris, E.J., Jagger, W.S., Johnson, J.H. (1967) Antibiotic-mediated transport of alkali ions across lipid barriers. *Proc. Natl. Acad. Sci. U.S.A.*, **58,** 1949-1956.

Racine, R.J. (1972) Modification of seizure activity by electrical stimulation II, motor seizure. *Electroencephalog. Clin. Neurophysiol.*, **32,** 281-294.

Racine, R.J., Coscina, D.V. (1979) Effects of midbrain raphe lesions or p-chlorophenylalanine on the development of kindled seizures in rats. *Brain Res. Bull.*, **4,** 1-7.

Ragsdale, D.S., Avoli, M. (1998) Sodium channels as molecular targets for antiepileptic drugs. *Brain Res. Rev.*, **26,** 16-28.

Ragsdale, D.S., Scheuer, T., Catterall, W.A. (1991) Frequency and voltage-dependent inhibition of type IIA Na+ channels, expressed in a mammalian cell line, by local anaesthetic, antarrhythmic, and anticonvulsant drugs. *Mol. Pharmacol.*, **40,** 756-765.

Rang, H.P., Dale, M.M., Ritter, J.M. (1995) *Pharmacology. Third Edition.* Churchill Livingstone, UK.

Ray, R., Julien, R., Gordon, J., Blosser, J. (1992) FPL 12495, a metabolite of remacemide, is a noncompetitive NMDA antagonist. *Soc. Neurosci. Abs.*, **17,** 391.

Reid, H.R., Edmonds, H.L., Schurr, A., Tseng, M.T., West, C.A. (1988) Pitfalls in the use of brain slices. *Prog. Neurobiol.*, **31,** 1-18.

Remmer, H. (1962) Drug tolerance. In: *Ciba Foundations Symposium on Enzymes and Drug Action.* Mongar, J.L., De Reuck, A.V.S. (Eds) Little Brown, Boston.

Remmer, H., Merker, H.J. (1963) Drug-induced changes in liver endoplasmic reticulum: association with drug metabolising enzymes. *Science,* **142,** 1657-1658.

Represa, A., Robain, O., Tremblay, E., Ben-Ari, Y. (1989) Hippocampal plasticity in chilhood epilepsy. *Neurosci. Lett.,* **99,** 351-355.

Reynold, E.H., Milner, G., Matthews, D.M., Chanarin, I. (1966) Anticonvulsant therapy, megaalobastic haemopoiesis and folic acid metabolism. *Quart. J. Med.,* **35,** 521-537.

Rho, J.M., Donevan, S.D., Rogawski, M.A. (1994) Mechanism of action of the anticonvulsant felbamate: opposing effects on *N*-methyl-D-aspartate and γ-aminobutyric $acid_A$ receptors. *Ann. Neurol.,* **35,** 229-234.

Richens, A., Mawer, G., Crawford, P., Harrison, B. (2000) A placebo-controlled, double-blind cross-over trial of adjunctive one month remacemide hydrochloride treatment in patients with refractory epilepsy. *Seizure,* **9,** 537-543.

Riley, R.J., Lambert, C., Cooper, A.E., et al. (1995) Induction of rodent hepatic drug-metabolizing activities by the novel anticonvulsant remacemide hydrochloride. *J. Pharmacol. Exp. Ther.,* **23,** 922-928.

Roberts, E., Frankel, S. (1950) γ-aminobutyric acid in brain: its formation from glutamic acid. *J.Biol. Chem.*, **187,** 55-63.

Rogawski, M.A., Porter, R.J. (1990) Antiepileptic drugs: Pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol. Revs.*, **42,** 223-286.

Ross, F.M., Brodie, M.J., Stone, T.W. (1998) The effects of adenine dinucleotides on epileptiform activity in the CA3 region of rat hippocampal slices. *Neuroscience,* **85,** 217-228.

Rostock, A., Tober, C., Rundfledt, C., et al. (1996) D-231129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. *Epilepsy Res.*, **23,** 211-223.

