HISTAMINE RECEPTORS MEDIATING VASCULAR RESPONSES IN BRAIN

by

PAUL MUNN GROSS
B.Sc., M.Sc.

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SUMMARY

Several facts suggest that histamine may influence the function of cerebral blood vessels. 1) Histamine has important vascular effects in non-neural organs; 2) Cerebral microvessels and arteries contain receptors for histamine; 3) Enzyme systems in brain capillaries, particularly adenylate cyclase, are sensitive to histamine; 4) Cerebral perivascular mast cells store histamine; 5) A non-mast cell pool of histamine is located within cerebral vascular smooth muscle; 6) Histaminergic nerves are present in brain, and may innervate blood vessels; and 7) Histamine may modulate noradrenergic nerve activity in cerebral vessels.

In the studies that form the basis for this thesis, I have examined several aspects of brain vascular responses to histamine. These include an examination of blood-brain barrier permeability during carotid arterial infusion of histamine, cerebral blood flow responses to arterial infusion of histamine and its agonists, and responses of pial arterioles and veins to 1) perivascular injection of histamine agents, and 2) the interaction of noradrenergic and histaminergic stimuli.

In the studies examining cerebrovascular permeability (blood-brain barrier), histamine was infused into the internal carotid artery of anaesthetised rats, and quantitative measurements were made of vascular permeability to sucrose and a neutral amino acid, alpha-aminoisobutyric acid. Histamine increased permeability up to 237% in several brain regions (e.g., diencephalon, caudate nucleus, hippocampus, cerebral cortex) and in different areas of cerebral cortex (e.g., visual, parietal, frontal, olfactory). Additional studies indicated that the permeability increase was reversible: it had persisted for at

least 30 minutes, but permeability had returned to normal within two hours. Pretreatment by intravenous injection with the histamine H_2 receptor antagonist, metiamide, blocked the increase in permeability, while antagonism at H_1 receptors with mepyramine was ineffective. Studies determining regional brain water content indicated that oedema in cortical grey matter occurred during histamine infusion.

Measurements of cerebral blood flow in anaesthetised rats, were made by the intracarotid ^{133}Xenon clearance technique and also by tissue sampling to determine the brain distribution of tracer after systemic administration of labeled iodoantipyrine. Blood flow was measured before and after disruption of the blood-brain barrier by carotid injection of hypertonic urea. When the blood-brain barrier was intact, histamine had no effect on cerebral blood flow. After barrier opening, histamine increased blood flow in a concentration-dependent manner (26% to 50%). The participation of both histamine H_1 and H_2 receptors in mediating increases in blood flow was demonstrated by studies with the respective receptor antagonists during histamine infusion, and by carotid infusion of specific receptor agonists after blood-brain barrier opening. Dilatation of cerebral vessels during histamine infusion was not a secondary result to increased brain metabolism: histamine infusion after blood-brain barrier disruption did not produce detectable increases in cerebral glucose uptake. These results suggest that histamine acted on receptors within vascular smooth muscle to produce increases in blood flow.

Pial arteriolar and venous responses to histamine and its pharmacological agonists were determined in anaesthetised cats by measuring vessel calibre before and after perivascular injection. Both H_1 and H_2

receptors could mediate dilatation in arterioles, but with different maximum effects. The rank order of potency in producing dilatation by the receptor stimulants, and maximum responses elicited, were H_2 (+43%) > histamine (+28%) > H_1 (+17%). By contrast, pial veins did not respond significantly to the histamine agents.

Whether histamine could influence vasoconstrictor effects of sympathetic nerve stimulation and exogenous noradrenaline was also studied in cat pial vessels. In arterioles, stimulation of H_1 receptors inhibited the constriction by nerve stimulation, but not that resulting from exogenous noradrenaline; H_2 receptors interfered with constrictor effects produced by both kinds of noradrenergic stimuli. In veins, both histamine receptor types inhibited the effects of nerve stimulation, but did not influence responses to noradrenaline. These experiments suggest that both H_1 and H_2 receptors inhibit noradrenergic activity by acting at prejunctional sites in cerebral arterioles and veins. In arterioles, noradrenergic postjunctional mechanisms are affected only by H_2 receptors, while in veins, histamine receptors appear to have no influence at this neuroeffector site.

From concepts developed in the separate parts of this thesis, I formulated a unifying hypothesis that proposes a spatial and differential orientation of receptor sites for histamine in cerebral vessels. The following is a possible profile of histamine receptor distribution within the cerebral vessel wall: 1) endothelia: predominantly H_2 receptors; 2) inner layers of smooth muscle: both H_1 and H_2 receptors; and 3) outer layers of smooth muscle: predominantly H_2 receptors.

PREFACE AND DECLARATION

The biogenic amine, histamine (β-imidazolylethylamine), has been the subject of considerable experimental interest since the pioneering experiments of Sir Henry Dale seventy years ago. Among its diverse physiological activities, the influence of histamine on the function of blood vessels is known, for peripheral organs, to be of cardinal importance to the regulation of local blood flow and exchange of materials in the microcirculation. In the brain, histamine has multiple storage sites and, putatively, a neurotransmitter role, but its effects on brain blood vessels have not been adequately described. This thesis addresses several aspects of histamine's influence and location of activity in brain vessels.

The first part of the thesis deals with the effect of histamine on vascular permeability in brain. New quantitative methods were used to study the regional nature of response, and the types of histamine receptors involved. The second topic concerns cerebral blood flow. Dose-response relationships, regionality, receptor involvement, and relation to metabolic response were included in this section. The third part of the thesis can be divided into two areas of investigation: an examination in situ of pial arteriolar and venous responses to histamine agents given by perivascular injection, an approach that provides information on cerebral vascular smooth muscle responses to direct histaminergic stimuli; and a determination of the influence exerted by histamine receptors on noradrenergic responses in pial vessels.

The studies of this thesis, therefore, deal with four vascular loci at which histamine may influence the cerebral circulation in vivo. 1) Vascular permeability studies focus on events occurring at the endothelial cell level of brain vessels. 2) and

3) Blood flow and pial vessel studies involve the action of histamine at possibly two sites within the smooth muscle layer of brain arteries and arterioles. 4) Studies pertaining to the interaction of histamine with noradrenergic stimuli concern a possible influence by histamine at the nerve-smooth muscle junction in brain vessels. From these concepts is synthesised an hypothesis that proposes a differential distribution of histamine receptors within the cerebral vessel wall.

The lines of inquiry, data, interpretations, and concepts of this thesis evolved from my previous experiences and my own original experiments. This material has not been presented before as a thesis in any form.

CHAPTER I. INTRODUCTION

Introduction I. Blood-Brain Barrier

Concepts

Nearly a century has passed since the seminal studies of Ehrlich (62) who showed that a restrictive vascular interface to circulating blood solutes exists in the brain. Within the last fifteen years, morphological, physiological, and biochemical studies have been completed so that basic features of a blood-brain barrier are now familiar (reviews: 24, 27, 39, 43, 45, 78, 175, 199). In general, blood constituents that are lipid-soluble rapidly reach equilibrium with endothelial cytoplasm and brain interstitial fluid (199, 200); lipid-insoluble substances, by contrast, passively diffuse across or between vascular endothelial cells by dissolving in aqueous phases in the endothelium (165, 199, 200). The rate of blood-to-brain transfer for lipid-insoluble particles approximates an inverse proportion to the molecular weight and hydrodynamic radius of the molecule (199, 200). Some substances, such as D-hexoses and L-amino acids, are transferred into the brain by special carriers (168).

Several unique characteristics associated with cerebral microvessels are 1) junctions between adjacent endothelial cells are tightly apposed and adhered by a matrix of fine fibres. In brain regions having barrier properties, there are no endothelial fenestrations; 2) there is normally a low level of contractile activity; 3) pinocytosis and vesicular transport are present but normally unimportant; 4) the mitochondrial density is relatively high; 5) regulatory enzymes are present in the endothelial cytoplasm; and 6) binding sites for neuro-transmitters are located within the endothelium. Because each of these functions may be influenced by, or be related to, a neurohumoral stimulus such as histamine, I will discuss relevant points in the following paragraphs.

Continuous bands of closely apposed endothelial cells

provide the unique characteristic of brain microvessels. These "tight" intercellular junctions have widths of about 12 Å (27, 78, 199) which physically limit the passage of plasma solutes having hydrodynamic radii greater than 5 Å (27, 199). Cell-to-cell mucoprotein adhesions may provide additional hindrance to intercellular diffusion (78, 180). These junctional gaps of endothelial cells in brain correspond to the small pore system of vessels in peripheral organs. Estimates of small pore widths in skeletal muscle capillaries are 40-100 Å (204). In brain microvessels, however, the widths of intercellular junctions may not be uniform throughout the entire microcirculation. Because metarterioles (181, 241) and, presumably, venules, can transport protein into the brain under normal conditions, and because these vascular segments are involved in pathological disruption of the blood-brain barrier during some conditions (91, 160, 181), it may be more appropriate to refer to "microvascular" rather than only "capillary" permeability. In skeletal muscle, the small pore system in venules appears to be especially vulnerable to histamine-like mediators (114, 148, 212).

In some regions of the brain, there is no vascular barrier to circulating solutes, and blood vessels are characterised by wide intercellular clefts and fenestrae (24, 27, 78, 199). Brain structures and regions in this category include the choroid plexus, hypophysis, ependymal cells, median eminence, area postrema, and pineal gland (78, 199). These areas are relatively so small and circumscribed that their high permeability does not produce effects in the brain as a whole from agents in blood (24, 199).

Although brain endothelial cells have low contractile activity under normal conditions (199), mechanisms having the potential to mediate contraction are present. Two factors that may be important to contraction, and are present within brain endothelial cells, are histamine-sensitive adenylate cyclase (8, 121, 123, 125)

and myofibrillar filaments (134, 174, 180). Adenylate cyclase activity influences the energy level in the cell by synthesis of cyclic AMP (123, 158), and actin and myosin are linked to mechanical function (76). Should these mechanisms be coupled in cerebral endothelia, then cellular contraction could occur through a histamine stimulus. Endothelial contraction appears to be an important mechanism to the increased permeability seen in muscle and mesentery vessels during histamine administration (79, 124, 148).

Pinocytosis and vesicular transport normally operate at a low level in brain endothelia (199). Although factors influencing these functions are not yet clear, there is evidence to suggest that vesicular activity is mediated through receptors (84) and may be related to the energy level in the cell (242). Cyclic AMP may be a critical intermediate in a mechanism producing increased vesicular activity following stimulation by such factors as serotonin (242), acute hypertension (95, 119), vascular injury (210) and histamine (121, 123).

In electron microscopic studies, Oldendorf and co-workers (170) demonstrated that endothelial cells in brain capillaries had higher mitochondrial volumes than in peripheral organ capillaries. Possible functions related to this unique characteristic of brain endothelia are (170) 1) active transport of ions between blood and cerebrospinal fluid; 2) extrachoroidal production of cerebrospinal fluid; 3) maintenance of the integrity of enzymes that regulate a barrier function; and 4) maintenance of the structural components of the blood-brain barrier. These last two mechanisms may be important to a histamine influence on cerebrovascular permeability. If histamine were to affect the energy level in endothelial cells, as discussed above, then histamine-sensitive enzymes, such as adenylate cyclase and, subsequently, vesicular activity, could be involved in a permeability change.

Two enzymes present in brain microvascular endothelia may be important in determining influences on permeability. Disposal of monoamines, such as dopamine (16), occurs through a degradative process involving a trapping and conversion function by dopa decarboxylase and subsequent catabolism by monoamine oxidase. This catabolic process is probably not important to histamine which is degraded principally by histamine-N-methyltransferase (52). This enzyme is present in brain endothelial cells (125), and is distributed widely throughout several brain structures (231). A second enzyme system that may be important to changes in cerebral microvascular permeability involves the formation of cyclic AMP from ATP by adenylate cyclase (123), which can be influenced in cerebral capillary fractions by many biogenic amines (8, 113, 162).

Finally, recent ligand-binding studies have demonstrated the presence of histamine receptors in cortical microvessels (179). Peroutka and co-workers showed that substantial levels of histamine, and adrenergic, receptors were present in both neuronal membranes and purified microvessels from bovine cortex (179). These results, therefore, confirm, and help to localise, the receptors for histamine previously identified in other neurochemical studies (220). Receptors at the plasma membrane of endothelial cells would represent the initial site for production of a permeability change by histamine.

Histamine

In qualitative studies published some thirty years ago, it was demonstrated that histamine could increase the passage from blood into brain of the dye, neutral red (115), and antibiotics, such as penicillin and streptomycin (74). Studies since then have failed to confirm that histamine can influence the permeability of cerebral vessels (24, 81, 199). Factors that may account for confusion in the literature are the route and concentration of histamine administered, the duration of administration,

physical characteristics of the tracer used, and differences in responses between species.

Several characteristics associated with histamine, however, suggest that it should be among endogenous substances that influence cerebral vascular permeability. 1) Histamine is a principal component of cerebral perivascular mast cells (58, 99, 116, 117), which, when degranulated, could effect vascular permeability from the outside of the vessel (99, 117, 206). 2) Histamine has been identified within the smooth muscle layers of brain blood vessels (67). This source of histamine could be liberated to influence endothelial permeability. 3) Histaminergic nerves are present in the brain (85, 220); if these nerves have vascular terminals, then activation of neural pathways could affect vascular permeability. 4) Histamine-sensitive enzymes are present within cerebral microvessels (8, 125). Enzymes linked to cyclic nucleotide formation, such as adenylate and guanylate cyclase, may be important to function of the blood-brain barrier during normal and pathological conditions (123). 5) Specific receptors for histamine are located within cerebreal microvessels (179). Histamine receptors located along the plasma membranes of cerebral endothelia would provide a site for binding by histamine in blood, or from the abluminal sources of histamine listed above.

In non-neural organs, histamine is clearly a potent mediator of increased permeability. Histamine-induced increases in vascular permeability to a variety of substances have been measured in skin (207), skeletal muscle (48, 72, 90, 212), and the mesentery (79, 124). It is not clear, however, by what mechanisms histamine produces its effect in these circulations. Widening of interendothelial pores (148, 157), increasing the number of pores per unit surface area (48), increasing the surface area (10), or stimulating vesicular transport (11, 203) are possible responses evinced by histamine.

Methodology

There are several methods available for the study of blood-brain barrier function. In general terms, these techniques can be categorised as either histological or physiological approaches to the measurement of vascular permeability in the brain; advantages and disadvantages in the use of several current methods have been reviewed recently by Blasberg (20). In this section, I will discuss techniques that have been used to estimate the influence of histamine on the blood-brain barrier, consider quantitative methods available for measuring blood-brain exchange, and introduce the technique used in the studies of this thesis.

Qualitative Methods. This technique is founded on the use of a tracer molecule that would not, under normal conditions, enter the brain from blood in significant amounts. The most common tracer, still used in contemporary research, is a dye, such as Evans blue, which binds almost completely to plasma proteins. Appearance of the dye in brain tissue, therefore, reflects the extent of protein extravasation into the brain interstitial space. This method was the first approach used to evaluate the influence of histamine on blood-brain barrier permeability (115); the dye, neutral red, was shown to enter brain tissue in mice after systemic injection of histamine. Studies at the same time by Földes and Kelentei (74) indicated that histamine could increase the brain penetration of circulating antibiotics. More recently, dyes have been used to evaluate function of the blood-brain barrier during several interventions, such as the effect of hypertonic solutions injected into the carotid artery (35, 198), and the influence of sympathetic nerves during acute arterial hypertension (103).

The principal advantage of qualitative methods is that they provide the opportunity to visualise an effect on barrier function by the colour of the dye used (103).

These methods have the obvious disadvantage in that they are not accurately quantifiable.

Electron Microscopy. Probably the most significant advances in understanding the nature of the blood-brain barrier have derived from studies using the electron microscope. This method involves use of an enzymatic tracer, such as horseradish peroxidase, which is given systemically and visualised microscopically at the endothelial cell level (202). The important concepts of "tight" interendothelial junctions and low vesicular activity in normal endothelium have evolved from this method (27, 202). The method has been extended to yield additional information concerning barrier function in pathological conditions. The appearance of coated vesicles in response to intravascular injection of mineral chlorides (120), the opening of tight junctions in response to carotid injection of urea (26), and increases in numbers of endothelial vesicles following irradiation (205), spinal cord compression (15), stab wounds (210), and systemic administration of hydrolytic enzymes (83) have been demonstrated with this technique. Electron microscopy offers the investigator the advantages of determining endothelial cell responses to stimuli on a focal level with extensive descriptive capacity. Pinocytotic activity is quantifiable (15, 181, 205), and information about related organelles and structures is readily available in the micrographs. The method does not, however, allow for determination of the rates of tracer transfer. Furthermore it requires considerable time, experience and skill in processing and interpreting the material.

Labeled Compounds. Tagging of vascular solutes with radioactive labels has introduced two important advantages to studies of brain vascular permeability. First, changes in permeability can be regionally quantified; second, the use of solutes with different molecular weights offers the potential advantage of discerning

different routes of passage into the brain. For example, low molecular weight solutes (small hydrodynamic radius), such as sodium (MW = 23; 21, 106, 138), and noradrenaline (MW = 168; 35) may pass through endothelial cell junctions and via vesicular transport, whereas larger particles, such as albumin (MW = $69x10^3$) may enter the brain only through vesicles (241). Studies using these methods have characterised regional permeability to changes to interventions such as drug-induced seizures (142) and carotid injection of hypertonic urea (35). The main disadvantage of this method is that rates of solute transfer into the brain have not been quantified.

Intra-carotid Injection Methods. Two methods involving simultaneous injection of test and reference substances into the carotid artery have added considerable information to the blood-brain barrier literature. In the method of Crone (37, 38), brain extraction of the test solute is compared to a nondiffusible reference tracer from the concentration of these substances appearing in cerebral venous blood after arterial injection. Many measurements are possible in the same preparation, and the method can be applied to humans (132). Because samples of blood are obtained from easily exposed blood vessels representing the arterial and venous circulations, the method does not provide values for regional permeability. This technique has been applied to yield the first measurements of vascular permeability in human brain during seizures (21), extraction of water (177), and penetration of lipid-soluble and carrier-mediated substances (108).

The brain uptake method of Oldendorf (167-169) compares the brain concentration of test solute with a freely diffusible reference tracer following arterial injection. This method is technically simple, and many animals can be processed in a short period of time. In recent years, the technique has been critically evaluated and modified

to include corrections for use of the reference tracer, ^{3}H-water (169), an intravascular component of radioactivity (169), injection volume (93), and circulation time following injection of the tracer mixture (93). A disadvantage of the method is that it provides only a measure of uptake of test solute, rather than a quantitative determination of permeability. This technique has been used extensively, and many important concepts concerning brain entry of amines, sugars, amino acids and steroids have evolved (167, 168, 175, 176).

Brain Tracer Washout. By initially loading the brain vascular compartment with a moderately permeable tracer, such as labeled water or alcohols, blood-brain barrier permeability can be determined by measuring tissue clearance of the tracer, either by direct tissue sampling (23) or by external scintillation detection (63). In the studies by Bradbury et al. (23), brain tracer concentrations were determined at various times after carotid injection of several test substances to describe washout curves; as only one measurement can be obtained from each animal, this technique has the disadvantage of requiring several animals to provide one data point. This limitation is overcome by the method using external detection of radioactivity. Eichling, Raichle and colleagues have demonstrated the dynamics of water permeability at cerebral microvessels during several interventions (63, 194-196). The method can generate multiple washout curves from one animal, and it can be adapted for use in humans (196). There are several important disadvantages with this technique, however. Regional data cannot be derived, relatively large experimental subjects are required, such as monkeys or humans, it is difficult to correlate the external radioactivity with that in brain, and the isotope typically used, $H_2{}^{15}O$, has a very short half-life (two minutes), requiring the co-ordinated use of a cyclotron.

Arterial Integral Method. This method involves intravenous administration of the test solute with subsequent arterial blood sampling to derive an integral of arterial radioactivity throughout the experiment. Brain tracer concentration at the end of the experimental period, divided by the arterial radioactivity over time, yields blood-to-brain transfer constants or permeability x surface area products that are more sensitive than those available using other techniques (20). Tracer may be administered to the animal by bolus injection (165) or by continuous infusion (44, 138). Tracer input by infusion has the limitation of leaving high intravascular isotope concentrations at the end of the study, leading to difficulty in separating brain from blood radioactivity. Also, it is technically cumbersome to maintain arterial tracer concentration throughout the experiment (44, 138). Accurate derivation of the intravascular radioactivity is also a problem in using the intravenous bolus method, but can be obviated by extending the experimental time period which allows for additional peripheral clearance of the tracer (201).

Other potential limitations of these techniques include reflux of the tracer from brain to blood, metabolism of the tracer by brain, and bulk flow of the tracer within the brain interstitial space. Back diffusion, or reflux does not seem to be an important problem because brain tracer concentrations are sufficiently low that a concentration gradient for tracer movement from brain to blood is negligible (19, 165, 201). Regarding tracer metabolism, appropriate test substances that are not metabolised by brain cells, such as sucrose (42, 137, 165), can be selected for this technique. Furthermore, bulk flow of sucrose is minimal within the time limit of the experiment because the measurable brain distribution volume is small (165).

As this method has several distinct advantages over other available methods, it was chosen to evaluate the

effects of histamine on brain vascular permeability in the studies of this thesis. The arterial integral method is quantitative, providing values for both permeability and intravascular volume on a regional basis (165, 201). The technique can be performed in small animals such as rats. The experimental period may be 10 to 40 minutes in duration (201), thereby averaging transitory changes in permeability and providing a representation of multiple passes of the tracer through the cerebral microcirculation. Several labeled substances could be used in this method (165). The choice of solute, in fact, may provide useful information concerning the physiological process of blood-to-brain transfer of the substances. For example, in the present studies, sucrose and a neutral amino acid were chosen as test solutes; their physical properties are similar to those of endogenous neurotransmitters, e.g., they are lipid-insoluble, carried in suspension in plasma water rather than bound to proteins, are stable under physiological conditions, and are not actively transported by a carrier across brain endothelial cells (165).

Introduction II. Cerebral Blood Flow

The level of cerebral tissue perfusion depends on a number of local and systemic factors. Neural, chemical, and intrinsic autoregulatory influences may dictate the rate of blood flow within brain regions or in the central nervous system as a whole. Several reviews, presenting an integrated discussion of these factors, have been published (32, 59, 96, 129, 131, 133, 192, 223, 224). Stimuli that may initiate or compound changes in cerebral blood flow can arise from alterations in the blood concentration of vasoactive amines (59) or from the metabolic demand by the brain (32, 87, 226). A focus of the inter-relationship of these latter two factors formulates the topic of this section.

Concepts

Controversy concerning the effect of circulating amines on cerebral vascular smooth muscle and brain metabolism has been present in available literature for many years (59, 223, 224). Species differences and the several methods that have been applied may account for some of the discrepancies in results (59). Another factor appreciated only recently, however, is the integrity of the blood-brain barrier. Because most amines are lipid-insoluble and polar (168,199), their passage from the circulation to vascular smooth muscle or brain where an effect can be mediated is severely restricted under normal conditions (59). When the blood-brain barrier is circumvented, however, the true vascular or metabolic effect of the particular amine becomes evident (147).

The above mechanisms, and relevance of the blood-brain barrier to their expression, were first demonstrated for the neurotransmitter substance, noradrenaline, by MacKenzie and co-workers (144, 145). Noradrenaline is a potent constrictor agent in peripheral blood vessels (1, 76), but, during carotid arterial infusion, it has no effect in the cerebral circulation when the blood-

brain barrier is intact (145). MacKenzie et al. demonstrated that transient disruption of barrier mechanisms by carotid injection of a hypertonic solution of urea allowed access of infused noradrenaline to brain; under these conditions, increases in both cerebral oxygen consumption and the blood flow were observed, suggesting that the infusion had produced, primarily, an increase in brain metabolic rate through the calorigenic action of noradrenaline (145).

Subsequent studies with other agents combining barrier disruption with carotid drug infusion were to identify theretofore undescribed metabolic and vascular responses in brain. In general, the evidence would suggest that most agents that alter cerebral blood flow following disruption of the blood-brain barrier do so by producing first an effect on brain metabolic rate. Substances in this category are 5-hydroxytryptamine (98), prostaglandins E_2 and $F_{2\alpha}$ (183), phenylethylamine (153), and vasoactive intestinal peptide (154). Dopamine and tyramine, which are probably degraded by enzymatic catabolism in cerebral endothelia during normal conditions (16, 174), may also be in this group of substances; but, their effects in the cerebral circulation have not been evaluated in conjunction with blood-brain barrier opening (151, 152). Amphetamine (150) and the dopamine agonists, apomorphine (151) and piribedil (155), appear to readily enter the central nervous system from blood, and are associated with marked increases in cerebral oxygen consumption and blood flow.

Some substances that have been examined produced larger changes in cerebral blood flow than in the index used to represent the metabolic response, usually either oxygen uptake or glucose consumption. Although inadequacies in methodology may account for these discrepancies in responses, it is possible that the agents may produce only a small effect, or have no influence, on brain metabolism, but have considerable stimulating effects directly

on vascular smooth muscle. Prostaglandins (182, 185), piribedil (155), and acetylcholine (105) are in this class of substances that may be principally of vascular nature.

Histamine

The effect produced by histamine in the cerebral circulation is still a matter of controversy despite interest that spans decades (77, 224). In an older review (224), and in more recent papers (52, 135), histamine has been reported to be a potent dilating agent in the brain circulation. Dilatation resulting from intravascular administration of this amine is difficult to understand, however, because histamine itself is impermeable at the blood-brain barrier (168).

In the last 10 years there have been several studies that reported an increase in cerebral blood flow resulting from histamine infusion. Critical appraisal of these reports, however, reveals the following potential limitations: 1) flow measurements were obtained from large extracranial vessels supplying the brain (217, 228), and may not accurately reflect parenchymal blood flow; 2) studies using perfused brain (159) or venous outflow (33) techniques require extensive preparatory surgery. Consequently, data from these experiments are probably not reliable (59, 102, 193); and 3) cerebral blood flow responses determined in dogs and cats (3, 33, 159, 217) are difficult to assess because the carotid vascular anatomy in these species is complex, possibly leading to errors in interpretation (118).

It is clear from the literature (171) that several characteristics of vascular response to histamine have been identified in peripheral organs but not in the brain. The role of the blood-brain barrier in determining what effect is produced by histamine in the cerebral circulation has not yet been examined. If

histamine were to evoke changes in cerebral blood flow, the regional nature of the response, the specific types of histamine receptor stimulated, and metabolic influences produced by this amine would constitute new information in the field. These questions were addressed in the studies of this part of the thesis.

