OPIATE MODULATION OF THE PREOVULATORY LUTEINIZING HORMONE SURGE IN THE RAT

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LIST OF ABBREVIATIONS

ACH	acetylcholine
ACTH	adrenocorticotrophic hormone
ADH	aldehyde dehydrogenase
ADR	adrenaline
AHA	anterior hypothalamic area
AR	aldehyde reductase
ARN	arcuate nucleus
BSA	bovine serum albumin
CA	catecholamine
CNS	central nervous system
COMT	catechol-O-methyltransferase
DA	dopamine
DHBA	dihydroxybenzylamine
DHPG	3,4-dihydroxyphenylglycol
DOMA	dihydroxymandelic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DOPEG	3,4-dihydroxyphenylglycol (DHPG)
DMN	dorsomedial nucleus
DUR	Duromorph
D+N	Duromorph and naloxone
E_2	oestradiol
ECD	electrochemical detection
EDTA	ethylenediaminetetra-acetic acid
β-END	β-endorphin
EOP	endogenous opioid peptide
EPI	epinephrine (ADR)
EW	egg white

FSH	follicle stimulating hormone
GABA	γ-aminobutyric acid
GnRH	gonadotrophin-releasing hormone (LHRH)
HA	histamine
HPLC	high performance liquid chromatography
5HIAA	5-hydroxyindoleacetic acid
5HT	serotonin
HVA	homovanillic acid
ICV	intracerebroventricular
IgG	immunoglobulin G
IP	intraperitoneal
IS	internal standard
LC	locus coeruleus
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
MAO	monoamine oxidase
MBH	medial basal hypothalamus
ME	median eminence
MHPG	3-methoxy,4-hydroxyphenylglycol
MOPEG	3-methoxy,4-hydroxyphenylglycol (MHPG)
MPO	medial preoptic area
MPS	modified physiological saline
NA	noradrenaline
NAL	naloxone
NaOH	sodium hydroxide
NE	norepinephrine (NA)
NM	normetanephrine
NMDA	N-methyl-D-aspartate
NPY	neuropeptide Y

NRS	normal rabbit serum
NSB	non-specific binding
6-OHDA	6-hydroxydopamine
OVX	ovariectomized
P	progesterone
PCP	phencyclidine
PeVN	periventricular nucleus
PNMT	phenylethanolamine-N-methyltransferase
POA	preoptic area
PPS	precipitating serum
PRL	prolactin
PVN	paraventricular nucleus
SAL	saline
SC	subcutaneous
SCH	suprachiasmatic nucleus
SD	serum diluent
SEM	standard error of the mean
SKF	SKF10047 (N-allylnormetazocine)
SON	supraoptic nucleus
S+N	SKF10047 and naloxone
SP	substance P
T	testosterone
THF	tetrahydrofuran
TIF	Tifluadom
T+F	Tifluadom and naloxone
VIP	vasoactive intestinal peptide
VNAB	ventral noradrenergic bundle
VMA	vanillylmandelic acid
VMN	ventromedial nucleus

SUMMARY

Endogenous opioid peptides have a profound inhibitory influence on the secretion of luteinizing hormone. Recently, it has been proposed that a reduction in the activity of these peptides in the hypothalamus may be the initial neural stimulus for the generation of the preovulatory luteinizing hormone surge which induces ovulation. Furthermore, this action may involve alterations in the activity of monoaminergic neurones which synapse on the luteinizing hormone-releasing hormone neurones in the hypothalamus. This investigation was undertaken to determine the effects of exogenous agonists of various opioid receptor subtypes on, (i) the magnitude of the preovulatory luteinizing hormone surge, and (ii) the activity of monoaminergic neurotransmitters in the hypothalamic areas which contain luteinizing hormone-releasing hormone neurones at the time when the surge is initiated.

First, agonists of μ-, κ- and σ-opioid receptors, and an opioid antagonist, were administered intraperitoneally to pro-oestrous rats at 12.30h. The animals were decapitated at either 14.30h or 18.00h on the same day. Trunk blood was collected, and the hypothalamic areas were microdissected.

In the second set of experiments, the minimum dose of each of the agonists which reduced the surge were co-administered (as above) with the minimum dose of the antagonist which

increased the surge. The rats were subsequently treated as in the first set of experiments.

The third set of experiments involved intraperitoneal injection of the opiates at 12.30h on pro-oestrus, and the rats being returned to the Animal Unit for daily vaginal lavages.

Finally, rats were injected, as in the second set of experiments, anaesthetized and serial blood samples collected throughout the late afternoon and early evening of pro-oestrus.

It was found that all three agonists could reduce the magnitude of the preovulatory surge. However, the σ-agonist failed to do so significantly. Prevention of the surge on pro-oestrus by administration of the agonists did not result in the surge being delayed by 24 hours. The antagonist markedly increase the surge amplitude, and this was inhibited by each of the agonists.

Inhibition of the luteinizing hormone surge was consistently associated with elevated noradrenaline activity in the medial preoptic area and the median eminence at 14.30h. Enhancement of the surge amplitude by the antagonist occurred in association with increased serotonin activity in the same two areas, and, on some occasions, with increased dopamine activity in the medial preoptic area.

In conclusion, there is a heterogeneous population of receptors which, when activated, suppress the secretion of luteinizing hormone-releasing hormone. It appears that this inhibition may involve alterations of the activity of noradrenaline projections to the luteinizing hormone-releasing hormone neurones in the medial preoptic area and median eminence. Furthermore, opioid antagonist enhancement of the surge may be mediated by serotonin projections to these neurones in the same two areas.

INTRODUCTION

OESTROUS CYCLE OF THE RAT

As in the human, the rat is a spontaneous ovulator, but has a very short oestrous cycle of four or five days. This is comprised of two or three days of dioestrus and one each of pro-oestrus and oestrus (Long and Evans, 1922).

Throughout early dioestrus plasma oestradiol-17β (E_2) secretion from the Graafian follicle is low, and increases slowly during late dioestrus. On the morning of pro-oestrus, E_2 reaches its peak circulating levels. This is the primary stimulus for the preovulatory luteinizing hormone (LH) surge from the anterior pituitary on the afternoon of pro-oestrus. The causal relationship between E_2 levels and the LH surge has been clearly demonstrated by the ability of antiserum to E_2 to abolish the surge (Ferin *et al*, 1970). The LH surge itself stimulates ovulation, which occurs soon after midnight between pro-oestrus and oestrus.

The profiles of secretion of LH, E_2 and progesterone (P) during the oestrous cycle are illustrated in Figure 1.

In general, follicle stimulating hormone (FSH) is secreted at low levels throughout the oestrous cycle, except for a marked increase which occurs around the time of ovulation. This FSH surge accelerates the development of follicles for the succeeding cycle.

If mating does not occur, the corpora lutea do not become functional and only a small amount of P is secreted. No luteal phase occurs and new follicles are ovulated as soon as they are sufficiently mature.

Luteinizing Hormone Secretion Profile During the Oestrous Cycle

There are two patterns of LH release that typify the secretion of this hormone over the oestrous cycle: basal (or episodic) and surge (or phasic) release. LH is not secreted at a steady rate, but rather as a series of ultradian pulses. The frequency and amplitude of these pulses depend upon the time of day (circadian) and the stage of the oestrous cycle (cyclic) (Leipheimer *et al*, 1985).

When rats are kept in a lighting schedule of 14 hours light and 10 hours dark, the frequency and amplitude of the LH pulses increases on pro-oestrus. This initiates a slow rise in plasma LH concentrations approximately seven hours after the onset of the light. The increase in plasma LH levels continues and accelerates to a plateau some two to four hours later (Blake, 1976). This is the characteristic preovulatory LH surge.

The Steroid-Primed Ovariectomized Rat as a Model for the Preovulatory LH Surge

Ovariectomy removes the gonadal steroid feedback to the hypothalamus and anterior pituitary and results in the loss of oestrous cycles. However, the administration of E_2 (and P) to ovariectomized (OVX) rats can stimulate a preovulatory-type LH surge, which can be timed more reliably than the spontaneous surge of intact animals. The E_2 + P-primed OVX model has been used extensively in investigations of the control of surge release of LH.

CENTRAL REGULATION OF LUTEINIZING HORMONE SECRETION

Neural regulation of anterior pituitary hormone secretion was first demonstrated by the ability of coitus to induce ovualtion in rabbits (Brook, 1938). Although the existence of a portal circulation between the hypothalamus and pituitary had earlier been demonstrated (Popa and Fielding, 1930), it was not until 1947 that the existence of a neurovascular link between the two was postulated (Green and Harris, 1947).

The involvement of a neural signal in the control of the rat LH surge was first demonstrated by pharmacological manipulation on the afternoon of pro-oestrus. The LH surge was delayed by 24 hours (Everett *et al*, 1949), but only if the drugs were injected during a particular time period. Thus the existence of a 'critical period' was postulated, during which the neural stimulus for the surge occurred. After this

time, the surge was found to be resistant to pharmacological blockade.

It has since become clear that the anatomical site of this neural stimulus lies within the hypothalamus. In 1955, the first demonstrations of hypothalamic releasing factors were reported, with the isolation of a peptide which could stimulate the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Guillemin, 1955; Saffran and Schally, 1955). Later, the existence of a hypothalamic peptide with a similar releasing action on LH was determined (Guillemin *et al*, 1963; Schally and Bowers, 1964).

The Hypothalamus

The hypothalamus consists of neural tissue situated beneath the thalamus and is composed of two major areas: the medial and lateral areas (Raisman and Field, 1971).

The medial area is itself subdivided into three groups:-

(i) an anterior group of nuclei consisting of the anterior hypothalamic area (AHA), medial preoptic area (MPO), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCH) and the supraoptic nucleus (SON),

(ii) a tuberal group of nuclei comprised of the ventromedial nucleus (VMN), dorsomedial nucleus (DMN), periventricular

nucleus (PeVN), arcuate nucleus (ARN) and the lateral tuberal nuclei.

(iii) a posterior group of nuclei formed by the posterior hypothalamic nucleus , the supramammillary and tuberomammillary nuclei and the periventricular system.

The lateral hypothalamic area forms the lateral border of the hypothalamus and is the site at which the medial forebrain bundle enters the hypothalamus. This nerve tract contains the projections between the hypothalamus and the rest of the brain.

The median eminence (ME) lies directly below the ARN in the hypothalamus and is the final common pathway through which the neural control of LH secretion is exercised (and that of the other anterior pituitary hormones). The nature of this control is addressed in detail below.

Figure 2 illustrates the anatomical relationship of the hypothalamus and the anterior pituitary.

Luteinizing Hormone-Releasing Hormone

The direct stimulus for the secretion of LH from the anterior pituitary, including the preovulatory surge, is luteinizing hormone-releasing hormone (LHRH or gonadotrophin-releasing hormone; GnRH) since immunoneutralization of LHRH results in abolition of the rat ovulatory cycle (Fraser *et al*, 1978).

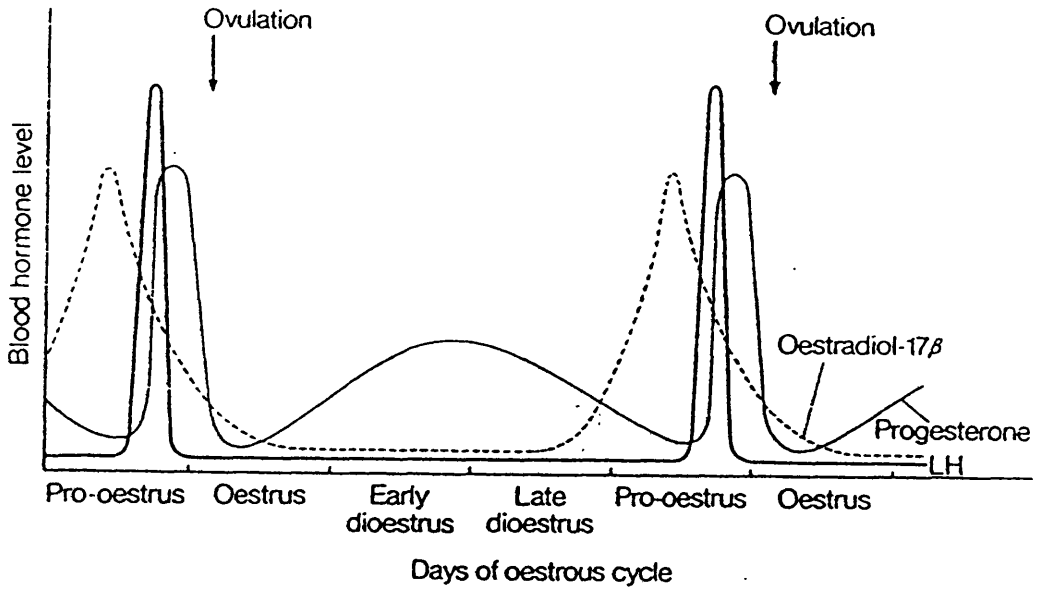


Figure 1. Schematic representation of the hormone changes in the systemic circulation of the rat throughout the oestrous cycle. From: Short, 1972.

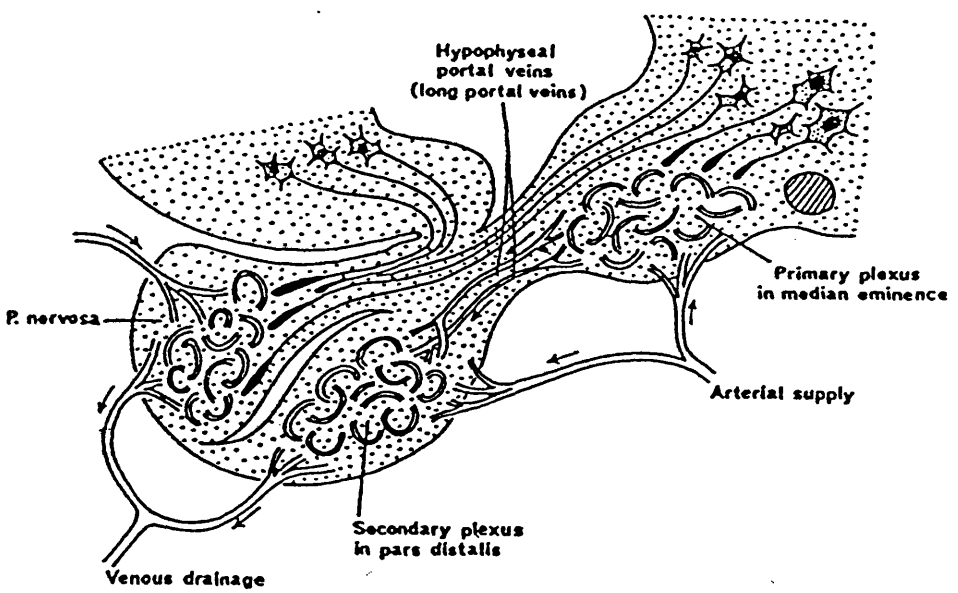


Figure 2. Diagram of the anatomical basis of the neuro-humoral relationship between the hypothalamus and the anterior pituitary (pars distalis). From: Perry, 1971.