Rowley, H.L., Marsden, C.A., Martin, K.F. (1995) Differential effects of phenytoin and sodium valproate on seizure-induced changes in γ-aminobutyric acid and glutamate release in vivo. *Eur. J. Pharmacol.*, **294,** 541-546.

Rundfeldt, C., Netzer, R. (2000) The novel anticonvulsant retagabine activates M-currents in Chinese hamster ovary-cells transfected with human KCNQ2/3 subunits. *Neurosci. Lett.*, **282,** 73-76.

Rutecki, P.A., Lebeda, F.J., Johnston, D. (1985) Epileptiform activity induced by changes in extracellular potassium in hippocampus. *J. Neurophysiol.*, **54,** 1363-1374.

Sachdeo, R.C., Sachdeo, S.K., Walker, S.A., Kramer, L.D., Nayak, R.K., Doose, D.R. (1996) Steady-state pharmacokinetics of topiramate and carbamazepine in patients with epilepsy during monotherapy and concominant therapy. *Epilepsia,* **37,** 774-780.

Salganicoff, L., De Robertis, E. (1965) Subcellular distribution of the enzymes of the glutamic acid, glutamine, and GABA cycles in rat brain. *J. Neurochem.*, **12,** 287-309.

Santangeli, S., McNeill, C., Sills, G.J., Brodie, M.J. (2000) Simultaneous determination of remacemide hydrochloride and desglycinyl-remacemide (AR-R12495XX) in brain tissue by high-performance liquid chromatography. *J. Chromatog. B.,* **746,** 325-329.

Schachter, S.C. (1998) Tiagabine. *Drugs of today,* **34,** 283-288.

Schachter, S.C., Tarsy, D. (2000) Remacemide: current status and clinical applications. *Exp. Opin. Invest. Drugs,* **9,** 871-833.

Schechter, P.J., Trainer, Y., Jung, M.J., Bohlen, P. (1977) Audiogenic seizure protection by elevated brain GABA concentrations in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. *Eur. J. Pharmacol.*, **45,** 319-328.

Scheyer, R.D. (1998) Involvement of glutamate in human epileptic activities. In: *Progress in Brain research.* Ottersen, O.P., Langmoen, I.A., Gjerstad, L. (Eds), pp 359-369. Elsevier Science.

Schumacher, T.B., Beck, H., Steinhauser, C., Schramm, J., Elger, C.E. (1998) Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. *Epilepsia,* **39,** 355-363.

Schwartz, J.R., Grigat, G. (1989) Phenytoin and carbamazepine: potential- and frequency-block of Na+ currents in mammalian myelinated nerve fibres. *Epilepisa,* **30,** 286-294.

Schwartzkroin, P.A. (1993) Too little inhibition and/or too much excitation: what is the truth? *Epilepsia,* **34,** 2-3.

Schwartzkroin, P.A., Prince, D.A. (1978) Cellular and field potential properties of epileptogenic hippocampal slices. *Brain Res.*, **147,** 117-130.

Schwid, S.R. (2001) A randomised, controlled trial of remacemide for motor fluctuations in parkinson's disease: Parkinson's study group. *Neurology,* **56,** 455-462.

Selzer, M.E. (1978) The action of phenytoin on a composite electrical-chemical synapse in the lamprey spinal cord. *Ann. Neurol.,* **3,** 202-206.

Seyfried, T.N., Glaser, G.H. (1985) A review of mouse mutants as genetic models of epilepsy. *Epilepsia,* **26,** 143-150.

Shank, R.P., Aprison, M.H. (1981) Present status and significance of the glutamine cycle in neural tissues. *Life Sci.,* **28,** 837-842.

Shank, R.P., Gardocki, J.F., Streeter, A.J., Maryanoff, B.E. (2000) An overview of the preclinical aspects of topiramate: pharmacology, pharmacokinetics, and mechanism of action. *Epilepsia,* **41 (suppl. 1),** S3-S9.

Shank, R.P., Gardocki, J.F., Vaught, J.L., et al. (1994) Topirmate: preclinical evaluation of a structurally novel anticonvulsant. *Epilepsia,* **35,** 450-460.