Methodology

Although there are several methods available for measuring cerebral blood flow in experimental animals (59, 193, 223), not all of the techniques have been adapted for studies in small animals, such as the rat. Reliable determinations of total or regional cerebral blood flow in rats have not been, or can not be, made from such techniques as venous outflow, isolated perfusion, electromagnetic flowmetry, or probe-detected gas or heat clearance. Four methods have proved useful for measuring blood flow in rat brain. These include the Kety-Schmidt and intracarotid injection techniques, which involve measurement of gas clearance from brain, and the radioactive microsphere and diffusible indicator methods, which involve fractionation and determination of tracer concentrations in brain regions.

Development for studies in rats of the Key-Schmidt method by Siesjö and co-workers (65, 164, 223) and the intracarotid gas injection method by Hertz et al. (107) has added important experimental advantages to the determination of blood flow and metabolic responses in rat brain. Of major importance is the facility with which to measure blood flow several times in the same rat; these methods, therefore, permit the determination of control blood flow and responses to successive stimuli during one experiment. Simultaneous evaluation of oxygen consumption is possible using these methods (107, 223). The methods have the limitations, however, of requiring rather extensive surgical preparation of the animal. In the Kety-Schmidt method, a representative cerebral vein must be cannulated; in the intra-

carotid injection method, carotid artery and scalp surgery are required. As both of these techniques involve gas clearance from brain, blood flow must be assumed to be in a steady-state at the time of measurement. Furthermore, particularly with the intracarotid injection method, there may be neuropathological damage resulting from multiple injections into the internal carotid artery (88). Control measurements of blood flow with these techniques, which reflect primarily cortical perfusion (65, 107, 110), are about 1.0 ml min^{-1} g^{-1} with the Kety-Schmidt method (65, 164), and between 1.4 and 1.6 ml min^{-1} g^{-1} with the intracarotid injection method (88,107). Values for the two methods are different because the intracarotid injection technique, by determination of flow from the initial slope of gas clearance (107), more closely represents the richer perfusion in cortical grey matter (110). Because the intracarotid injection technique permits several determinations of blood flow in one animal, and because this method was extensively evaluated during pilot studies for the thesis (88), the technique was applied in the present studies to characterise blood flow responses to histamine. A technique was required to evaluate possible regional responses in brain to histamine. Both the microsphere and diffusible indicator methods allow for assessment of flow on a regional basis by either tissue sampling (112, 166) or autoradiographic (216) determination. The theory and application of particulate (109, 149, 213) and diffusible (80, 127, 128) indicators for blood flow measurement have been thoroughly described.

With the radioactive microsphere method, a bolus of spheres is injected into the heart, distributed according to the partition of the cardiac output, and, because the spheres are too large to pass through microvessels, they lodge primarily at the metarteriolar level (109). Up to five determinations of cerebral blood flow can be

made in large animals, such as dogs (149). In rats, however, the technique has proved to be technically difficult (112) and small sample sizes from rat brain may undermine the sensitivity of the method. Furthermore, the microsphere method has not been properly evaluated for application to small animal brain as it has in dog brain (149), and in other organs (86, 109). Questions concerning appropriate sphere size, arteriovenous shunting, and neuropathology need to be answered before this technique can be accepted as a reliable method for cerebral blood flow measurement in rats. Blood flows for cortex obtained from microspheres in the studies of Horton et al. (112) were 1.0 to 1.3 ml min^{-1} g^{-1}.

The diffusible indicator method, which depends on the principles governing exchange between blood and brain of inert substances (127), has been applied to measure regional cerebral blood flow in large and small animals (80, 216). By obtaining thin sections of brain after systemic administration of the tracer, and exposing these to film, measurement of blood flow in very small brain regions can be determined by densitometry (216). This approach has the major disadvantage, however, of requiring several days for data processing, and involves the use of expensive, sophisticated equipment. A simpler approach, which yields less data but is based on the same principles, is to determine blood flow in macroscopic brain regions dissected by direct sampling of the tissue. This technique has been used previously to measure brain blood flow in rats with the tracers ^{14}C-ethanol (66) and ^{14}C-iodoantipyrine (112, 166). Whether the data are derived by autoradiography or by tissue sampling, however, the diffusible indicator method can provide only one measurement of blood flow in a single animal. In anaesthetised rats, this technique gives blood flow values for cortex of 1.0 to 1.2 ml min^{-1} g^{-1} (112).

The diffusible indicator technique, using the tracer, iodoantipyrine, was applied in the present studies for three reasons. First, the method is based on sound principles (127, 216); it has been shown that values for cerebral blood flow, obtained with iodoantipyrine, are similar to those derived from use of radioactive gas (216). Second, the technique provides regional blood flow data to complement the studies using the intracarotid gas injection method, which does not yield accurate data for regional perfusion. Third, regional blood flows determined by tissue sampling represent a compatible technical approach to data derivation in the blood-brain barrier studies. Comparisons of blood flow and vascular permeability responses determined from tissue sampling may provide additional insight to the regional effect of histamine in the cerebral circulation.

Introduction III. Pial Vessels in Situ

To study further effects of histamine in the cerebral circulation, experiments were undertaken to evaluate the response of pial arterioles and veins to specific stimulation of histamine receptors by pharmacological agonists. Three approaches were followed: 1) to determine responses of pial arterioles to perivascular injection of histamine and its H_1 and H_2 receptor agonists; 2) to determine respones of pial veins to stimulation of histamine receptors; and 3) to examine how these receptor mechanisms might apply *in vivo*, studies were conducted to determine the interaction between histaminergic and noradrenergic stimuli.

Concepts

Examination of exposed pial vessels is one of the oldest quantitative methods used in brain vascular research. Experiments reported by Forbes and co-workers 52 years ago (77) provided an important foundation for the development of several concepts (224). Three manipulations have mainly been used to provoke changes in pial vessel calibre; vasoactive drugs have been given intravascularly, drugs have been administered perivascularly, or endogenous mechanisms have been stimulated, such as by nerve activation or by altering arterial pressure. Determination of pial vascular responses to intra-arterial or intravenous drug administration has not been very helpful generally because it is not known to what extent circulating agents penetrate the blood-brain barrier. Observation of a pial arteriolar response during intravascular drug infusion, for example, may be explained by direct stimulation of vascular smooth muscle (the drug penetrated the blood-brain barrier) or may arise secondarily from changes in systemic arterial pressure. It is not clear how permeable vascular endothelia are at the level of arterioles and veins. Enzymatic mechanisms may be absent in large brain vessels (16), but a structural barrier is probably present in

pial arterioles (144, 241).

Determination of pial vascular responses to perivascular administration of vasoactive solutions is an attractive experimental technique for characterising sensitivity to specific stimuli. A large number of studies concerning the vascular effects in exposed brain vessels of several chemical and autonomic stimuli has been reviewed recently (131). For studies of histamine, this approach has three important advantages: 1) perivascular injection of histamine agents mimics the route by which histamine may influence brain vascular tone *in vivo*, either by release from mast cells (58, 116), or by activation of histaminergic nerves, which may have vascular terminals (85, 220); 2) it is possible that histamine has a metabolic influence on neurones (85), and that alteration of their metabolism may affect brain vessels. The perivascular injection technique, however, obviates this potential problem; and 3) injection of histamine onto the outside of blood vessels may provide information regarding the vascular orientation of histamine receptors. There is evidence from studies using rabbit ear arteries *in vitro* (82), that a predominance of histamine H_2 receptors may exist on the outside of blood vessels. Comparison of responses to stimulation of histamine receptors, as determined in other parts of this thesis, is important to the formulation of hypotheses concerning the spatial distribution of histamine receptors within the cerebral vascular wall.

An important limitation in studying pial vessels is that no information about cerebral blood flow is gained. In response to drugs, changes in pial vascular calibre may not accurately represent the response of intraparenchymal vessels which are more important to the regulation of brain perfusion (208).

The capability of histamine to interact with, and modulate, noradrenergic activity can be examined readily in the exposed pial vessel preparation by

ombining perivascular injection of histamine agents
ith stimulation of noradrenergic effector mechanisms.
, dense innervation of sympathetic fibres in pial
essels exists, extending throughout the hemibrain
from the ipsilateral superior cervical ganglion (173).
Modest pial arteriolar and venous constriction results
from stimulation of sympathetic nerves (7, 130, 240).
Concentration-response curves to perivascular injection
of noradrenaline in arterioles have been determined by
Wahl and Kuschinsky (130, 235, 237). There is no inform-
ation concerning noradrenaline effects in pial veins.

Histamine

Very few studies have been reported describing pial vascular responses to histamine. In the earliest experiments using the exposed brain technique, Forbes and his contemporary colleagues (77, 224) measured pial artery calibres during systemic administration of histamine. Their results cannot be properly interpreted, however, because histamine produced changes in arterial pressure which would affect pial artery responses. A number of studies involving histamine responses, as reviewed by Sokoloff (224), can be similarly criticised.

Differences in the manner in which histamine has been introduced to the pial vasculature have produced conflicting results. In studies involving superfusion of histamine over the entire cortical field, Raper et al. (197) found increases in arteriolar calibre in cats, and Rosenblum (209) found no effect on cortical vessels in mice. It is possible that histamine may have produced effects on cerebral metabolism which masked vascular responses in these studies. The superfusion technique does not seem appropriate for examination of histamine or other agents that might affect both neuronal metabolism and vascular smooth muscle. When histamine is administered by perivascular injection, pial arterioles always respond with dilatation which is concentration-dependent (238). As determined from

studies using histamine receptor blocking agents, the dilatation produced by histamine in pial arterioles is mediated almost exclusively by H_2 receptiors (238).

What response veins have to histamine *in vivo* is an important determinant to capillary blood flow, pressure and filtration, as discussed for peripheral organs by Haddy, Diana and associates (46, 47, 49, 89). Studies in peripheral vascular beds of vasoactive agents, including histamine, indicate that the precapillary resistance vessels are generally more responsive to these stimuli than capacitance vessels (46, 221), and, in fact, the vascular segments may have the opposite responses to the same stimulus (89, 221). In skeletal muscle and skin preparations, histamine may constrict (89, 189) or dilate (46, 47) veins. These findings collectively imply that such factors as vascular wall structure, wall tension, receptor distribution within the vessel wall, and receptor density may be important to differences in response to histamine between arteries and veins. In the only study to date in which brain arterial and venous responses to drug stimulation have been compared, vasoactive intestinal peptide produced significant dilatation of arterioles, but responses in veins were not different than those produced by the injection vehicle (154).

Interaction of Histamine With Noradrenergic Mechanisms

Histamine is synthesised and/or stored in autonomic nerves (214). It is richly concentrated in post-ganglionic sympathetic nerves (214), and is avidly sequestered by sympathetic axons in rabbit iris (61). Although not yet demonstrated histochemically, these findings suggest that histamine may be present in cerebro-vascular sympathetic nerves, and may play a role in modulating nervous activity at the sympathetic neuro-effector junction. Using rabbit basilar arteries *in vitro*, Bevan et al. (17) showed that histamine could modify vascular responses to transmural nerve

stimulation and exogenous noradrenaline. Similar responses have been found in the dog saphenous vein (156), rabbit ear artery (73), and rabbit ganglia (28) in vitro. In each of these studies, a potentiating effect of histamine on noradrenergic responses, linked to the H_1 class of receptors, was identified. The organisation and function of histamine receptors at sympathetic nerve endings appear to be complex because H_2 receptors, in contrast to the H_1 class, inhibit adrenergic mechanisms in heart (141), blood vessels (156, 191) and ganglia (28). How several transmitter substances may affect neurotransmission has been discussed by Brimble and Wallis (28) and Starke et al. (229).

Methodology

Two methods currently in use to study brain surface vessels in situ are the open skull method of Wahl et al. (236) and the cranial window preparation of Levasseur et al. (136). The methods differ technically in two ways: in the open skull technique, the brain is exposed, and is irrigated with mineral oil. The cranial window method involves use of an air-tight glass canopy under which artificial cerebrospinal fluid is circulated. In recent studies by Navari et al. (163), the two methods were critically and extensively evaluated. Their analysis indicates that the open skull method may be vulnerable to loss of carbon dioxide by diffusion from the brain surface, rendering the preparation unphysiological and leading to enhanced vascular responsiveness to some stimuli. Responses to arterial hypercapnia and superfused noradrenaline were greater in the open skull preparation, but there were no differences in vascular responses in the two methods during arterial hypotension (163). Although the importance of a stable concentration of carbon dioxide and H^+ ion is recognised (131), it seems that the open skull preparation of Navari et al. did not satisfactorily replicate the depth of oil over

the brain as produced by Wahl et al. (236) and others who have adopted this method (146, 154). A thin layer of oil (1-2 mm) as used by Navari et al. would permit gas diffusion, while more oil (10-20 mm) probably reduces this problem. Measurements of pH in the subarachnoid space by Wahl's group (218) indicate that there is not measurable loss of carbon dioxide during typical experiments. Furthermore, prolonged and complicated studies using the open skull method have been performed with no appreciable loss in vascular responsiveness over several hours (146). Another difference between the two techniques is the extra-vascular pressure, which, in the cranial window method was established at 5 mmHg, and, in the open skull method, was at, or slightly above, zero. This difference, however, appeared not to be a factor in determining vascular responses (163).

The mode of drug delivery in the two methods would appear to be an important difference in choice of technique. In the cranial window method, vasoactive agents are perfused over the brain surface (197); if these drugs also affect neuronal metabolism, then it would be difficult to discern vascular responses. For this reason, the cranial window method was deemed unsuitable for studies relating to histamine. In the open skull method, vasoactive agents can be administered by peri-vascular micro-injection, which limits the volume of agents being studied and would not be expected to reach brain tissue in sufficient concentration to influence cellular metabolic rate (235). The principal limitation to this approach is that it is difficult to determine the extent or duration of receptor stimulation from the drug injected.

Introduction IV. Histamine Receptors.

Histamine is an autocoid with ubiquitous distribution in mammalian tissues. Within the first decade (1910-1920) of investigation into physiological and pathological mechanisms that involve histamine, it was recognised, primarily through the efforts of Dale (41), that this amine was important to inflammatory and anaphylactic phenomena. Other mechanisms, reactions, and substances now known to be related to histamine include allergies and hypersensitivity syndromes, pain and itch, tissue response to injury, tissue growth and repair, systemic response to venoms and toxins, autonomic control of the circulation, cluster (Horton's) headache, and mast cells and basophils. Excellent reviews concerning the early history of histamine research, and extension of physiological understanding of this amine, have been published by Douglas (52), Beaven (13) and Rossi (211).

Recognition of responses mediated by histamine prompted considerable pharmacological development of agents that could inhibit its activity. In 1948, Folkow and co-workers (75) demonstrated in cats that histamine-induced depressor responses were only partially attenuated by mepyramine (pyrilamine), a prototype among many histamine antagonists available at that time. These findings led the authors to believe that there may be two kinds of histamine receptors in blood vessels (75). This concept was given further description through the studies of Ash and Schild (6) who characterised several biological responses to histamine. These investigators identified responses that could be blocked by mepyramine, and related anti-histamines, as effects mediated by an H_1 class of receptors; non-H_1 receptors (defined later as H_2 receptors) mediated responses to histamine that were refractory to mepyramine. Synthesis of the first histamine H_2 receptor antagonist, burimamide, emerged from the exhaustive tests over eight years by Black and associates (18) of Smith, Kline and French

Laboratories.

With the concept and pharmacological entity of two distinct receptors for histamine being established, it is now possible to categorise many physiological phenomena as H_1 or H_2 receptor responses (171, 219). Specific stimulating or blocking agents have been developed to examine various mechanisms (13, 52, 54, 55, 211).

Prototype H_1 receptor response: contraction of extra-vascular smooth muscle.

H_1 receptor agonists: histamine, 2-methylhistamine, 2,2-pyridylethylamine, 2,2-aminoethylthiazole.

H_1 receptor antagonists: mepyramine maleate, diphenhydramine.

Prototype H_2 receptor response: stimulation of gastric acid secretion.

H_2 receptor agonists: histamine, 4-methylhistamine, dimaprit, impromidine.

H_2 receptor antagonists: burimamide, metiamide, cimetidine.

CHAPTER II. PROCEDURES

General Surgery and Animal Preparation.

Rats

Experiments were performed with male Sprague-Dawley rats weighing 275 to 425 g. Anaesthesia was induced with 3% halothane in oxygen, the trachea was cannulated, and mechanical ventilation with 0.4% halothane and N_2O-O_2 (70-30%) was used to maintain anaesthesia. Skeletal muscle paralysis was achieved with gallamine triethiodide (10 mg $kg^{-1}h^{-1}$, iv), and heparin (750-1000 U kg^{-1}, iv) was used for anticoagulation.

Polyethylene catheters were placed in both femoral arteries and veins for continuous measurement of arterial pressure, for withdrawal of arterial blood, and for administration of drugs and tracers. Arterial blood gases and pH were determined frequently. Corrections for arterial PCO_2 and pH were made by adjustments in tidal volume or by intravenous injection of sodium bicarbonate (8.4%). Body temperature was maintained at 37 $\pm$ 0.5 ^{o}C by external heating.

In most experiments, a catheter was inserted retrogradely into the external carotid artery, and advanced to the origin of the internal carotid artery. I ligated remaining arterial branches near the carotid bifurcation, including the large pterygopalatine artery which branches from the internal carotid artery (100, 106). In other studies, an additional catheter was inserted into the right axillary artery, and advanced retrogradely through the subclavian artery to the brachiocephalic trunk. Correct positioning of the catheter in the subclavian artery was verified by injection of Evans blue (about 50 µl), and by immediate appearance of the dye in the common carotid artery. The external carotid and subclavian artery catheters, therefore, provided access for drug

administration to the brain via the common and internal carotid arteries. In the rat, the internal carotid artery does not have anastomoses with extracranial structures distal to the pterygopalatine artery (100). Drugs were administered into the internal carotid artery by constant pump infusion at a rate of 45 μl min^{-1}.

Some of the experiments required dissection of the rat brain at the end of the study. The intact brain was removed from the skull, and dissection was completed with a small curved scalpel blade. Topological landmarks, described by Zeman and Innes (246), were followed to obtain bilateral samples of hypothalamus, thalamus, caudate nucleus, hippocampus, frontoparietal cortex, and three samples of cerebrum. Cerebrum samples were comprised of parts of the hemisphere remaining after the discrete structures listed above were removed. Midbrain, pons, medulla, cerebellum, and, for one study, cerebellar cortex, were also obtained. Samples weighed from 12 mg (hypothalamus) to 175 mg (cerebrum).

In experiments requiring measurement of brain and arterial blood radioactivity, the samples were placed in either glass (for tissue) or plastic (for plasma) scintillation vials. One to two ml of Soluene was added to the vials containing tissue for maceration, and vials were heated in a water bath (50 ^{o}C) until the sample was dissolved. Acetic acid (2 N, 0.5 ml) was added to inhibit chemiluminescence, and 10 ml of scintillation fluid (Instagel) was added before the samples were placed in a cooled counter for scintillation analysis. Samples were counted for four to ten minutes, depending on the level of radioactivity. Plasma samples from arterial blood, and Instagel, were combined in vials and counted for four minutes. Triplicate readings were obtained and averaged for each vial. Raw counts per minute were converted to

disintegrations per minute from a linear calibration curve using both external standardisation and the channels-ratio method for efficiency determination (178).

Cats

Mongrel cats of both sexes weighing 2-5 kg were used. Anaesthesia was induced by intravenous injection of Saffan (alphaxone, 6.75 mg kg^{-1} and alphadolone acetate, 2.25 mg kg^{-1}). The trachea was intubated, and the cats were mechanically ventilated with air and oxygen to maintain arterial PO_2 greater than 100 mm Hg, and arterial PCO_2 between 30 and 35 mm Hg. Anaesthesia was maintained by intravenous injection of alpha-chloralose in doses of 50 mg kg^{-1}, given as needed during the study. Cannulae were inserted into a femoral artery and vein, and were advanced to the descending aorta and inferior vena cava. Throughout the study, end-tidal CO_2, arterial blood pressure, and body temperature were measured continuously, and samples of arterial blood were frequently analysed for blood gas and pH determination. When required, skeletal muscle paralysis was achieved with gallamine triethiodide (10 mg $kg^{-1}h^{-1}$, iv).

Methods I. Blood Brain Barrier Studies

For these studies, 108 rats were used, prepared as described above. In each set of experiments described in this section, saline or histamine was infused unilaterally into one internal carotid artery. Responses in the hemisphere ipsilateral to infusion were compared to the contralateral (control) side of the brain. I completed three series of studies which were designed to examine different aspects of the influence of histamine on cerebrovascular permeability: 1) permeability studies using sucrose. This series involved determination of dose-response relationships, reversibility, and the class of histamine receptor involved in mediating changes in permeability; 2) permeability studies using a neutral amino acid, alpha-aminoisobutyric acid. These experiments involved use of autoradiographic and densitometric techniques that permitted a more precise identification of brain regions in which permeability changes occurred; and 3) studies concerning brain water content to determine whether histamine caused oedema in brain.

Permeability to Sucrose

In these experiments (n = 70), saline or histamine was infused for 15 minutes into the internal carotid artery. Five minutes after the start of infusion, 10 μCi of ^{14}C-sucrose (165) in 0.5 ml saline was injected intravenously. For the next ten minutes, femoral arterial blood was sampled repeatedly. The samples were centrifuged, and 20 μl aliquots were removed and analysed for radioactivity to describe the history of arterial $[^{14}C]$ over the course of the experiment (Figure 1). At the end of the ten minute blood sampling period, the rat was sacrificed and brain samples were obtained by dissection, and were weighed. In additional experiments, used to derive

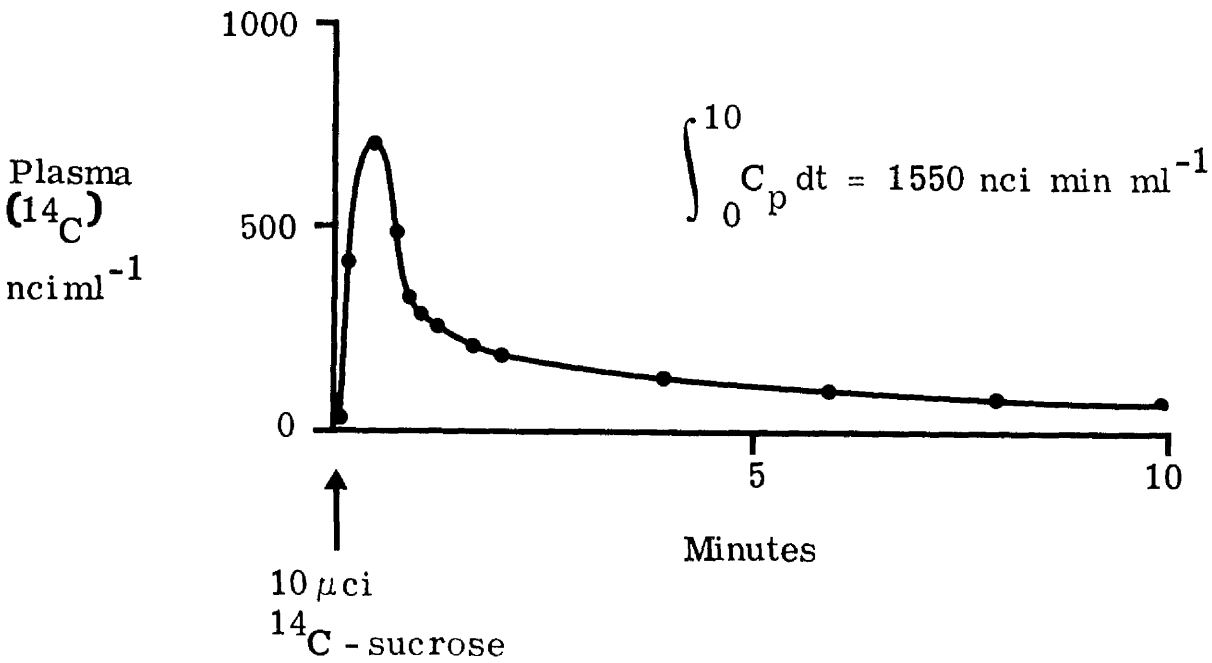

Figure 1. Derivation of arterial ^{14}C integral after intravenous injection of radioactive sucrose. Blood samples (filled circles) were obtained from a femoral artery, and radioactivity was measured by liquid scintillation counting.

estimates of intravascular volumes for the brain regions, the experiments were only two minutes in duration. Radioactivity levels were measured by liquid scintillation counting; brain samples were counted in duplicate for 10 minutes each, and plasma samples were counted in triplicate for four minutes each.

The method of analysis for quantitative measurement of brain vascular permeability is based on the theory of unidirectional transfer of sucrose from blood across endothelial cell membranes into brain extracellular spaces (165, 201). Because sucrose is lipid-insoluble and polar (37, 42, 165, 199), its rate of transfer under normal conditions is very slow; likewise, because brain levels remain low, reflux of sucrose from brain back to blood can be regarded as negligible (165). Permeability x surface area products (PA) for sucrose were derived from the relationship (165):

$$dC_{i}/dt = PA \cdot C_{p} \qquad (1)$$

where C_i = brain parenchymal tracer concentration at time t and C_p = arterial plasma tracer concentration. By integrating the above equation, the operational equation of the method derives:

$$PA = \frac{C_i(t)}{\int_0^t C_p \, dt} \qquad (2)$$

The amount of tracer in brain (C_i) was determined by correcting the total measured brain tracer concentration for the amount of tracer estimated to be within the intravascular space. The estimated vascular volumes from the two minute experiments, together with the final whole blood tracer concentration, determined this correction (165).

Estimates for blood volume were determined from two-minute experiments which were conducted for each series

under conditions identical to the ten-minute studies. Brain tracer content in the two-minute experiments was converted to a blood volume estimate by correcting for the amount of tracer estimated to be in parenchyma (165). This was accomplished by subtracting from total brain tracer concentration the product of permeability and arterial ^{14}C integral, as follows:

$$BV = \frac{C_i(\underline{t})^* - PA \cdot \int_0^{\underline{t}} C_p \, d\underline{t}^*}{C_b^*} \qquad (3)$$

where: values denoted by * are obtained from the two-minute experiment, and PA is determined from the ten-minute study. C_b indicates the final tracer concentration in whole blood.

Dose-Response Relationship. To examine whether an increase in permeability was sensitive to the concentration of histamine infused, four sets of studies were completed involving continuous infusion of saline or one of three doses of histamine, 2, 6, or 20 $\mu g \ min^{-1} \ kg^{-1}$. For the permeability studies, five, four, six and seven rats, respectively, were used for the four conditions; to estimate blood volumes, five, two, three, and five rats were used. In rats in each of the groups, brains were dissected regionally and bilaterally to determine if responses were consistent from structure to structure, and to examine side-to-side differences.