LHRH is released from the ME into the primary plexus of the hypophyseal portal blood system, where it is transported through portal veins to a second capillary bed in the anterior pituitary. Here it enters the anterior pituitary and stimulates the release of both LH and FSH from gonadotrophs.

The peptide sequence of LHRH was first determined from porcine hypothalami (Schally *et al*, 1971) and, soon afterwards, from ovine hypothalami (Burgess *et al*, 1971) as the decapeptide: pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. To date only one molecular form of mammalian LHRH has been isolated, despite the characterization of several molecular forms from non-mammalian vertebrates (King *et al*, 1988).

In the rat, high numbers of perikarya and fibres containing LHRH have been visualized in the MPO and high concentrations of LHRH itself have been found in the ARN, SCH and ME; in the latter two areas the LHRH was restricted to fibres alone (Palkovits *et al*, 1974; Shivers *et al*, 1983a; Witkin *et al*, 1982).

LHRH levels in portal blood have been measured and correlated to the LH surge in anaesthetized (Sarkar *et al*, 1976) and conscious rats using the push-pull perfusion technique (Levine and Ramirez, 1982). These experiments demonstrate clearly that LHRH release is increased on the afternoon of pro-oestrus and that this increase precedes the LH surge.

A luteinizing hormone-release inhibiting factor has been isolated from rat hypothalamus and shown to prevent LHRH-stimulated LH release (Hwan and Freeman, 1987). It is postulated that progesterone (P) may increase the amplitude of the LH surge by freeing the pituitary gonadotrophs from tonic restraint of the release-inhibiting factor. However, administration of a P antagonist has been shown to enhance the activation of LHRH neurones on the afternoon of pro-oestrus (Lee *et al*, 1990). It would appear that P increases the amplitude of the E_2-elicited surge by enhancing LHRH neurone response to E_2 and, perhaps, also by reducing the outflow of an LH-release inhibiting factor.

In addition to its effects at the hypothalamic level, E_2 also increases pituitary responsiveness to the LHRH signal at the time of the preovulatory LH surge. This is achieved by increasing LHRH receptor numbers, at least partially, by increasing the number of LHRH-receptive cells (Lloyd and Childs, 1988).

NEUROTRANSMITTERS CONTROLLING LHRH SECRETION

According to one theory, the LHRH neurones intrinsically release LHRH and the profile of secretion is modified by external influences (Estes *et al*, 1982). However, much evidence now points to basal and surge release of LHRH being orchestrated by inputs from other neurones.

LHRH neurones concentrate neither E_2 (Shivers *et al*, 1983b) nor P (Fox *et al*, 1990) and so the influence of these steroids must be indirect. E_2 excites some non-tuberoinfundibular neurones in the ARN (Nishihara and Kimura, 1989) and spontaneous electrical activity in the ARN is increased on the afternoon of pro-oestrus (Yeoman and Jenkins,1989). Presumably, the ARN is the site of interneurones which are responsible for the monitoring, and transmitting of information on the steroid milieu to the LHRH neurones.

Noradrenaline

A large volume of evidence suggests that noradrenaline (NA) may be the major neurotransmitter which directly stimulates LHRH neurones to initiate the LH surge.

Three major NA systems project to the hypothalamus; the locus coeruleus (LC), an ascending NA tract with projections to the MPO and ME via the dorsal noradrenergic bundle (Jones and Moore, 1977), and the dorsal and lateral tegmental systems which innervate many areas of the hypothalamus including the SCH, MPO, ME and ARN through the ventral noradrenergic bundle (VNAB; see Figure 3).

There is close apposition of NA nerve terminals and LHRH cells in the MPO and to a lesser extent in the ME and ARN (McNeill and Sladek, 1978), and catecholaminergic neurones innervate LHRH neurones in the MPO (Watanabe and Nakai, 1987). Thus, it appears that there are direct functional

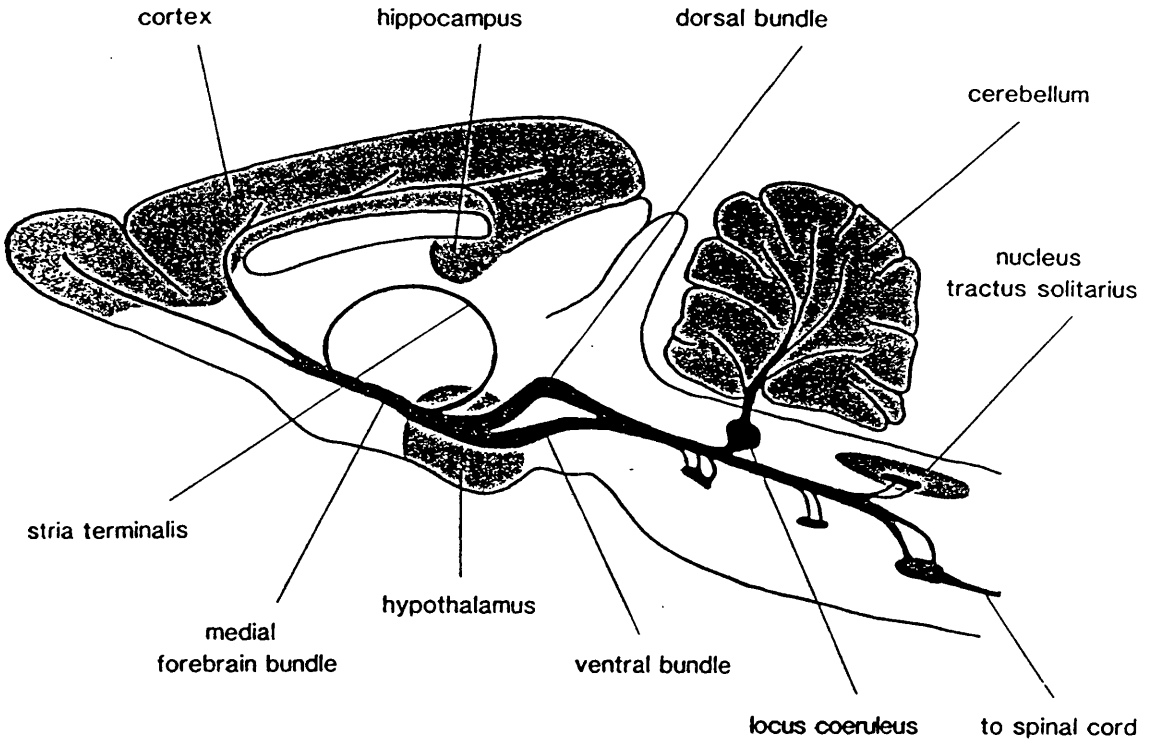


Figure 3 (a). Saggital section showing the noradrenergic projections in the rat brain. From: Rang and Dale, 1987.

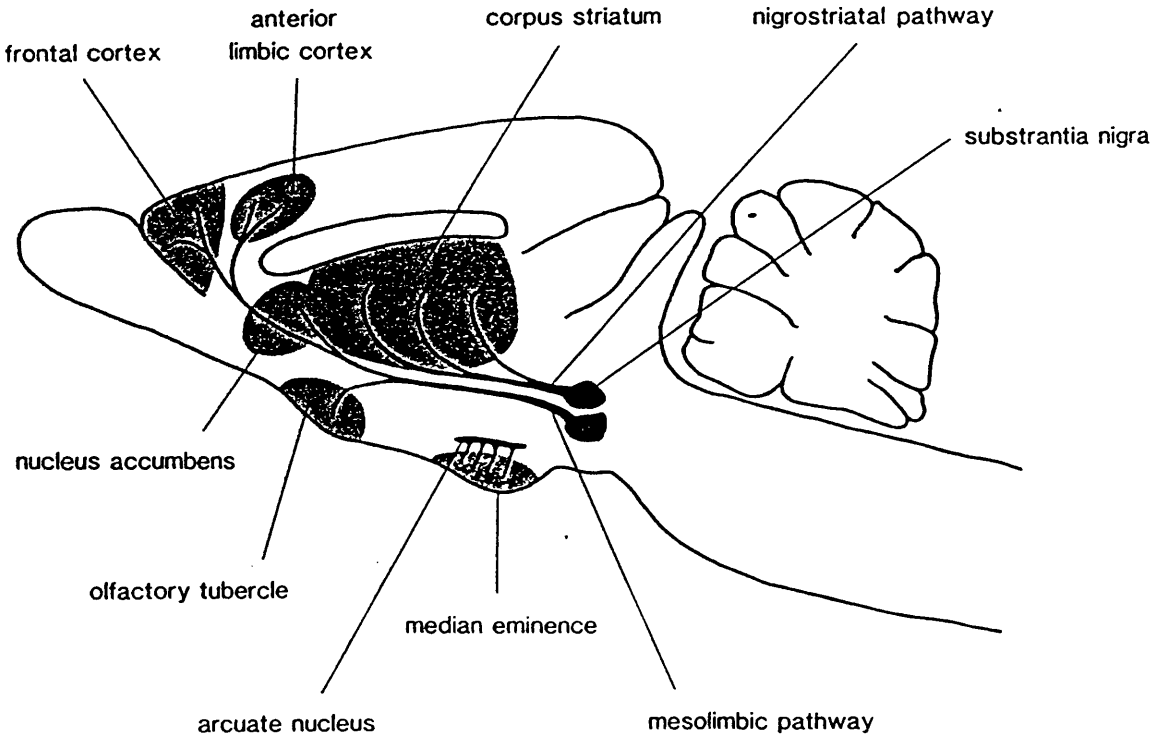


Figure 3 (b). Saggital section showing the dopaminergic projections in the rat brain. The main groups of perikarya and fibre tracts are shown in black. Grey areas show the location of axon terminals. From: Rang and Dale, 1987.

connections between the two systems. Receptors for NA are divided into two major classes, α- and β-adrenoreceptors, based on the relative affinities of various adrenergic agonists and antagonists for each. These are further subdivided into α_1- and α_2- and β_1- and β_2-adrenoreceptors on the same basis. All four of the above classes of adrenoreceptors have been visualized in the hypothalamus (Leibowitz *et al*, 1982). Both types of α-adrenoreceptor were found in highest concentrations in the MPO, ME and ARN while β-adrenoreceptors (predominantly β_2) were most common in the MPO.

NA activity in discrete hypothalamic nuclei was first investigated by Crowley and co-workers (Crowley *et al*, 1978). Later, it was shown that, during the E_2-induced LH surge in OVX rats, increased NA turnover in the SCH, MPO, ME and ARN was associated with rising or peak levels of circulating LH (Wise *et al*, 1981). This relationship was subsequently found to hold for NA turnover in the ME and the preovulatory LH surge, while in the MPO increased NA turnover was associated with peak but not rising LH levels (Rance *et al*, 1981). Furthermore, prevention of increased NA turnover using phenobarbital was shown to be associated with failure of the LH surge on pro-oestrus (Rance and Barraclough, 1981).

There are also reports of inhibitory effects of NA on the LH surge. Electrochemical stimulation of the locus coeruleus (LC), on pro-oestrus blocked the LH surge (Dotti and Taleisnik, 1982); an effect which could be prevented by

administration of a β-adrenergic antagonist (Dotti and Taleisnik, 1984).

Similar work later showed that the release of LHRH induced by direct stimulation of the MPO could be enhanced by LC electrical stimulation (Gitler and Barraclough, 1987) and that this could be prevented by α- but not β-adrenoreceptor antagonists (Gitler and Barraclough, 1988). Furthermore, a stimulatory β-adrenergic component has been identified in the control of the LH surge (Al-Hamood *et al*, 1985). It would appear that NA can influence LHRH secretion in both a positive and a negative fashion, the former action being mediated by α- and β-, and the latter by β-adrenoreceptors. The current consensus is that NA is stimulatory to LHRH secretion in the presence of E_2 but inhibitory in its absence (for review see Ramirez *et al*, 1984).

Our understanding of synaptic transmission cannot explain this change in NA actions on LHRH secretion since excitatory synapses cannot become inhibitory and *vice versa*. Therefore, other influences must be postulated to explain the complex behaviour of NA in the control of LHRH secretion; these will be reviewed later in the Introduction.

While there can be little doubt of the influence of NA on LHRH secretion, it is difficult to reconcile the temporal relationship between increasing NA turnover in hypothalamic nuclei containing LHRH neurones with the premise that this is the initial neural stimulus for the preovulatory LH surge.

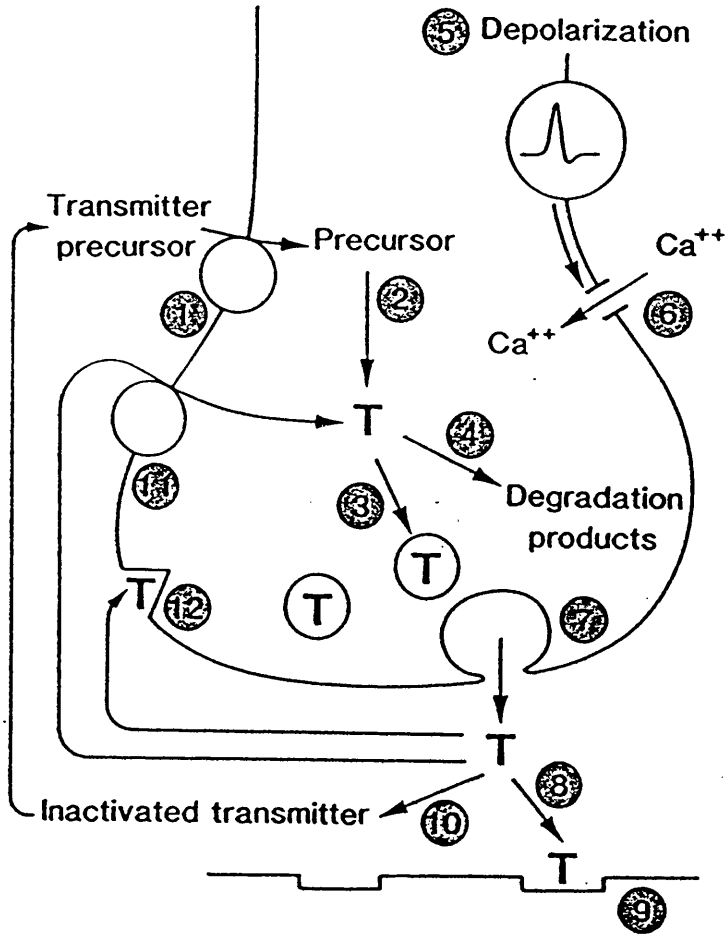


Figure 4. The main processes involved in synthesis, storage and release of the aminergic neurotransmitters (including NA, DA and 5HT): (1) Uptake of transmitter precursor by nerve terminals, (2) Synthesis of transmitter in nerve terminals, (3) Storage of transmitter in releasable form, (4) Degradation of surplus transmitter within nerve terminals, (5) Depolarization of nerve terminal by propagated action potential, (6) Influx of Ca^{2+} ions in response to depolarization, (7) Release of transmitter, (8) Diffusion of transmitter to post-synaptic receptors, (9) Interaction with receptors and production of post-synaptic effect, (10) Inactivation of transmitter within synaptic cleft, (11) Reuptake of transmitter or of degradation product by nerve terminals, (12) Interaction of transmitter with pre-synaptic receptors. From: Rang and Dale, 1987.