Sherwin, A.L., Gelder, N.M. (1986) Amino acid and catecholamine markers of metabolic abnormalities in human focal epilepsy. *Adv. Neurol.,* **44,** 1011-1032.

Shoulson, I., Greenmyre, T., Kieburtz, K., et al. (2000) A multicentre randomised controlled trial of remacemide hydrochloride as monotherapy for PD. *Neurology,* **54,** 1583-1588.

Shouse, M.N., Langer, J., Bier, M., et al. (1996) The α_2-adrenoceptor agonist clonidine suppresses seizures, whereas the α_2-adrenoceptor antagonist idazoxan promotes seizures in amygdala-kindled kittens: a comparison of amygdala and pontine microinfusion effects. *Epilepsia,* **37,** 709-717.

Sills, G.J., Leach, J.P., Fraser, C.M., Forrest, G., Patsalos, P.N., Brodie, M.J. (1997) Neurochemical studies with the novel anticonvulsant levetiracetam in mouse brain. *Eur. J. Pharmacol.,* **325,** 35-40.

Sills, G.J., Patsalos, P.N., Butler, E., Forrest, G., Ratnaraj, N., Brodie, M.J. (2001) Visual field constriction: acummulation of vigabatrin but not tiagabine in the retina. *Neurology,* **57,** 196-200.

Skrede K.K.R., Westgaard R.H. (1971) The transverse hippocampal slice: a well defined cortical structure in vitro. *Brain Res.,* **35,** 589-593.

Sloviter, R.S. (1987) Decreased hippocampal inhibition and a selective loss of interneurones in experimental epilepsy. *Science,* **235,** 73-76.

Snead, O.C. III (1978) γ-Hydroxybutyrate in the monkey; I. EEG, behavioural and pharmacokinetic studies. *Neurol.* **28,** 636-642.

Snead, O.C. III (1983) On the sacred disease: the neurochemistry of epilepsy. *Int. Rev. Neurobiol.,* **24,** 93-180.

Snead, O.C. III (1988) γ-Hydroxybutyrate model of generalised absence seizures: Further characterisation and comparison with other absence models. *Epilepsia,* **29,** 361-368.

Snead, O.C. III (1996) Presynaptic $GABA_B$ and γ-Hydroxybutyric acid-mediated mechanisms in generalised absence seizures. *Neuropharmacol.,* **35,** 359-367.

Song, J.H., Nagata, K., Huang, C.S., Yeh, J.Z., Narahashi, T. (1996) Differential block of two types of sodium channel by anticonvulsants. *Neuro-report,* **7,** 3031-3036.

Southam, E., Kirkbay, D., Higgins, G.A., Hagan, R.M. (1998) Lamotrigine inhibits monoamine uptake in vitro and modulates 5-hydroxytryptamine uptake in rats. *Eur. J. Pharmacol.*, **358,** 19-24.

Spiegel, E.A. (1937) Quantitative determination of the convulsive reactivity by electrical stimulation of the brain with the skull intact. *J. Lab. Clin. Med.*, **22,** 1274-1276.

Srinivasan, J., Richens, A., Davies, J.A. (1995) The effect of the desglycinyl metabolite of remacemide (FPL 12495AA) and dizocilpine (MK801) on endogenous amino acid release from mouse cortex. *Br. J. Pharmacol.*, **116,** 3087-3092.

Stables, J.P., Bialer, M., Johannessen, S.I., et al. (1995) Progress report on new antiepileptic drugs. A summary of the second Eilat conference. *Epilepsy Res.*, **22,** 235-246.

Stagnitto, M.L., Palmer, G.C., Ordy, J.M., et al. (1990) Preclinical profile of remacemide: a novel anticonvulsant effective against maximal electroshock seizures in mice. *Epilepsy Res.*, **7,** 11-28.

Stanton, P.K., Jones, R.S.G., Mody, I., Heinemann, U. (1987) Epileptiform activity induced by lowering magnesium in combined hippocampal-entorhinal cortex slices: modulation by receptors for noradrenaline and NMDA. *Epilepsy Res.*, **1,** 53-62.