Reversibility. Upon finding an increase in permeability with histamine, I attempted to determine whether the response was a reversible phenomenon. Histamine was infused for 15 minutes (20 $\mu g \ min^{-1} \ kg^{-1}$), but ^{14}C-sucrose was not injected until 30 minutes or two hours after the end of infusion. For each of these conditions, five rats were used in the permeability experiments, and three rats were used in the

estimation of blood volume.

Receptor Identification. To determine which histamine receptors were involved in the permeability response, separate groups of rats were pretreated with either mepyramine maleate (H_1 receptor antagonist) or metiamide (H_2 receptor antagonist) before histamine was infused (20 $\mu g\ min^{-1}\ kg^{-1}$). Each antagonist was given intravenously 10 minutes before the start of histamine infusion in a dose of 5 mg kg^{-1}. For mepyramine, six rats were used to determine permeability responses, and three rats were used to estimate blood volumes; for metiamide, five and three rats were used for the two groups.

Permeability to Alpha-Aminoisobutyric Acid (AIB).

In these studies, saline (n=5) or histamine (n=5, 20 $\mu g\ min^{-1}\ kg^{-1}$) was infused for 15 minutes. ^{14}C-AIB (55 μCi in 0.8 ml saline) was injected intravenously five minutes after the start of infusion, and blood samples were obtained repeatedly over the following 20 minutes. A 20 minute sampling period was required in these studies to allow intravascular tracer concentration to reach levels that were only a fraction (less than 6%) of brain concentration. Blood volume estimates have not been incorporated into this technique (19).

Samples of plasma were analysed for radioactivity by liquid scintillation counting. Following sacrifice, the brain was carefully removed and immersed in isopentane at -50 ^{o}C. Sections of brain were cut coronally (20 μm thick) on a cryostat at -22 ^{o}C, were placed on cover-slips, and were dried on a hot-plate. Autoradiographs were made of the brain sections and of plastic ^{14}C standards by exposure to film for 14 to 21 days. Optical densities were obtained using a frame size of 0.09 mm^2 on a densitometer. By reference to the plastic standards, optical densities could

be converted to nCi units, which, with the arterial ^{14}C integral, were used to compute blood-to-brain rate constants (K_i) for AIB (19) using the equation:

$$K_i = \frac{C_i(\underline{t})}{\int_0^{\underline{t}} C_p \, d\underline{t}} \qquad (4)$$

Brain Water Content

Twenty-eight rats were used to determine if changes in brain water content resulted from histamine infusion. Unilateral infusion of saline (n=14) or histamine (n=14, 20 $\mu g \ min^{-1} \ kg^{-1}$) was made for 15 minutes. An additional five minute period followed the infusion to allow blood pressure to return to normal and stabilise before the rat was sacrificed. Brain regions obtained bilaterally were hippocampus, parietal cortex, cerebrum, and hindbrain, which included the brainstem and cerebellum. The brain samples were placed individually in tared aluminium trays, and were weighed within five minutes of being removed from the skull. The samples were placed in an oven at 50 ^{o}C for several days until desiccated. Percentage water content was then calculated from the dry and wet weights, and right and left regions were compared.

Methods II. Cerebral Blood Flow Studies

Intracarotid ^{133}Xenon Clearance Technique.

Sixteen rats were used to estimate cerebral blood flow from the clearance of ^{133}Xenon injected into the internal carotid artery (107). For these rats, a longitudinal scalp incision was made to expose the skull and temporal muscles, and the right temporal muscle and overlying skin were reflected. A fine needle, with an outside diameter slightly smaller than the inside diameter of the catheter in the external carotid artery, was inserted until its tip lay at the mouth of the catheter. The catheter and needle were secured in place with skin sutures, and the needle was connected to a microsyringe by a screw fitting.

A sodium-iodide scintillation crystal was placed over the exposed parietotemporal calvaria immediately ventral to the crista frontalis, and was angled (45^{o}) to avoid extracranial sources of xenon, such as the trachea. The crystal, with an internal diameter of 12 mm, was thickly collimated with lead to an aperture diameter of 4 mm. Other characteristics of the crystal, and its relationship to cranial landmarks, are shown in Figure 2. The crystal was coupled to a photomultiplier, a scaler and a ratemeter which integrated the counts with a one-second time constant. Output was recorded on chart paper at a speed of 1 cm sec^{-1}. The pulse-height analyser was calibrated to the photopeak of ^{133}Xenon at 81 keV with a gate width of $\pm$ 20%.

For each determination of cerebral blood flow, a 10 µl bolus (10 µCi) of ^{133}Xenon was injected from the microsyringe over one second, and the washout was monitored continuously until it had decreased to about 40% of the highest count rate.

Cerebral blood flow was calculated from the initial

slope of a semilogarithmic plot of the clearance curve (107, 110) using the following equation:

$$CBF = \frac{(\ln 2)\ (\lambda)\ (60)}{t_{\frac{1}{2}}} \qquad (5)$$

where: CBF = cerebral blood flow in ml min^{-1} g^{-1}; λ = brain/blood partition coefficient for xenon in grey matter of rat brain (0.80; 164); $t_{\frac{1}{2}}$ = half-time of the ^{133}Xenon clearance in seconds. Flow measurements during normal conditions were possible every few minutes because the rate of gas clearance was rapid and background counts were minimal (less than 5% of the highest count rate).

Arterial blood pressure and blood gases were measured at the time of each blood flow estimate. Cerebral vascular resistance was calculated by dividing mean arterial pressure by blood flow.

Three sets of studies were conducted to determine effects of intra-arterial infusion of histamine before and after disruption of the blood-brain barrier, and to evaluate the participation of specific histamine receptors.

First, histamine was infused into the axillary artery catheter at a rate of 45 μl min^{-1} and in a dose of 20 μg min^{-1} kg^{-1}. This dose was chosen because preliminary trials with various doses of histamine indicated that higher doses produced arterial hypotension that may have exceeded the lower limit of autoregulation. Duplicate determinations of blood flow were made when arterial pressure had stabilised, usually about 5 minutes after the beginning of histamine infusions.

Second, the blood-brain barrier was first disrupted by carotid injection of hypertonic urea (2M). This technique produces a transient increase in cerebrovascular permeability to circulating blood solutes (described in more detail below). Histamine infusion

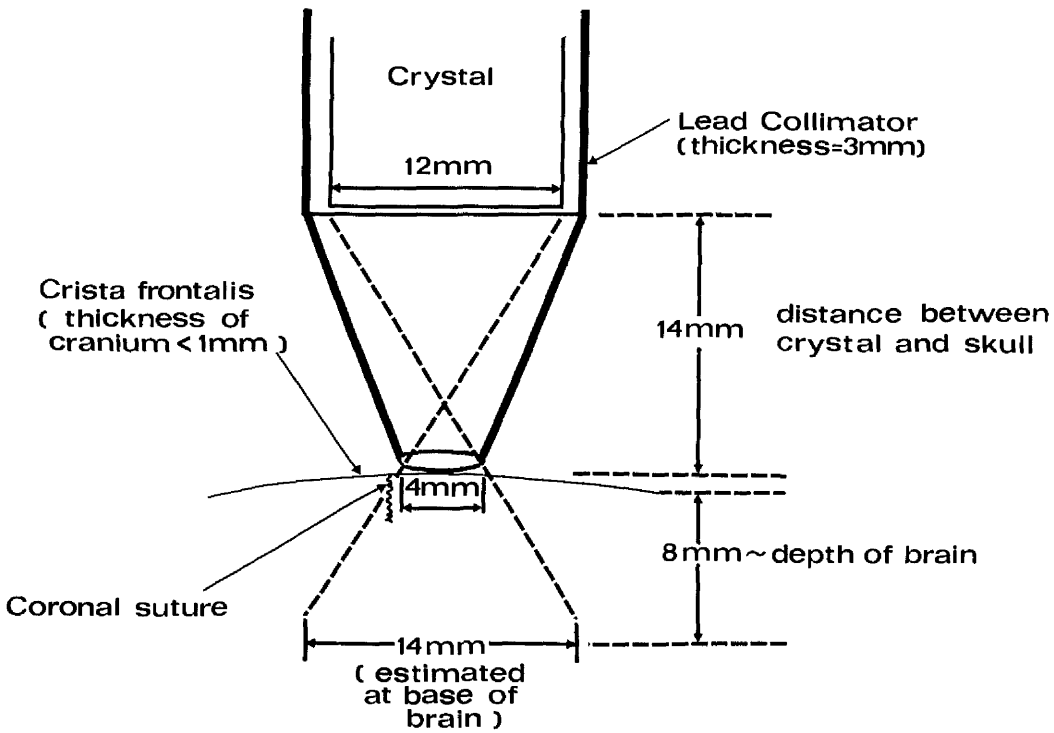

Figure 2. Schematic diagram of scintillation crystal and its relationship to the rat head for external detection of ^{133}Xenon after carotid injection. Characteristics of the crystal viewing area are shown.

was begun 10-15 minutes after urea injection, and blood flow determinations were made 5 minutes later. Histamine was infused in four concentrations: 2, 6, 20 and 60 $\mu g\ min^{-1}\ kg^{-1}$, and duplicate measurements were obtained at each dose. If the values did not differ by more than 10%, they were averaged; if there was variability in the duplicated measurement, the first determination was accepted. In some rats, it was necessary to inject a second bolus of urea so that additional blood flow determinations could be secured. Preliminary studies indicated that two injections of urea in the same animal did not have deleterious effects on the preparation.

Third, because higher doses of histamine produced increases in cerebral blood flow after blood-brain barrier opening, I administered one of two histamine receptor antagonists before histamine infusion, but after injection of urea (to assure similar opportunity for the antagonists to reach cerebrovascular smooth muscle and brain). Mepyramine maleate (H_1 receptor antagonist) or metiamide (H_2 receptor antagonist) was given intravenously (each 5 $mg\ kg^{-1}$) to test for effectiveness in blocking histamine-related increases in cerebral blood flow. Measurements of flow were obtained during administration of ascending doses of histamine in animals treated with each of the blocking agents.

^{14}C-iodoantipyrine

In 34 rats, measurements of regional cerebral blood flow were obtained by systemic administration of ^{14}C-iodoantipyrine and regional dissection of fresh brain. A method using this tracer for autoradiographic determination of cerebral blood flow in conscious rats has been published recently (216). Iodoantipyrine is a diffusible substance at cerebral vessels with a brain tissue:blood partition coefficient of 0.79 (216). Values for cerebral blood flow obtained with this tracer

are similar to those derived from the radioactive gas, iodine-labeled trifluoroiodomethane (216). ^{14}C-iodoantipyrine (20 μCi in 1.5 ml saline) was infused intravenously during 30 second experiments on a "ramp" schedule, i.e., the rate of infusion was increased progressively so that arterial tracer concentration would be highest at the end of the experiment. This infusion schedule was used to provide optimal conditions for separating rates of blood flow between high and low flow structures. During administration of the tracer, arterial blood was allowed to drip freely from a partially occluded catheter in the femoral artery, and 12 to 18 samples of blood were obtained separately on preweighed filter discs. The times of sampling were recorded and were used with the radioactivity concentration of each blood sample to describe the history of arterial radioactivity during the experiment. The rat was killed by decapitation immediately at the end of tracer infusion, the brain was removed, and regional samples were dissected and placed in tared scintillation vials for weighing. The brain and blood samples were then processed for liquid scintillation counting to determine radioactivity levels. Brain and blood samples were each counted for four minutes. Computations of blood flow for the brain regions were performed according to the equations of Kety (128) as applied by Sakurada et al. (216):

$$K = \frac{C_i(T)}{\lambda \int_0^T C_a e^{-k(T-t)} dt} \qquad (6)$$

where: $C_i(T)$ is the tracer concentration in the brain sample at a given time, T; λ is the brain:blood partition coefficient; C_a is the concentration of tracer in arterial blood; t is time; and K is a constant that incorporates the rate of blood flow per unit weight of tissue.

The assumptions in using iodoantipyrine for measurement of cerebral blood flow are satisfied because 1) equilibration between the tracer and tissue is limited by delivery, not by diffusion; 2) arterio-venous shunts in the brain microcirculation are either not present or not important; and 3) small regions of brain have relatively homogenous levels of perfusion (80, 216).

Three sets of paired experiments were conducted: First, control measurements of blood flow were obtained in rats which had only the carotid artery surgery performed (no drug infusion). Additional studies were completed in rats given histamine infusion (20 $\mu g\ min^{-1}\ kg^{-1}$) into the internal carotid artery, but without blood-brain barrier disruption by urea injection. Second, urea was first injected to disrupt blood-brain barrier mechanisms, then, 15 minutes later, saline or histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) was infused into the internal carotid artery catheter. Third, comparisons in response were made between rats infused with the histamine receptor agonists, either 2,2-pyridylethylamine (H_1) or dimaprit (H_2) (each 200 $\mu g\ min^{-1}\ kg^{-1}$) following carotid injection of urea. All substances infused into the internal carotid artery were dissolved in saline and given at 45 $\mu l\ min^{-1}$.

Because histamine given by intracarotid infusion produeed arterial hypotension, which, due to autoregulatory mechanisms, might interfere with vasodilator responses, I examined blood flow responses in three additional rats whose blood pressure was maintained by aortic occlusion. In these animals, both axillary arteries were used for measuring blood pressure and for sampling arterial blood during the experiment, and histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) was infused via a catheter in the external carotid artery. I placed a ligature around the descending aorta at the level of the diaphragm. Iodoantipyrine was infused via a catheter in either the

jugular or axillary vein. During infusion of histamine, when arterial pressure decreased, an assistant gently pulled on the ligature to occlude the aorta and maintain arterial pressure at levels approximating normal.

^{14}C-2-deoxyglucose

Sixteen rats were used to determine whether carotid arterial infusion of histamine increased the rate of glucose utilisation in the brain. ^{14}C-2-deoxyglucose (10 μCi in 1 ml saline) was injected intravenously and arterial blood was sampled repeatedly over the following 30 minutes. From the plasma of each blood sample, a 20 μl aliquot was obtained to determine the radioactivity concentration in the blood sample, and a 15 μl aliquot was used to measure the glucose concentration. Either histamine (20 μg min^{-1} kg^{-1}) or saline was infused continuously throughout the experiment. The infusions were begun 10-15 minutes following carotid injection of urea. Continuous infusion of the agents was applied to provide a "constant" stimulus, which is one of the assumptions of the deoxyglucose method (225).

At the end of the 30 minute blood sampling period, the rat was killed, and brain samples were obtained by dissection, were weighed, and were analysed for radioactivity concentration by liquid scintillation counting. Samples were counted for four minutes. Derivations of glucose consumption in each brain region were computed according to the equations of Sokoloff et al. (225).

Blood-Brain Barrier Disruption

Because vascular endothelia in the cerebral circulation may form a barrier to circulating histamine or its agonists, a method was needed which would disrupt barrier mechanisms without damaging the brain. A technique involving use of hypertonic solutions injected into the carotid circulation has been devised by Rapoport and co-workers (198, 201) and extended for use

in baboons and rats by Pickard, MacKenzie and co-workers (60, 145, 147, 184). One ml of 2M urea (filtered and warmed to 37 ^{o}C) was injected into the carotid artery over 30 seconds. This technique has been shown previously in rats to increase brain vascular permeability to noradrenaline (60,92), and to maintain an open barrier for at least 30 minutes (94).

In preliminary studies, I examined the effect of hypertonic urea injection on cerebral blood flow by using the intracarotid ^{133}Xenon technique, which permitted serial measurements of blood flow over the space of several minutes after urea was given. These studies demonstrated that brain blood flow was transiently increased by 15-20% for approximately 15 minutes after urea. This phenomenon of increased cerebral blood flow following urea injection has been confirmed in rats by other investigators (93). All intracarotid drug infusions in my studies were begun, therefore, 15 minutes after injection of urea to avoid the complicating effects that urea had on the brain circulation.

In additional studies in four rats, cerebrovascular permeability to labeled sucrose was determined after intracarotid urea injection. These studies were designed to quantify the extent of blood-brain barrier opening using the urea injection technique. ^{14}C-sucrose (10 μCi in 1 ml saline) was injected intravenously 10 minutes after urea, and arterial blood was sampled for the following 10 minutes when the rat was killed for brain sampling. Brain and blood samples, and calculations for regional permeability x surface area products were evaluated as described above in Methods I. Estimates for blood volumes were taken from the data of Ohno et al. (165).

Methods III. Pial Vessels in Situ

The methods in this section are adapted from the original technique of Wahl and associates (236). Thirty-two cats were used for these experiments. The head of the cat was positioned in a stereotaxic frame, and a longitudinal incision was made along the scalp. Skin, fasciae and muscle were cleared from the bone and retracted with ligatures onto a metal ring so that the skin formed a pool over the skull. Using a dental drill cooled with saline, I made a craniectomy measuring 2 x 1 cm over the parietal calvaria. The exposed dura and skull surface were then bathed in heated mineral oil (liquid paraffin) which flowed continuously across the surgical field at a depth of 15 mm. A thermistor positioned near the craniectomy permitted the constant monitoring of bath temperature which was maintained at 37-38^{o}C throughout the experiment. I cut the dural membrane along the borders of the craniectomy and controlled bleeding with strips of oxidised cellulose.

The field was illuminated with fiber optic lights and visualised with a stereomicroscope at x40 or x70 magnification. The image of vessels viewed was passed through a television camera mounted on the microscope and displayed on a television monitor in the laboratory. The diameter of a given vessel could be determined from an image-splitting device (9) connected to the microscope and television camera. The amount of shear of the image was recorded continuously on a strip-chart; the degree of shear required to appose tangentially the split images of a vessel was directly proportional to the size of the vessel. Filaments of known diameter were used to calibrate the system before each study.

Several pial arterioles and veins were selected randomly at the beginning of each experiment. At normal arterial pressure (between 90 and 120 mmHg), the resting diameters

of the vessels in these studies were in the range of 35 to 260 μm.

Drugs were made fresh the day of each experiment. Mock cerebrospinal fluid (CSF) was used as the solvent vehicle for the injection solutions. Composition of the mock CSF (in mM) was: NaCl 144; K^+ 3; Ca^{2+} 5; HCO_3^- 12. pH was adjusted to 7.18. The drugs used in these experiments were histamine dihydrochloride, 2,2-pyridylethylamine, (H_1 receptor agonist), impromidine (H_2 receptor agonist), mepyramine maleate (H_1 receptor antagonist) and noradrenaline. Glass pipettes with tip diameters of 8-10 μm were filled with the agents and mounted on a micromanipulator. The position of the pipette tip could be precisely controlled and was placed through the arachnoid membrane into the perivascular space of the selected vessel. Approximately 3 μl of fluid was injected and resulting changes in vascular calibre were followed for up to 12 minutes. The observed changes in vascular diameter were expressed as a percentage change from the preinjection control diameter of the vessel.

Two series of experiments were completed using this method.

Perivascular injection in arterioles and veins

In these studies, several does of the histamine agents were injected to determine dose-response relationships in both arterioles and veins. Vessels were selected randomly, and there was no order of the dosages used for a particular drug. In some cases, a given vessel was used for subsequent injections but there was always a period of a least 30 minutes between two injections.

Noradrenergic stimulation

In these studies a bipolar platinum electrode was positioned on the cervical sympathetic nerve trunk on the same side as the surgery. During stimulation, the electrical parameters were 15V, 2ms, 20 Hz.

Stimulation was verified as effective when the ipsilateral nictitating membrane retracted and the pupil showed marked dilatation.

When sympathetic stimulation was examined in the presence of histamine agonists, the protocol was designed to examine first the response of the vessel to nerve stimulation. Vascular diameters were recorded between 30 and 45 seconds after the start of stimulation. The stimulator was turned off, and the vessel was allowed to recover. Measurements were taken to verify when the vessel had returned to control diameter. Usually, this period was 2-3 minutes. The pipette containing the histamine agent was then positioned next to the vessel, the stimulator was turned on, response of the vessel to stimulation was checked on the television monitor and the eye, and the drug was injected. Measurements were recorded beginning at 30 seconds after the start of stimulation, and were continued for the following 15 seconds. Comparisons were made between the vessel diameter before stimulation (control), during stimulation, and in the presence of the histamine agent during stimulation.

In studies involving simultaneous application of the histamine agents with noradrenaline, the solutions were made to their respective designated concentrations and were combined in the same pipette. The final concentration of the individual agent in the pipette was corrected for volume. Comparisons were made between the responses to the agents alone, and their response in combination.

Statistical Analysis

Data were analysed by several statistical procedures. Matched and independent comparisons by t-test, one-and two-way analysis of variance, Scheffé and Dunnett multiple comparison tests, and nonparametric tests were performed as described by Steel and Torrie (230),

Winer (244) and Siegel (222). In all cases, a minimum level of significance was accepted at $p < 0.05$.

Drugs and Tracers

Several pharmacological agents and radioactive tracers were used in these studies. Because molecular weight (MW) may be an important consideration for some of the substances, the value in daltons is included where relevant.

Metiamide (MW = 244), 2,2-pyridylethylamine (MW = 194), dimaprit (MW = 234), and impromidine (MW = 431) were obtained from Smith, Kline and French, Welwyn Garden City, England. Mepyramine maleate (MW = 401) was obtained from May and Baker, Dagenham, England. Other drugs used were histamine dihydrochloride (MW = 184), (Sigma Chemicals, Dorset, England), urea (Hopkin and Williams, Chadwell Heath, England), heparin (Boots, Nottingham, England) gallamine triethiodide (May and Baker), alpha-chloralose (BDH Chemicals, Poole, England), and Saffan (Glaxovet, Greenford, England).

Isotopes used were ^{14}C-sucrose (MW = 342) (Radiochemical Centre, Amersham, England), ^{14}C-alpha-aminoisobutyric acid (MW = 103) (Radiochemical Centre), ^{133}Xenon (Radiochemical Centre), ^{14}C-iodoantipyrine (New England Nuclear, Boston, Massachusetts), and ^{14}C-2-deooxyglucose (New England Nuclear).

CHAPTER III. RESULTS

Results I. Blood-Brain Barrier

Permeability to Sucrose

The values obtained for permeability x surface area products (PA) for several brain regions will be presented in the following tables. Values in the "non-infused" brain regions represent the PA product contralateral to the infusion, and, therefore, serve as the control for the experimental "infused" structure. Differences were calculated as (experimental - control ÷ control) x 100.

Dose-Response Relationship. Infusion of saline or histamine in various doses did not influence arterial blood gases, but, in the animals receiving histamine, arterial blood pressure was lower during the experiment (Table 1).

Unilateral carotid infusion of saline did not produce any significant changes in regional PA products (Table 1; Figure 3). Infusion of histamine in a low dose (2 $\mu g\ min^{-1}\ kg^{-1}$) was associated with no significant change in permeability in any brain region except hippocampus in which an increase of 33% occurred (Table 1; Figure 3). At higher concentrations, (6 and 20 $\mu g\ min^{-1}\ kg^{-1}$), histamine produced significant increases in several regions in the distribution of the middle cerebral artery. Structures affected were thalamus, caudate nucleus, hippocampus, parietal cortex, and cerebrum (Table 1). For the specific grey matter regions, the range of permeability change was from 50 to 81% for 6 $\mu g\ min^{-1}\ kg^{-1}$, and from 70 to 237% for 20 $\mu g\ min^{-1}\ kg^{-1}$ (Figure 3). In regions in the distribution of the basilar artery, e.g., midbrain and cerebellum, there were no side-to-side differences (Table 1).

Measurements of blood volume from the two-minute

Table 1. EFFECTS OF HISTAMINE INFUSION ON REGIONAL PERMEABILITY X SURFACE PRODUCTS FOR SUCROSE

	Saline	2 μg $min^{-1}kg^{-1}$	6 μg $min^{-1}kg^{-1}$	20 μg $min^{-1}kg^{-1}$
Mean arterial pressure (mm Hg)	95 ± 3	81 ± 10	80 ± 4	62 ± 3
Arterial blood gases and pH				
PO_2 (mm Hg)	113 ± 2	115 ± 5	111 ± 3	113 ± 5
pH	7.42 ± 0.03	7.45 ± 0.01	7.45 ± 0.02	7.42 ± 0.02
PCO_2 (mm Hg)	39 ± 1	38 ± 1	37 ± 1	36 ± 1
N	5	4	6	7

Regional permeability 10^{-6} X Sec^{-1}	Non Infused	In-fused	Non Infused	In-fused	Non Infused	In-fused	Non Infused	In-fused
Thalamus	7.3 ± 1.6	7.3 ± 1.8	8.8 ± 1.8	8.6 ± 2.9	8.5 ± 1.3	15.4 ± 4.6*	5.1 ± 1.7	12.5 ± 1.8*
Caudate Nucleus	8.8 ± 1.5	10.0 ± 1.5	7.2 ± 2.3	5.8 ± 3.4	7.8 ± 1.1	12.2 ± 0.7*	4.5 ± 1.5	10.6 ± 2.5*
Hippocampus	6.4 ± 1.8	4.2 ± 1.8	11.0 ± 1.3	14.3 ± 0.7*	9.0 ± 0.8	13.5 ± 2.6*	4.0 ± 0.8	6.8 ± 1.5*
Cortex	10.7 ± 1.1	7.7 ± 2.2	11.1 ± 2.1	12.5 ± 3.1	12.9 ± 3.5	20.0 ± 2.6*	6.8 ± 2.6	22.9 ± 3.9*
Cerebrum	9.3 ± 1.3	9.0 ± 1.2	14.0 ± 2.4	16.6 ± 1.8	11.9 ± 1.2	14.7 ± 1.7	13.2 ± 1.3	16.7 ± 2.6
Midbrain	7.8 ± 1.6	7.0 ± 2.1	11.8 ± 1.6	11.6 ± 0.8	11.5 ± 1.3	12.0 ± 1.0	7.4 ± 1.5	7.2 ± 2.0
Cerebellum	7.1 ± 2.1	8.5 ± 2.1	13.2 ± 2.3	13.0 ± 1.5	8.3 ± 2.6	9.5 ± 2.3	8.2 ± 2.8	8.4 ± 2.6

Values are mean ± SE. * Significantly different from corresponding non-infused side ($p < 0.05$).