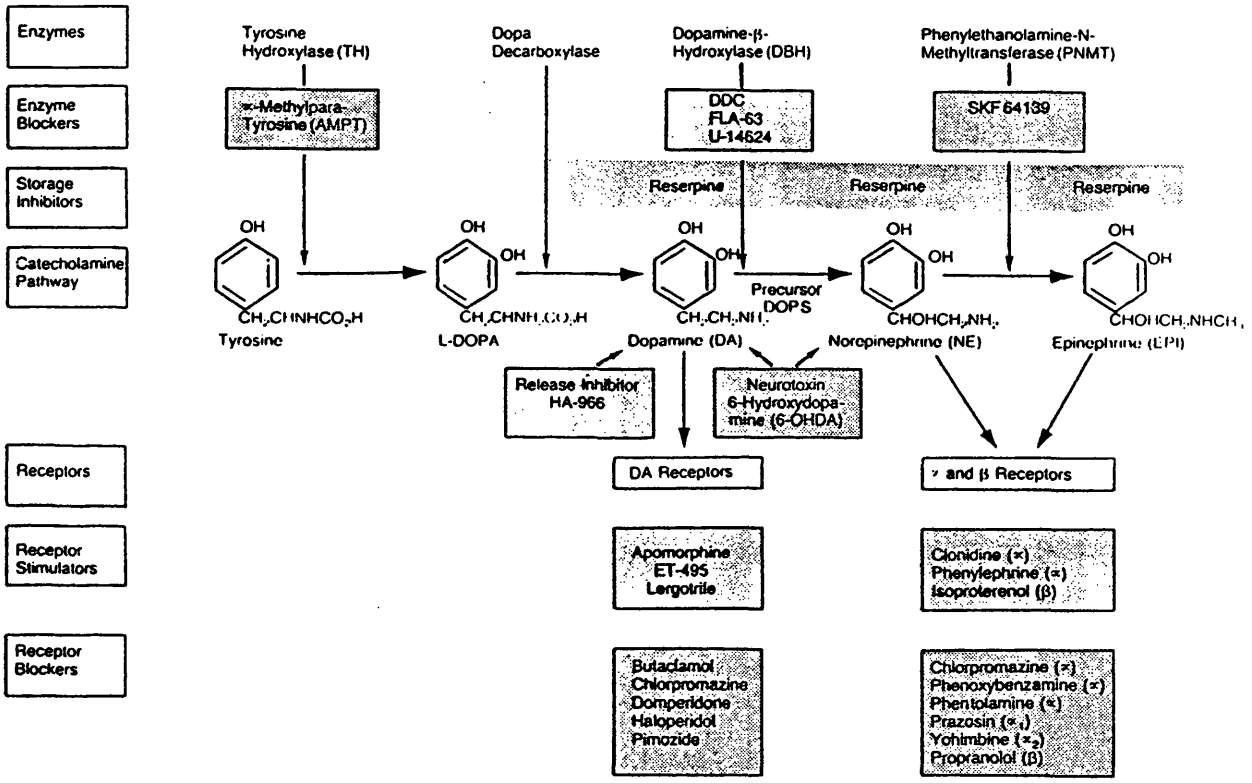


Figure 5. Biosynthetic pathway of the catecholamines showing some drug manipulations that can alter their synthesis or action. From: Ramirez *et al*, 1984.

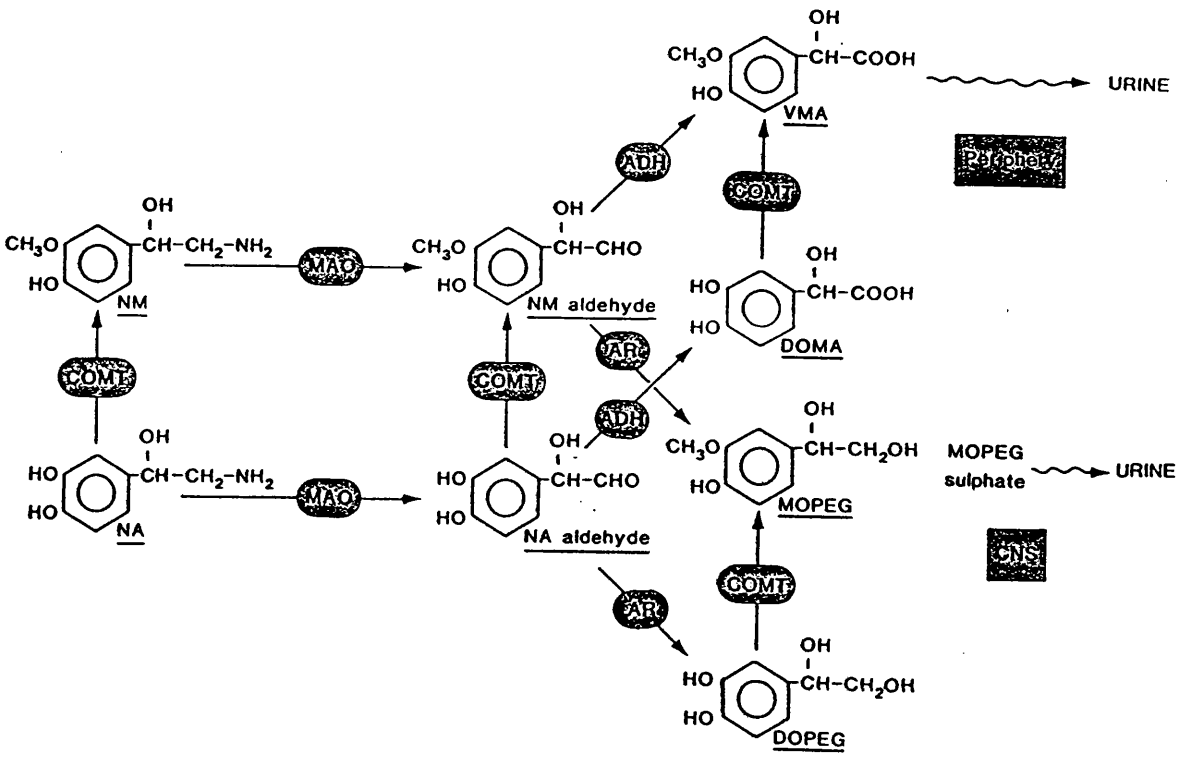


Figure 6 (a). The main pathways of NA metabolism in the brain and periphery. From Rang and Dale, 1987.

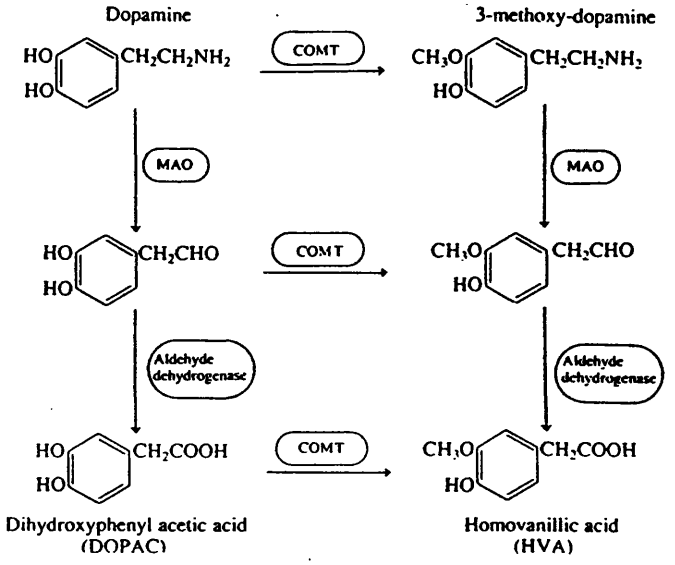


Figure 6 (b). The main pathways of DA metabolism in the brain. From: Rang and Dale, 1987.

Dopamine

Neurones of the tuberoinfundibular dopamine (DA) system originate in the ARN and PeVN, and project to the internal and external ME (Lindvall and Bjorklund, 1982). Those that terminate in the external layer secrete DA into the hypophyseal blood system to inhibit prolactin (PRL) secretion from anterior pituitary lactotrophs (MacLeod, 1976). However, those terminating in the internal layers appear to be involved in controlling the secretion of releasing hormones, including LHRH (Sawyer and Clifton, 1980). Ninety per cent. of the tyrosine hydroxylase-containing cells of the ARN express P receptors (Fox *et al*, 1990), which suggests that these cells may be involved in mediating the feedback effects of P to the hypothalamus.

A second group of DA neurones project to the hypothalamus; the incertohypothalamic DA tract. This is of extrahypothalamic origin and projects principally to the MPO. E2 + P priming has been demonstrated to have a positive feedback effect on incertohypothalamic DA neurones (Sanghera *et al*, 1991). Thus both DA systems may be involved in the feedback effects of steroids to elicit the LH surge.

Receptors for DA are currently divided into three classes: D_1, D_2 and D_3. DA receptors have been visualized in the hypothalamus (Leibowitz *et al*, 1982). The ME and ARN were found to contain a moderate density of these receptors, while the SCH and MPO were only sparsely populated.

Conflicting evidence has been provided concerning the role of DA in the regulation of LHRH secretion, with both stimulatory and inhibitory actions being reported (Kalra and Kalra, 1983, for review). There appears to be an anatomical basis for the differential actions of DA on the release of LHRH. DA is stimulatory in the MPO (Kawakami *et al*, 1975) and zona incerta (ZI) (MacKenzie *et al*, 1988), but inhibitory in the ARN and ME (Fuxe *et al*, 1977). This suggests that the incertohypothalamic tract is stimulatory, and the tuberoinfundibular tract is inhibitory to LHRH release.

Decreased spontaneous activity of DA neurones has been found in the ME on the afternoon of pro-oestrus (Kerdelhue *et al*, 1989) without administration of drugs altering DA metabolism. Furthermore, E2 + P treatment has a positive feedback effect on incertohypothalamic neurones (Sanghera *et al*, 1991). From this a physiological role for DA in the control of the LH surge can be inferred; a decrease in its inhibitory activity, and an increase in its stimulatory activity both contributing to the stimulation of the surge.

Serotonin

The hypothalamus is densely innervated by 5HT projections from the raphe nuclei (Steinbusch, 1981). Populations of 5HT receptors have been visualized in the MPO and ARN in addition to several other hypothalamic areas (Biegon *et al*, 1982), including the ME (Villar *et al*, 1984). 5HT axons terminate on

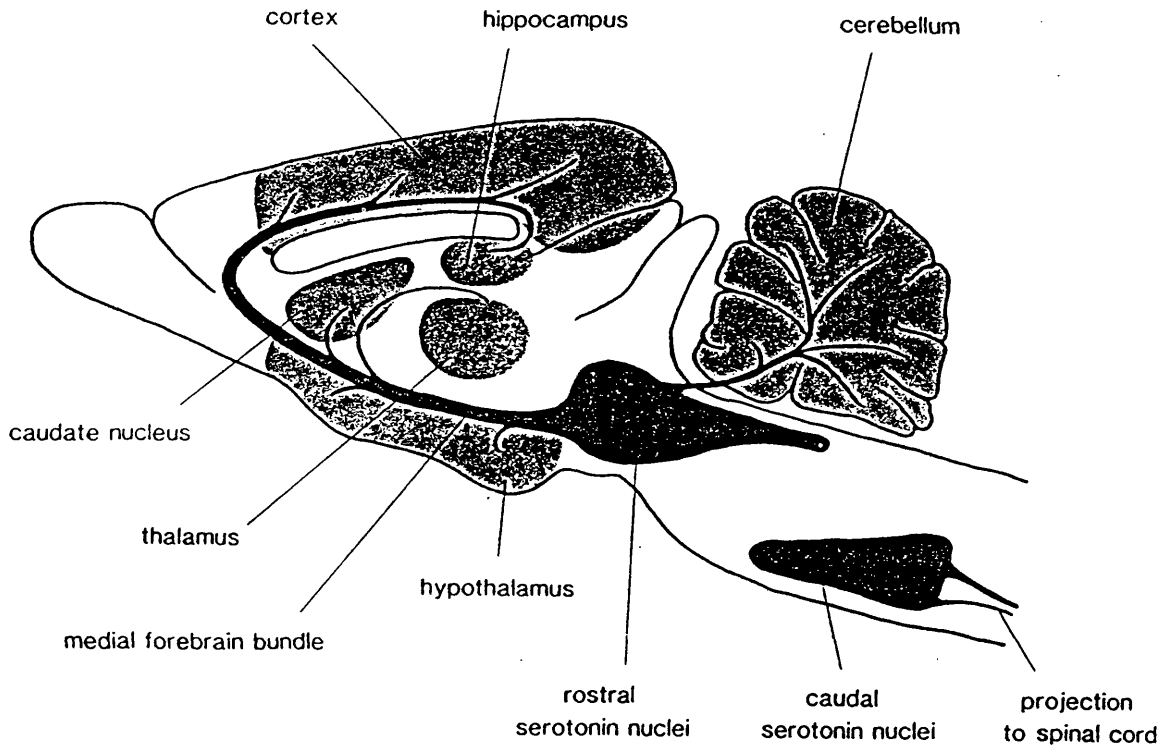


Figure 7. Saggital section through the rat brain showing the serotoninergic pathways. The main groups of 5HT cell bodies and fibre tracts are shown in black. Grey areas show the locations of 5HT nerve terminals. From: Rang and Dale, 1987.

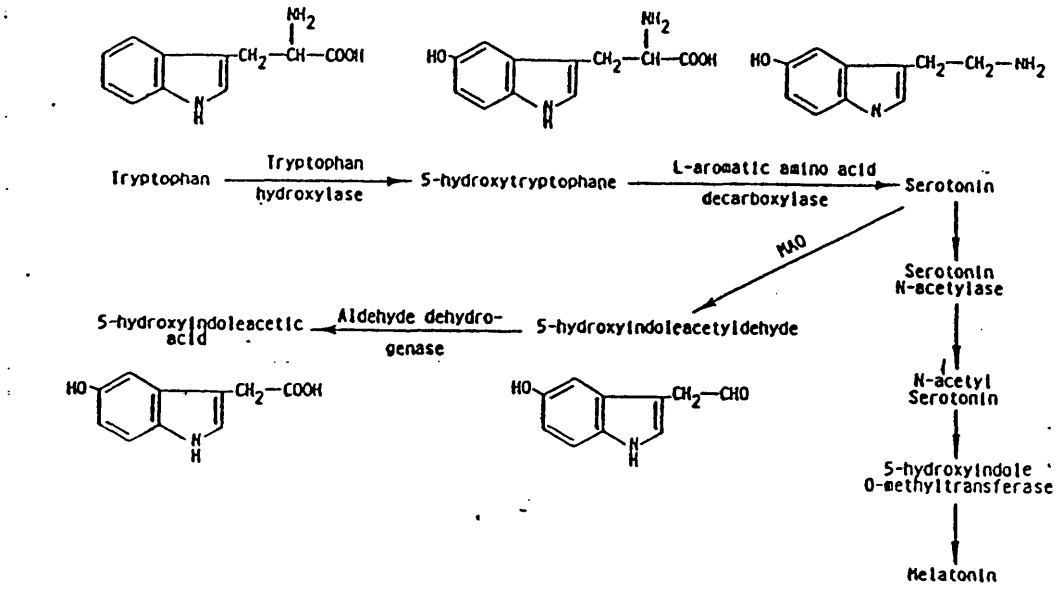


Figure 8. Metabolic pathways of the biosynthesis and breakdown of 5HT. From: Johnston, 1990.