Starr, M.S. (1996). The role of dopamine in epilepsy. *Synapse*, **22,** 159-194.

Stefani, A., Pisani, A., De Murtas, M., et al. (1995) Action of GP 47779, the active metabolite of oxcarbazepine, on the corticostriatal system. II. Modulation of high-voltage-activated calcium currents. *Epilepsia,* **336,** 997-1002.

Stefani, A., Spadoni, F., Bernardi, G. (1997) Voltage activated calcium channels: targets of antiepileptic drug therapy? *Epilepsia,* **38,** 959-965.

Stefani, A., Spadoni, F., Siniscalchi, A., Bernadi, G. (1996) Lamotrigine inhibits Ca^{2+} currents in cortical neurones: functional implications. *J. Pharmacol. Exp. Ther.,* **277,** 121-127.

Stephen, L.J., Sills, G.J., Brodie, M.J. (1998) Lamotrigine and topiramate may be a useful combination. *Lancet,* **351,** 958-959.

Stephen, L.J., Sills, G.J., Brodie, M.J. (2000) Topiramate in refractory epilepsy: a prospective study. *Epilepsia,* **41,** 977-980.

Stone, T.W. (1989) Electrophysiological methods in neuropharmacology. In: *Neurotransmitters, drugs and disease.* Webster, R.A., Jordan, C.C. (Eds) pp 48-57. Oxford: Blackwell Scientific.

Stone, T.W. (1995) CNS neurotransmitters. In: *Neuropharmacology.* pp 46-64. Oxford: Freeman.

Stone, T.W., Javid, M.J. (1983) Effects of anticonvulsants and other agents on seizures induced by intracerebral L-glutamate. *Brain Res.*, **264,** 165-167.

Storm, J.F. (1988) Temporal integration by a slowly inactivating K+ current in hippocampal slice. *Nature,* **336,** 379-381.

Stringer J.L., Lothman E.W. (1992) Reverbatory seizure discharges in hippocampal-parahippocampal circuits. *Exp. Neurol.*, **116,** 198-203.

Su, T-Z., Lunney, E., Campbell, G., Oxender, D.L. (1995) Transport of gabapentin, a γ-amino acid drug, by system 1 α-amino acid transporters: a comparative study in astrocytes, synaptosomes, and CHO cells. *J. Neurochem.*, **64,** 2125-2131.

Subramaniam, S., Donevan, S.E., Rogawski, M.A. (1996) Block of the N-methyl-D-aspartate receptor by remacemide and its des-glycine metabolite. *J. Pharmaol. Exp. Ther.*, **276,** 161-168.

Sucher, N.J., Awobuluyi, M., Choi, Y.B., Lipton, S.A. (1996) NMDA receptors: From genes to channels. *TIPS,* **17,** 348-355.

Sveinbjornsdottir, S., Sander, J.W.A.S., Upton, D., et al. (1993) The excitatory amino acid antagonist D-CPP-ene (SDZ EAA-494) in patients with epilepsy. *Epilepsy Res.*, **16,** 165-174.

Swinyard, E.A., Sofia, R.D., Kupferberg, H.J. (1986) Comparative anticonvulsant activity and neurotoxicity of felbamate and four prototype antiepileptic drugs in mice and rats. *Epilepsia,* **27,** 27-34.

Swinyard, E.A., Woodhead, J.H. (1989) General principles. Experimental selection, quantification, and evaluation of anticonvulsants. In: *Antiepileptic Drugs (3rd Edition).* Levy, R.H., Mattson, R.H., Meldrum, B.S., Penry, J.K., Dreifuss, F.E. (Eds) pp 85-102. New York: Raven Press.

Szente, M.B., Pongracz, F. (1979) Aminopyridine induced seizure activity. *Electroencephalogr. Clin. Neurophysiol.,* **46,** 605-608.