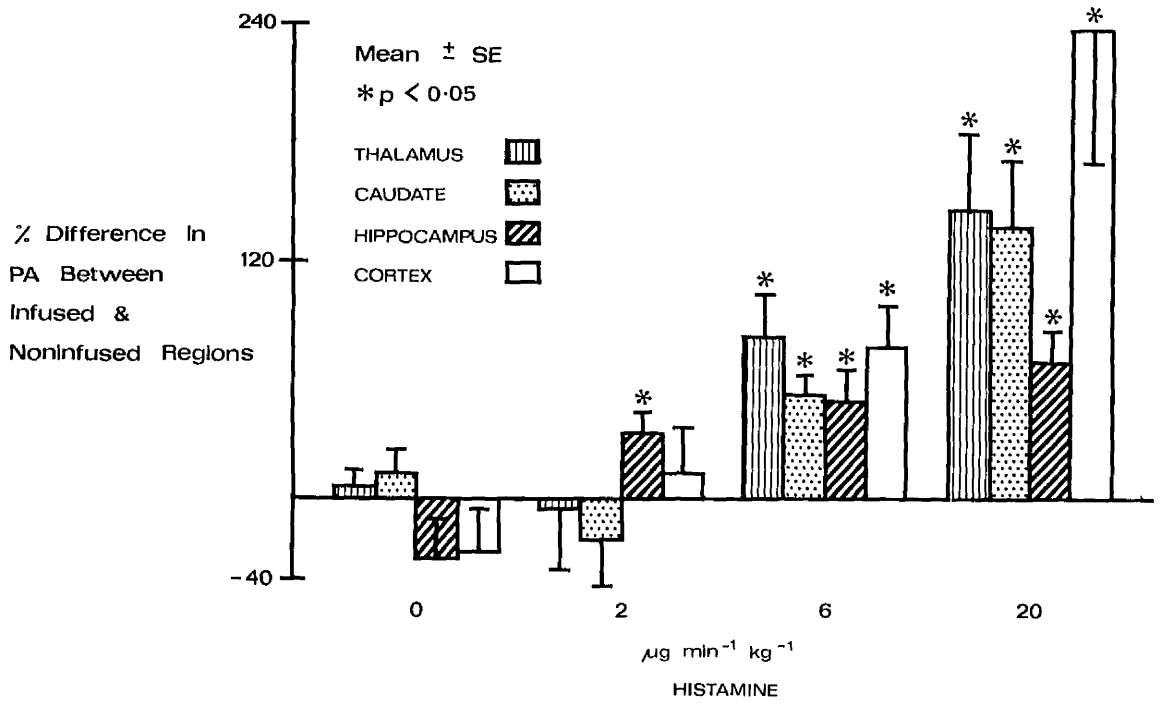

Figure 3. Differences in permeability x surface area products (PA) for sucrose between non-infused (left) and infused (right) brain regions during histamine infusion.

experiments indicated that there was a tendency for histamine to decrease vascular volume in brain regions ipsilateral to infusion (Table 2). A significantly smaller blood volume was found only in cerebral cortex during infusion at the 20 $\mu g\ min^{-1}\ kg^{-1}$ dose (Table 2).

Table 2. Effects of histamine infusion on regional blood volumes (BV)

Regional BV ($ml\ g^{-1} \times 100$)	Saline		Histamine ($20\ \mu g\ min^{-1} kg^{-1}$)	
	L	R	L	R
Thalamus	2.6±0.3	2.5±0.3	2.1±0.5	1.5±0.5
Caudate nucleus	1.6±0.2	1.5±0.2	1.6±0.3	1.2±0.3
Hippocampus	2.1±0.3	2.5±0.3	2.0±0.3	1.6±0.3
Cortex	2.0±0.1	3.0±0.2	2.9±0.5	1.2±0.2*
Cerebrum	2.1±0.3	2.0±0.2	1.9±0.3	1.8±0.3
Midbrain	2.3±0.3	2.7±0.4	1.9±0.3	2.2±0.4
Cerebellum	3.2±0.3	3.0±0.3	3.0±0.4	3.0±0.4

Values are $\bar{x} \pm SE$ for 5 animals in each group. * $p < 0.05$. Histamine was infused into the right internal carotid artery for five minutes before the beginning of the experiment, and continuously throughout the two minute blood sampling period. ^{14}C-sucrose was used as tracer (165).

Reversibility. In these experiments, both groups of rats were allowed to recover from the histamine infusion before the permeability experiment was conducted. Arterial blood gases and pH were normal, and arterial blood pressure had returned to normal levels before the blood sampling was begun (Table 3).

In the rats allowed to recover for 30 minutes after the infusion, PA products were still higher ipsilateral to infusion in caudate nucleus, hippocampus, and cortex (Table 3). The range of increased permeability was from 62 to 95% (Figure 4). In thalamus, which had displayed an increased permeability during infusion, there was no difference between the infused and non-infused side 30 minutes following infusion (Table 3). Likewise, there were no significant differences in PA between left and right cerebrum, midbrain and cerebellum (Table 3).

By contrast to the 30 minute post-infusion condition, at two hours after infusion, there were no regions which indicated a side-to-side difference in PA products (Table 3; Figure 4).

Receptor Identification. Administration of the histamine receptor antagonists, mepyramine or metiamide, produced transient changes in arterial pressure which had stabilised before the permeability experiment was started. Arterial blood gases in both groups of rats were normal (Table 4).

Pretreatment with the H_2 receptor blocking agent, metiamide, prevented the increase in permeability seen in untreated animals (Table 4); there were no significant differences in PA products in any region examined (Table 4, Figure 5).

By contrast, pretreatment with the H_1 antagonist, mepyramine, failed to alter the increase in permeability in all regions except hippocampus, in which there was

Table 3. EFFECTS OF TIME AFTER HISTAMINE INFUSION ON REGIONAL PERMEABILITY X SURFACE AREA PRODUCTS FOR SUCROSE

	30 min Post-infusion		2 hours Post-infusion	
Mean Arterial pressure (mm Hg)	83 ± 12		99 ± 13	
Arterial blood gases and pH				
PO_2 (mm Hg)	123 ± 7		123 ± 6	
pH	7.43 ± 0.01		7.46 ± 0.02	
PCO_2	39 ± 1		35 ± 1	
N	5		5	
Regional permeability 10^{-6} X Sec^{-1}	Non-infused	Infused	Non-infused	Infused
Thalamus	10.0 ± 1.9	10.1 ± 1.3	12.3 ± 1.6	14.4 ± 2.4
Caudate Nucleus	8.6 ± 1.8	16.8 ± 2.5*	13.3 ± 1.3	13.2 ± 1.8
Hippocampus	7.2 ± 1.1	11.7 ± 2.1*	7.7 ± 0.8	7.1 ± 1.2
Cortex	9.8 ± 1.9	18.6 ± 1.3*	12.0 ± 1.6	8.3 ± 2.4
Cerebrum	10.8 ± 0.9	13.7 ± 1.0	8.9 ± 0.9	8.1 ± 1.7
Midbrain	11.8 ± 1.1	11.4 ± 0.8	5.1 ± 1.6	6.6 ± 1.2
Cerebellum	8.7 ± 1.2	10.6 ± 0.9	5.6 ± 1.5	5.4 ± 1.2

Values are mean ± SE. * Significantly different from corresponding non-infused side ($p < 0.05$). Histamine had been infused at a dose of 20 μg min^{-1} kg^{-1} for 15 minutes.

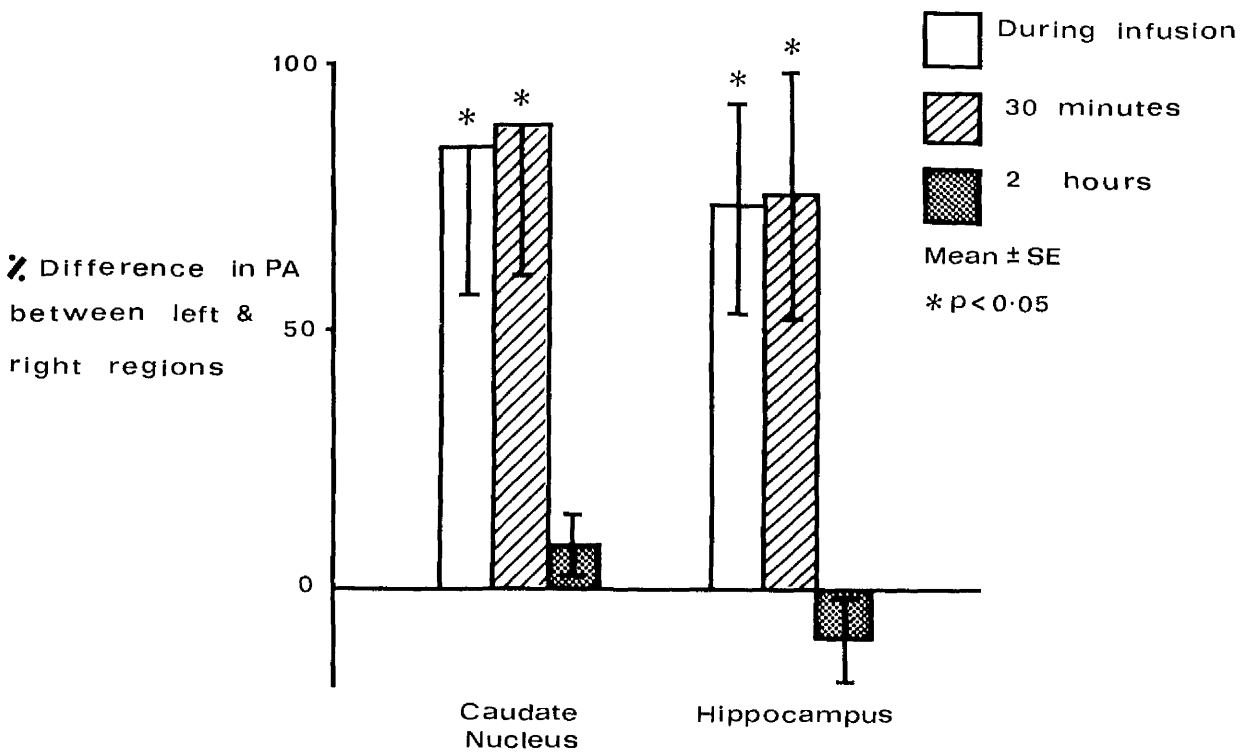

Figure 4. Reversibility of permeability response for sucrose following histamine infusion.

Table 4. EFFECTS OF HISTAMINE ON REGIONAL PERMEABILITY X SURFACE AREA PRODUCTS FOR SUCROSE AFTER SELECTIVE HISTAMINE RECEPTOR BLOCKADE

	Metiamide		Mepyramine	
Mean arterial pressure (mm Hg)	80 ± 4		78 ± 2	
Arterial blood gases and pH				
PO_2 (mm Hg)	122 ± 4		127 ± 5	
pH	7.42 ± 0.02		7.44 ± 0.02	
PCO_2 (mm Hg)	37 ± 1		37 ± 1	
N	5		6	
Regional permeability 10^{-6} X Sec^{-1}	Non-infused	Infused	Non-infused	Infused
Thalamus	13.2 ± 1.1	11.8 ± 1.6	11.5 ± 1.7	15.1 ± 1.3*
Caudate Nucleus	8.6 ± 1.1	9.3 ± 1.4	11.3 ± 1.5	19.5 ± 1.9*
Hippocampus	9.8 ± 1.6	8.1 ± 1.9	10.4 ± 0.7	12.9 ± 1.1*
Cortex	10.8 ± 2.0	9.8 ± 2.5	12.7 ± 1.8	18.7 ± 2.2*
Cerebrum	8.5 ± 1.8	8.9 ± 1.7	10.9 ± 1.1	14.3 ± 0.9*
Midbrain	10.3 ± 1.8	10.3 ± 1.9	11.4 ± 0.9	11.0 ± 1.1
Cerebellum	10.5 ± 2.2	12.5 ± 2.0	10.0 ± 1.4	10.6 ± 0.9

Values are mean ± SE. * Significantly different from corresponding non-infused side ($p < 0.05$).
Dose of histamine was 20 $\mu g\ min^{-1} kg^{-1}$.

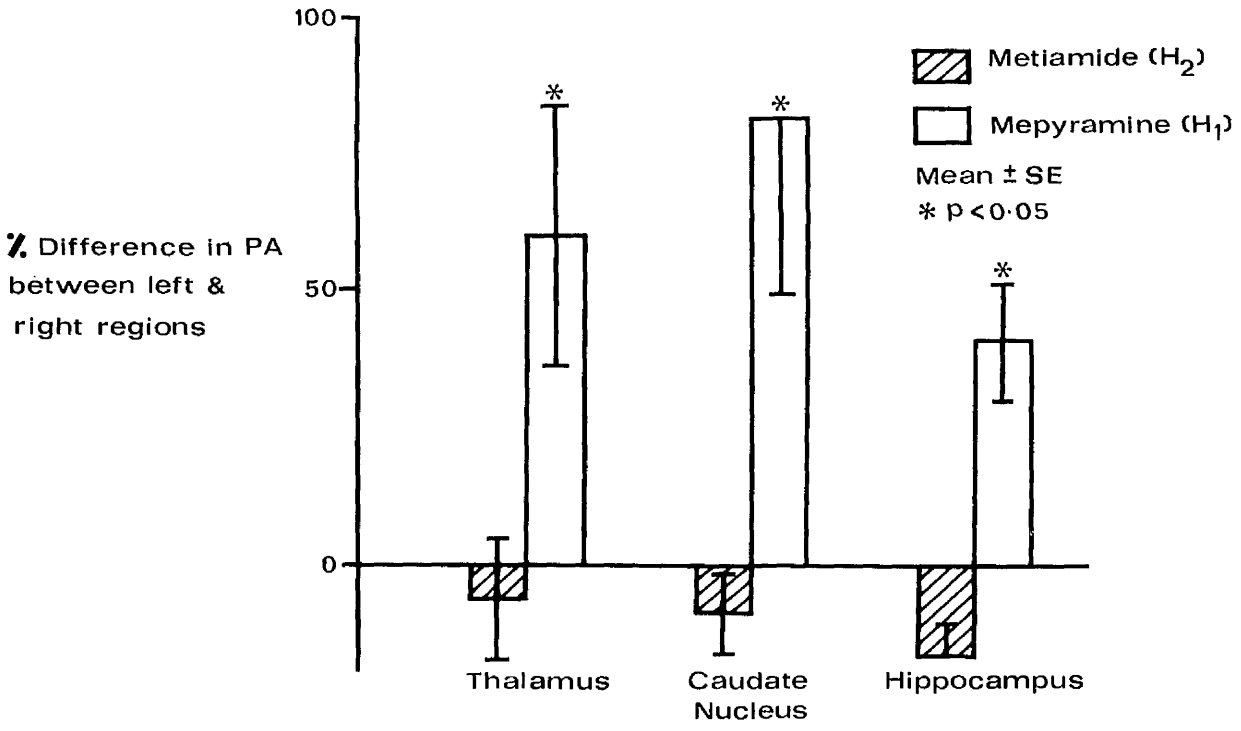

Figure 5. Receptor involvement in permeability response for sucrose during histamine infusion.

some attenuation of the permeability increase (Table 4, Figure 5). In midbrain and cerebellum, there were no differences in PA products (Table 4).

Permeability to Alpha-Aminoisobutyric Acid (AIB)

In this series, using an autoradiographic method, optical densities in cortex were generally higher than in deep brain regions, such as caudate nucleus and midbrain. There was difficulty in identifying other brain structures because the brain concentration of AIB was low, and anatomical features were not highlighted in the autoradiographs. Identification of the structures presented was based on their relative location within the frontal plane.

Physiological measurements obtained within five minutes of the end of the experiment indicated that arterial blood gases and blood pressure in both groups were normal (Table 5). Blood pressures were lower in the histamine group probably because the animals had not fully recovered from the hypotensive effects of histamine.

Infusion of saline did not produce any significant differences in AIB transfer (K_i) between the infused and non-infused hemispheres (Table 5). Infusion of histamine, however, did produce increases in K_i in several regions of cerebral cortex. Visual, parietal, sensory-motor, frontal and olfactory cortices demonstrated increases in K_i of 40 to 111% ($p < 0.05$, Table 5, Figure 6). In regions within the distribution of the anterior cerebral artery, e.g., rhinal and anterior cingulate cortices, K_is were increased similarly in both hemispheres (Table 5). In auditory cortex, caudate nucleus and midbrain, there were no significant increases in AIB permeability (Table 5). Also, there was no effect of histamine infusion in choroid plexus, which had relatively high transfer constants under normal conditions (Table 5).

Table 5. EFFECTS OF HISTAMINE INFUSION ON REGIONAL BLOOD-TO-BRAIN TRANSFER CONTENTS FOR ALPHA-AMINOISOBUTYRIC ACID

	Saline		Histamine	
Mean Arterial pressure (mm Hg)	112 ± 10		94 ± 13	
Arterial blood gases and pH				
PO_2 (mm Hg)	112 ± 9		133 ± 9	
pH	7.43 ± 0.02		7.39 ± 0.01	
PCO_2 (mm Hg)	37 ± 2		40 ± 2	
Regional permeability 10^{-5} X Sec^{-1}	Non-infused	Infused	Non-infused	Infused
Cortex				
Visual	7.5 ± 1.4	7.8 ± 1.1	7.8 ± 1.2	13.7 ± 2.8*
Auditory	8.4 ± 1.2	7.7 ± 1.1	9.7 ± 1.7	13.0 ± 2.7
Parietal	11.0 ± 1.3	9.7 ± 1.3	9.4 ± 1.6	13.2 ± 2.4*
Sensory-motor	9.4 ± 1.2	9.3 ± 1.9	10.1 ± 1.7	18.8 ± 4.1*
Frontal	10.5 ± 1.6	10.3 ± 1.5	10.2 ± 1.9	15.5 ± 2.9*
Olfactory	6.0 ± 0.8	5.8 ± 0.6	7.7 ± 1.4	15.5 ± 3.3*
Rhinal	6.9 ± 0.9	6.5 ± 0.8	14.0 ± 1.8	14.6 ± 1.3
Anterior cingulate	9.7 ± 1.5	9.3 ± 1.0	18.6 ± 3.7	19.8 ± 4.8
Caudate nucleus	3.2 ± 0.2	3.5 ± 0.3	4.2 ± 0.6	5.1 ± 1.0
Midbrain	3.6 ± 0.4	3.6 ± 0.3	4.6 ± 0.7	4.8 ± 0.4
Choroid plexus	70.4 ± 12.1	70.5 ± 13.3	55.3 ± 4.6	57.5 ± 6.3
Pineal Gland	87.7±9.0		69.8±5.1	

Values are mean ± SE for 5 animals in each group. * Significantly different from corresponding non-infused side ($p < 0.05$). Dose of histamine = 20 $\mu g\ min^{-1}\ kg^{-1}$ for 15 minutes.

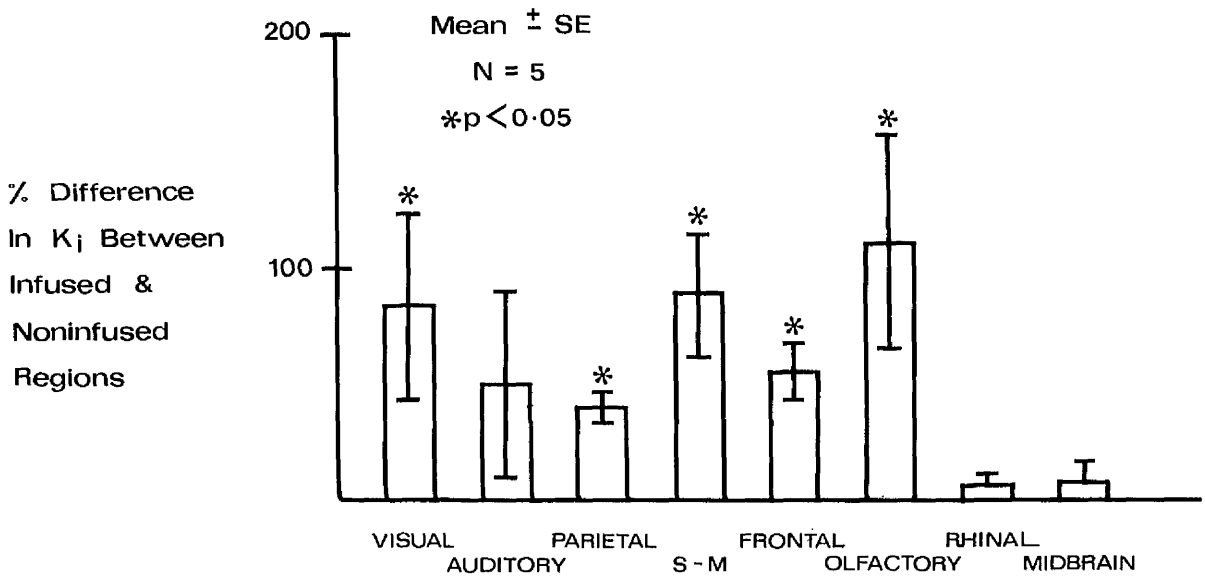

Figure 6. Regional effect of histamine on blood-to-brain transfer of alpha-aminoisobutyric acid in cerebral cortex. S-M = sensory-motor cortex.

Brain Water Content

Arterial blood pressure and blood gases determined at the end of these experiments were normal (Table 6).

The sampled regions showed differences in water content from structure to structure: grey matter structures, such as cortex and hippocampus, contained more water than did regions of mixed grey and white matter, such as brainstem and cerebellum (hindbrain) (Table 6). In the rats infused with saline, there were no significant differences in water content between the infused and non-infused sides of the brain (Table 6). In the histamine group, water content was greater in cortex ipsilateral to infusion, but, in hippocampus, cerebrum or hindbrain, there were no side-to-side differences.

Table 6. EFFECTS OF HISTAMINE INFUSION ON REGIONAL BRAIN WATER CONTENT

	Saline			Histamine		
Mean arterial pressure (mm Hg)	98 ± 6			113 ± 5		
Arterial blood gases and pH						
PO_2 (mm Hg)	116 ± 6			117 ± 6		
pH	7.39 ± 0.01			7.39 ± 0.01		
PCO_2 (mm Hg)	39 ± 1			38 ± 1		
Regional water content (% fresh weight)	Non-infused	Infused	%Δ	Non-infused	Infused	%Δ
Hippocampus	84.0 ± 0.9	83.8 ± 0.9	-0.2 ± 0.9	82.9 ± 0.5	83.0 ± 0.5	0.2 ± 0.4
Cortex	84.6 ± 0.5	84.9 ± 0.7	0.4 ± 0.4	83.0 ± 0.7	84.1 ± 0.8*	1.3 ± 0.3**
Cerebrum	78.9 ± 0.2	79.0 ± 0.1	0.2 ± 0.2	78.7 ± 0.1	78.8 ± 0.1	0.2 ± 0.1
Hindbrain	75.9 ± 0.1	76.0 ± 0.1	0.1 ± 0.1	75.6 ± 0.2	75.6 ± 0.2	0.1 ± 0.1

Values are mean ± SE for 14 animals in each group. Histamine (20 $\mu g\ min^{-1} kg^{-1}$) or saline was infused unilaterally into an internal carotid artery for 15 minutes. The animals were sacrificed 5 minutes after the end of infusion.
** Significantly different from response to saline infusion ($p < 0.05$). * Significantly different from corresponding non-infused side ($p < 0.05$).

Results II. Cerebral Blood Flow.

Quantification of Blood-Brain Barrier Disruption

Cerebrovascular permeability to labeled sucrose was evaluated after carotid injection of hypertonic urea. The hemisphere contralateral to urea injection served as the control for these studies.

In brain regions contralateral to urea injection, there was a range of permeability x surface area products of 7.8 to 12.6 x 10^{-6} sec^{-1}. Ipsilateral to urea, there was a substantial increase in sucrose penetration to the brain. Results, expressed as ratios of ipsilateral/contralateral, were: caudate nucleus: 4.2; hippocampus: 4.5; cerebrum: 4.6; thalamus: 3.9; and medulla: 1.3

Responses to Histamine: Xenon Clearance

Intracarotid infusion of histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) decreased arterial pressure from 104 to 79 mmHg, but did not alter cerebral blood flow when the blood-brain barrier was intact (Table 7). At doses greater than 6 $\mu g\ min^{-1}\ kg^{-1}$, histamine significantly reduced cerebral vascular resistance (Table 7). After urea, infusion of histamine in a low dose (2 $\mu g\ min^{-1}\ kg^{-1}$) did not significantly increase cerebral blood flow, but higher concentrations (6, 20, 60 $\mu g\ min^{-1}\ kg^{-1}$) were effective (Table 7). The increases in blood flow produced by these higher doses of histamine were 27 to 50% above pre-infusion control levels of flow (Figure 7).

Additional flow measurements were obtained during histamine infusion after intravenous administration of the specific histamine receptor antagonists, mepyramine maleate (H_1) or metiamide (H_2) (Table 8). Histamine was infused at the doses which produced increases in blood flow before receptor blockade. The blood-brain barrier was disrupted with urea before the

Table 7. EFFECTS OF HISTAMINE ON CEREBRAL BLOOD FLOW AND VASCULAR RESISTANCE (^{133}XENON CLEARANCE)

		Histamine infusion after urea injection					
	Pre-infusion control	Histamine 20 μg min^{-1} kg^{-1}	2 μg min^{-1} kg^{-1}	6 μg min^{-1} kg^{-1}	20 μg min^{-1} kg^{-1}	60 μg min^{-1} kg^{-1}	Post-infusion Control
Mean arterial pressure (mm Hg)	104±4	79±5*	90±12	99±7	91±4*	78±4*	100±5
Arterial blood gases and pH							
PO_2	128±5	116±15	133±12	138±7	120±8	134±9	133±9
pH	7.42±0.01	7.41±0.02	7.44±0.02	7.43±0.02	7.40±0.02	7.44±0.03	7.40±0.02
PCO_2 (mm Hg)	38±1	39±1	39±2	38±1	39±1	38±1	36±1
Hct (%)	42±1						
N	16	9	4	7	7	7	7
CBF^1, ml min^{-1} g^{-1}	1.54±0.05	1.61±0.08	1.58±0.05	2.00±0.16*	2.38±0.19*	2.36±0.34*	1.53±0.05
Cerebral vascular resistance, mm Hg ml min^{-1} g^{-1}	67.8±2.2	49.2±1.8*	57.4±8.6	51.1±4.6*	39.4±2.6*	36.9±4.9*	65.4±5.8

Values are mean ± SE. CBF^1 is cerebral blood flow estimate derived from initial slope of ^{133}Xe clearance. Histamine was infused into the internal carotid artery. * Significantly different from pre-infusion control ($p < 0.05$).