LHRH neurones in the POA (Kiss and Halasz, 1985). There is also evidence of 5HT innervation of tuberoinfundibular neurones in the ARN (Willoughby and Blessing, 1987). Thus 5HT may have both direct and indirect effects on LHRH neurones.

Inhibition of LH secretion has been reported after injection of 5HT into the third ventricle of female rats (Schneider and McCann, 1970), and the LH surge has been blocked after stimulation of the medial raphe nucleus on pro-oestrus (Morello and Taleisnik, 1985); an effect which appears to be mediated by GABAergic neurones (Morello *et al*, 1989). However, 5HT activity in the ME is increased on the afternoon of pro-oestrus (Kerdelhue *et al*, 1989), and E2 + P treatment of ovariectomized (OVX) rats increases 5HT synthesis in the MPO (King *et al*, 1986). This suggests a stimulatory role for 5HT on LH release. *In vitro,* P enhancement of the E_2-induced LH surge in OVX rats can be blocked by 5HT-antagonists (King and Kang, 1988). It may be that the progesterone enhancement of the LHRH neurone response to E_2 is mediated by 5HT.

Endogenous Opioid Peptides

The opium alkaloid, morphine, was first isolated from the poppy, Papaver somniferum, by Seturner in 1803 and has long been known to have widespread actions on many physiological systems (Bernard, 1864).

In the early 1970's a stereospecific binding site for morphine was identified (Goldstein *et al*, 1971) which led to

the isolation and characterization of the first endogenous opioid peptides (EOP's) discovered, leu- and met-enkephalin (Hughes *et al*, 1975). EOP's have since been extensivley investigated and many more have been identified; the major classes being roughly divided as endorphins, enkephalins and dynorphins/neo-endorphins which are derived from three precursors: pro-opiomelanocortin (POMC), pro-enkephalin A and pro-enkephalin B (for review see Hollt, 1983).

In addition to the multiplicity of EOP's, there are various subtypes of opioid receptor: μ-, κ-, σ-, δ- and ε. There is further support for additional divisions of the μ- and κ-classes (for review, see Dougall, 1988).

The opioid nature of the σ-receptor has been brought into question since cocaine also binds to these receptors (Sharkey *et al*, 1988), and a non-opioid σ-receptor has been identified in the guinea-pig myenteric plexus (Roman *et al*, 1988). However, more recent evidence suggests that a σ-opiate receptor does exist and is distinct from the non-opioid σ-receptor (Holtzman, 1989). These opioid (high affinity) σ-receptors have been visualized in high concentrations in the hypothalamus, while PCP-receptors were found to be relatively sparse (Largent *et al*, 1986).

EOP's are ubiquitous neurotransmitters throughout the central nervous system (CNS). High concentrations of β-endorphin (β-END) and leu- and met-enkephalin have been found in the SCH, MPO, ME and ARN, as well as in many other hypothalamic and

Endogenous Ligand	μ	κ	δ
β-Endorphin	+++	+++	+++
leu-enkephalin	+	+++	0
met-enkephalin	++	+++	0
dynorphin A	++	+	+++

Table 1. Affinity of the most common EOPs for the major opioid receptor subtypes (high: +++; moderate: ++; low: +; none: 0).

extrahypothalamic sites. In the SCH and MPO the enkephalins were found to be contained in perikarya of EOP neurones (Hokfelt *et al*, 1977; Watson *et al*, 1982). In the ME, the enkephalins (Watson *et al*, 1982; Williams and Dockray, 1985) and β-END (Mezey *et al*, 1985) were visualized solely in fibres. Perikarya and fibres of enkephalin- (Hokfelt *et al*, 1977; Watson *et al*, 1982; Williams and Dockray, 1985) and β-endorphin-containing (Mezey *et al*, 1985) cells have been visualized in the ARN. Several other less common EOPs have also been found in these areas (for review see Palkovits, 1988).

Opioid receptors are widespread in the CNS. High numbers of κ-receptors have been shown to exist in the preoptic area with fewer numbers of μ- and δ-receptors present. In the ME

CNS Region	μ	κ	δ
Telencephalon			
Frontal cortex	+++	++	+
Piriform cortex	++	++	++
Entorhinal cortex	++	++	++
Amygdala			
Central nucleus	0	0	++
Medial nucleus	+++	++	++
Lateral nucleus	++++	+++	+++
Hippocampal formation			
Hippocampus	+++	++	+
Dentate gyrus	+++	+	+
Olfactory tubercle	+	+++	+++
Nucleus accumbens	++++	++++	+++
Caudate-putamen	++++	++++	+++
Globus pallidus	+	+	+
Medial septum	+++	+	+
Bed nucleus stria terminalis	++	++	+++
Preoptic area	+	+	++++
Diencephalon			
Hypothalamus			
Supraoptic nucleus	0	0	++
Paraventricular nucleus	0	0	++
Arcuate nucleus	0	0	++
Ventromedial nucleus	0	+	+++
Dorsomedial nucleus	+	0	+++
Lateral hypothalamic area	+	0	++
Thalamus			
Periventricular area	0	0	+++
Central-medial nucleus	++++	+	++
Reuniens nucleus	++++	+	++
Medial habenula	+++	+	+++
Mesenencephalon			
Interpeduncular nucleus	++++	+++	+++
Substantia nigra			
Pars compacta	+++	0	0
Pars reticulata	++	+	+
Ventral tegmental area	++	0	+
Periaqueductal grey	+	0	++
Superior/Inferior colliculi	++++	+	++
Dorsal raphe nucleus	++	0	++
Pons/medulla			
Parabrachial nucleus	+++	0	++
Nucleus raphe magnus	++	0	+
Nuc. retic. gigantocellularis	+	0	+
Nucleus tractus solitarius	++++	+	+++
Lateral reticular nucleus	+	0	+
Spinal trigeminal nucleus	+++	0	++
Spinal Cord			
Substantia gelatinosa	+++	+	++

Table 2. Regional distribution of opioid receptors in the rat brain (very dense: ++++; dense: +++; moderate: ++; low: +; undetectable;0). From: Mansour, 1988.

and ARN only κ-receptors have been found (Mansour, 1988). P treatment in the presence of E2 increases μ-receptor numbers in the MPO 27 hours after its administration (Mateo *et al*, 1992). Thus the increase in opioid receptor numbers on oestrous may result from the action of gonadal steroids and so enhance the inhibitory influence of EOPs on oestrus.

EOP perikarya in the ARN concentrate E_2 (Jirikowski *et al*, 1986) and synaptic connections exist between EOP neurones projecting from the ARN and LHRH neurones in the MPO (Leranth *et al*, 1988). Thus, EOP neurones could provide the functional link between circulating E_2 and LHRH neurones. β-END is one of the most biologically active EOP's and inhibits LHRH secretion from the ME (Bicknell, 1985). Morphine prevents release of LHRH but does not alter the hypothalamic content of LHRH (Mehmanesh *et al*, 1988). Both morphine and β-END are able to block the preovulatory LH surge when administered on the morning of pro-oestrus (Leadem and Kalra, 1985). The ability of the opiate antagonist, naloxone, to advance the surge when administered on the morning of pro-oestrus led to the postulation that a decrease in EOP tone may be the initial neural stimulus for the preovulatory LH surge (Allen and Kalra, 1986).

Large variations in hypophyseal β-END levels occur over the rat oestrous cycle (Sarkar and Yen, 1985). Fluctuations are also evident in the β-END concentrations in the MPO, ME and ARN; its levels being minimal on the morning and afternoon of pro-oestrus (Parnet *et al*, 1990). These fluctuations are

dependent on the gonadal steroid milieu since they are eliminated by ovariectomy and reinstituted following E_2 administration (Genazzani *et al*, 1990). Specific implantation of E_2 in the MPO of dioestrous rats increases the β-END levels in the ARN-ME region (Sturzebecher *et al*, 1988). Thus there is a large volume of evidence suggesting that a decrease in EOP tone in response to high circulating E_2 may indeed be the initial neural stimulus for the preovulatory LH surge. There is also evidence that this reduction in EOP tone may be partly attributable to a decline in the responsiveness of the opiate receptor mechanism during the E_2-induced LH surge (Berglund *et al*, 1988).

Much work has been carried out concerning opiate effects on LHRH secretion, but relatively little has been published which attempts to identify the receptor subtypes involved. This can be attributed to the lack of specific agonists and antagonists available until recently. κ-agonists increase prolactin (PRL) secretion and reduce LH secretion when administered centrally, but the latter effect could be blocked by a specific μ-antagonist and so was attributed to cross-specificity of the agonist employed (Pfeiffer *et al*, 1987). However, more recently, the highly specific κ-agonist, Tifluadom, has been shown to be as effective as morphine in reducing plasma LH concentrations on the early afternoon of pro-oestrus (Gopalan *et al*, 1989), which indicates that κ-receptors may indeed be involved in opioid suppression of LH release.

Although direct connections between EOP and LHRH neurones have been established (Leranth *et al*, 1988), there is extensive evidence that EOP effects on LHRH secretion may be indirect. Naloxone stimulation of LH release can be blocked by α-adrenoreceptor antagonists (Van Vugt *et al*, 1981) and inhibitors of dopamine-β-hydroxylase (Kalra and Crowley, 1982). Further, α-adrenergic agonists are able to elicit LH release when administered centrally to morphine-treated rats (Kalra and Gallo, 1983). The amount of LH released in response to naloxone has been directly related to the turnover of NA in the SCH, MPO and ME of OVX, E_2-injected rats at the time of treatment; the responses being least when NA turnover is at its lowest (Akabori and Barraclough, 1986). Ionotophoretically applied naloxone enhances the response of neurones in the MPO to stimulation of the ventral NA bundle (VNAB) (Dyer and Grossman, 1988). Since opioid peptides inhibit release of NA from the MPO (Diez-Guerra *et al*, 1987) it would appear that some NA axon terminals in the MPO are pre-synaptically inhibited by EOP's. However, a small proportion of MPO cells increased their firing rate on application of ionotophoretic naloxone, but did not respond to electrical stimulation of the VNAB (Dyer and Grossman, 1988). Thus some EOP neurones make functional synapses directly upon cells of the MPO to inhibit their activity.

Tyrosine hydroxylase and met-enkephalin have been co-localized in many neurones of the A1 NA cell group which project to the MPO (Ceccatelli *et al*, 1989). This leads to the intriguing possibility that the pre-synaptic opiate

receptors on NA terminals in the MPO may be autoreceptors on neurones that contain NA and EOP as co-transmitters; the principal site of action of NA being post-synaptic and of EOP's being pre-synaptic.

Recent work has indicated that μ-, but not κ- nor δ-receptors, mediate the inhibitory regulation of NA release from the cortical, cerebellar and hippocampal projections of the rat LC (Werling *et al*, 1987). This indicates that there may be specific subpopulations of opiate receptors involved in the modulation of NA release.

In addition to their many effects on NA neurochemistry within the hypothalamus, opiates also alter that of DA. Morphine reduces the turnover of DA in the ME of male rats (Deyo *et al*, 1979). Recently, κ- and μ-agonists have been shown to inhibit DA release from the MBH *in vitro* while leaving the outflow of NA unaffected (Heijna *et al*, 1991), and tuberoinfundibular neurones are tonically inhibited in dioestrus by κ- but not μ-receptor mediated mechanisms (Manzanares *et al*, 1992). These effects on tuberoinfundibular DA neurones are likely to be involved in the regulation of PRL secretion, but would also provide for κ-stimulation of LH secretion should some of these DA neurones be involved in control of LHRH rather than PRL.

Morphine activates incertohypothalamic neurones projecting to the SCH and MPO in the male rat, as evidenced by its ability to increase the concentration of the DA metabolite, 3,4-

dihydroxyphenylacetic acid (DOPAC) in these regions (Lookingland and Moore, 1985). This again, may provide for opioid stimulation of LHRH if a similar action results in the female.

In the dorsal raphe nucleus, activation of κ- but not μ-receptors reduces the response of 5HT-sensitive neurones to electrical stimulation (Pinnock, 1992) suggesting that, here, EOPs are presynaptic inhibitors at 5HT terminals.

Thus it appears that the effects of EOPs on LHRH secretion may be mediated in large part by NA, DA and 5HT neurones.

α-adrenergic activation stimulates accumulation of met-enkephalin in the ME (George *et al*, 1990) and long term DA agonist treatment reduces EOP immunoreactivity in the MBH and ME of male rats (Locatelli *et al*, 1983), suggesting that there may be a reciprocal relationship between catecholamine and EOP systems in the hypothalamus.

Other Aminergic Neurotransmitters

(i) Adrenaline (ADR)

ADR administered intracerebroventricularly (ICV) stimulates LH release (Vijayan and McCann, 1978), while inhibitors of phenylethanolamine-N-methyltransferase (PNMT) block both the steroid-induced surge in OVX rats (Crowley and Terry, 1981) and the spontaneous LH surge in intact rats (Coen and Coombs,

1983). This would point to an essential stimulatory role for ADR in the regulation of the LH surge. However, the very existence of ADR as a classical neurotransmitter in the rat CNS has recently been called into question with the claim that PNMT-containing neurones are not necessarily ADRergic (Sved, 1989). Evidence now seems to favour the view that the appearance of ADR in the rat CNS is either as a co-transmitter with NA or as a post-synaptic product of NA catabolism (Mefford, 1987). The effects of ADRergic drugs on the LH surge may be a result of activating receptors in NA neuronal systems or by altering the metabolism of NA itself.

(ii) Acetylcholine (ACH)

ACH muscarinic receptor numbers in the POA vary over the oestrous cycle, being highest at pro-oestrus (Olsen *et al*, 1988) and administration of muscarinic agonists increase hypothalamic E_2 binding (Lauber and Whalen, 1988). However, an ACH effect on LH secretion has not been established, whereas an E_2-mediated effect on lordotic behaviour has been demonstrated (Dohanich and Clemens, 1981). Thus ACH may be of importance in the behavioural aspects of the oestrous cycle, but have little or no involvement in the central regulation of the LH surge.

(iii) Histamine (HA)

HA stimulates LHRH release from the steroid-primed rat MBH *in vitro* (Miyake *et al*, 1987); an action which is independent of NA stimulation of the surge (Ohtsuka *et al*, 1989). Thus HA may have a facilitatory role in the induction of the LH surge. Blockade of the LH surge by centrally administered HA antagonists or synthesis blockers has not been demonstrated; therefore the physiological relevance of HA in the control of the surge may be minimal.

Other Peptide Neurotransmitters

(i) Neuropeptide Y (NPY)

NPY inhibits LH secretion when injected ICV into OVX rats (McDonald *et al*, 1989). However, the opposite is the case in steroid-primed rats, where stimulation occurs (Kalra and Crowley, 1984). *In vitro*, NPY stimulates release of LHRH from the MBH of steroid-primed rats (Crowley and Kalra, 1987) and ICV administration of antiserum to NPY blocks the LH surge in steroid-primed rats (Wehrenberg *et al*, 1989). These results indicate that NPY may be important in the stimulation of the LH surge on pro-oestrus. Indeed, NPY levels in the ME, but not SCH, MPO nor ARN, peak approximately one hour prior to the LH surge on pro-oestrus (Sahu *et al*, 1989), lending support to a physiological role for this neuropeptide in the control of ovulation.