Tauboll, E., Gjerstad, L. (1998) Effects of antiepileptic drugs on the activation of glutamate receptors. In: *Progress in brain research.* Ottersen, O.P., Langmoen, I.A., Gjerstad, L. (Eds), pp 385-393. Elsevier Science.

Taylor, C.P., Gee, N.S., Su, T-Z., et al. (1998) A summary of the mechanistic hypotheses of gabapentin pharmacology. *Epilepsy Res.,* **29,** 233-249.

Taylor, C.P., Vartanian, M.G., Andruszkiewicz, R., Silverman, R.B. (1992) 3-alkyl GABA and 3-alkylglutamic acid analogues: two new classes of anticonvulsant agents. *Epilepsy Res.,* **11,** 103-110.

Thesleff, S. (1980) Aminopyridines and synaptic transmission. *Neurosci.,* **5,** 1413-1419

Tieney, B., Bresnick, E. (1981) Differences in the binding of 3-methylcholantrene and phenobarbital to rat liver cytosolic and nuclear protein fractions. *Arch. Biochem. Biophys.*, **210,** 729-739.

Traub, R.D., Colling, S.B., Jefferys, J.G.R. (1995) Cellular mechanism of 4-aminopyridine-induced synchronised after-discharges in the rat hippocampal slice. *J. Physiol.*, **489,** 127-140.

Traub, R.D., Jefferys, J.G.R., Whittington, M.A. (1994) Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. *J. Physiol.*, **478,** 379-393.

Traynelis, S.F., Dingledine, R. (1988) Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J. Neurophysiol.*, **59,** 259-276.

Treiman, D.M., Meyers, P.D., Walton, N.Y., Collins, J.F., Colling, C., Rowan, A.J., et al. (1998) A comparison of four treatments for generalized convulsive status epilepticus. *New Eng. J. Med.*, **339,** 792-798.

Twyman, R.E., Green, R.M., Macdonald, R.L. (1992) Kinetics of open channel block by penicillin of single $GABA_A$ receptor channels from mouse spinal cord neurones in culture. *J. Physiol.*, **445,** 97-127.

Twyman, R.E., Rogers, C.J., Macdonald, R.L. (1989) Differential regulation of γ-aminobutyric acid receptor channels by diazepam and phenobarbital. *Ann. Neurol.*, **25,** 213-220.

Udenfriend, S. (1950) Identification of γ-aminobutyric acid in brain by the isotope derivative method. *J. Biol. Chem.*, **187,** 65-69.

Ullrich, V., Weber, P. (1972) The O-dealkylation of 7-ethoxycoumarin by liver microsomes. *Hoppe-Seyler's Z. Physiol. Chem.*, **353,** 1171-1177.

Upton, N. (1994) Mechanisms of action of new antiepileptic drugs: rational design and serendipitous findings. *Trends Pharmacol. Sci.*, **15,** 456-463.

Urenjak, J., Tegtmeier, F., Beile, A., Khan, S., Peters, T. (1991) Synaptosomal respiration: A potent indicator of veratridine-induced Na^+ influx. *Pharmacology,* **43,** 26-35.

Van Luijtelaar, E.L.J.M., Coenen, A.M.L. (1995) Effects of remacemide and its metabolite FPL 12495 on spike-wave discharges, EEG and behaviour in rats with absence epilepsy. *Neuropharmacol.*, **34,** 419-425.

Voskuyl, R.A., Albus, H. (1985) Spontaneous epileptiform discharges in hippocampal slices by 4-aminopyridine. *Brain Res.*, **342,** 54-66.

Waldmeier, P.C., Baumann, P.A., Wicki, P., Feldtrauer, J.J., Stierlin, C., Schmutz, M. (1995) Similar potency of carbamzepine, oxcarbazepine, and lamotrigine in inhibiting the release of glutamate and other neurotransmitters. *Neurology,* **45,** 1907-1913.

Wallace, R.H., Wang, D.W., Singh, R., et al. (1998) Febrile seizures and generalised epilepsy associated with a mutation in the sodium channel β1 subunit gene SCN1B. *Nat. Genet.,* **19,** 366-370.