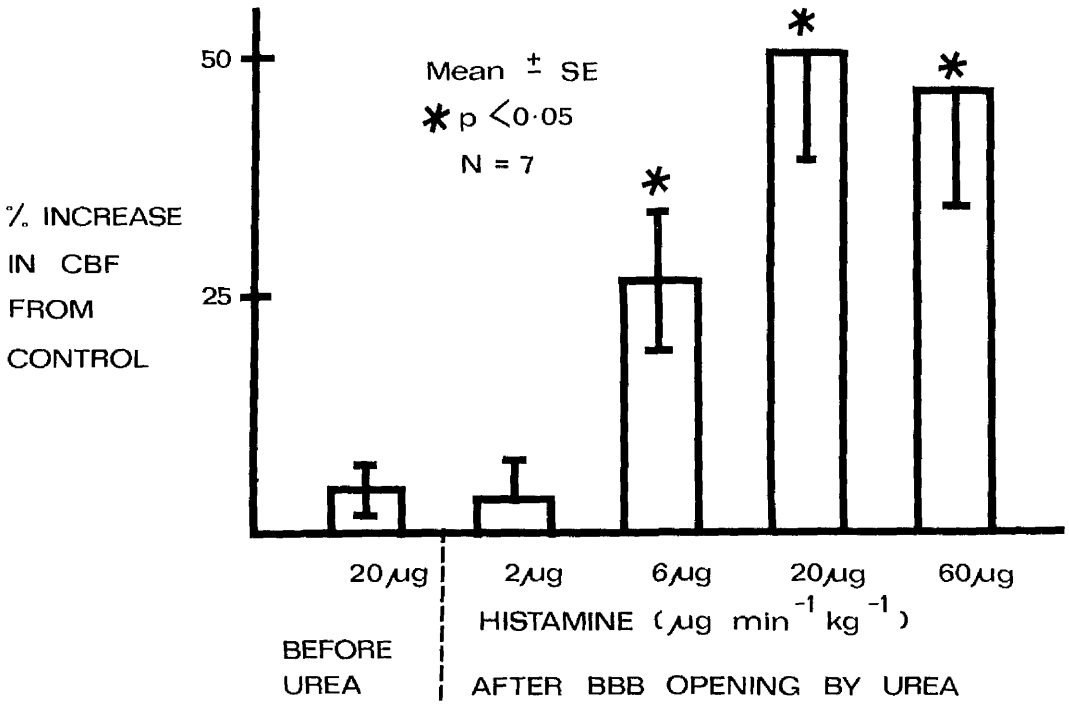

Figure 7. Cerebral blood flow responses (^{133}Xenon clearance) to histamine infusion before and after blood-brain barrier (BBB) disruption.

Table 8. Effects of Histamine Infusion on Cerebral Blood Flow After Specific Receptor Blockade

	H_1 Blockade				H_2 Blockade			
	Before Blockade		After Mepyramine		Before Blockade		After Metiamide	
	MAP	CBF[1]	MAP	CBF[1]	MAP	CBF[1]	MAP	CBF[1]
Control	103±4	1.54±0.08	100±13	1.51±0.17	105±3	1.44±0.06	116±10	1.62±0.14
6 μg $min^{-1}kg^{-1}$	102±6	2.20±0.23‡	100±6	1.63±0.06	‡ 87±10	1.89±0.18‡	107±6*	1.57±0.15*
Control	116±6	1.40±0.10	103±8	1.45±0.08	100±7	1.53±0.10	98±5	1.52±0.09
20 μg $min^{-1}kg^{-1}$	83±4‡	2.14±0.34‡	100±9	1.77±0.07*‡	88±4‡	2.24±0.24‡	93±4	1.58±0.11*
Control	99±5	1.59±0.13	95±11	1.53±0.09	104±10	1.53±0.15	113±5	1.63±0.12
60 μg $min^{-1}kg^{-1}$	76±7‡	2.05±0.23‡	72±18‡	1.77±0.09*‡	76±3‡	2.45±0.36‡	107±2*	1.59±0.14*

Values are means ± SE; 5 animals represented for each mean. Mepyramine and metiamide were given i.v. (5 mg kg^{-1}) to separate groups of rats. MAP = mean arterial pressure in mmHg; CBF' = cerebral blood flow estimate from ^{133}Xe clearance, and expressed as ml min^{-1} g^{-1}. Histamine was infused into the internal carotid artery after blood-brain barrier disruption with urea. ‡ Significantly different from control value ($p < 0.05$); * Significantly different from response before blockade ($p < 0.05$).

administration of antagonist and histamine. Both antagonists attenuated blood pressure and blood flow responses to histamine (Table 8); metiamide was more effective.

Responses to Histamine and its Receptor Agonists: Iodoantipyrine

In control animals, there were no significant side-to-side differences in blood flow in any brain region (Table 9). Infusion of histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) in animals not given urea to disrupt the blood-brain barrier also did not produce side-to-side differences in blood flow (Table 9). In additional studies designed to examine whether the arterial hypotension caused by histamine was important to the blood flow result, I found that when arterial pressure was maintained near control levels during histamine infusion, there were no effects on blood flow in the ipsilateral hemisphere. Blood pressure before histamine infusion in three rats was 118±13 mmHg; during infusion, it was 93±13 mmHg; and, 30 seconds after the start of the experiment during histamine infusion and aortic occlusion, blood pressure was 115±7 mmHg. Blood flow in the non-infused cerebrum was 1.34±0.09 $ml\ min^{-1}\ g^{-1}$, and, in the cerebrum ipsilateral to histamine, blood flow was 1.26±0.09 $ml\ min^{-1}\ g^{-1}$.

Following carotid injection of urea, infusion of saline was associated with no change in regional cerebral blood flow (Table 10); there was a tendency for blood flow to be lower in several regions ipsilateral to infusion, but these differences were not significant ($p > 0.05$, Table 10). Administration of histamine after blood-brain barrier disruption, however, produced significant increases in blood flow in hypothalamus (+9%), thalamus (+10%), parietal cortex (+31%), and cerebrum (+9%) (Table 10). There was a tendency for blood flow to be increased in caudate nucleus and hippocampus, but these changes did not attain statistical significance

($p > 0.05$, Table 10). In regions not perfused primarily by internal carotid artery blood, such as brainstem and cerebellum, there were no significant side-to-side differences in blood flow during histamine infusion (Table 10).

Carotid infusion of the histamine receptor agonists, pyridylethylamine (H_1) and dimaprit (H_2), after urea injection, produced significant flow increases in several brain regions (Table 11, Figure 8). Stimulation of H_1 receptors by pyridylethylamine was associated with increased blood flow in hypothalamus (+29%), caudate nucleus (+20%), parietal cortex (+35%), and cerebrum (+10%) (Table 11). Dimaprit evoked increases in blood flow (range of +12 to +29%) in all territories examined in the distribution of the middle cerebral artery (Table 11).

Effect of Histamine on Glucose Consumption

Saline or histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) was infused for 30 minutes into the internal carotid artery following carotid injection of urea. Regional brain glucose consumption was measured by the 2-deoxyglucose method and tissue sampling.

Neither agent produced any regional or side-to-side change in the rate of glucose consumption (Table 12, Figure 9). Some of the regional values were lower in the animals given histamine; this effect may have arisen because of the prolonged infusion of histamine (30 minutes) which probably stimulated release of catecholamines from adrenal tissue, and increased levels of circulating glucose. Higher blood glucose concentrations occurred in the animals given histamine, and this result may explain the lower values for glucose utilisation in the histamine-infused rats.

To establish whether the method could detect changes in glucose consumption, two additional animals were given an intra-carotid infusion of noradrenaline (6 μg

min^{-1} kg^{-1}) after blood-brain barrier disruption by urea. Noradrenaline produced an average increase in glucose utilisation in caudate nucleus and parietal cortex by 31% and 43%, respectively; in mid-brain, there was no effect of noradrenaline infusion.

Table 9. Effect of carotid artery surgery and histamine infusion on regional cerebral blood flow (iodoantipyrine).

	Control			Histamine		
Mean arterial pressure (mm Hg)	121±4			82±6*		
Arterial Blood Gases and pH						
PO_2 (mm Hg)	122±6			107±8		
pH	7.43±0.01			7.44±0.01		
PCO_2 (mm Hg)	38±1			38±1		
N	6			5		
Regional cerebral blood flow ($ml.min^{-1}g^{-1}$)	Left	Right	% R-L	Left	Right	% R-L
Cerebrum	1.21±0.13	1.19±0.12	-1±4	1.04±0.14	1.02±0.08	-5±15
Diencephalon						
Hypothalamus	0.89±0.08	0.97±0.08	11±11	0.85±0.03	0.87±0.08	3±9
Thalamus	1.21±0.12	1.19±0.11	-1±4	1.18±0.11	1.15±0.08	-1±9
Hippocampus	1.00±0.07	0.95±0.07	-5±2	0.85±0.08	0.88±0.10	3±2
Cerebral Grey Matter						
Caudate nucleus	1.20±0.09	1.18±0.09	-2±2	1.12±0.06	1.17±0.04	5±6
Cortex	1.05±0.10	1.12±0.07	11±13	1.23±0.13	1.19±0.14	-1±10
Brainstem						
Midbrain	1.12±0.07	1.00±0.07	-10±5	1.07±0.07	1.01±0.05	-5±5
Pons	0.99±0.04	1.00±0.05	1±2	0.90±0.05	0.96±0.08	7±6
Medulla	0.88±0.04	0.88±0.05	1±3	0.95±0.09	0.95±0.09	1±2
Cerebellum	0.76±0.05	0.73±0.05	-4±3	0.84±0.06	0.85±0.06	2±2

Values are means ± SE. Cerebral blood flow is compared in the right hemisphere in which surgery was performed (both groups) and histamine was infused (20 $\mu g\ min^{-1}kg^{-1}$), and in the left, control hemisphere. There were no significant differences in either group ($p > 0.05$). * Significant difference in blood pressure ($p < 0.05$).

Table 10. Effect of saline and histamine infusion on regional cerebral blood flow (iodoantipyrine) after disruption of the blood-brain barrier.

	Saline			Histamine		
Mean arterial pressure (mm Hg)	111±9			94±12		
Arterial blood gases and pH						
PO_2 (mm Hg)	126±7			130±6		
pH	7.46±0.02			7.42±0.01		
PCO_2 (mm Hg)	36±2			35±2		
N	5			5		
Regional cerebral blood flow ($ml.min^{-1}g^{-1}$)	Left	Right	%Δ R-L	Left	Right	%Δ R-L
Cerebrum	1.13±0.21	1.06±0.15	-3±5	1.25±0.06	1.37±0.07*	9±3
Diencephalon						
Hypothalamus	0.75±0.05	0.78±0.03	6±5	1.05±0.09	1.14±0.07*	9±2
Thalamus	1.16±0.08	1.05±0.06	-8±6	1.23±0.13	1.34±0.14*	10±2
Hippocampus	1.15±0.16	1.02±0.14	-11±4	0.91±0.08	1.12±0.11	26±15
Cerebral grey matter						
Caudate nucleus	1.19±0.13	1.09±0.08	-6±5	1.24±0.17	1.45±0.15	23±19
Cortex	1.24±0.18	1.09±0.16	-11±9	1.23±0.08	1.62±0.19*	31±14
Brainstem						
Midbrain	0.91±0.10	0.93±0.11	2±5	1.16±0.05	1.16±0.03	0±6
Pons	0.85±0.09	0.83±0.09	-3±1	1.05±0.06	1.01±0.06	-3±2
Medulla	0.68±0.07	0.69±0.06	2±2	0.98±0.07	1.02±0.05	5±3
Cerebellum	0.62±0.05	0.64±0.06	4±2	0.94±0.06	0.96±0.05	2±1

Values are means ± SE. Saline and histamine (20 $\mu g.min^{-1}kg^{-1}$) were infused into the right internal carotid artery for five minutes. The infusions were begun 15 minutes after urea was injected into the carotid artery.
*Significantly different from left side ($p < 0.05$).

Table 11. Effect of H_1- and H_2-receptor agonists on regional cerebral blood flow (iodoantipyrine) after disruption of the blood brain-barrier

	Pyridylethylamine (H_1)			Dimaprit (H_2)		
Mean arterial pressure (mm Hg)	98±13			103±18		
Arterial blood gases and pH						
PO_2 (mm Hg)	121±6			108±18		
pH	7.45±0.01			7.42±0.03		
PCO_2 (mm Hg)	37±1			39±2		
N	5			5		
Regional cerebral blood flow ($ml.min^{-1}g^{-1}$)	Left	Right	%Δ R-L	Left	Right	%Δ R-L
Cerebrum	1.06±0.14	1.18±0.17*	10±4	1.22±0.16	1.38±0.19*	12±4
Diencephalon						
Hypothalamus	0.88±0.06	1.13±0.11*	29±10	0.99±0.10	1.14±0.09*	15±5
Thalamus	1.12±0.16	1.29±0.14	19±13	1.18±0.14	1.36±0.15*	15±7
Hippocampus	0.90±0.10	1.00±0.12	11±8	0.92±0.13	1.13±0.13*	24±3
Cerebral grey matter						
Caudate nucleus	1.12±0.10	1.35±0.16*	20±8	1.14±0.15	1.41±0.18*	25±7
Cortex	1.08±0.18	1.41±0.22*	35±11	1.09±0.11	1.43±0.19*	29±6
Brainstem						
Midbrain	0.95±0.08	1.00±0.06	6±6	1.17±0.08	1.23±0.09	7±10
Pons	0.93±0.05	0.95±0.04	1±4	1.08±0.09	1.09±0.08	2±7
Medulla	0.96±0.12	0.92±0.11	-4±4	1.12±0.07	1.06±0.07	-5±5
Cerebellum	0.85±0.04	0.86±0.07	0±3	0.89±0.06	0.95±0.08	3±3

Values are means ± SE. The agonists (each 200 $\mu g.min^{-1}kg^{-1}$) were infused into the right internal carotid artery for five minutes. The infusions were begun 15 minutes after urea was injected into the carotid artery.
* Significantly different from left side ($p < 0.05$).

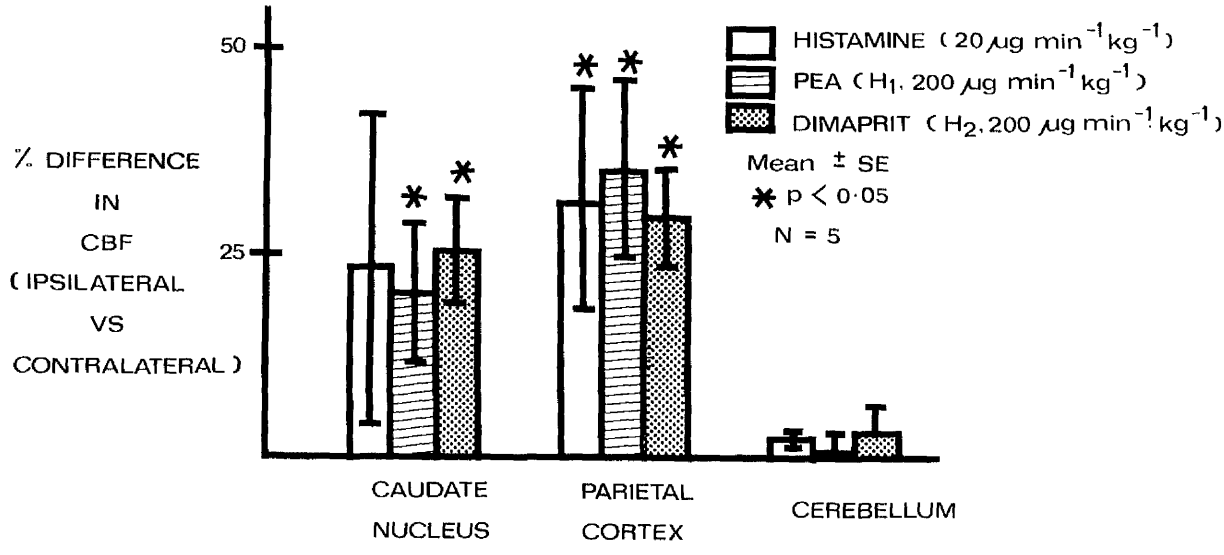

Figure 8. Comparison of cerebral blood flow responses (iodoantipyrine) to histamine receptor agonists after blood-brain barrier disruption. PEA = pyridylethylamine.

Table 12. EFFECTS OF HISTAMINE INFUSION ON REGIONAL CEREBRAL GLUCOSE CONSUMPTION AFTER DISRUPTION OF THE BLOOD-BRAIN BARRIER.

	Saline		Histamine	
Mean arterial pressure (mm Hg)	98 ± 7		98 ± 5	
Arterial blood gases and pH				
PO_2 (mm Hg)	122 ± 14		133 ± 7	
pH	7.42 ± 0.02		7.44 ± 0.02	
PCO_2 (mm Hg)	38 ± 1		36 ± 1	
Regional glucose consumption ($\mu M\ min^{-1}\ g^{-1}$)	Non-infused	Infused	Non-infused	Infused
Thalamus	0.67 ± 0.08	0.65 ± 0.06	0.64 ± 0.06	0.63 ± 0.04
Caudate nucleus	0.84 ± 0.07	0.86 ± 0.09	0.73 ± 0.06	0.69 ± 0.05
Hippocampus	0.51 ± 0.06	0.50 ± 0.05	0.43 ± 0.03	0.45 ± 0.04
Cerebral cortex	0.72 ± 0.02	0.74 ± 0.04	0.60 ± 0.05	0.59 ± 0.03
Midbrain	0.58 ± 0.04	0.60 ± 0.03	0.54 ± 0.04	0.51 ± 0.04
Cerebellar cortex	0.43 ± 0.02	0.44 ± 0.03	0.38 ± 0.04	0.40 ± 0.03

Values are mean ± SE for 30 minute experiments. Saline n = 6; Histamine n = 8. Saline or histamine (20 $\mu g\ min^{-1}\ kg^{-1}$) was infused unilaterally and continuously for 30 minutes. Infusion was begun 15 minutes after blood-brain barrier opening by carotid injection of hypertonic urea. There were no significant differences between infused and non-infused brain regions in either group ($p > 0.05$).

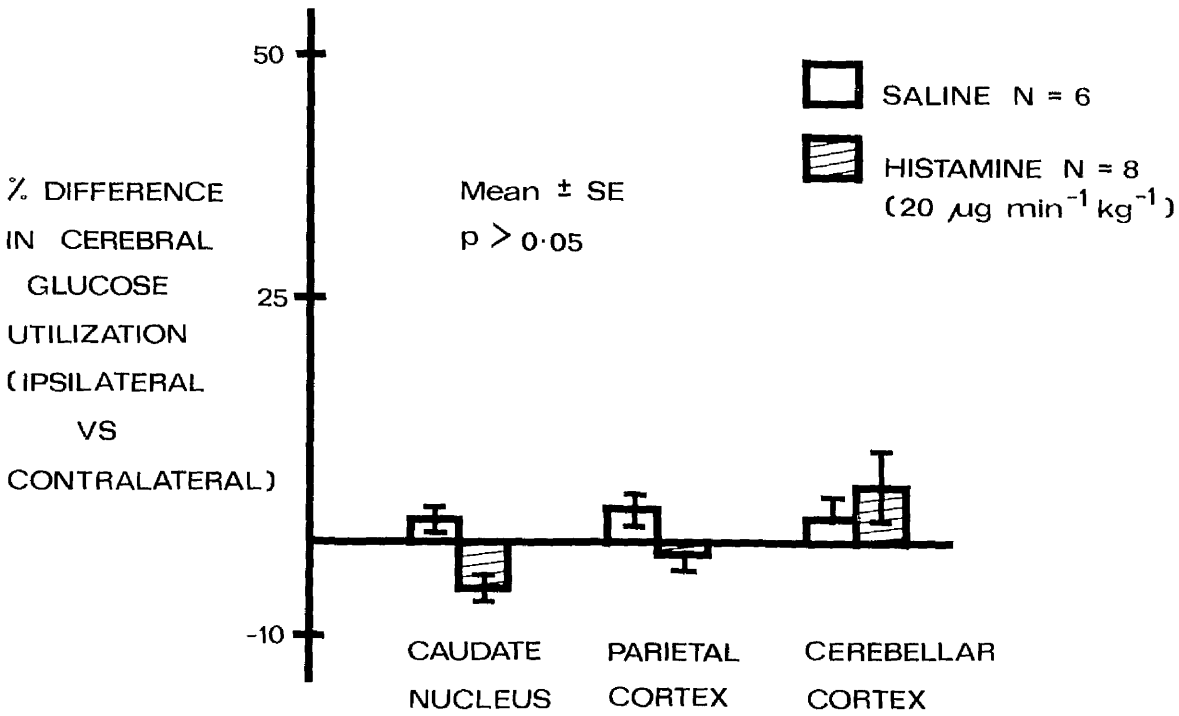

Figure 9. Effects of histamine infusion on regional cerebral glucose consumption after disruption of the blood-brain barrier.

Results III. Pial Vessels in Situ.

Responses to Perivascular Injection of Histamine and Its Agonists.

Arterioles

Perivascular injection of mock cerebrospinal fluid (CSF) had no significant effect on arteriolar diameter (% change from control = $-2\pm2\%$, n = 82 vessels in 30 cats.

Responses to histamine were established over a range of four doses from 10^{-10}M to 10^{-4}M. Small dilatations resulted at doses of 10^{-10} and 10^{-8}M but responses were greater at 10^{-6} and 10^{-4}M (Figure 10). The maximum response demonstrated with histamine occurred at 10^{-4}M (28% increase in vessel diameter from control).

The H_1 receptor agonist, pyridylethylamine, was studied at five concentrations between 10^{-7}M and 10^{-3}M. This drug produced only weak dilatatory responses at all doses examined. The threshold for dilatation with pyridylethylamine was at 10^{-6}M (10% increase in calibre), and the maximum response occurred at 10^{-4}M (17%) (Figure 10).

Six concentrations of the H_2 receptor agonist, impromidine, were studied. This drug produced significant dilatations of arterioles at concentrations as low as 10^{-10}M (16% increase in vessel calibre). The maximum dilatation produced by impromidine occurred at 10^{-6}M (43% increase in vessel calibre) (Figure 10).

Because impromidine proved to be an active and potent drug at pial arterioles, a further analysis was completed to describe a possible relationship of vessel size to magnitude of response. At the concentrations producing the largest increases in vessel calibre (10^{-7} to 10^{-4}M, n = 31 vessels), there was no significant relationship between initial vessel size and the magnitude of response that resulted from impromidine. However, when vessel responses were partitioned between

the lowest size limit (< 80 μm) and the highest size limit (> 155 μm), there was a tendency for difference with regard to size and response. Small vessels had an increase in vessel calibre of 45±4% and large vessels had an increase in vessel calibre of 35±4% (n = 9 each).

Veins

Veins did not respond significantly to perivascular injection of mock CSF (+4±2%, n = 15 vessels in six cats).

For each of the histamine agents, venous responses were examined at two concentrations. For histamine itself, there was no significant effect on venous calibre at either of the concentrations studied. At 10^{-6}M and 10^{-4}M, vein responses to perivascular injection were 2% and 4%, respectively ($p > 0.05$ with respect to pre-injection control calibre of the vessel) (Figure 11).

For the H_1 receptor agonist, pyridylethylamine, which was studied at concentrations of 10^{-5}M and 10^{-3}M, responses were 1% and 5%, respectively ($p > 0.05$) (Figure 11).

Similarly, impromidine, the H_2 receptor agonist, did not produce significant changes in venous calibre. Responses to 10^{-8}M and 10^{-6}M were 0% and 5%, respectively ($p > 0.05$) (Figure 11).

Interaction with Noradrenergic Stimuli

Sympathetic Stimulation

Six sets of experiments were conducted using 12 cats in this series. Sympathetic nerve stimulation was studied for its effect on both arterioles and veins in the presence of histamine, pyridylethylamine, and impromidine.

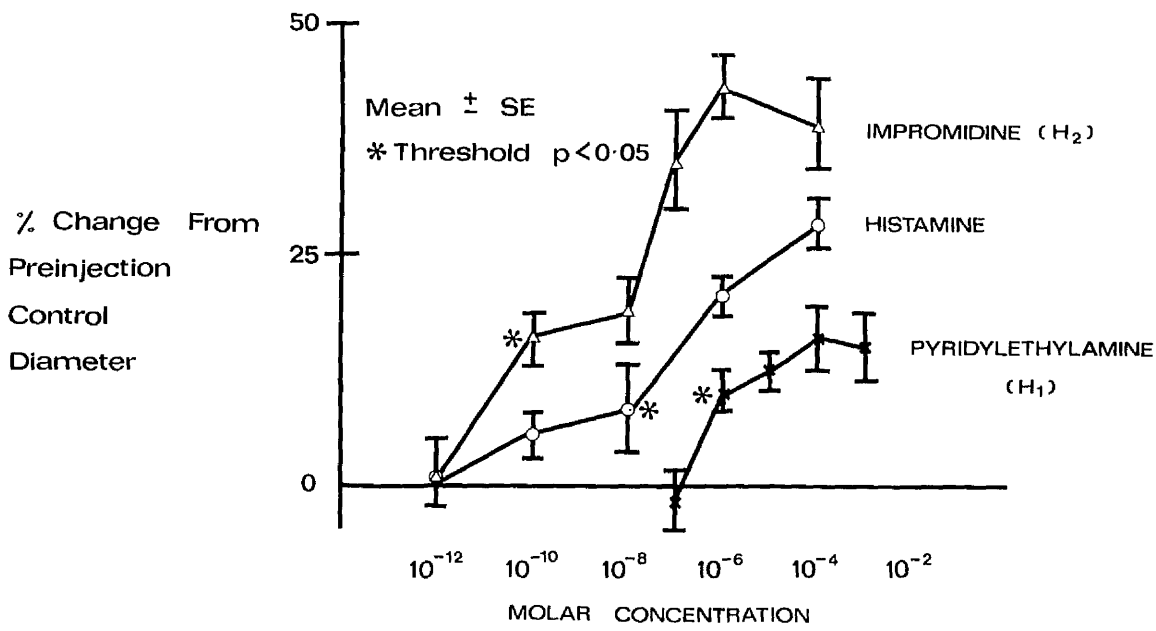

Figure 10. Effects of histamine agonists on the calibre of pial arterioles. Numbers Represented: impromidine = 60 vessels in 11 cats; histamine = 40 vessels in 14 cats; pyridylethylamine = 42 vessels in 13 cats.