There is evidence to suggest that NPY may act on the LHRH neurones as a co-transmitter with NA (Everitt *et al*, 1984), and/or as an independent transmitter on which NA neurones make direct appositions (Guy and Pelletier, 1988) to alter LH secretion (Allen and Kalra, 1987).

(ii) Substance P (SP)

SP may be involved in the neural circuitry regulating LHRH secretion since its levels in the ME vary throughout the oestrous cycle (Antonowicz *et al*, 1982), and it elicits LHRH secretion from the MBH *in vitro* (Ohtsuka *et al*, 1987). Furthermore, SP innervation of LHRH neurones in the POA of rats has recently been reported (Tsuruo *et al*, 1991).

Again, however, very little work has been carried out to elucidate the involvement of this neuropeptide in the regulation of LH secretion and its participation may only be of marginal importance.

(3) Vasoactive Intestinal Peptide (VIP)

VIP administered centrally reduces the magnitude of the steroid-induced LH surge in OVX rats (Weick *et al*, 1992). Thus this neuropeptide curtails LHRH secretion during the LH surge. However, the physiological significance of its ability to do so has yet to be assessed.

Excitatory Amino Acids

Glutamate, aspartic and cysteic acid all stimulate LH release following ICV infusion (Ondo *et al*, 1976; Ondo, 1981; Scheibel *et al*, 1980). Infusion of the specific agonist, N-methyl-D-aspartate (NMDA) into discrete hypothalamic areas identified the MPO as the sole site of action of the excitatory amino acids (Ondo *et al*, 1988). NMDA stimulation of LH secretion is markedly increased by E_2 + P pre-treatment of OVX rats (Carbone *et al*, 1992) suggesting that the excitatory amino acids may have some involvement in the transduction of the steroid signal to the LHRH neurones. However, the significance of these neurotransmitters in the control of the LH surge has yet to be fully determined.

γ-Aminobutyric Acid

γ-aminobutyric acid (GABA) neurones are extensively distributed throughout the hypothalamus (Sakaue *et al*, 1988), and electron microscopy has shown synaptic contacts between GABA neurones and LHRH neurones in the MPO (Leranth *et al*, 1988b).

GABA is an inhibitory neurotransmitter, but has both stimulatory and inhibitory effects on LHRH secretion depending on its site of action. In the MPO, GABA is inhibitory to LHRH release (Demling *et al*, 1985). Stimulation of NA neurones projecting to the MPO induces release of GABA when E_2 levels are low but, not when they are high (Herbison *et al*, 1990); i.e. their inhibitory action is eliminated on

pro-oestrus. It appears that GABA neurones here are inhibitory interneurones between the NA and LHRH neurones that lose their sensitvity to NA on pro-oestrus, thus freeing the LHRH neurones from tonic restraint. This mechanism may be involved in NA stimulation of the surge.

GABA also reduces NA turnover in the MPO concomitant with its inhibition of LH release (Adler and Crowley, 1986). This suggests that, quite apart from its role as a relay for the NA signal, GABA is a presynaptic inhibitor of NA activity in this area. Indeed, it has recently been proposed that it is a reduction in GABAergic tone which synchronizies the activity of LHRH neurones to stimulate the LH surge (Jarry *et al*, 1991). This is supported by subsequent work which demonstrated that decreased GABA activity in the MPO and MBH precedes the surge (Seltzer and Donoso, 1992).

GABA is also inhibitory to LHRH release in the zona incerta (ZI) by a different mechanism. Here, its effects can be attributed to its inhibition of the stimulatory input to the LHRH system from the incertohypothalamic DA tract (Wilson *et al*, 1990). However, GABA produces a stimulatory effect on LHRH secretion in the ARN/ME. This is believed to be a result of its negative influence on the activity of the inhibitory tuberoinfundibular DA system (Nikolarakis *et al*, 1988).

Finally, the inhibtion of the LH surge elicited by stimulation of the medial raphe nucleus appears to be mediated by GABA (Morello *et al*, 1989).

In general, the wide-ranging actions of GABA on LHRH secretion result from direct inhibition of LHRH neurones in the MPO or from inhibition of both stimulatory and inhibitory inputs to the LHRH neurones.

Summary

It is clear that the secretion of LH, including the preovulatory LH surge, is regulated by a complex neural network involving several classes of neurotransmitters. These modulate the secretion of LH by altering the activity of LHRH neurones within the hypothalamus.

The neurotransmitters which appear to be of paramount importance in this neural circuitry are NA, DA, 5HT, EOPs, NPY and GABA; there is a complex interplay between these neurones with the effects of some being mediated by others, and this is represented in Figure 9.

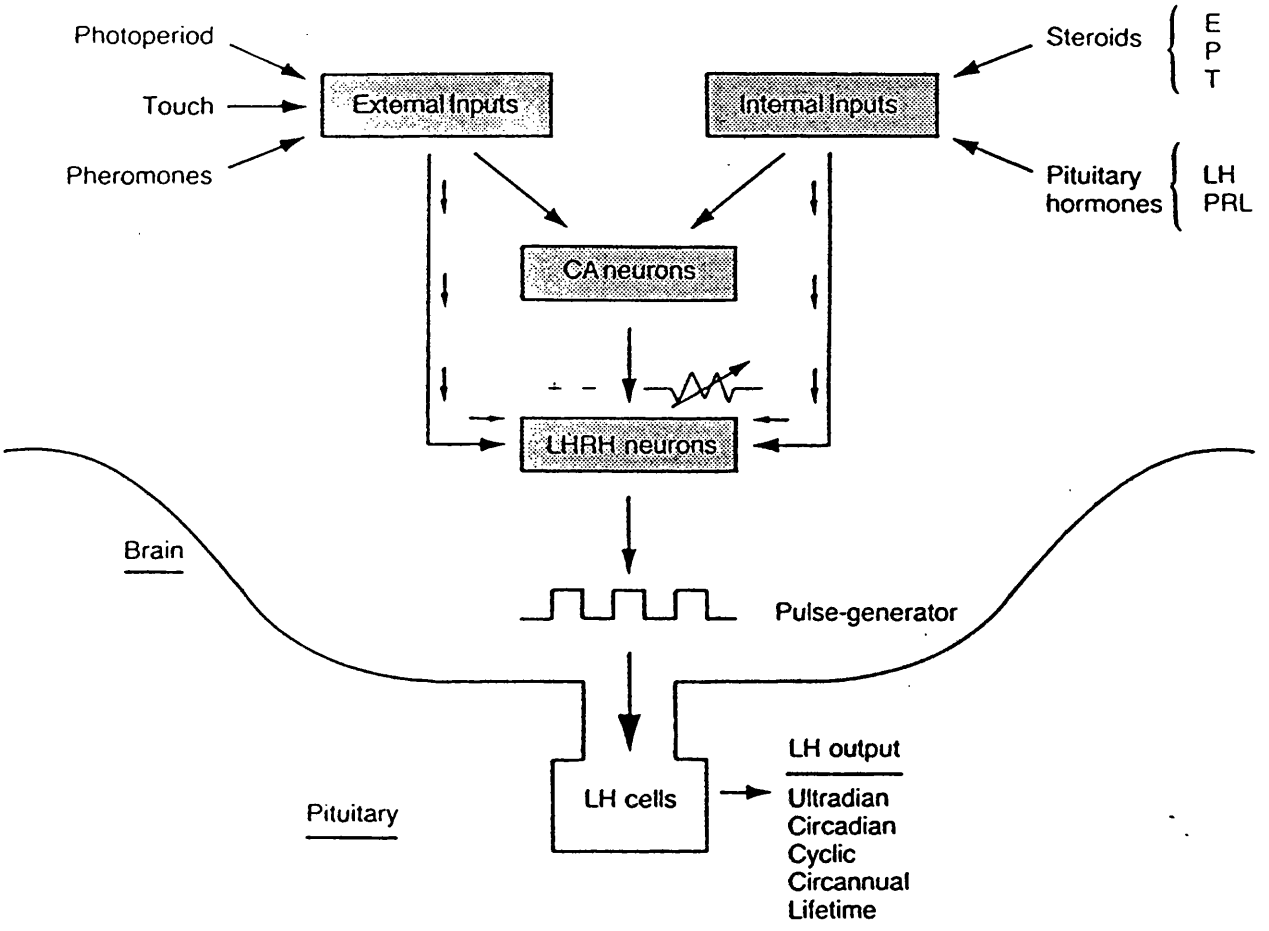


Figure 9. Schematic representation of the organization of the neural circuitry regulating the secretion of LHRH from the ME. Short arrows represent interneurone pathways and continuous lines depict direct neural connections. From: Ramirez *et al*, 1984.

AIMS

As outlined in the Introduction, there is a wealth of information published on opiate modulation of the central control of the preovulatory LH surge. Much of this work has concentrated on the effects of the μ-agonist, morphine, and the general opiate antagonist, naloxone. Relatively little attention has been paid to the possible actions mediated by other receptor subtypes.

This investigation was designed to determine the possible involvement of κ- and σ-receptors in the central opiate effects on the surge, and to compare these to the responses produced by the μ-agonist, morphine.

Furthermore, it was decided to investigate the mediation of opiate effects on the LH surge by the biogenic amines, NA, DA and 5HT. To achieve this objective, the effects of the opiates on biogenic amine neurotransmission were measured in the hypothalamic areas which contain LHRH neurones (SCH, MPO, ME and ARN) on the early and late afternoon of pro-oestrus.

Studies of amine turnover in discrete hypothalamic nuclei have involved the measurement of amine depletion after administration of a synthesis inhibitor (Rance *et al*, 1982). These inhibitors themselves alter LH secretion (Kalra and McCann, 1974), and so could alter the response which could be evoked by administration of further pharmacological agents.

The measurement of neurotransmitters and their metabolites, as used here, eliminates the need for pharmacological

manipulations to determine the activity in the nerves under investigation. The concentration of metabolite reflects neuronal activity (Lookingland *et al*, 1987) and the ratio of the metabolite to its respective transmitter provides a qualitative index of the turnover rate of the latter (Commissoing, 1985).

MATERIALS AND METHODS

Animals

An in-house colony of female Sprague-Dawley rats was established from stock purchased from Tuck and Sons, Battlesbridge, Essex. The animals were housed in a light- and temperature-controlled environment; lights on 06.00h-20.00h, temperature 21±1°C. Food and water were supplied *ad libitum*.

Vaginal lavages were performed on the rats between 09.00h-10.30h each morning. The morphology of the cells sloughed from the vagina were used to identify the stage of the oestrous cycle of each rat (Mandl, 1951).

Those rats (200-330g) that had exhibited at least two consecutive four-day oestrous cycles were selected for experimentation on the morning of pro-oestrus. The records kept for animals that satisfied this criterion but, were not used for experimentation, showed that there was approximately a 97% probability that oestrus follow on the next day, i.e. the preovulatory LH surge occurred in 97% of the animals used in the experiments.

Drugs

The properties of the drugs are summarized in Table 3.

Naloxone is a specific opiate antagonist which has greatest affinity for the μ-receptor, but also possesses substantial affinity for both δ- and κ-receptors.

Morphine is a highly specific μ-agonist and displays very little activity at other opioid receptor types. However, it has a low efficacy for μ-receptors, and so can act as an antagonist in tissues with low receptor densities (Kenakin and Beek, 1980). In this study a long-acting preparation of morphine, Duromorph, was used which exhibits potent analgesic effects for up to 24 hours after administration (Johnston, 1990).

Drug	Receptor Properties
Naloxone	Opiate antagonist
Duromorph	μ-agonist
Tifluadom	κ-agonist
N-allylnormetazocine	σ-agonist/μ-antagonist

Table 3: Drugs used in the study and their receptor properties.

Tifluadom is a specific κ-agonist, being over x100 more potent at the κ-receptor than at any other opioid receptor (Burkhard, 1984).

N-allylnormetazocine (SKF 10047), as a racemic mixture, exhibits several pharmacological actions. The (+)-enantiomer binds at two sites in the rat brain; a high affinity σ-opioid receptor and a low affinity phencyclidine(PCP)-receptor

(Largent *et al*, 1986) which has also been designated a non-opioid σ-receptor. (-)-N-allylnormetazocine binds to μ-receptors, where it acts as an antagonist (Compton *et al*, 1987), in addition to PCP-receptors.

I am grateful to the following establishments for the gifts of the drugs used in the study: Laboratories for Applied Biology Ltd. (London, U.K.) for the gift of Duromorph, Kalie-Chemie (Hannover, F.R.G.) for Tifluadom and the National Institute on Drug Abuse (U.S.A.) for N-allylnormetazocine (SKF 10,047). Naloxone was purchased from Sigma Chemicals Corporation (Poole, Dorset, U.K.).

Decapitation Procedure

Trunk blood was collected immediately upon decapitation, centrifuged (3000rpm, 5mins, 4°C) and the separated plasma stored at -80°C until LH determination. The brains were rapidly removed, frozen on dry ice and stored at -80°C to prevent *post mortem* degradation of the brain aminergic neurotransmitters.

Brain Microdissection

The SCH, MPO, ME and ARN were isolated by a modification of the Palkovits Technique (Paxinos and Watson, 1986):

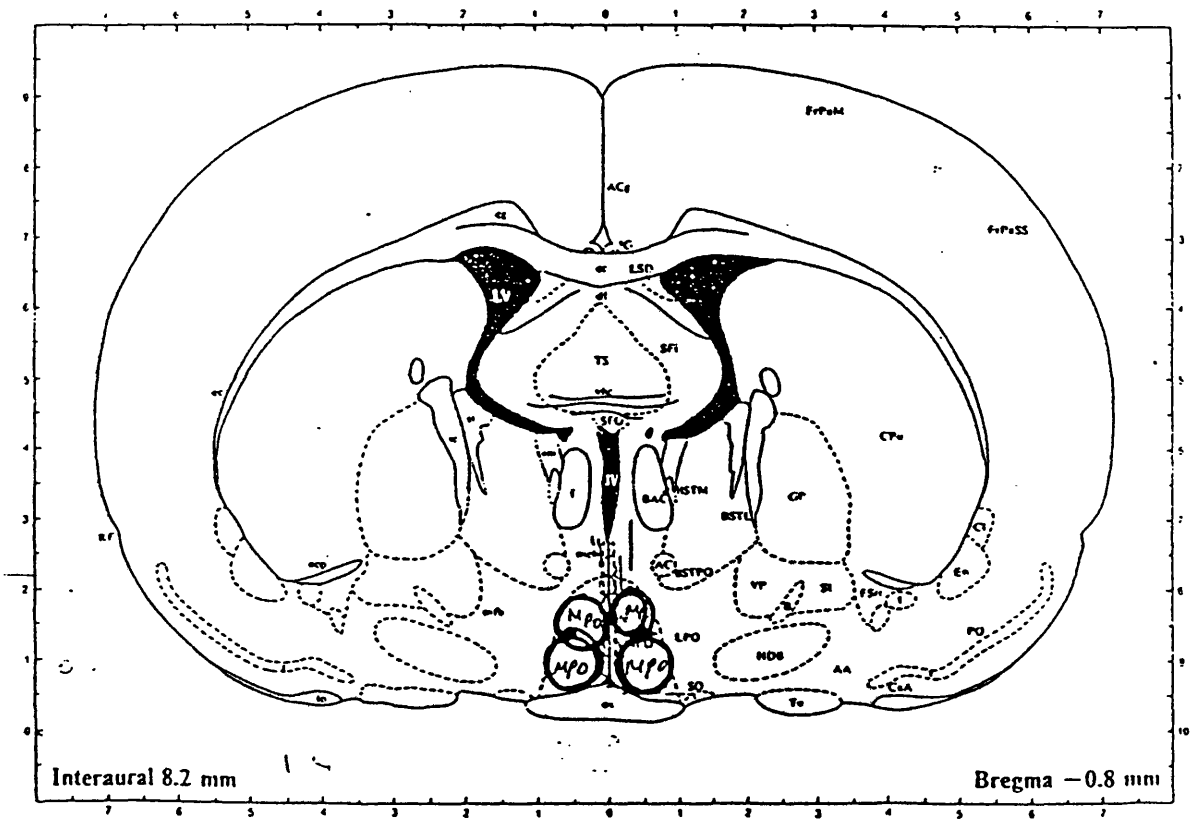


Figure 10. Coronal section (number 5) through the rat brain showing the location of the hypothalamic areas to be isolated. From: Paxinos and Watson, 1986.