Wamil, A.W., Cheung, H., Harris, E.W., McLean, M.J. (1996) Remacemide HCl and its metabolite, FPL 12495AA, limit action potential firing and block NMDA responses of mouse spinal cord neurons in cell culture. *Epilepsy Res.,* **23,** 1-14.

Wamil, A.W., McLean, M.J. (1994) Limitation by gabapentin of high frequency action potential firing by mouse cerebral neurones in cell culture. *Epilepsy Res.,* **17,** 1-11.

Wang, S.J., Huang, C.C., Hsu, K.S., Tsai, J.J., Gean, P.W. (1996) Inhibition of N-type calcium currents by lamotrigine in rat amygdalar neurones. *Neuro-report,* **7,** 3037-3040.

Wattenberg, L.W., Leong, J.L., Strand, P.J. (1962) Benzpyrene hydroxylase activity in the GI tract. *Cancer Res.,* **22,** 1120-1125.

Watts, A.E., Jefferys, J.G.R. (1993) Effects of carbamazepine and baclofen on 4-aminopyridine-induced epileptic activity in rat hippocampal slices. *Br. J. Pharmacol.*, **108,** 819-823.

Wauquier, A., Zhou, S. (1996) Topirmate: A potent anticonvulsant in the amygdala-kindled rat. *Epilepsy Res.*, **24,** 73-77.

Waxman, D.J., Azaroff, L. (1992) Phenobarbital induction of cytochrome P450 gene expression. *Biochem. J.*, **281,** 577-592.

Weinberger, J., Nicklas, W.J., Berl, S. (1976) Mechanisms of action of anticonvulsants. *Neurology,* **26,** 162-166.

Westbrook, G.L., Lothman, E.W. (1983) Cellular and synaptic basis of kainic acid-induced hippocampal epileptiform activity. *Brain Res.*, **273,** 97-109.

Westergaard, N., Drejer, J., Schousboe, A., Sonnewald, U. (1996) Evaluation of the importance of transamination versus deamination in astrocyctic metabolism of [U-^{13}C]glutamate. *Glia,* **17,** 160-168.

White, H.S. (1999) Comparative anticonvulsant and mechanistic profile of established and newer antiepileptic drugs. *Epilepsia,* **40 (suppl. 5),** S39-S40.

White, H.S., Harmsworth, W.L., Sofia, R.D., Wolf, H.H. (1995) Felbamate modulates the strychnine-insensitive glycine receptor. *Epilepsy Res.*, **20,** 41-48.

White, H.S., Skeen, G.A., Edwards, J.A. (1992) Pharmacological regulation of astrocytic calcium channels: implications for the treatment of seizure disorders. In: *Progress in Brain Research, Vol 94.* Yu, A.C.H., Hertz, L., Norenberg, M.D., Sykova, E., Waxman, S.G. (Eds) pp 77-87. Amsterdam: Elsevier.

Whitlock, J.P. (1986) The regulation of cytochrome P450 gene expression. *Ann. Rev. Pharmacol. Toxicol.*, **26,** 333-369.

Whittington, M.A., Jefferys, J.G.R. (1994) Epileptic activity outlasts disinhibition after intrahippocampal tetenus toxin in the rat. *J. Physiol.*,**481,** 593-604.

Willetts, J., Balster, R.L., Leander, J.D. (1990) The behavioural pharmacology of NMDA receptor antagonists. *Trends Pharmacol. Sci.*, **11,** 423-428.

Willow, M., Catterall, W.A. (1982) Inhibition of binding of [^{3}H]batrachotoxin A 20-alphabenzoate to sodium channels by the anticonvulsant drugs diphenylhydantoin and carbamazepine. *Mol. Pharmacol.*, **22,** 627-635.

Willow, M., Gonoi, T., Catterall, W.A. (1985) Voltage clamp analysis of the inhibitory actions of phenytoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. *Mol. Pharmacol.*, **27,** 549-558.

Willow, M., Kuenzel, E.A., Catterall, W.A. (1984) Inhibition of voltage-sensitive sodium channels in neuroblastoma cells and synaptosomes by the anticonvulsant drugs diphenylhydantoin and carbamazepine. *Mol. Pharmacol.*, **25,** 228-234.