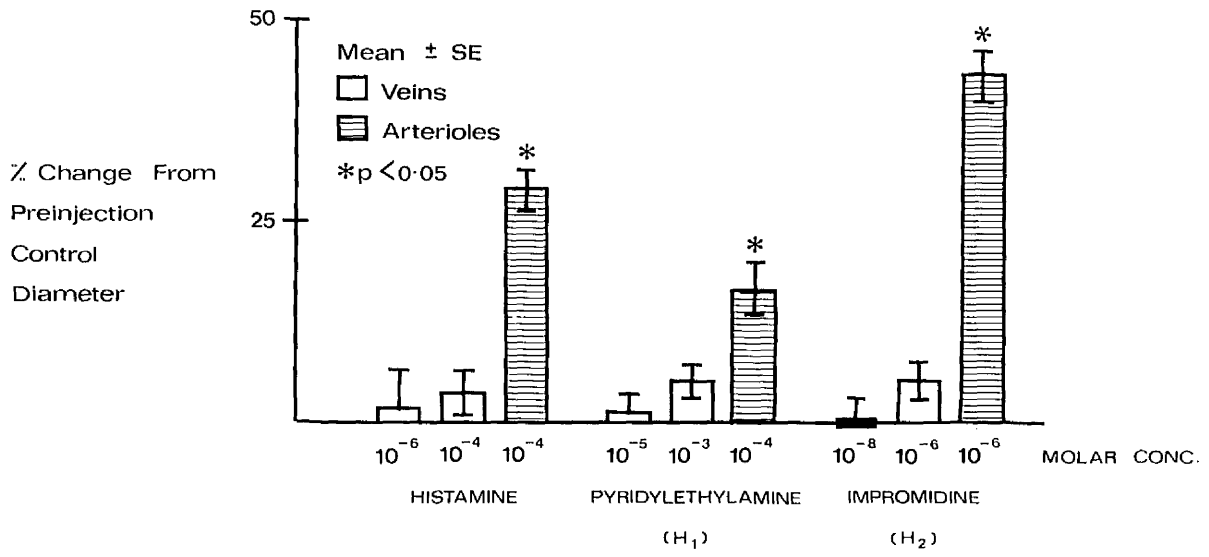

Figure 11. Effects of histamine agonists on the calibre of pial veins. Responses in veins are contrasted with the maximal response found in arterioles. Numbers represented: histamine = 15 veins in 4 cats, 15 arterioles in 7 cats; pyridylethylamine = 24 veins in 5 cats, 12 arterioles in 5 cats; impromidine = 30 veins in 5 cats, 17 arterioles in 6 cats.

Arterioles

Sympathetic nerves were stimulated before each trial with a histamine agent. Before histamine was injected, stimulation of sympathetic nerves produced a decrease in arteriolar calibre of -6% (n = 8) (Figure 12). In vessels not under stimulation, and at concentrations of 10^{-6}M and 10^{-4}M, histamine produced dilatations of 22% (n = 12) and 31% (n = 5), respectively. During sympathetic stimulation, injection of histamine in these same doses produced dilatations that were considerably smaller than before stimulation. Vessel responses to the two doses were +7% (n = 7) and +11% (n = 6), respectively (Figure 12).

Before injection of pyridylethylamine in 10 arterioles, sympathetic stimulation produced a mean decrease in vessel calibre of -6% (Figure 13). Pyridylethylamine, in doses of 10^{-5}M and 10^{-3}M, produced dilatations of 11% (n = 8) and 21% (n = 9) (Figure 13). In the presence of nerve stimulation, these responses were not changed. At 10^{-5}M, there was still an increase in vessel size of 12% (n = 6); at 10^{-3}M, there was a mean dilatation of 20% (Figure 13).

Prior to injection of impromidine, sympathetic stimulation reduced vessel calibre by -11% in 16 arterioles (Figure 14). At doses of 10^{-8}M and 10^{-6}M, impromidine increased vessel calibre by 12% (n = 13) and 36% (n = 8), respectively (Figure 14). During nerve stimulation, the constriction normally seen during sympathetic stimulation was abolished by simultaneous injection of impromidine. Vessel calibres were increased by 13% (n = 6) and 28% (n = 5) for the two concentrations, respectively (Figure 14).

Veins

In veins, I studied sympathetic nerve influences on vessel calibre before and during administration of only one concentration of each histamine agent. Before histamine in 11 vessels, stimulation of nerves

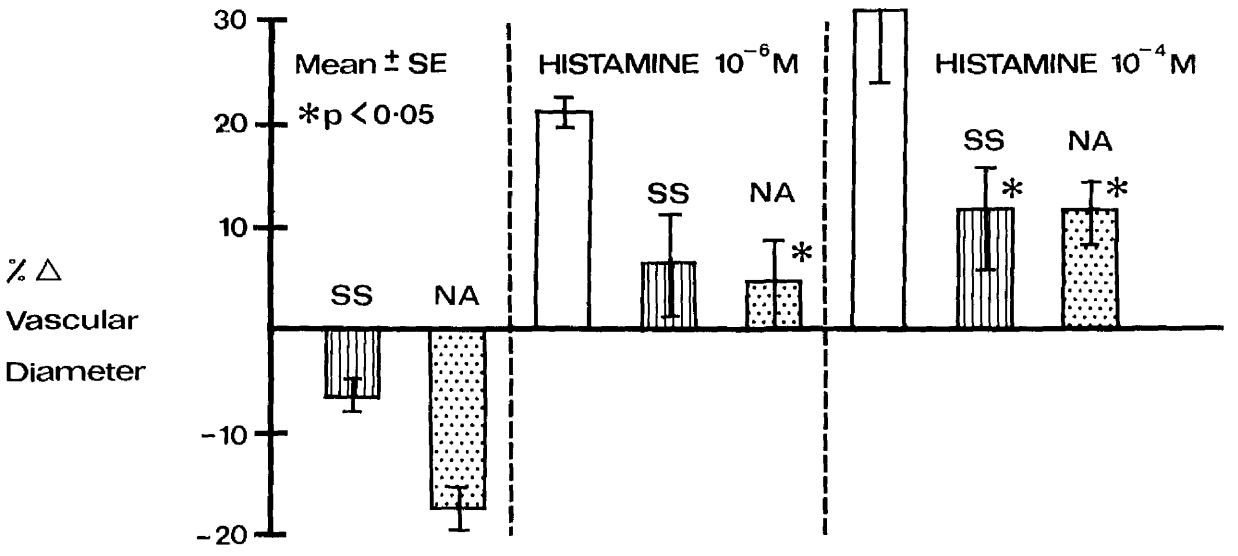

Figure 12. Effects of histamine on responses of pial arterioles to noradrenergic stimuli. Values are mean ± SE; see text for number of vessels and cats. HA = histamine; SS = sympathetic nerve stimulation; NA = noradrenaline. *$p < 0.05$ for comparison of vessel response between individual and combined stimuli.

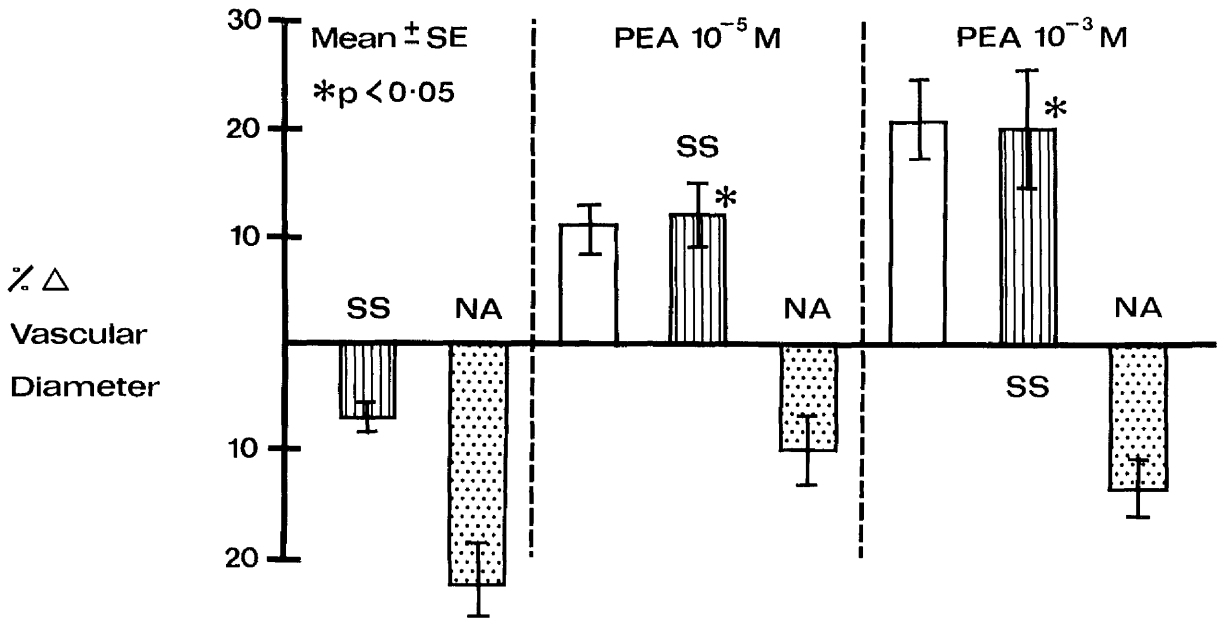

Figure 13. Effects of pyridylethylamine (PEA, H_1 agonist) on responses of pial arterioles to noradrenergic stimuli. Abbreviations and symbols as in Figure 12.

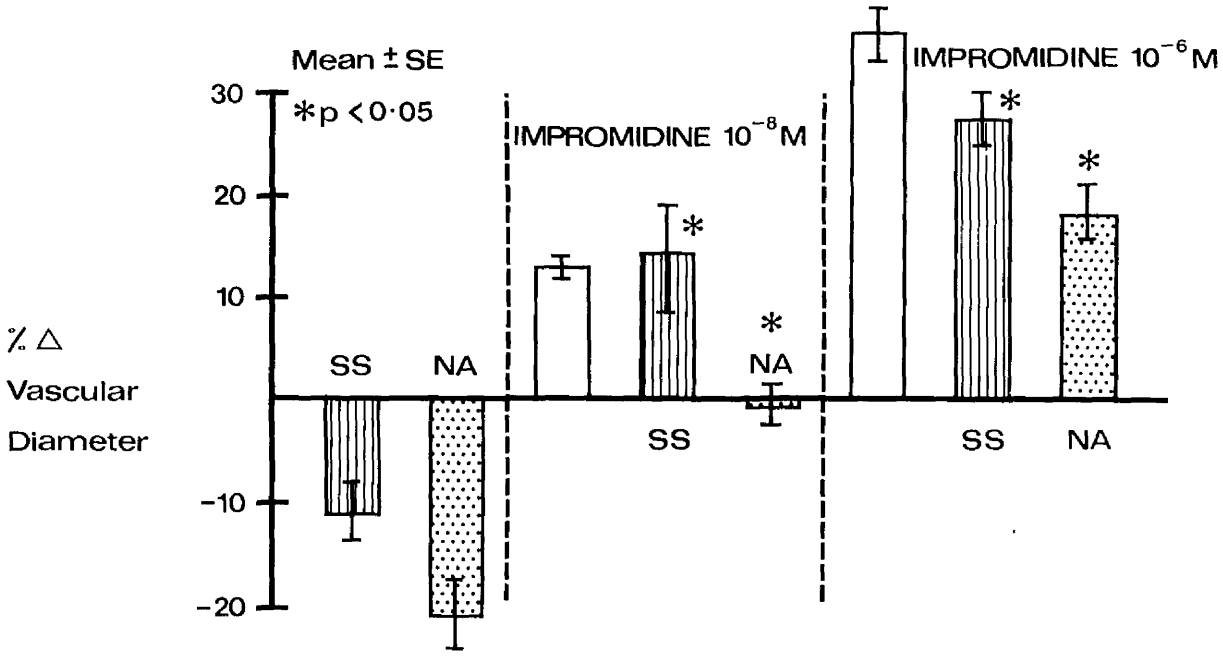

Figure 14. Effects of impromidine (IMP, H_2 agonist) on responses of pial arterioles to noradrenergic stimuli. Abbreviations and symbols as in Figure 12.

produced a 9% decrease in vessel calibre. Histamine, in a dose of 10^{-6}M, increased vein calibre by 2% (n = 9). When combined, constriction of the veins was not seen; a 5% increase in venous calibre was observed (n = 11) (Figure 15).

Before pyridylethylamine, sympathetic stimulation produced a constriction of veins by -8% (n = 8) (Figure 16). In a dose of 10^{-5}M, pyridylethylamine increased vessel calibre by 1% (n = 13). During sympathetic stimulation, pyridylethylamine prevented the constriction (+4% change in vein calibre, n = 8) (Figure 16).

Before impromidine was given, stimulation decreased vein calibres in 9 vessels by -14% (Figure 17). There was no change (0%, n = 14) in vessel calibre when impromidine (10^{-8}M) was given. When combined, however, the constriction seen with nerve stimulation was prevented by impromidine (+1% change in vessel calibre, n = 9) (Figure 17).

Noradrenaline

The effects of noradrenaline were examined alone and in the presence of each of the histamine agents in both arterioles and veins.

Arterioles

Arteriole responses to each of the histamine agents alone were the same as in the series involving sympathetic nerve stimulation.

Noradrenaline (2.5×10^{-4}M) was given perivascularly by itself before each of the histamine agents. Before histamine, noradrenaline produced a mean decrease in vessel calibre of -18% in nine vessels (Figure 12). When combined with histamine in two doses (10^{-6}M and 10^{-4}M), vessel calibres showed small increases (+4% and +11%, respectively, each n = 12) (Figure 12).

Before pyridylethylamine, noradrenaline injection

produced reductions in arteriolar diameter by -22% (n = 9) (Figure 13). In the presence of pyridylethylamine, the noradrenaline constriction was only partly attenuated. At 10^{-5}M, vessel response to the two drugs was -10% (n = 9); at 10^{-3}M, the response was -14% (n = 8) (Figure 13).

For the group of vessels in which responses to impromidine were examined, noradrenaline produced constriction of -22% (n = 7) (Figure 14). When combined, the noradrenaline-impromidine mixture produced a -1% change in vessel calibre at 10^{-8}M impromidine (n = 18), and an 18% increase in vessel diameter with 10^{-6}M impromidine (n = 8) (Figure 14).

Veins

Vein responses to each of the histamine agents alone were the same as in the sympathetic stimulation series. The same dose of noradrenaline (2.5×10^{-4}M) was given perivascularly to veins alone and in combination with one concentration of each of the histamine agents. In the histamine experiments, noradrenaline produced a mean decrease in vessel diameter of -18% (n = 11) (Figure 15). When combined with 10^{-6}M histamine, the mixture containing noradrenaline produced a decrease in vessel calibre of -15% (n = 11) (Figure 15).

Before pyridylethylamine, a reduction in venous calibre of -13% (n = 8) was produced by noradrenaline. In combination, the mixture produced a decrease in vessel diameter of -20% (n = 8) (Figure 16).

In the impromidine studies, noradrenaline by itself decreased vessel calibre by -13% (n = 8). When given together, the noradrenaline-impromidine mixture produced a decrease in vein calibre of -12% (n = 8) (Figure 17).

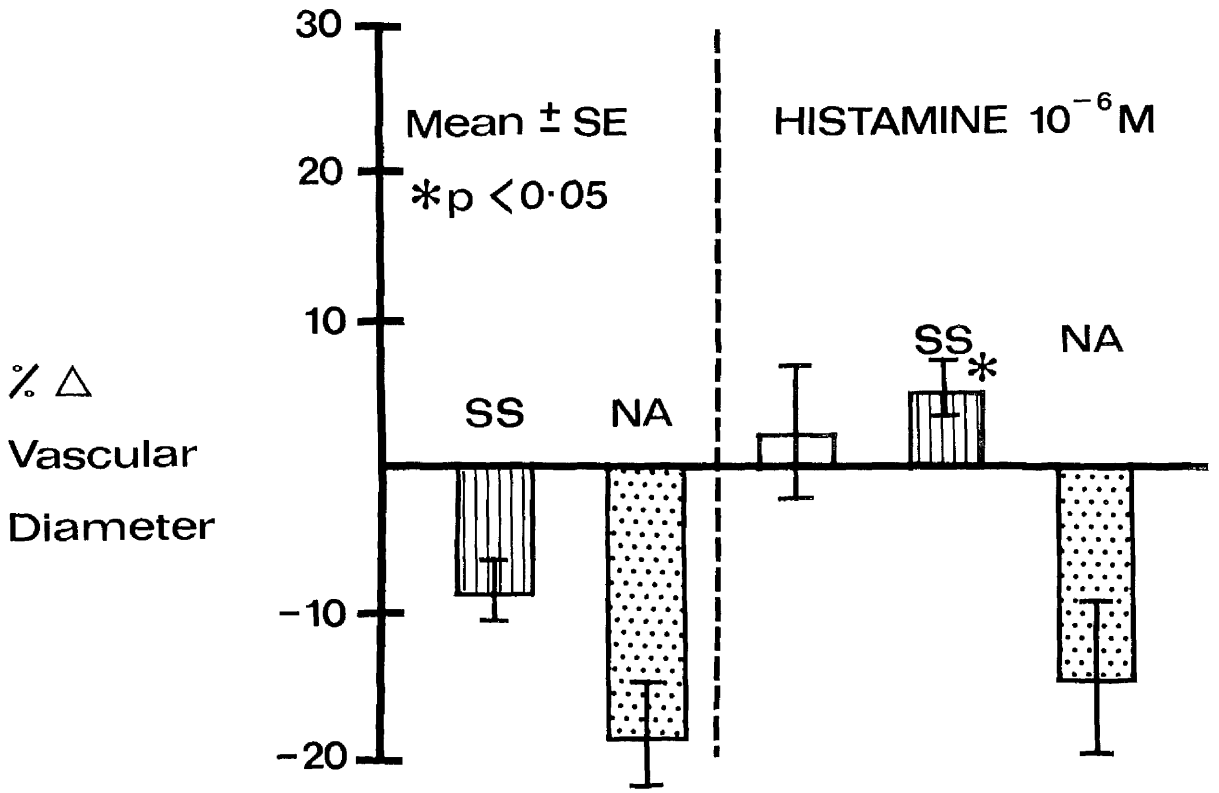

Figure 15. Effects of histamine on responses of pial veins to noradrenergic stimuli. Abbreviations and symbols as in Figure 12.

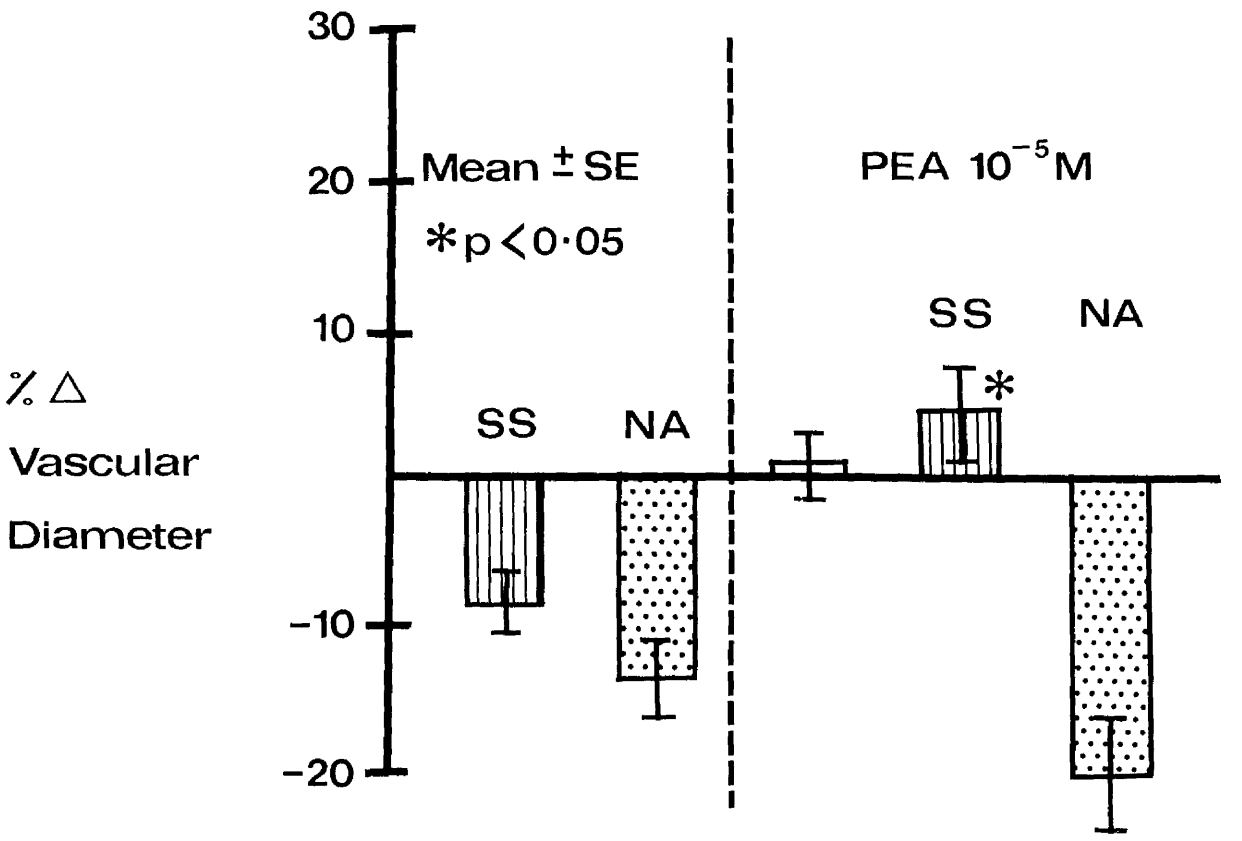

Figure 16. Effects of pyridylethylamine (PEA, H_1 agonist) on responses of pial veins to noradrenergic stimuli. Abbreviations and symbols as in Figure 12.

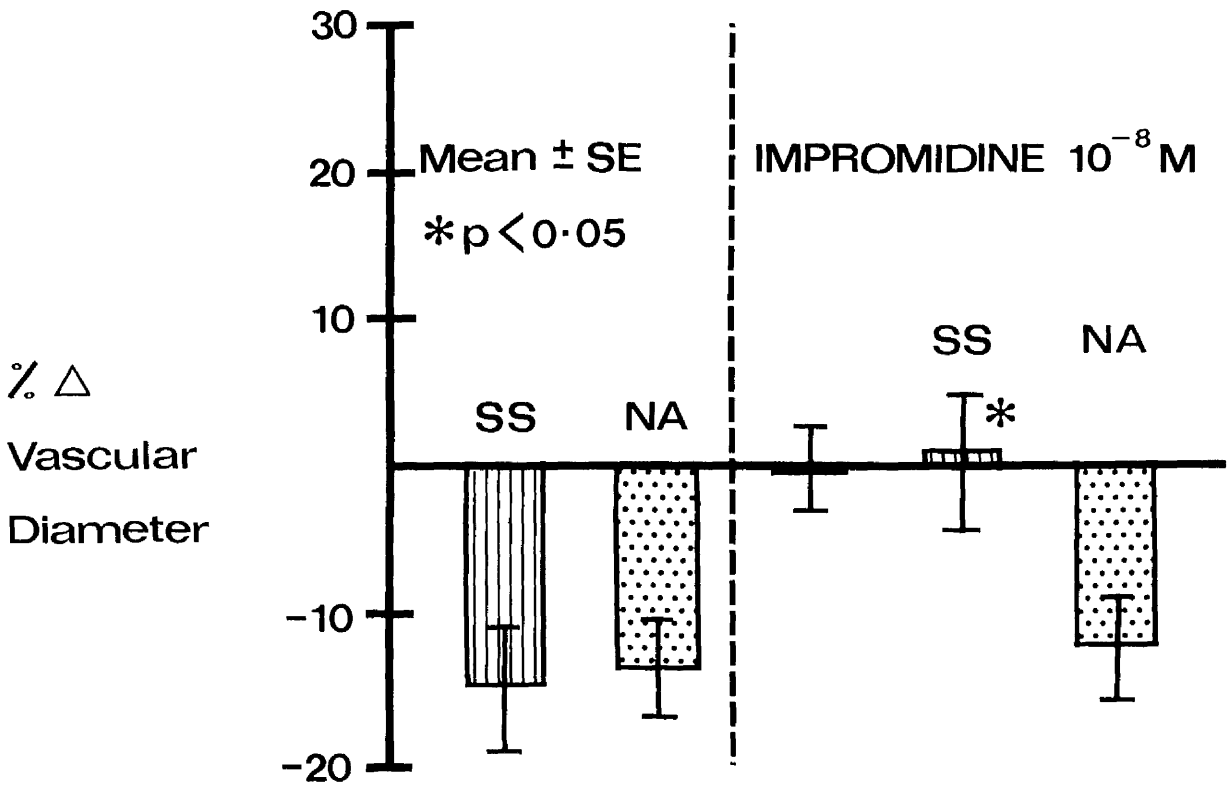

Figure 17. Effects of impromidine (IMP, H_2 agonist) on responses of pial veins to noradrenergic stimuli. Abbreviations and symbols as in Figure 12.

CHAPTER IV. DISCUSSION

Discussion I. Blood-Brain Barrier.

Intra-arterial infusion of histamine produced an increase in permeability of the blood-brain barrier. Several new concepts emerged from these studies: 1) the increase in permeability was sensitive to the concentration of histamine administered; 2) the permeability of several brain regions was increased; 3) the increase in permeability due to histamine was a reversible phenomenon; 4) histamine H_2 receptors were predominantly responsible for the permeability effect; and 5) the increase in permeability was associated with formation of oedema in grey matter.

In this discussion, I will focus on six points that encompass the above characteristics of the permeability alteration produced by histamine in brain vessels: the regionality of the response; the reversibility of the phenomenon; receptor involvement; the magnitude of response; possible mechanisms to explain the change in permeability; and implications the studies have for blood-brain barrier responses under various conditions *in vivo*.

Regionality

During histamine infusion, increases in permeability to sucrose occurred in several brain structures (Table 1) and, as demonstrated by the AIB studies, several regions of cerebral cortex were affected (Table 5). Permeability increases within relatively homogeneous tissue, i.e., cerebral cortex, were variable (Table 5). The effective concentration of histamine, and anatomical factors related to drug delivery and receptor distribution in the cerebral microcirculation, may account for these differences.

Concerning the concentration of histamine in the microcirculation, it is difficult to calculate or argue

how a relatively higher concentration reached areas such as caudate nucleus and cerebral cortex where the largest increases in permeability occurred. These regions are highly vascularised (246) which could explain a permeability change based on a larger surface area for contact at endothelial cell layers by histamine. Furthermore, there may be heterogeneity of vascular metabolic activity within different brain regions (36), viz, vessels in grey matter may have a higher metabolic rate than vessels in mixed regions or in white matter. Intrinsically, therefore, vessels in structures such as caudate nucleus and cerebral cortex may have a greater capacity to react to stimuli in the blood.