On removal of the brain from storage, the frontal lobes and hindbrain were separated by coronal cuts. The dorsal cut surface was placed on the mounting stage of a freezing microtome (-80°C) and the tissue held firmly in place by encasement of the base in Cryo-Gel. 50 μm coronal brain slices were cut until the fused portion of the corpus callosum could be seen. Subsequently, 500 μm sections were taken, numbered 0-12 and stored on microscope slides resting upon dry ice. The remainder of the brain was discarded.

When all thirteen sections had been cut, section numbers 4-11 which contained the areas of interest were, in turn, transferred to a cold plate (-20°C) under a binocular microscope (x16 magnification). The areas were punched from the sections using modified hypodermic needles of 0.66mm internal diameter. A different needle was used for each area and the needles were rinsed in alcohol between samples. The ME was isolated by punches of half the needle cross-sectional area.

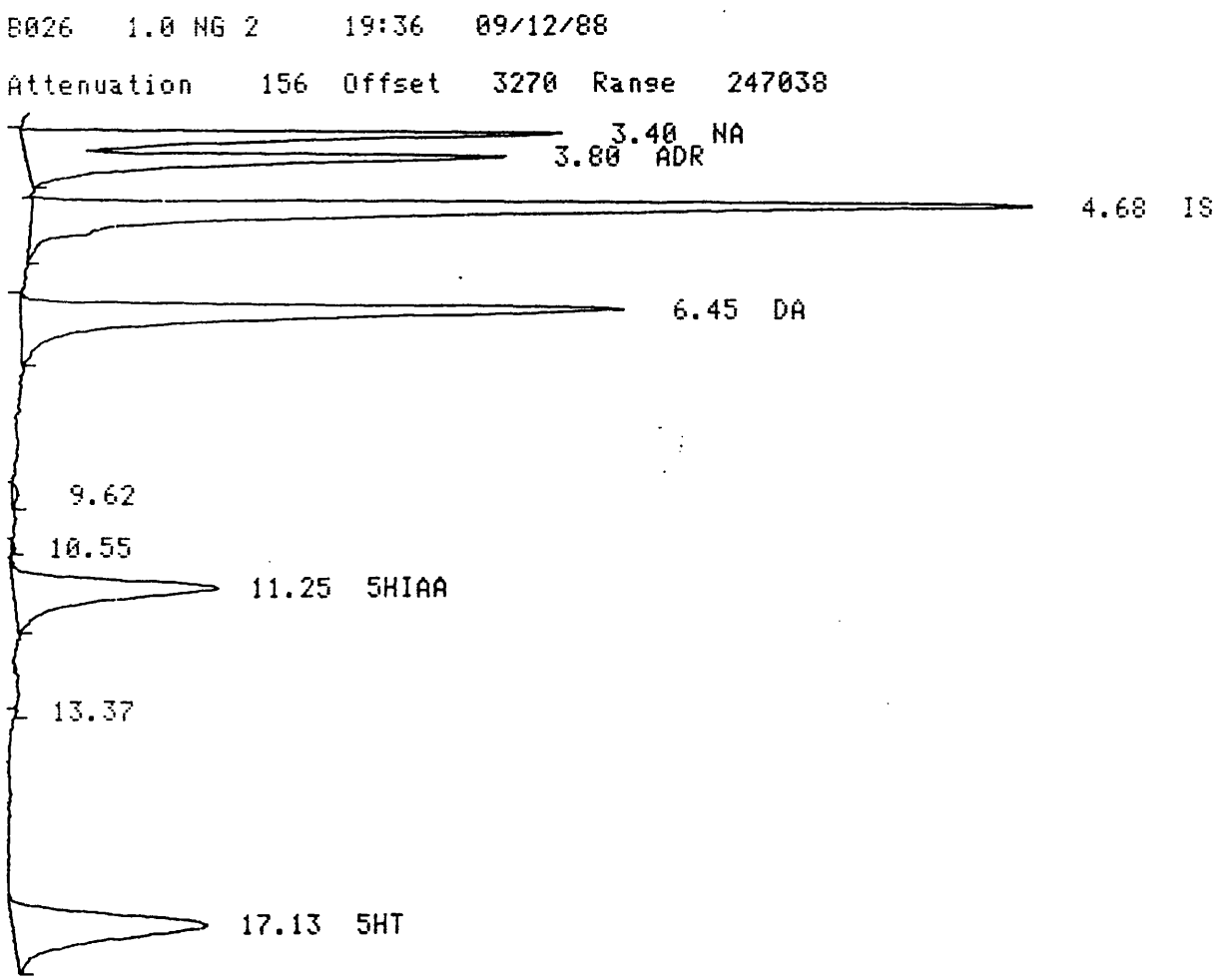
As the hypothalamic areas were isolated by multiple punches it was possible for a fraction of each area to be lost. Therefore a record of the success of the punches was kept.

Chromatography

The specific hypothalamic areas were collected separately in microtubes (2ml) kept cold on dry ice. The isolated tissue was stored at -80°C until assayed. At a later date, 50μl of

0.1M hydrochloric acid containing 2.5ng of 3,4-dihydroxybenzylamine (DHBA) was added to each sample which was then centrifuged (3000rpm, 5 mins., 4°C) in a Denley BR401 Refrigerated Centrifuge to ensure all the tissue was at the bottom of the tube. The sample was sonicated for 10 seconds to disperse the tissue through the liquid and then re-centrifuged (as above). 20µl aliquots of supernatant were drawn off and injected on to a high performance liquid chromatography (HPLC) system and the content of NA, DHPG, MHPG, ADR, DA, DOPAC, HVA, 5HT and 5HIAA were simultaneously measured by electrochemical detection (ECD); the full chemical names are given in the 'List of Abbreviations' and their chemical properties are summarized in Table 4. The internal standard to analyte peak area method (described below) was used to quantify the content of the monoamines in each of the areas. The minimum detectable quantity of each of the biogenic amines was 0.05-0.10ng/20µl of supernatant, dependent on the noise level of the system.

The response of the system was checked by running mixtures of the amines in known concentrations from 0.2-2.0ng/20µl. The system demonstrated a linear response to all of the amines measured over these concentrations. A standard mixture containing known concentrations (1ng/20µl) of the amines under investigation was run on the HPLC column each morning. The amines in the samples were identified by the coincidence of their retention times on the column with the known amines in the standard mixture. The ratio of the amine peak areas to the internal standard peak area in the standard mixture



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Method BASLC3AB
Method Text BASLC3AB

Sample
Weight 1.00 Volume 1.00

Report Retention Time	Intall Relative Time	Peak Area	Response Factor	Peak Conc	Peak Name	Peak Code
3.40	0.73	1441063	1.493	0.60	NA	
3.80	0.81	1495842	1.252	0.52	ADR	
4.68	1.00	3582568	1.000	1.00	IS	I.S.
6.45	1.38	2390724	0.978	0.65	DA	X
9.62	2.05	19039	1.000	0.01		X
10.55	2.25	9661	1.000	0.00		
11.25	2.40	1129780	1.362	0.43	5HIAA	
13.37	2.85	2808	1.000	0.00		
17.13	3.66	1383064	1.400	0.54	5HT	
		11454549		3.76		

Figure 11. HPLC trace of the standard solution containing 1ng/20µl of each of the identified compounds.

indicates the percentage recovery at the detector of the amines per unit of internal standard. This ratio is known as the response factor. Addition of the same amount of internal standard to the samples thus allows an integrating computer to calculate the content of the amines in the samples by taking the ratio of the peak areas with the internal standard and incorporating the response factor. The mean amine content of each sample was scaled up by a factor of 50/20 to give the total amine content in each hypothalamic region.

Amine	Description
DHBA	Internal Standard
NA	Transmitter
DHPG	NA metabolite
MHPG	NA metabolite
ADR	Transmitter
DA	Transmitter
DOPAC	DA metabolite
HVA	DA metabolite
5HT	Transmitter
5HIAA	5HT metabolite

Table 4. Designation of the amines measured by the HPLC system described in the text. The full chemical names of the amines are given in the 'List of Abbreviations'.

Once the samples had been run on the column in duplicate, the remaining protein pellet was stored at -80°C until the protein assay was carried out.

The HPLC equipment used was: a Gilson Model 302 pump fitted with a Gilson 5.SC pump head, a Gilson Model 802 manometric module, a Rheodyne 7125 injection valve, a Beckman Ultrasphere IP 4.6mm x 4.5cm guard column, a Beckman Ultrasphere 4.6mm x 15cm column; both columns were packed with 5mm ODS Hypersil (octadodecylsilane),a BAS MF-1000 working electrode, a BAS MF-2020 auxiliary electrode , a BAS RE-1 Ag/AgCl reference electrode coupled to a BAS LC-3A amperometric detector set at a potential of +0.70V. The system was connected to a Trivector Trio Chromatography Computing Integrator. The flow rate was set at 0.6ml/min. The mobile phase was 6.74g/l citric acid, 4.81g/l sodium citrate, 47mg/l EDTA, 100mg/l heptanesulphonic acid, 1.15ml/l glacial acetic acid, 3ml/l tetrahydrofuran (THF), 5.0-7.5% (V/V) methanol. The pH was adjusted to 4.9 with 10M NaOH. Methanol and THF were added as necessary in the routine running of the system.

Protein Estimation

A standard curve of protein content against absorbance at 595nm after reaction with Coomassie Blue G250 (Pierce, Life Science Laboratories Ltd., Luton, U.K.) was first prepared. Known weights of bovine serum albumin (BSA) (0-40mg) were

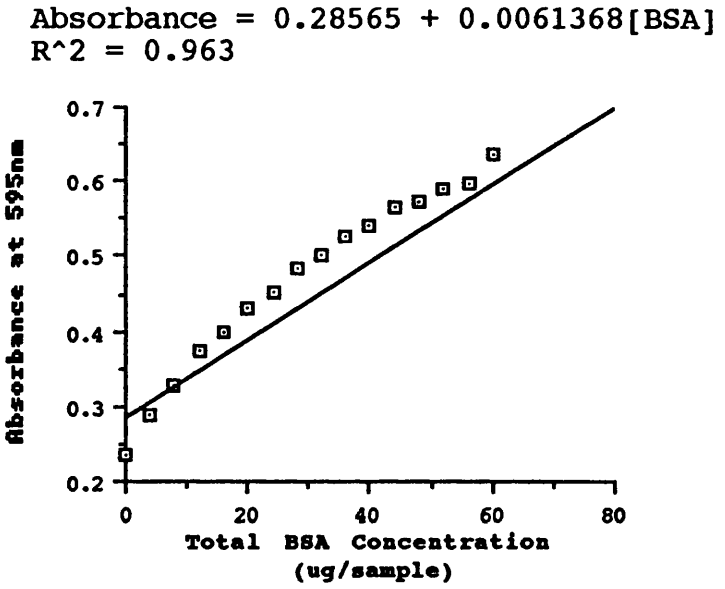

Figure 12. Standard curve of absorbance at 595nm after reaction with Coomassie Blue G250 versus concentration of Bovine Serum Albumin (BSA).

first dissolved in 100µl of 0.1m sodium hydroxide (NaOH) and incubated overnight at 4°C. The following morning the BSA was diluted to 1ml with distilled water. Next, 1ml of Coomassie Blue G250 was added and the absorbance at 595nm read immediately on an LKB Biochrom Ultrospec 4050 spectrophotometer using 200µl of 0.1M NaOH plus 1800µl of distilled water as the blank solution. The absorbance of each tube was read in duplicate and the mean used for the calculation (see Figure 12).

The protein pellet remaining after HPLC was dispersed in 210µl 0.1M NaOH by sonication and incubated overnight at 4°C. The next morning, two 100µl samples were transferred to separate tubes and each treated as described above for the standard curve. The protein concentration in each tube was calculated by substitution of the absorbance into the regression equation of the standard curve. The protein content of the duplicate tubes was summed to give the total protein content of the original sample.

If the sample tissue content lay below two standard deviations of the mean and no tissue had been lost in the micropunch procedure itself, then the protein was deemed to have been lost in the course of assay/storage/transfer of the samples and was replaced by the mean protein content of the area for the calculation of the amine concentrations.

Amine Concentration Calculation

The total amine content of the individual amines in each sample was divided by the total protein content of the sample, or the mean protein content for the particular area, to give the concentration of the amines in the original tissue in ng amine/μg protein.

Femoral Artery Cannulation

Surgical anaesthesia (complete cessation of the hind limb flexor withdrawal reflex) was induced as described in Experiment 4 and a cannula (0.63mm outside diameter) was inserted into the femoral artery.

Blood samples (200μl) were withdrawn through the cannula at 30 minute intervals throughout the afternoon and early evening of pro-oestrus. The animals were killed by an overdose of ether when the experiment was completed.

The blood samples were immediately centrifuged (3000rpm, 5mins., 4°C) and 100μl plasma separated and stored at -80°C until LH determination. The red blood cells were reconstituted with 100μl of warmed, heparinized saline (10 units/ml) and reinjected through the cannula. Animals were kept warm throughout the procedure either by radiant heat or by the use of a thermostat-controlled, heated dissection table.

LH Radioimmunoassay

Reagents:

(i) Modified physiological saline (MPS) buffer; 8.8g NaCl (0.15M) and 100mg Thiomersal were mixed well in 1 litre of distilled water on a stirrer, and the pH was adjusted to 7.0 using 1M sodium hydroxide (NaOH). 20ml was discarded and 7.8ml 0.5M NaH_2PO_4 was added. Once made up, the solution was stored at room temperature.