Wilson E.A., Brodie, M.J. (1996) New antiepileptic drugs. In: *Balliere's Clinical Neurology. Modern Management of Epilepsy.* Brodie M.J., Treiman, D.M. (Eds) pp 723-747. Balliere-Tindall, London.

Wilson E.A., Brodie, M.J., Wong, I.C., et al. (1997) Severe persistent visual field constriction associated with vigabatrin. *Br. Med. J.*, **314,** 1693-1695.

Wilson, T.C.M., Eisman, M.S., Machulskis, G.E. (1992) Quantitation of the novel anticonvulsant remacemide in rat and dog plasma and urine: application of the plasma methodology to measure the plasma protein binding of remacemide. *J. Chromatog. B.*, **582,** 195-202.

Wong, R.K.S., Prince, D.A. (1979) Dendritic mechanisms underlying penicillin-induced epileptiform activity. *Brain Res.*, **204,** 1228-1231.

Wood, J.H., Lake, C.R., Ziegler, M.G., Sode, J., Brooks, B.R., Van Buren, J.M. (1977) Cerebrospinal fluid norepinephrine alterations during electrical stimulation of cerebellar, cerebral surfaces in epileptic patients. *Neurology*, **27,** 716-724.

Yaari, Y., Konnerth, A., Heinemann, U. (1986) Non-synaptic epileptogenesis in the mammalian hippocampus in vitro. II Role of extracellular potassium. *J. Neurophysiol.* **56,** 424-438.

Yaari , Y., Pincus J.H., Argov, Z. (1977) Depression of synaptic transmission by diphenylhydantoin. *Ann. Neurol.,* **1,** 334-338.

Yamaguchi, S., Rogawski, M.A. (1992) Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. *Epilepsy Res.,* **11,** 9-16.

Yamamoto C., McIlwain H. (1966) Electrical activities in thin sections from the mammalian brain maintained in chemically-defined media in vitro. *J. Neurochem.,* **13,** 1333-1343.

Yan, Q.-S., Jobe, P.C., Dailey, J.W. (1993) Noradrenergic mechanisms for the anticonvulsant effects of desipramine and yohimbine in genetically epilepsy-prone rats: studies with microdialysis. *Brain Res.*, **640,** 24-31.

Yan, Q.-S., Jobe, P.C., Dailey, J.W. (1994) Evidence that a serotonergic mechanism is involved in the anticonvulsant effect of fluoxetine in genetically epilepsy-prone rats. *Eur. J. Pharmacol.*, **252,** 105-112.

Yan, Q.-S., Mishra, P.K., Burger, R.L., Bettendorf, A.F., Jobe, PC., Dailey, J.W. (1992) Evidence that carbamazepine and antiepilepsirine may produce a component of their anticonvulsant effect by activating serotonergic neurons in genetically epilepsy prone rats. *J. Pharmacol. Exp. Ther.*, **261,** 652-659.

Yonekawa, W.D., Kapetanovic, I.M., Kupferberg, H.J. (1995) The effects of anticonvulsant agents on 4-aminopyridine induced epileptiform activity in rat hippocampus in vitro. *Epilepsy Res.*, **20,** 137-150.

Yudkoff, M., Nissim, I., Hummeler, K., Medow, M., Pleasure, D. (1986) Utilization of [^{15}N] glutamate by cultured astrocytes. *Biochem. J.*, **234,** 185-192.

Yudkoff, M., Nissim, I., Nelson, D., Lin, Z.P., Erecinska, M. (1991) Glutamate dehydrogenase reaction as a source of glutamic acid in synaptosomes. *J. Neurochem.*, **57,** 153-160.

Zis, A.P., Nomkos, G.G., Brown, E.E., Damsa, G., Fiberger, H.C. (1992) Neurochemical effects of electrically and chemically induced seizures: an in vivo microdialysis study in hippocampus. *Neuropsychopharmacol.*, **7,** 189-195.