Two anatomical factors may account for regional differences in the permeability response to histamine. First, arterial "boundary" zones, where late-generation branches of two arteries converge, are known to be vulnerable to vascular insults such as the ischaemic process (245). In the AIB series, substantial changes in cortical permeability occurred in the visual area (+84%; a boundary zone of the posterior and middle cerebral arteries) and the olfactory area (+111%; a boundary zone of the anterior and middle cerebral arteries). Precisely what factors account for the vulnerability of boundary zone regions are not known.

Second, neurochemical studies have demonstrated that the concentration of histamine elements in the brain is non-uniform (85, 219, 220). The anatomical distribution of histamine receptors within the microcirculation of different brain regions, therefore, may be an important factor. A high and heterogeneous density of histamine receptors in cerebral cortical microvessels, for example, may explain the large and variable increase in vascular permeability in this region.

Reversibility

Arterial infusion of histamine produced an increase in permeability of the blood-brain barrier that persisted for 30 minutes, but, within two hours, barrier permeability had recovered to normal. The persistence of the permeability increase for at least 30 minutes after the end of histamine infusion may be related to the activated state of enzymes participating in the response. Studies of nucleotide systems in cerebral microvessels (113) have demonstrated that, once stimulated, adenylate cyclase activity may be sustained for periods up to 30 minutes, even following washing and sedimentation. A high energy turnover in brain endothelia, reflected by dense mitochondrial content (170), significant metabolic activity (36), and facility for synthesis of cyclic AMP when stimulated by histamine (123, 125), may account for the restorative properties of the blood-brain barrier. In thalamus, which had normal permeability 30 minutes after infusions (Table 3), these mechanisms may be relatively more important.

Several other interventions transiently increase vascular permeability in brain vessels. Acute arterial hypertension, convulsions, arterial hypercapnia, and carotid perfusion with lipid-insoluble hypertonic solutions are manoeuvers that produce reversible increases in barrier permeability lasting from 15 minutes to several hours (24, 199). Carotid perfusion with lipid-soluble hypertonic solutions, such as propylene glycol, increases blood-brain barrier permeability irreversibly (198). This result may be related to destruction of cell membranes in endothelia or to cytotoxic effects on endothelial metabolism (198). By contrast, the concentrations of histamine used in the present studies were within the physiological limit of repair by the blood-brain barrier.

Receptors

The results demonstrate that H_2 receptors were predominantly responsible for the increase in permeability produced by histamine; the H_2 receptor blocking agent, metiamide, reduced the permeability response to histamine, while mepyramine, an H_1 receptor antagonist, was mostly ineffective. This discussion will focus on three points concerning receptors: first, evidence for histamine receptors in cerebrovascular endothelia; second, the localisation of action by metiamide; and third, comparisons with other studies which have examined histamine receptor involvement in vascular permeability changes.

Evidence for the presence of histamine receptors in brain microvessels has emerged almost entirely from the work of Joó and colleagues (123). Over several years of research effort, these investigators have identified a histamine-sensitive cyclic nucleotide system in brain capillaries, and have proposed that histamine and cyclic AMP are important to normal and pathological function of the blood-brain barrier (121, 123). In a recent report, Karnushina et al. (125) showed that stimulation of adenylate cyclase by histamine was mediated by H_2 receptors. H_1 receptors were present but not important to adenylate cyclase activity. The presence of histamine-sensitive enzymes has been confirmed in other laboratories (8, 113), but specific receptors mediating their effects were not identified.

Several studies have provided evidence for histamine receptors in brain slices or homogenates (85, 220). It is not known, however, whether these receptors are incorporated in neuronal tissue or within blood vessels and endothelia. Three of the brain regions studied in the present experiments, diencephalon, hippocampus, and cerebral cortex, have relatively

high concentrations of both H_1 and H_2 receptors (85, 188, 219, 220). A high concentration of histamine elements in certain brain regions may indicate that vascular responses are more readily detectable. It is interesting, from this line of rationale that attenuation of increased permeability in hippocampus by mepyramine (Figure 5) may have occurred because this brain region is relatively rich in H_1 receptors. Generally, however, H_2 receptors were primarily responsible for the increase in brain vascular permeability during histamine infusion.

The above conclusion extends from the finding that metiamide blocked the histamine effect. Metiamide is a water-soluble protonated compound, and has poor penetration from blood into the central nervous system (13, 171, 211). Nevertheless, in a previous study, metiamide was effective in reducing oedema caused by exposure to radiation (122). In the present experiments, metiamide was given before histamine infusion, and, therefore, would have been deposited at H_2 receptors before the beginning of histamine stimulation. It is worthwhile to consider, therefore, at what site in the cerebral circulation metiamide could exert its blocking effect on histamine. Two loci, neither involving complete penetration of the drug from blood to the central nervous system, are possible. First, metiamide may occupy H_2 receptor binding sites on the luminal plasma membrane of endothelial cells. This locus has been identified as an important structural site for binding by circulating hormones (176). Second, metiamide may reach significant intraendothelial concentrations by simple dissolution and diffusion within the aqueous phase of plasma membranes. Within the endothelial cytoplasm, metiamide may inhibit histamine-sensitive adenylate cyclase and prevent the formation of cyclic AMP, which could influence vascular permeability mechanisms (123).

Previous studies in skeletal muscle and skin preparations have identified the receptors involved in vascular responses produced by histamine. Thermal injuries in limbs of rats cause systemic levels of histamine to increase (111); resulting local increases in blood flow and oedema formation are mediated by H_2 receptors (29). In blood vessels of human skin, both H_1 and H_2 receptors mediate erythemic responses to intradermal injection of histamine (207). Furthermore, it has been clearly shown in cat skeletal muscle studies (71, 72) that both classes of receptors mediate increases in vascular permeability and oedema formation produced by arterial infusion of histamine. In rat brain in my studies, mediation of increased permeability to histamine only by H_2 receptors may indicate a species difference, or may indicate a regional organ difference in distribution of histamine H_1 and H_2 receptors. These possibilities have been discussed previously by several authors (13, 72, 171, 211).

Magnitude

From intra-carotid infusion of histamine, increases in cerebrovascular permeability of up to 237% were determined from the two quantitative methods applied in the present studies (Figures 3 and 6). There are no available studies with which to directly compare the permeability results with histamine: previous reports examining the effect of histamine at the blood-brain barrier have not used quantitative methodology (74, 81, 115), and there are no other studies in which agents that might influence vascular permeability in the brain have been administered by intra-arterial infusion.

There are, however, several studies that have applied quantitative methods to examine blood-brain barrier permeability in pathological conditions that could

involve histamine release. Among these reports, low level irradiation (205) and hypoxic ischaemia (181) were associated with alterations in the permeability of cerebral microvessels of about the same magnitude as the present studies. Both of the experiments, however, used quantitative morphological technique (counting of endothelial vesicles) which does not provide information concerning the rate of solute passage into the brain.

Other perturbations that affect blood-brain barrier permeability, and have been quantitatively studied, include convulsive seizures (142), acute arterial hypertension (104), gliosarcoma tumours (139), and carotid perfusion with hypertonic solutions (201). These conditions are associated with very severe disruption of barrier function, with four to 20-fold increases in permeability occurring. The increase in permeability produced by histamine, therefore, represents a change in properties of cerebral endothelia that is considerably more subtle than has been previously determined.

It is interesting to compare the results of the present studies in brain with those obtained in a peripheral organ circulation. In the isolated dog hindlimb, intra-arterial infusion of histamine in doses of 1-5 $\mu g\ min^{-1}\ kg^{-1}$ did not significantly increase capillary filtration coefficients, but at doses of 20 - 60 $\mu g\ min^{-1}\ kg^{-1}$, similar, therefore, to the present experiments, filtration coefficients were increased 100 - 200% (48). As this measurement in muscle primarily reflects the rate of fluid movement across microvessels (49), the results of the two studies are not directly comparable, but, nevertheless, provide an example of similarity in response between two very

different microvascular beds.

Mechanism.

Increases in permeability of the blood-brain barrier to circulating solutes are typically the result of widened interendothelial junctions and/or activation of pinocytosis and vesicular transport (24). It is useful to consider also the possible mechanisms ascribed to increased vascular permeability in non-neural organs. For these tissues, in addition to the above responses, an increase in capillary surface area, an increase in the number of pores (open cell junctions) per unit surface area, and the formation of transcellular channels from chains of vesicles are possibilities (27, 204). These items will be considered in this section.

The increase in permeability x surface area product seen with histamine infusion in several brain regions may simply be a reflection of increased tissue perfusion, i.e.,increased capillary surface area. Two of my findings in separate studies however, do not support this argument. First, there was no detectable increase in local blood flow to the regions of brain in which increases in permeability occurred (Table 9). This finding would suggest that histamine infusion did not increase the effective perfusion area in the vascular bed. Second, histamine infusion tended to decrease, rather than increase, regional vascular volume (Table 2). Thus, the area within a given unit length of microvessel appeared not to be larger. Taken together, these findings suggest that histamine did not cause "capillary dilatation", a condition that would be associated with increased surface area for blood-to-brain solute transport.

Histamine may cause widening of tight junctions at apposing brain vascular endothelial cells. Previously, the prototype condition for this kind of barrier opening involves adminstration into the carotid artery of

hypertonic, lipid-insoluble solutions (198, 201). An osmotic effect on endothelial cells ensues, involving loss of cell water to blood and shrinking of cellular volume (26, 199). The histamine solution in the present studies was not hypertonic, and was unlikely to produce an osmotic effect in the cerebral vascular bed.

It is possible, however, that histamine stimulates contractile mechanisms that are present in brain microvessels (134, 174). Contraction of endothelia in capillaries or venules could lead to widening of interendothelial junctions. This response to histamine, associated with reversible deformation of endothelial nuclei, has been observed previously in blood vessels of skeletal muscle (148) and mesentery (79, 124). Whether this response occurs uniformly throughout the microvascular bed, to involve an increase in the number of open interendothelial junctions (pores) per unit surface area, has not been examined. In thermal injuries, circulating histamine levels are increased (111), and may be a factor in the genesis of blood-brain barrier disruption and cerebral oedema from widening of interendothelial junctions that accompany severe burns (12).

Histamine may stimulate pinocytotic mechanisms and vesicular transport. Activation of this response could lead to formation of endothelial channels or chains of contiguous vesicles (143, 199, 204). Previously, Joó and colleagues have proposed that histamine stimulates vesicular activity by activation of adenylate cyclase in brain endothelia (121, 123). From this enzyme system, cyclic AMP, which may determine the energy level in the cell, is synthesised (125). Vesicular activity, and contractile processes, in brain endothelia, therefore, may be dependent on the formation of cyclic AMP. This mechanism has been discussed previously for the biogenic amine, serotonin (242).

Influences by circulating active agents on the permeability of water at cerebral blood vessels have not been well defined. Intra-arterial infusion of histamine in the present studies produced oedema in grey matter, which may reflect a change in water permeability. Four processes may be involved to explain an increase in brain tissue water content during histamine administration. First, histamine may directly affect membrane properties for water transport in cerebral endothelia. This mechanism has been proposed previously for skeletal muscle vessels (11) and for sheep lung (25). Second, histamine may stimulate elaboration of vasopressin which regulates sequestration of water from blood (76, 135). Vasopressin release is sensitive to histamine given systemically (51, 233), but it has not been shown that high intra-arterial levels of histamine, such as during carotid infusion, cause vasopressin release. Possible vascular mechanisms evoked by circulating histamine, as related to drinking behaviour and water balance, have been discussed by Leibowitz (135). Third, an increase in microvascular hydrostatic pressure would increase blood-to-tissue filtration (69, 90). A decrease in the ratio of pre- to post-capillary resistance, as is observed in skeletal muscle preparations during histamine infusion (46, 47, 89), could account for increased water movement by bulk flow from blood into the brain. These microcirculatory hemodynamic alterations, however, should be reflected by a change in the rate of tissue perfusion (11,46). Since an increase in cerebral blood flow was not seen during histamine infusion when the blood-brain barrier was intact, this mechanism, by itself, does not seem likely. Fourth, because histamine increases vascular permeability to circulating solutes, the osmotic pressure balance between plasma and interstitial fluid will favour a gradient toward brain. In this case, plasma water driven by vascular hydrostatic pressure, and osmotic

solutes entrained in the convective stream, would act synergistically to produce a "vasogenic" oedema (69).

Implications

A multiple capacity for forming, storing, and releasing histamine is present in the brain, especially in the vicinity of blood vessels (99, 117). In this section, I will discuss five circumstances in which histamine could have a role in influencing cerebral vascular permeability in vivo. The first four items (Figure 18) relate to sources of histamine that may be involved in initiating permeability changes. Finally, I will discuss the implications of histamine's effect on the permeability of brain blood vessels with reference to its participation in pathological and immunological responses.

Figure 18 depicts four possible conditions in which histamine could initiate changes in vascular permeability in vivo. First, histaminergic neurones are present in several brain regions (85, 219, 220). If activation of these neuronal path-ways produced significant changes in metabolic activity, then vascular permeability could be affected by vasoactive metabolites. Additionally, central neurones may innervate cerebral micro-vessels (56), and influence the level of local blood flow and vascular permeability (195). If histaminergic neurones have vascular innervation, then permeability of the blood-brain barrier may be under this form of neural regulation.

Second, histamine is present in cerebral perivascular mast cells (58, 116, 117), and may be released locally by adjacent nerve stimulation or by the presence of mast-cell releasing factors, such as antigens, kinins, and histamine itself (22, 187, 206). There is evidence from studies in skin that mast cell degranulation occurs explosively, and that the resulting

effects on vascular permeability and blood flow are reversible with time (68). Furthermore, it appears that mast cell histamine may influence vascular responses entirely through the H_2 class of histamine receptors located on the albuminal side of the vessel (30, 190). It is interesting that characteristics of the effects of histamine on brain vascular permeability as identified in the present studies, viz, reversibility within two hours, and predominant participation by H_2 receptors, are similar to those associated with mast cell degranulation in other organs.

Third, histamine has been identified within smooth muscle layers of brain blood vessels (67). This store of histamine, which may be important to neurovascular effects of histaminergic nerves, could serve as an instantaneous supply of histamine, participating in vascular responses during several conditions. Local smooth muscle levels of histamine may be sensitive to the metabolic state of the smooth muscle cells. If the catabolic enzyme for histamine, histamine-N-methyltransferase, were inhibited during a metabolic disturbance, then local levels of histamine may increase sufficiently to influence vascular permeability.

Fourth, focal and/or systemic levels of histamine may be increased during some pathological conditions. Migraine headache (5) and peripheral thermal injuries (111) are conditions in which increased circulating levels of histamine have been measured. Additionally, it is known that histamine is released from circulating basophils during immune and allergic reactions (187). Under these conditions, histamine may increase vascular permeability by stimulation of endothelial membrane receptors. The present experiments indicate that this effect would be mediated through H_2 receptors.

If histamine were to increase cerebrovascular permeability during such conditions as migraine headache, then

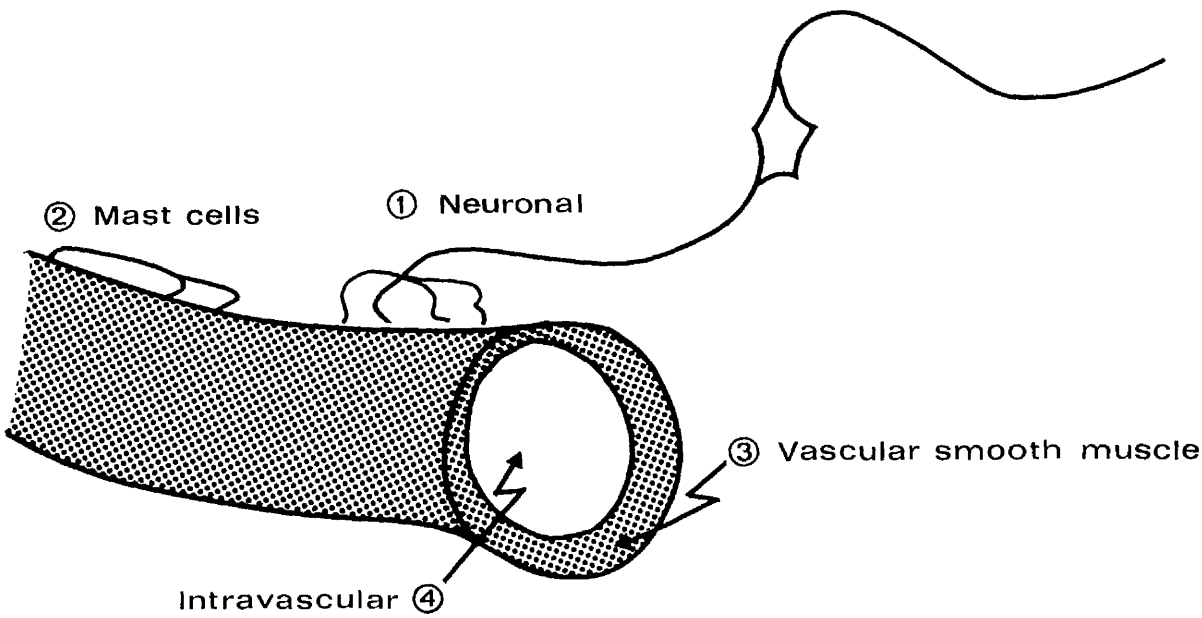

Figure 18. Possible sources of histamine that may influence cerebrovascular permeability in vivo. See corresponding text for comments and references.

the concentration in brain of other vasoactive, or metabolically active, substances may become important. An hypothesis involving the relevance of blood-brain barrier integrity has been proposed by Harper and associates (97) to explain the possible participation of several agents that might influence cerebral vascular or metabolic states during migraine.

The participation of histamine in inflammatory and immune reactions has been recognised for many years (22, 114, 140, 227, 243, 247). Although histamine may be involved as a modulator of cerebral vascular permeability in several pathological conditions (e.g., anaphylaxis, headache, head injury, ischaemia, tumour invasion), I will focus this part of the discussion on its role in the inflammatory process (e.g., for brain, meningitis, encephalomyelitis). The events occurring during inflammation resemble, or may be a part of, the sequelae of cerebral vascular responses associated with various types of brain pathology.

Inflammatory reactions typically involve histamine release from perivascular mast cells and circulating basophils (114, 187). The available histamine appears to evoke two types of effects that influence vascular permeability: "pro-inflammatory" and "anti-inflammatory" actions (187). Pro-inflammatory actions of histamine are principally designed to increase vascular permeability to facilitate the migration of complement, primarily leukocytes and immunoglobins, from blood to the abluminal site of inflammation (22, 186, 187). The anti-inflammatory action of histamine operates as a negative-feedback mechanism, such as by inhibiting further histamine release from mast cells and basophils, and by retarding lysosomal enzyme release from neutrophils (187). It is interesting that the two classes of histamine receptors can mediate individually separate steps in the inflammation process (186, 187). Brain vascular H_1 receptors, although not participating directly in permeability responses to circulating

histamine, may therefore have an important role in inflammation regulation.

If histamine were an important mediator of brain vascular permeability responses in inflammatory-like conditions, then it should be possible to clinically treat these diseases with specific histamine-blocking agents. For conditions in which anti-histamines have been used, such as in migraine headache (161), it has been disappointing that the drugs do not satisfactorily alleviate symptoms. Possible explanations for the failure of histamine antagonists in these conditions are: 1) mediators other than histamine, such as serotonin, may be more important to the vascular response and symptoms than histamine; 2) local levels of histamine may be higher than the effective capacity for blockade by the histamine antagonists in the doses used; 3) therapeutic blockade at only one type of receptor may not be sufficient. The present experiments show that H_2 receptors blockade can prevent permeability increases to arterially infused histamine. Under different conditions, however, both receptors may need to be blocked simultaneously for effective antagonism (40, 171); 4) the site from which histamine exerts its effect may not be readily determined or reached by clinical administration. If histamine is released perivascularly, for example, then pharmacological blockade by currently available H_2 receptor antagonists may not be possible because these agents do not readily pass the blood-brain barrier (219).

Discussion II. Cerebral Blood Flow.

The principal findings in the blood flow studies were 1) intra-arterial infusion of histamine, when the blood-brain barrier was intact, did not affect cerebral blood flow. Because arterial pressure decreased significantly, the maintenance of cerebral blood flow must be ascribed to autoregulatory dilatation of brain resistance vessels. Cerebral vascular resistance decreased significantly during histamine infusion; 2) after disruption of the blood-brain barrier, infusion of histamine caused dose-dependent decreases in cerebral vascular resistance, and increases in blood flow. Measurement of local brain blood flow with iodoantipyrine indicated that increases in blood flow occurred in several regions; 3) the increase in blood flow was mediated by both H_1 and H_2 receptors; and 4) the increase in blood flow with histamine was due to stimulation of vascular histamine receptors mediating dilatation, rather than a result of stimulation of cerebral metabolism which would have produced increases in blood flow secondarily.

Relevance of the Blood-Brain Barrier

The importance of the blood-brain barrier to circulating amines has been recognised for many years (59, 147). In the present studies, no effect of intra-arterial histamine on blood flow could be observed unless blood-brain barrier mechanisms were first disrupted by carotid injection of hypertonic urea. The failure of histamine to exert an effect on cerebrovascular smooth muscle when the blood-brain barrier was intact may be ascribed to two reasons. First, sufficient concentrations of histamine to produce vasomotor responses may not be obtained because histamine does not readily pass the morphological components of the blood-brain barrier, i.e., either through interendothelial junctions or via vesicular

transport. Second, circulating histamine that penetrates to the cytoplasm of brain vascular endothelial cells may be catabolised by degradative enzymes. This enzymatic mechanism of the blood-brain barrier has been clearly shown for the monoamines, dopamine, noradrenaline, and 5-hydroxytryptamine (16, 174). Monoamine precursors are actively transported into microvascular endothelial cells where they are decarboxylated to the respective amine, then deaminated by monoamine oxidase (174), a process which renders the amine inactive. For histamine, however, oxidation by monoamine oxidase is not a major pathway in the catabolic process (52). Ring methylation, by histamine-N-methyltransferase, and oxidative deamination, by diamine oxidase, are the principal routes for catabolism (13, 52, 211). Although histamine enzyme systems are present in the brain (85, 220, 231), it is not known to what extent they are localised to the vascular endothelium where they would have an important function in forming a blood-brain barrier to histamine.

In preliminary studies, I demonstrated that carotid injection of hypertonic urea caused a four-fold increase in vascular permeability to sucrose (molecular weight = 342 daltons). In a previous study in rats (92), it was shown that carotid injection of urea increased the brain uptake of noradrenaline (molecular weight = 168 daltons) by approximately four-fold. Thus, because histamine dihydrochloride has a similar molecular weight (184 daltons) to noradrenaline, vascular permeability to histamine in the present studies was probably increased by about four-fold. An increase in the permeability to histamine at the level of arterioles and metarterioles could explain the increases in cerebral blood flow during histamine infusion. Stimulation of histamine receptors on inner layers of vascular smooth muscle would appear to be the most likely mechanism for producing

dilatation. The possibility that histamine passed from blood to the outside of cerebral vessels to evoke an effect seems unlikely because 1) brain arterioles appear to have predominantly H_2 receptors distributed along outer surfaces (238), and 2) histamine infusion was not associated with detectable increases in brain uptake of glucose (see below). Because both classes of receptors participated in dilatatory responses to infused histamine. I speculate that H_1 and H_2 receptors are distributed equally along inner layers of smooth muscle in brain arterioles.

Several previous studies in baboons have demonstrated the importance of blood-brain barrier mechanisms in determining cerebral circulatory responses to arterial administration of amines. Noradrenaline, 5-hydroxytryptamine, and low doses of phenylethylamine are without significant effect on cerebral blood flow unless blood-brain barrier mechanisms are first disrupted by hypertonic solutions of enzymatic inhibitors (98, 145, 153). From the results of the present studies, histamine can be added to this category of putative neurotransmitter substances having cerebral vasomotor effects that depend on the integrity of the blood-brain barrier (147).

Regionality

The regional pattern of cerebral blood flow responses to arterial infusion of histamine may derive from two anatomical factors: the distribution of histamine delivery in blood to the cerebrovascular bed, and the distribution of vascular histamine receptors in each brain structure.

Infusion of histamine into the internal carotid artery in rats provides for delivery of the drug primarily to brain regions in the distribution of the middle cerebral artery (100, 246). In the present studies,

hypothalamus, thalamus, caudate nucleus, hippocampus, and parietal cortex were the structures in which increases in blood flow occurred during histamine infusion. Each of these regions is supplied by branches of the middle cerebral artery (246). It cannot be determined from the present studies whether there were differences in magnitude of vascular response between white and grey matter structures. In regions not supplied by blood from the internal carotid and middle cerebral arteries, i.e., brainstem and cerebellum, there were no responses to histamine infusion. In summary, therefore, these studies indicate that histamine increases blood flow in brain regions where the blood-brain barrier is disrupted and where histamine is infused.

In brain structures where blood flow responses to histamine were detectable, there was no particular uniformity in the blood flow increase according to structure. A difference in regional responsiveness may arise from differential localisation of vascular receptors for histamine in the brain regions examined. For example, for each of the histamine agents infused (Tables 10-11), parietal cortex showed the greatest increase in blood flow. An approximate order of responsiveness may be ranked as follows: parietal cortex > caudate nucleus > hippocampus > diencephalon. During stimulation of H_1 receptors by pyridylethylamine, however, relatively larger increases in blood flow to hypothalamus and thalamus occurred (Table 11). This pattern may suggest a denser population of H_1 receptors in blood vessels of the diencephalon.

Receptors

Identification of receptors involved in cerebral blood flow responses to histamine was approached along two methodological lines. In the first series involving the xenon clearance method, it was demonstrated that histamine could produce increases in blood flow after

the blood-brain barrier was disrupted with urea. Blood flow responses to histamine were reduced when animals were pretreated with specific blocking agents, either the H_1 receptor antagonist, mepyramine, or the H_2 receptor antagonist, metiamide. Both of these blocking agents are known to be specific antagonists for their respective receptors (52). In the second series of studies using the iodoantipyrine tissue sampling method, the results showed that carotid infusion of histamine or specific receptor agonists after blood-brain barrier disruption increased cerebral blood flow. Regional responses to histamine, pyridylethylamine (H_1 receptor agonist) and dimaprit (H_2 receptor agonist) were of approximately the same magnitude. Taken together, the results of the above two studies indicate that both H_1 and H_2 receptors are present in cerebral vessels, and that both classes of receptors can mediate vascular dilatation.

Several studies have been undertaken to determine whether histamine receptors are present in brain tissue (85, 219), but it is not clear if the receptors are partitioned between neuronal elements and the vasculature. Both receptor types have been identified in brain homogenates and slices (220), and, in a recent study, it was shown that stimulation at both receptors could activate adenylate cyclase (188). Formation of cyclic AMP may be important to molecular mechanisms that could influence vascular smooth muscle (1, 4). Histidine, and the metabolic enzymes for histamine, which would be important to the regulation of receptor activity in vivo, are present in all of the brain regions which demonstrated blood flow responses to histamine in the present studies (231).