(ii) 1% egg white (EW); this was prepared by dissolving 1g of egg albumin in 100ml MPS and filtering through acetone-washed glass wool. The pH was adjusted to 7.0 using 1M NaOH when necessary. This solution was stored at 4°C and made up fresh each week as required.

(iii) Serum diluent (SD); a 1:400 dilution of normal rabbit serum (NRS) was prepared; a 0.25ml volume of NRS was dissolved in MPS containing 0.05M EDTA. The was pH adjusted to 7.0 with 1M NaOH and the volume was made up to 100ml with MPS. The solution was stored at 4°C.

(iv) NIH-RP-3-rLH Standard; a standard of 5mg/ml in PBS was prepared by reconstitution in 1ml of distilled water. This solution was aliquoted into 25µl volumes, frozen and stored at -20°C. A standard curve was made up by adding 756µl EW/MPS to the 25µl aliquot. In ice, eight eppendorfs were set out in two rows, and 200µl of EW/MPS aliquoted into each. 200µl was

removed from the standard tube and serially double diluted down. This gave eight points on the standard curve.

(v) NIH-rLH-S-10 Antibody; a 1:20 solution was provided. Of this, a 10ml aliquot was withdrawn and added to 990ml of SD (x100 dilution) to give a dilution of 1:2000. This was aliquoted into 200µl volumes and stored at -20°C. When required, a 200µl aliquot of the 1:200 dilution was added to 5.8ml of SD (x30 dilution) to give a concentration of 1:60000, which was subsequently diluted by three to give the recommended final working concentration of 1:180000.

Protocol:-

The standards, non-specific binding (NSB), zero and totals were included at the beginning of each assay and repeated at the end. On the second day, the label ^{125}I-LH was made up in EW/MPS to give counts of 15000-20000/50ml. On the third day, precipitating serum (PPS) was prepared; 1ml donkey-anti rabbit immunoglobulin G (IgG) was made up in 23 ml EW/MPS. On the final day, 500ml EW was added to all of the tubes with the exception of the totals (n=5) prior to centrifugation. After one hour of spinning, the tubes were drained and counted; the totals were neither spun nor drained. The tubes were kept at 4°C for the duration of the assay.

The sensitivity of the assay was 0.4ng per sample and the intra- and interassay coefficients of variation were 10.5% and 8.7% respectively.

Statistics

Scheffe's One-way Analysis of Variance and Covariance was performed on the data. When the 'F'-test was significant, it was followed by the repeated measure, multiple comparison t-test between means.

All statistical tests were carried out on the Glasgow University Mainframe (Glasgow VME) using the P1V programme of the statistical software package BMDP (Copyright: Regents of University of California, Los Angeles) and converted for use on ICL VME by the Program Library Unit, University of Edinburgh.

EXPERIMENT 1

This investigation was planned to examine the effects of the four opiate drugs administered during the critical period, at various doses, on the plasma LH concentration immediately prior to the LH surge, and during the plateau phase of LH secretion.

The method was selected such that it would further allow an investigation of any relationship between neurotransmitter concentrations in the SCH, MPO, ME and ARN at the end of the critical period with surge levels of LH later in the day.

Materials and Methods

Experimental animals were injected intraperitoneally (IP) at 12.30h with either Duromorph, tifluadom, N-allylnormetazocine or naloxone dissolved in a 0.05ml/15g volume of physiological saline. The doses used were 1,3,10,30 and 50 mg/kg except for Duromorph for which the highest doses were 40 and 60 mg/kg respectively. Control animals were injected with vehicle alone. The rats were subsequently divided into two groups; the first being decapitated at 14.30h and the second at 18.00h. The number of animals in each group is shown in Tables 5(a) and 5(b).

Results

(1) 14.30h Decapitations

These results are summarised in Tables 5(a) and 6-9.

(a) Luteinizing Hormone

For control animals the plasma LH concentration at 14.30h was 2.1±0.3 ng/ml.

None of the four drugs significantly altered plasma LH levels at any of the doses used.

(b) Noradrenaline

The control values found for NA in the SCH, MPO, ME and ARN were 40.1±7.2, 47.6±8.9, 22.4±4.1 and 30.1±3.3 ng amine/μg protein respectively (results expressed ± SEM).

(i) Naloxone

No dose of naloxone significantly affected the NA concentrations in the SCH. The 1 mg/kg dose of naloxone significantly elevated NA levels in the MPO ($p<0.001$) and ARN ($p<0.01$)but, no other dose had any significant effect in these two areas. The 1, 3 ($p<0.001$) and 30 ($p<0.01$) mg/kg doses of naloxone significantly raised NA

concentrations in the ME, but the other two doses caused no significant alterations.

(ii) Duromorph

The 1 mg/kg dose of Duromorph significantly elevated ($p<0.01$) NA concentrations in the SCH; the other doses used were ineffective. The 1 and 10 mg/kg doses also caused significant increases ($p<0.05$) in the NA concentrations in the MPO. The 1, 3 ($p<0.001$) and 40 ($p<0.05$) mg/kg doses significantly elevated NA concentrations within the ME. The 1 ($p<0.05$) and 3 ($p<0.001$) mg/kg doses resulted in significant increases in NA concentrations in the ARN.

(iii) Tifluadom

Tifluadom had no significant effect on NA concentrations in the SCH at any of the doses used, but the 10 mg/kg dose significantly elevated NA concentration in the MPO ($p<0.01$). The 1, 3 ($p<0.001$), 10 ($p<0.05$) and 30 ($p<0.01$) mg/kg doses all caused significant increases in NA concentrations in the ME. The 1 ($p<0.01$), 10 ($p<0.001$) and 30 ($p<0.01$) mg/kg doses significantly raised NA concentration in the ARN.

(iv) N-allylnormetazocine

None of the doses of N-allylnormetazocine used had a significant effect on NA concentrations in the SCH, nor in the MPO. The 1 ($p<0.01$) and 3 ($p<0.001$) mg/kg doses of N-allylnormetazocine significantly increased NA concentrations in the ME. The 3 ($p<0.01$) and 30 ($p<0.05$) mg/kg doses of N-allylnormetazocine significantly raised the NA concentrations of the ARN.

(c) Dopamine

The concentrations of DA in the SCH, MPO, ME and ARN of control animals were 2.4 ± 0.5, 3.5 ± 0.6, 21.4 ± 6.0 and 5.8 ± 1.8 ng amine/μg protein respectively (values given $\pm$ SEM).

(i) Naloxone

The 1 ($p<0.05$), 3 ($p<0.01$) and 30 ($p<0.05$) mg/kg doses of naloxone caused significant elevations of DA concentrations in the SCH. The 1 and 3 mg/kg doses of naloxone significantly increased DA concentrations in the MPO ($p<0.001$ and $p<0.01$ respectively), and in the ME (both $p<0.05$). However, no dose of naloxone significantly altered DA concentrations in the ARN.

(ii) Duromorph

The 1 and 10 mg/kg doses of Duromorph significantly increased the DA concentrations of the SCH (both $p<0.001$) and the MPO ($p<0.01$ and $p<0.001$ respectively). No dose of Duromorph had a significant effect on the DA concentrations of the ME or of the ARN.

(iii) Tifluadom

None of the doses of Tifluadom used significantly altered the DA concentrations in the SCH or in the ME. Nevertheless, the 1 ($p<0.05$) and 10 ($p<0.001$) mg/kg doses of Tifluadom significantly raised DA concentrations in the MPO. The latter dose also significantly increased ($p<0.01$) the DA concentrations in the ARN.

(iv) N-allylnormetazocine

The 1 ($p<0.05$) and 30 ($p<0.001$) mg/kg doses of N-allylnormetazocine significantly elevated DA concentrations in the SCH. In addition, the 3 ($p<0.05$) and 10 ($p<0.01$) mg/kg doses of N-allylnormetazocine significantly raised DA concentrations in the MPO. However, this opiate had no significant effect on DA concentrations either in the ME or in the ARN at any of the doses studied.

(d) 5-Hydroxyindoleacetic Acid

The values for 5HIAA concentrations in the SCH, MPO, ME and ARN of controls were 8.3±2.0, 8.7±1.3, 5.1±0.8 and 11.3±3.4 ng amine/μg protein respectively (results expressed ± SEM).

(i) Naloxone

The 1 ($p<0.01$), 10 and 30 (both $p<0.05$) mg/kg doses of naloxone significantly elevated 5HIAA concentrations in the SCH compared to those in the controls. Furthermore, the 1, 3 and 10 mg/kg doses of naloxone significantly increased 5HIAA concentrations in the MPO and the ME (all $p<0.05$). The 30 mg/kg dose of naloxone also significantly raised 5HIAA levels in the ME ($p<0.01$). However, naloxone had no significant effect on 5HIAA levels in the ARN at any dose administered.

(ii) Duromorph

The 1 ($p<0.01$), 40 and 60 (both $p<0.05$) mg/kg doses of Duromorph significantly elevated 5HIAA concentrations in the SCH compared to those in controls. The 1 ($p<0.001$), 3 ($p<0.01$), 10 ($p<0.05$) and 60 ($p<0.01$) mg/kg doses of Duromorph caused significant increases in the 5HIAA concentrations of the MPO. The 3 mg/kg dose of Duromorph significantly elevated 5HIAA concentrations in the ME and ARN, as did the 60mg/kg dose in the ARN (all $p<0.05$).

(iii) Tifluadom

The 1 ($p<0.01$), 3 ($p<0.001$), 10 and 30 (both $p<0.05$) mg/kg doses of Tifluadom significantly increased 5HIAA concentrations in the SCH at 14.30h compared to controls. The 1 ($p<0.001$), 3 ($p<0.01$) and 10 ($p<0.001$) mg/kg doses of Tifluadom significantly increased the 5HIAA concentrations in the MPO. Only the 3 mg/kg dose of Tifluadom significantly affected ($p<0.01$) the 5HIAA concentrations in the ME, causing an increase. The 1 ($p<0.05$) and 10 ($p<0.01$) mg/kg doses of Tifluadom caused significant elevations in the 5HIAA concentration of the ARN.

(iv) N-allylnormetazocine

None of the doses of N-allylnormetazocine administered had significant effects upon 5HIAA concentrations in the SCH, MPO, ME or ARN.

(e) Serotonin

The 5HT concentrations in the SCH, MPO, ME and ARN of control animals were 23.1 ± 3.3, 23.0 ± 2.4, 13.1 ± 2.2 and 23.1 ± 3.6 ng amine/μg protein respectively (results expressed $\pm$ SEM).

(i) Naloxone

The 1 ($p<0.01$) and 10 ($p<0.05$) mg/kg doses of naloxone significantly raised the 5HT concentrations of the SCH compared to controls. However, no dose of naloxone significantly altered the 5HT concentrations of the MPO. The 1 ($p<0.001$) and 3 ($p<0.01$) doses of naloxone significantly elevated 5HT concentrations in the ME, while only the 1 mg/kg dose did so in the ARN ($p<0.01$).

(ii) Duromorph

The 1 mg/kg dose of Duromorph caused a significant rise in the 5HT concentrations of the SCH, MPO (both $p<0.001$), ME and ARN (both $p<0.01$) compared to controls. In addition, the 40 ($p<0.05$) mg/kg dose of Duromorph also significantly raised 5HT concentrations in the ME.

(iii) Tifluadom

The 1 mg/kg dose of Tifluadom significantly elevated 5HT concentrations in the SCH ($p<0.01$), MPO ($p<0.001$), ME ($p<0.05$) and ARN ($p<0.01$) compared to the levels in the controls. The 10 mg/kg dose of Tifluadom also significantly increased 5HT concentrations in the SCH ($p<0.01$), MPO ($p<0.05$) and the ARN ($p<0.01$), and the 30mg/kg dose significantly raised the 5HT concentrations in the SCH and ME (both $p<0.01$).

(iv) N-allylnormetazocine

No dose of N-allylnormetazocine significantly altered 5HT concentrations in any of the four regions studied with respect to control animals.

(v) Ratio of 5HIAA/5HT

The controls values for the ratio of 5HIAA/5HT in the SCH, MPO, ME and ARN were 0.368±0.069, 0.472±0.109, 0.461±0.065 and 0.478±0.106 respectively (results expressed ± SEM).

(i) Naloxone

Naloxone had no significant effect on the ratio of 5HIAA/5HT in any of the four areas examined.

(ii) Duromorph

Only the 10 mg/kg dose of Duromorph significantly increased the ratio of 5HIAA/5HT in the SCH compared to that seen in controls. No significant effects of Duromorph were evident in the MPO, ME or ARN.

(iii) Tifluadom

None of the doses of Tifluadom had significant effects on the ratio of 5HIAA/5HT in the SCH or the MPO. Nevertheless, the 50mg/kg dose of Tifluadom significantly elevated the

ratio of 5HIAA/5HT in the ME ($p<0.05$) and the ARN ($p<0.001$) compared to those of controls.

(iv) N-allylnormetazocine

No dose of N-allylnormetazocine used significantly affected the ratio of 5HIAA/5HT in any of the four areas studied.

(2) 18.00h Decapitations

These results are summarized in Tables 5(b) and 10-13.

(a) Luteinizing Hormone

The plasma LH concentration at 18.00h in control animals was 9.5 ± 2.2 ng/ml.

(i) Naloxone

The 10 mg/kg dose of naloxone significantly elevated ($p<0.05$) plasma LH concentrations at 18.00h compared to those seen in controls.

(ii) Duromorph

Both the 40 and 60 mg/kg doses of Duromorph significantly reduced ($p<0.05$) plasma LH levels in comparison to control values.

(iii) Tifluadom

The 3 ($p<0.01$), 10, 30 and 50 mg/kg ($p<0.05$) doses of Tifluadom significantly reduced plasma LH concentrations from those seen in the controls.

(iv) N-allylnormetazocine

N-allylnormetazocine had no significant effect on plasma LH levels at any of the doses used.

(b) Noradrenaline

The NA concentrations in the SCH, MPO, ME and ARN of control animals at 18.00h were 22.1 ± 5.5, 29.1 ± 7.0, 28.7 ± 4.4 and 26.8 ± 3.5 ng amine/μg protein respectively (results expressed $\pm$SEM).

(i) Naloxone

None of the doses of naloxone significantly affected NA levels in either the SCH, MPO or ARN. However, the 3 ($p<0.01$), 10 and 50 ($p<0.05$) mg/kg doses of naloxone significantly increased NA concentrations in the ME compared to controls.

(ii) Duromorph

Duromorph did not significantly affect NA concentrations in the SCH, MPO, ME or ARN at any of the doses administered.

(iii) Tifluadom

No dose of Tifluadom significantly altered NA concentrations in the SCH, MPO, ME or ARN compared to the values recorded in the controls.

(iv) N-allylnormetazocine

N-allylnormetazocine had no significant effect on NA concentrations in the SCH, MPO or in the ME. However, the 30 mg/kg dose of N-allylnormetazocine significantly elevated ($p<0.05$) NA concentrations in the ARN at 18.00h.

(c) Dopamine

The control values for the DA concentrations in the SCH, MPO, ME and ARN were 2.6 ± 0.7, 3.8 ± 0.4, 36.3 ± 8.0 and 4.4 ± 1.3 ng amine/μg protein respectively (values expressed $\pm$ SEM).