Mechanism

Increases in organ blood flow may result from one or both of two possible mechanisms. The metabolic demand of the organ may increase, which would produce

secondarily an increase in organ perfusion, or resistance vessels in the organ may dilate under extrinsic neural or chemical stimulation (1, 76). Previously, for most of the putative neurotransmitters examined to date, it has been demonstrated that increases in cerebral blood flow in response to arterial infusion of the agents have been associated with concomitant increases in cerebral metabolic rate. Noradrenaline (145), dopamine receptor agonists (151, 155), and vasoactive intestinal peptide (154) can be categorised as agents which appear to stimulate cerebral metabolism, producing, secondarily, increases in cerebral blood flow. Acetylcholine, which is also a possible neurotransmitter in the central nervous system, increases cerebral blood flow without detectable increases in metabolic rate (105); presumably, this amine stimulates cholinergic receptors in cerebrovascular smooth muscle to produce vascular dilatation.

The results of the present studies suggest that histamine causes dilation of cerebral vessels without influencing metabolic rate in the brain. The increases in blood flow probably resulted from direct stimulation of vascular histamine H_1 and H_2 receptors. Infusion of histamine into the internal carotid artery after blood-brain barrier disruption did not increase brain up-take of glucose, as determined by the 2-deoxyglucose method. Infusion of noradrenaline, however, did produce increases in glucose utilisation, indicating that the method could detect changes in metabolic demand if they occurred. The magnitude of change in glucose uptake evoked by noradrenaline is similar to that determined previously in baboons (145), and to the effects on cerebral blood flow in rats after blood-brain barrier disruption (60).

In available literature concerning the effect of histamine in the cerebral circulation, there is

considerable confusion about the effect this amine may have on vessels supplying, and within, the brain. Several earlier reports indicated that intra-arterial infusion of histamine increases cerebral blood flow in different species (Table 13). In these studies, measurements were obtained 1) from extracranial vessels supplying the brain, and/or 2) in species such as dogs and cats, animals in which major vessels serving the brain are known to anastamose with extracranial tissues (118). Both of these circumstances may lead to errors in interpretation. Concerning the first point, histamine-induced dilatation of the internal carotid artery, for example, would not necessarily be associated with dilatation of vessels distal to the circle of Willis. This separation of vascular responses has been clearly shown for the vaso-constrictor substance, 5-hydroxytryptamine (98); in baboons, intracarotid infusion of 5-hydroxytryptamine caused marked constriction of the internal carotid artery, but did not reduce cerebral blood flow unless the blood-brain barrier was first disrupted. Cerebral vascular autoregulatory mechanisms would explain this phenomenon. Likewise, a similar series of vascular events may apply to histamine: intracarotid infusion probably dilates the internal carotid artery, but, as shown in the present studies, and in those by Eidelman et al. (64), cerebral blood flow does not increase. Concerning the second point above, species differences in vascular response may account for some of the confusion. These differences may involve the distribution and density of histamine receptors in cerebral vessels, blood-brain barrier permeability, or vascular anatomy. In studies using dogs, however, there are serious limitations in examining cerebral blood flow responses to intra-arterial drug administration (105).

The molecular mechanisms by which histamine induces vascular smooth muscle responses are not yet clear (34).

Table 13. STUDIES EXAMINING EFFECTS OF HISTAMINE ON CEREBRAL BLOOD FLOW

Investigators	Reference	Species	Method	Route	Dose	Flow Response	Receptors
1. Anderson & Kubicek, 1971	(3)	Dog	Electromagnetic (e-m) flowmeter (basilar)	i.v.	20 µg/kg	145% increase	Not tested (NT)
2. Watters, 1971	(239)	Monkey	Angiography	i.v.	50 µg/kg	"marked" dilatation	NT
3. Carpi et al. 1972	(33)	Dog	Venous outflow	i.v.	4 µg	increase	NT
4. Tindall & Greenfield, 1973	(232)	Man	e-m flowmeter (common carotid)	Common carotid	14 µg	59% increase	NT
5. Saxena, 1975	(217)	Dog	e-m flowmeter (common carotid)	Common carotid	6 µg/kg	400% increase	H_2*
6. Muravchick & Bergofsky, 1976	(159)	Cat	Perfused brain	Basilar	3 µg/kg	About 75% increase	NT
7. Spira et al. 1978	(228)	Monkey	e-m flowmeter (Common & external carotid)	Common carotid	4 µg/kg/min	-30% vascular resistance	H_1 and H_2
8. Eidelman et al. 1979	(64)	Monkey	^{133}Xe clearance	Internal carotid	10 µg/kg/min	No change	NT
9. Present study		Rat	^{133}Xe clearance	Internal carotid	6-60 µg/kg/min	27-50% * increase	H_1 and H_2
10. Present study		Rat	^{14}C-iodoantipyrine + tissue sampling	Internal carotid	20 µg/kg/min	10-30% * increase	H_1 and H_2

* After blood-brain barrier disruption.

In vitro studies in cultured neural cells and nerves indicate that stimulation of H_1 receptors is associated with facilitated Ca^{2+} influx, glycogenolysis, and synthesis of cyclic GMP (219). Stimulation of H_2 receptors is strongly linked to cyclic AMP (85, 158, 219). In the rabbit ear artery preparation, responses to stimulation of H_1 receptors are mediated by efflux of K^+ and release of Ca^{2+} from intracellular stores, while responses to stimulation of H_2 receptors are not dependent on K^+ flux and are associated with diminished release of Ca^{2+} from intracellular stores (34).

Implications

The interactions of histamine with cerebral vascular smooth muscle in vivo appear to be complex. They depend on three types of cellular storage, two distinct receptors, the integrity of the blood-brain barrier, and the particular brain region stimulated.

Histamine is richly concentrated in mast cells located along the adventitial border of brain blood vessels (58, 116). Should histamine be released from these cells, potent vasoactive effects could ensue. A nonmast cell pool of histamine is found within cerebrovascular smooth muscle (67). No specific function has yet been associated with this source of histamine but it is possible that nonmast cell histamine modifies effects of cerebrovascular nerves. Histamine is also found within neural tissue (85, 220). There is strong evidence that this source of histamine participates in synaptosomal transmission (85, 220). If histaminergic nerves innervate cerebral blood vessels, then vasomotor regulation could be influenced by neural withdrawal or stimulation, as has been proposed for peripheral reflexes (14, 31, 190), and central adrenergic neurones (195).

The present studies suggest that histamine may be

important to cerebral vascular function in pathological conditions where its release is likely to occur. Brain tissue trauma (247) or inflammation (114, 227) could involve liberation of histamine. Under these circumstances, vascular dilatation may result from stimulation of both classes of histamine receptors.

Discussion III. Pial Vessels in Situ

Two series of studies were performed to characterise the responses of pial arterioles and veins to stimulation of histamine receptors. In the first series, comparisons were made between responses of arterioles and veins to application of histamine agents. Two important results emerged from these studies: 1) arterioles responded to injection of histamine H_1 and H_2 receptor agonists, suggesting the presence of H_1 and H_2 receptors in brain resistance vessels, and 2) veins were unresponsive to histamine agents, in contrast to arterioles which showed distinct responses. Part of the discussion will focus on factors that could account for these results.

In the second series, vasoconstrictor responses to noradrenergic stimuli were examined during simultaneous application of the histamine agents to determine whether significant interaction occurred between the putative neurotransmitters, noradrenaline and histamine. Stimulation of histamine receptors interfered with noradrenergic effects at pial arterioles and veins. The loci of interaction, and the implications of these results for neural control of cerebral vessels, will be discussed in this section.

Histamine Agonists

Arterioles. The results demonstrate that histamine, and each of the histamine receptor agonists, produces dilatation of pial arterioles when the drug is given by perivascular microinjection. Histamine produced increases in arteriolar calibre up to +28% at a concentration of 10^{-4}M, results that confirm previous findings in another laboratory (238). The new information in this study, however, was that an order of potency for dilatation can be established when the three agents for stimulation of histamine receptors are considered together: H_2 > histamine > H_1. Thus,

in contrast to the conclusion of Wahl and Kuschinsky (238), the present experiments provide experimental evidence for the location of histamine H_1 receptors on the outside of brain arterioles. Furthermore, specific stimulation of H_2 receptors produced stronger dilatations of pial arterioles than did histamine itself.

Because histamine H_1 receptors have been previously identified in brain tissue preparations (219), it is not surprising that H_1 receptors may be localised in pial arterioles. Both H_1 and H_2 receptors have been functionally identified in most preparations for studying cardiovascular responses to histamine (171). The conclusion by Wahl and Kuschinsky (238) that only H_2 receptors mediate histaminergic dilatation of brain arterioles was made on the basis of studies using histamine receptor blocking agents. In the present experiments, specific receptor agonists were used. The smaller response of pial arterioles to H_1 receptor stimulation may be related to the density of this type of receptor on the outside of the vessel wall. It is possible that H_2 receptors are more densely populated, and H_1 receptors more sparsely populated, along the outer layers of smooth muscle in brain arterioles. A suggestion for this kind of arrangement of histamine receptors in skeletal muscle and cutaneous vessels has been made previously (82, 190).

In these studies, histamine produced smaller responses to perivascular injection than did the H_2 receptor agonist, impromidine. Two factors may account for this difference. First, histamine may act preferentially at H_1 receptors (30, 54), which, due to their more sparse distribution or lower potency, mediate smaller responses. Second, impromidine has very high and specific affinity for H_2 receptors (55). Previous studies have demonstrated that impromidine is substantially more potent than histamine for

producing responses in the cardiovascular system (172). It is interesting that, at the doses of impromidine and histamine producing maximal effects on pial arteriolar diameter (10^{-6}M and 10^{-4}M, respectively), there is also near maximal activation of adenylate cyclase in preparations of hippocampal homogenates or slices and in cortical microvessel fractions (101, 125, 234). The potential importance of histamine-sensitive nucleotide systems in brain, and possibly cerebral vascular regulation, has been reviewed recently (220).

Veins. Pial veins were not responsive to stimulation of histamine receptors. Over the two concentrations studied, each of the histamine agents produced very weak dilatations or no response. This absence of vascular responsiveness contrasts with the results in pial arterioles, which proved to be very responsive to stimulation of histamine receptors (Figure 11). Cerebral venous responses to histamine receptor stimulation also differ somewhat from responses in peripheral veins. In skeletal muscle and skin vein preparations, histamine has been shown to be vasoactive, although the nature of vascular response has not been consistent, even within the same species. In hindlimb preparations in dogs, for example, histamine can increase (89, 189) or decrease (46, 47) venous resistance. In skin, muscle and mesentery, histamine produces contraction of veins (79, 124, 221).

Three factors may explain the difference in vascular response between cerebral arterioles and veins, and between vein responses to histamine in brain and peripheral organs. First, the amount of smooth muscle available for vasomotor control is clearly less in venous segments (2, 76). Thus, if all other factors are not important, thickness of the smooth muscle wall may be critical to the magnitude of

vascular response to histamine. Second, veins may lack tone, due to lower luminal pressure which may passively render these vessels unresponsive. It is also possible that exposure of the pial circulation to atmospheric pressure, as in the open skull preparation, contributes to reduction of wall tone, more in veins than in arterioles. In vessel preparations in vitro, histamine mediates contraction of cerebral vessels with low tone, and relaxation of vessels precontracted with serotonin (57). In vivo, however, veins normally have low wall tension (2, 76) which may be important to their response to circulating and perivascular agents. Third, histamine receptors in cerebral veins may be poorly distributed, or absent. The capacity for response to agents which stimulate histamine receptors may not exist in the cerebral venous circulation.

Interaction of Histamine Receptors With Noradrenergic Stimuli

These studies indicate that stimulation of histamine receptors interferes with the effects of noradrenaline in pial arterioles and veins. Histamine and noradrenaline mediated opposite effects on pial arteriolar calibre; histamine produced dilatation of pial arterioles, and noradrenaline, either from sympathetic nerve stimulation or exogenous administration, constricted vessels. When the stimuli were applied simultaneously, histamine inhibited noradrenergic constriction of arterioles via both H_1 and H_2 receptors during sympathetic stimulation, and via only H_2 receptors during application of noradrenaline.

In veins, stimulation of histamine receptors prevented constriction during sympathetic stimulation, but had no influence on the venous response to injected noradrenaline. This discussion will consider lines of evidence from previous studies to suggest loci of interaction at the neuroeffector junction, and how

this mechanism may operate *in vivo*.

Inhibition of arteriolar constriction during sympathetic nerve stimulation was produced by simultaneous application of either the H_1 or the H_2 receptor agonist. This result could occur from one or both of two mechanisms: the contrictor effect of nerve stimulation, and the dilator effect of histamine receptor stimulation, may have been additive, thereby cancelling their respective effects on vascular smooth muscle; or, histamine receptors on presynaptic nerve endings may inhibit the release or enhance the re-uptake of the transmitter, noradrenaline. Although the first possibility cannot be ruled out, there is evidence from other studies that histamine can interfere with noradrenergic mechanisms at sympathetic nerve endings. In dog saphenous vein strips *in vitro*, McGrath and Shepherd (156) demonstrated that the amount of radioactive noradrenaline released during sympathetic stimulation could be reduced by activation of histamine H_2 receptors. Enhancement of noradrenaline reuptake via histaminergic mechanisms could not be demonstrated because cocaine, which inhibits noradrenaline uptake, did not alter the responses seen with H_2 receptor stimulation alone (156). Inhibition by histamine of vasconstrictor effects to sympathetic stimulation has also been observed in the dog gracilis muscle and hindpaw preparations (191). In these studies, Powell (191) demonstrated that H_2 receptors were responsible for interference in noradrenergic transmission at pre-junctional sites. Powell also showed that vasoconstrictor responses in the hindpaw to injected noradrenaline were attenuated by histamine, suggesting a postjunctional site of inhibition (191).

These conclusions differ from those of Bevan and co-workers (17) who suggested that histamine potentiated, rather than inhibited, sympathetic effects in rabbit

basilar arteries in vitro. These investigators showed that contractile responses to transmural stimulation and serotonin were greater when histamine was added to the bath medium. The histamine effect could be blocked by an H_1 receptor antagonist (17). It is not clear from their studies, however, whether the vessels were in a contracted state at the time of measurement. It has been shown previously that histamine mediates relaxation of cerebral arteries that are precontracted, and contraction of vessels that are not given tone (57). Thus, the results of Bevan et al.(17) may have been influenced by the level of tone in their vessels before study. Support for this argument is provided by results for veins in the present studies. Veins have less wall tension (tone) than arterioles (2, 76); application of histamine agents simultaneously with noradrenaline in cerebral veins did not prevent the noradrenergic constrictor effect. Indeed, in some vessels, responses to noradrenaline were larger during combination with histamine or the H_1 agonist (Figures 15 and 16).

Venous constrictor responses to sympathetic stimulation were abolished by each of the histamine agents. Because veins did not respond to injection of histamine agonists alone, these results provide further support for the presence of inhibitory histaminergic mechanisms, involving both receptor types, at sympathetic presynaptic sites.

The involvement of histamine in autonomic nervous mechanisms in peripheral vascular beds has been illustrated previously by Ryan, Brody and colleagues (31, 215). In Figure 19 are presented schematic diagrams to summarise the potential relationships between histamine receptors and noradrenergic stimuli in cerebral vessels.

In Panel A is shown the anatomical arrangement from which interaction between histamine and noradrenaline

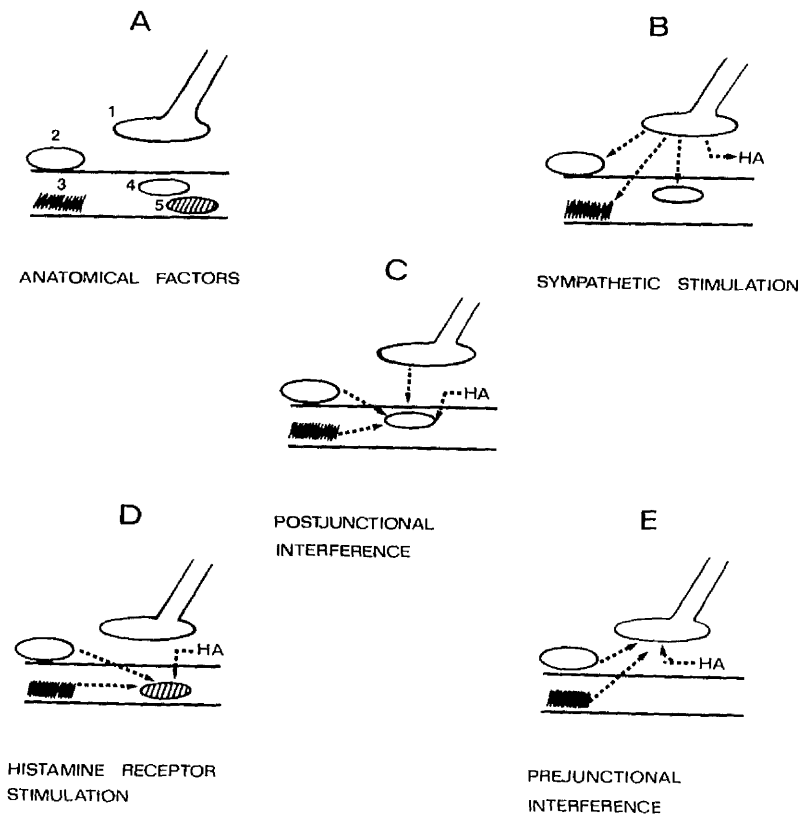

Figure 19. Schematic diagrams depicting possible mechanisms of interaction between histamine and sympathetic nerves on cerebral vessels. See text for corresponding discussion. 1: sympathetic nerve; 2: mast cell; 3: non-mast cell pool of histamine; 4: noradrenergic post-junctional receptor; and 5: histamine receptor.

could occur. A sympathetic nerve ending, perivascular mast cell, nonmast cell histamine within vascular smooth muscle (58, 67, 173), and receptor sites for noradrenaline (130) and histamine (238; Figures 12-14) are present. Panel B demonstrates a possible endogenous mechanism which could involve liberation of histamine resulting from sympathetic nerve stimulation. Histamine is contained in sympathetic nerves (61, 214), and may be released with noradrenaline during activation of the nerves. The released noradrenaline may disrupt mast cell membranes (206), liberating histamine, and may have a role in activating mobilisation of the nonmast cell store of histamine from vascular smooth muscle (215). Noradrenaline produces constriction of pial arterioles by interaction with adrenergic receptors on the outside of the vascular wall (130). In Panel C, the available histamine interferes with noradrenaline stimulation at the adrenergic receptor site. As demonstrated in the present studies, and in those by Powell (191), this response is mediated by H_2 receptors in arterioles, but does not apply to veins. Panel D shows histamine interacting with histamine receptors in smooth muscle. By itself, this mechanism always produces dilatation of pial arterioles (238; Figures 12-14), but no response in veins (Figures 15-17). Panel E depicts the prejunctional inhibitory mechanism involving histamine. Histamine liberated from any of the three possible sources by sympathetic nerve stimulation interacts at prejunctional sites, probably inhibiting the release of transmitter (191). H_2 receptors (156, 191), and H_1 receptors, as shown in the present experiments, participate in this mechanism in both arterioles and veins.

Implications

Sympathetic nerves and histamine stores are located in close proximity to cerebral blood vessels.

Although sympathetic innervation is dense (173), the effects of nerve activation under normal conditions may not be important (102, 193). It is conceivable that histamine release accompanies stimulation of sympathetic nerves and modulates the resulting influence on vascular tone.

Possible roles for the store of nonmast cell histamine found within the smooth muscle wall have been difficult to identify (30). In vascular studies in the dog, Powell and Brody (190) provided evidence that release of the smooth muscle store of histamine involved activation of both H_1 and H_2 receptors. Mast cell histamine, however, may interact only with H_2 receptors (190). These findings, and the results of the present studies, provide a speculative basis for the concept that nonmast cell histamine interacts with prejunctional adrenergic mechanisms in cerebral vessels.

Discussion IV. Hypothesis: Differential Localisation of Histamine Receptors in Cerebral Vessels.

In recent years, there has been a growing interest and experimental evidence for specialised localisation of receptors within the walls of blood vessels (50, 53, 126). Identification of receptor sites in blood vessels could provide an important basis for clarifying physiological mechanisms and facilitating pharmacological manipulation in the treatment of vascular disorders. To date, available reports have addressed only the distribution of adrenergic receptors in peripheral blood vessels (50, 53, 70). In this section, findings from the three series of studies will be synthesised to produce a unifying concept for the thesis, viz, there is nonuniform spatial distribution of histamine H_1 and H_2 receptors within the cerebral vascular wall (Figure 20).

In the studies evaluating the effect of histamine on the blood-brain barrier, histamine was infused into the internal carotid artery of rats, and vascular permeability was determined using radioactive sucrose and an arterial integral method. Histamine increased permeability of the blood-brain barrier in several brain regions. By use of histamine receptor blocking agents, it was demonstrated that the effect of histamine was mediated through H_2 receptors, located probably within the vascular endothelium. An H_1 receptor blocking agent did not significantly affect the permeability response to histamine. The studies demonstrate, therefore, that intravascular histamine can increase the permeability of cerebral vessels by stimulation of H_2 receptors in endothelial cells. This conclusion is supported by recent neurochemical studies which demonstrated that H_2 receptors primarily mediate the activation by histamine of adenylate cyclase in cerebral capillaries (125). H_2 receptors,

therefore, may be predominantly localised in cerebral endothelia (Figure 20).

Two lines of evidence from the present studies indicate that H_1 and H_2 receptors are distributed equally within layers of cerebral vascular smooth muscle closest to the lumen (Figure 20). Following blood-brain barrier opening, histamine infusion into the internal carotid artery of rats produced increases in blood flow that could be attenuated by either an H_1 receptor antagonist or an H_2 receptor antagonist. Also, following blood-brain barrier opening, infusion into the internal carotid artery of histamine H_1 or H_2 receptor agonists produced increases in cerebral blood flow that were of similar magnitude. The results suggest that increases in brain blood flow produced by circulating histamine could be mediated by stimulation of both H_1 and H_2 receptors. The response was unrelated to cerebral metabolism, suggesting that the effect of histamine involved stimulation of vascular receptors. Both classes of receptors mediate vascular responses to intra-arterial histamine in peripheral organs (30, 171).

A greater sensitivity for dilatation to histamine on the outside of blood vessels is mediated primarily through H_2 receptors. In cat pial arterioles (238), and in rabbit ear arteries (82), perivascular stimulation of histamine H_2 receptors produces greater responses than stimulation from the luminal side of the vessel (82). These findings were confirmed in the present studies by use of specific histamine receptor agonists which were applied perivascularly to pial arterioles in cats. Stimulation of H_2 receptors produced larger dilatatory responses than did stimulation of H_1 receptors. The results suggest that H_2 receptors are preferentially localised in the outer layers of cerebral vascular smooth muscle (Figure 20).

In summary, the studies suggest the following profile of histamine receptor distribution in cerebral vessels: 1) endothelia: predominantly H_2 receptors; 2) "inner" smooth muscle: both H_1 and H_2 receptors; and 3) "outer" smooth muscle: predominantly H_2 receptors (Figure 20).

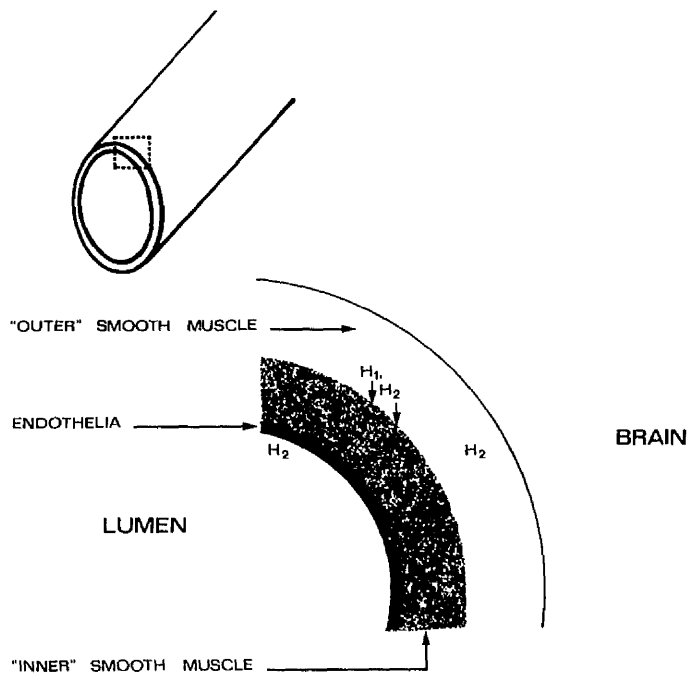

Figure 20. Differential localisation of histamine receptors within the cerebral vessel wall.

Conclusions

Infusion of histamine into the internal carotid artery in rats increased the permeability of brain blood vessels to circulating solutes. Characteristics of the permeability increase were: 1) the response occurred in several brain structures, and in several areas of cerebral cortex; 2) the response was mediated through histamine H_2 receptors; 3) the response was reversible within two hours; and 4) cerebral oedema accompanied the increase in solute permeability.

When the blood-brain barrier was intact, infusion of histamine into the internal carotid artery in rats did not affect cerebral blood flow. When the blood-brain barrier was open, histamine produced concentration-dependent increases in cerebral blood flow. Blood flow responses to histamine were mediated through both classes of histamine receptors (H_1 and H_2). Increases in blood flow were the result of direct stimulation of vascular histamine receptors, and were not due to stimulation of cerebral metabolism.

Perivascular injection of specific histamine receptor agonists in pial arterioles of cats revealed the presence of both H_1 and H_2 receptors. Stimulation of H_2 receptors produced more potent responses than stimulation of H_1 receptors. Pial veins did not respond to the histamine agents.

Stimulation of histamine receptors interfered with noradrenergic constriction of pial arterioles and veins. Constriction of arterioles and veins during sympathetic nerve stimulation was prevented by simultaneous stimulation of H_1 or H_2 receptors. Arteriolar constriction to exogenous noradrenaline was prevented by H_2 receptors, but not by H_1 receptors. Neither class of histamine receptor affected venoconstrictor responses to exogenous noradrenaline.

The studies indicate several loci at which histamine receptors can mediate or modify vascular responses in the brain. In cerebral endothelial cells, H_2 receptors are important; in smooth muscle, both receptor types mediate dilatation, but vascular responses may depend on the direction (intravascular or perivascular) from which the histamine stimulus arises; finally, both receptor types can modify adrenergic neurovascular mechanisms.

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