(i) Naloxone

No dose of naloxone significantly altered DA concentrations in any of the four areas studied compared to values observed in control animals.

(ii) Duromorph

No dose of Duromorph significantly altered DA concentrations in the SCH, ME and ARN compared to control values. However, the 3 mg/kg dose of Duromorph did significantly increase ($p<0.001$) DA concentrations in the MPO.

(iii) Tifluadom

Only the 50 mg/kg dose of Tifluadom significantly increased ($p<0.001$) DA concentrations in the SCH at 18.00h compared to controls. In the other three areas studied, no dose of Tifluadom significantly altered DA concentrations.

(iv) N-allylnormetazocine

The 50 mg/kg dose of N-allylnormetazocine also significantly elevated ($p<0.05$) DA concentrations in the SCH compared to controls. DA concentrations in the MPO, ME and ARN were not significantly affected by any of the doses of N-allylnormetazocine administered.

(d) 5-Hydroxyindoleacetic Acid

Control values for 5HIAA in the SCH, MPO, ME and ARN were 8.7 ± 2.6, 10.4 ± 2.7, 5.9 ± 1.3 and 7.7 ± 1.2 ng amine/μg protein respectively (results expressed $\pm$ SEM).

(i) Naloxone

Naloxone had no significant effects on 5HIAA concentrations in the SCH, MPO and ME compared to values recorded in controls. However, in the ARN, the 1 and 10 mg/kg doses of naloxone significantly elevated (both $p<0.01$) 5HIAA levels above those observed in the controls.

(ii) Duromorph

No dose of Duromorph significantly altered 5HIAA concentration in any of the four regions studied.

(iii) Tifluadom

Tifluadom had no significant effects on 5HIAA concentrations in any of the four areas studied.

(iv) N-allylnormetazocine

The 50 mg/kg dose of N-allylnormetazocine significantly increased 5HIAA concentrations in the SCH, while the 3 and 30 mg/kg doses significantly increased 5HIAA concentrations in the ME (all $p<0.05$). No dose of N-allylnormetazocine had a significant effect on 5HIAA concentrations in the MPO and the ARN.

(e) Serotonin

The 5HT levels for controls at 18.00h were 18.9±3.1, 21.2±4.3, 14.8±2.0 and 19.6±3.0 ng amine/μg protein for the SCH, MPO, ME and ARN respectively (values given ± SEM).

(i) Naloxone, (ii) Duromorph and (iii) Tifluadom

Neither naloxone, Duromorph nor Tifluadom had significant effects on 5HT concentrations in any of the four areas studied at any of the doses administered.

(iv) N-allylnormetazocine

The 3 and 30 mg/kg doses of N-allylnormetazocine significantly increased ($p<0.01$) 5HT concentrations in the SCH. However, N-allylnormetazocine did not significantly alter 5HT concentrations in the MPO, ME or ARN at any of the doses used.

(f) Ratio of 5HIAA/5HT

The results for the ratio of 5HIAA/5HT in control animals in the SCH, MPO, ME and ARN were 0.447±0.081, 0.717±0.227, 0.453±0.087 and 0.425±0.049 respectively (values expressed ± SEM).

(i) Naloxone, (ii) Duromorph and (iii) Tifluadom

None of these drugs had any significant effect on the ratio of 5HIAA/5HT in any of the four areas studied regardless of the dose administered.

(iv) N-allylnormetazocine

In the group of animals receiving 50 mg/kg N-allylnormetazocine, the 5HIAA/5HT ratio in the SCH ($p<0.001$) and ARN ($p<0.05$) was significantly greater than that seen in the controls. The 30mg/kg dose also significantly increased the ratio of 5HIAA/5HT in the ARN compared to that of controls ($p<0.001$). However, N-allylnormetazocine had no significant effects on the ratio of 5HIAA/5HT in the MPO, nor in the ME.

Discussion **- Luteinizing Hormone**

The ability of the 10mg/kg dose of naloxone to increase the LH surge amplitude may reflect an enhanced response of pituitary gonadotrophs to the LHRH signal on the afternoon of pro-oestrus as a result of the administration of the antagonist. Similarly, the reduced LH surge magnitude evident after the injection of the higher doses of Duromorph and Tifluadom could be explained by inhibition of pituitary responsiveness to LHRH caused by the μ- and κ-agonists. However, the hypothalamus contains only a low density of opioid receptors (Khachaturian *et al*, 1985) and opioids have been shown to have no direct effect on secretion of LH from the anterior pituitary (for review,

see Bicknell, 1985). Thus, it is probable that the effects of the opiates in this experiment do not result from alterations of the pituitary responsiveness to LHRH, but rather from effects on the secretion of LHRH itself. This hypothesis is dealt with in more detail in the General Discussion under the section entitled 'Site of Action of Opiates'.

The higher doses of naloxone administered increased the amplitude of the preovulatory LH surge. This indicates that, although reduced, EOP tone is not totally eliminated during the critical period; the increased amplitude resulting from antagonism of EOP secretion at this time.

Paradoxically, the lowest dose of the antagonist depressed the surge release of LH (although not significantly), suggesting the existence of a population of opiate receptors which have a positive influence on the LHRH surge-generating system. These stimulatory receptors would appear to be more sensitive to naloxone since their actions predominate at the lowest dose. Their effects are over-ridden by activation of the inhibitory receptors as the higher doses of naloxone result in increased LH release. It is possible that the EOP neurones involved in the surge-generating system possess autoreceptors that, when activated, limit the outflow of EOP. Hence, naloxone antagonism at these receptors would increase the release of EOPs and thus inhibit LH secretion. However, it may be that

two separate opioid systems exist, one stimulatory and the second inhibitory to the surge.

The stimulation of the LH surge that resulted from administration of the lowest dose of Duromorph implies that the stimulatory (auto)receptors postulated above may be of the μ-opioid type.

The highest two doses of Duromorph completely abolished the LH surge, providing confirmation of μ-opiate suppression in this situation. However, as stated in the Introduction, morphine has a low efficacy for the μ-receptors and so can act as an antagonist in regions where receptors are sparse, as they are in these areas (see Table 2). The low doses of Duromorph may be displacing more potent EOPs from post-synaptic receptors and removing their inhibition. At the higher doses, sufficient Duromorph could be present to maximally activate the post-synaptic receptors and produce its inhibitory action.

Tifluadom, administered at 12.30h, generally reduced plasma LH levels at both 14.30h and 18.00h which suggests that κ- as well as μ-opioid receptors participate in opiate suppression of the LH surge, contrary to the findings of Pfeiffer *et al* (1987). However, κ-receptors do not appear to be involved in the stimulatory EOP mechanism suggested above as all doses of Tifluadom reduced the LH surge amplitude.

Previously, work from this laboratory had been unable to demonstrate σ-opiate inhibition of LH release at 14.30h on pro-oestrus after administration of the σ-agonist, N-allylnormetazocine, at 12.30h (Gopalan *et al*, 1989). In the former experiments drugs were injected under light ether-induced anaesthesia which was itself found to lower LH levels (although not significantly). The ether-induced decrease may have masked any further reduction that could have been caused by the σ-agonist. In the present experiments, all doses of N-allylnormatazocine, injected at 12.30h, reduced plasma LH concentration at 14.30h compared to controls. However, the reductions were not significant, leaving the involvement of σ-receptors unclear.

The results, both within and between groups decapitated at 18.00h, exhibited a wide variance. This is likely to have resulted from the use of N-allylnormetazocine as a racemic mixture; as pointed out earlier, the (+)enantiomer is a σ-agonist while the second is a μ-antagonist. Furthermore, the (+)enantiomer may also act at non-opioid σ-receptors. It is possible that, similar to naloxone, the μ-antagonist properties are responsible for the stimulatory effects of N-allylnormetazocine. Thus the σ-agonist properties would appear to cause inhibition of the preovulatory LH surge since two of the doses used reduced the surge levels of LH (again non-significantly). However, the possibility that the stimulatory actions of this drug are mediated by its actions at non-opioid σ-receptors cannot be discounted.

Aminergic Activity at 14.30h

Of the monoamines measured, 5HIAA was the only metabolite which appeared within the detectable limits of the HPLC-ECD system. When raised concentrations of 5HT were found, increased levels of 5HIAA were almost always observed. This indicates that, at times when 5HT content is elevated, the release of this monoamine may also be increased. Since the enzyme monoamine oxidase (MAO) is involved in the catabolism of all three monoamines measured it is probable that a similar relationship may exist between concentrations of the catecholamines and their secretion. This premise is reviewed in more detail in the General Discussion, under the section entitled, 'Catecholamine Concentrations and Neuronal Activity'.

The only dose of naloxone which significantly altered the magnitude of the preovulatory LH surge (10mg/kg) had no effect on the concentrations of NA or DA in any of the four areas studied. Thus, it appears that the catecholamines may be of little importance in the mediation of naloxone-enhancement of the LH surge amplitude.

The 10mg/kg dose of naloxone did, however, increase 5HIAA concentrations in the SCH, MPO and ME. Taken on their own, the results suggest that the naloxone-induced increase in the LH surge may be mediated by antagonism at presynaptic opioid receptors on 5HT neurones which are known to make connections with LHRH neurones in this area (Kiss and

Halasz, 1985), and believed to stimulate LHRH release (King *et al*, 1986).

However, several other doses of naloxone also increased 5HIAA concentrations in these three regions but, without the same effects on LH. These doses also generally increased DA concentrations in the MPO and ME. DA is thought to be stimulatory to LHRH secretion by an action in the MPO (Kawakami *et al*, 1975) and to inhibit LHRH release at the level of the ME (Fuxe *et al*, 1977). This latter action may account for the failure of the increased 5HT (and DA) activity in the MPO, produced by the lower doses, to enhance the LH surge. This hypothesis is further supported by the finding that levels of DA in the ME similar to those of controls are associated with elevated LH concentrations during the surge. DA projections to the external layer of the ME secrete this amine into the hypophyseal portal blood system as PRL inhibiting factor (PIF) (Ben-Jonathan, 1985). Naloxone suppresses PRL secretion by increasing the outflow of DA from the ME (Pfeifer *et al*, 1987). The possibility that the increased DA concentration in the ME found in the present study is a reflection of naloxone's actions on the regulation of PRL secretion cannot be discounted.

The lowest dose of naloxone markedly elevated NA concentrations in the MPO, ME and ARN, but reduced LH surge levels (although not significantly). Only in the ME did any other dose of naloxone alter NA concentrations. Elevated NA

levels were associated with both high and low circulating LH concentrations during the surge and this was dependent on dose administered. Hypothalamic NA is generally believed to be stimulatory to LHRH neurones when circulating gonadal steroid concentrations are high, such as during pro-oestrus. However, inhibitory actions have also been reported at this time (Dotti and Taleisnik, 1984). If the increased NA concentrations found in the work presented here are mediating the effects of naloxone, then it would appear that NA is exerting an inhibitory influence on LHRH secretion. The failure of the higher dose of naloxone, which increased NA concentrations in the ME, to reduce the surge amplitude may be attributable to the absence of concurrent DA inhibition in the ME. Thus, it may be the cumulative effects of NA and DA that mediate the negative influence of naloxone on the surge.

Consequently, the results are consistent with the hypothesis that naloxone enhancement of the LH surge is primarily mediated by 5HT in the MPO. This effect of 5HT may be reinforced by a similar stimulatory action of DA in the MPO. There is also evidence that naloxone activates an inhibitory action of DA in the ME which could negate the above stimulation. Furthermore, naloxone's reduction of the surge may also involve the stimulation of an inhibitory NA input to the LHRH neurones.

Similarly to naloxone, the lower doses of Duromorph generally elevated biogenic amine concentrations in the

four areas examined. As previously indicated, this can be attributed to Duromorph's ability to act as an antagonist in areas sparsely populated with μ-receptors. However, unlike naloxone, the lower doses of Duromorph elevated the LH surge amplitude. This was associated with increased 5HT activity in all four areas studied and elevated DA levels in the MPO, but with no alteration of DA activity in the ME. Hence, the apparent stimulation of the LH surge caused by the lower doses of Duromorph may result from its μ-antagonist activity increasing the stimulatory biogenic amine activity but, only in the absence of the inhibitory influence exerted through DA in the ME.

The highest two doses of Duromorph completely abolished the LH surge, but in association with elevated 5HT activity in the MPO and ME. From the above, such activity would be expected to cause increased rather than reduced secretion of LH. However, this was in combination with both elevated NA concentrations in the ME and unaltered DA concentrations in the MPO. It could be that the stimulatory action of 5HT is not sufficient to over-ride the inhibitory influence of NA without the support of DA stimulation in the MPO. This suggestion implies that a combination of the stimulatory inputs from 5HT and DA neurones may be required to produce LHRH hypersecretion.

The effects of the higher doses of Duromorph are likely to result from its agonist properties, since increasing its concentration by an order of magnitude will greatly

increase its receptor occupancy rate. It is difficult to reconcile the similar inceases of NA concentrations in the ME resulting from both the low and high doses of Duromorph. Such increases could be explained if the agonist effects on NA were not direct, but were mediated by inhibitory interneurones (possibly GABA) that possessed a relatively dense population of receptors. The antagonist activity of Duromorph would then not be evident in this system, and elevation of NA concentrations would occur regardless of dose. Thus, the paradoxical stimulation of the surge by Duromorph may be mediated by a μ-antagonist-induced increase of stimulatory 5HT activity in the MPO and ME which may be enhanced by DA activity in the MPO. Furthermore, μ-inhibition of the surge may be mediated by an inhibitory action of NA in the ME.

The κ-agonist, Tifluadom, was very successful in reducing the LH surge amplitude at all but the lowest dose. The action of Tifluadom on LH concentrations was clearly mirrored by its ability to elevate NA levels, most commonly in the ME. This result is consistent with the previous findings that increased NA activity in the ME after opiate administration is associated with a decreased surge amplitude. It would appear that the inhibitory NA pathway has the greatest influence on the LHRH neurones, since the increased 5HT and DA activity in the MPO failed to enhance LH release in the face of this NA inhibition. Thus, the opioid receptor population which activates the inhibitory

NA pathway appears to be composed of a heterogenous group of μ- and κ-receptors.

The putative σ-agonist had least effect on biogenic amine concentrations in the areas studied, and had no significant effects on LH concentrations during the surge. This provides qualitative support for the hypothesis that opioid effects on the LH surge are mediated by aminergic neurones within the hypothalamus. However, the lower doses of N-allylnormetazocine did increase NA concentrations in the ME, and this was associated with a decreased LH surge magnitude for one of the doses. This indicates that the opioid receptor population which activates the inhibitory NA pathway might include σ-receptors. However, given the high degree of activity this drug possesses for several receptor types, it is possible that N-allylnormetazocine has both positive and negative influences on the surge; these actions negating each other. The results, taken on their own, do not provide stong support for the putative role of opioid σ-receptors in mediating EOP effects on the preovulatory LH surge.

In conclusion, it appears the opioid antagonist enhancement of the LH surge involves stimulatory actions of 5HT and DA in the MPO while the agonist inhibition involves the μ- and κ-opioid activation of an inhibitory NA pathway projecting to the ME.

Aminergic Activity at 18.00h

The results indicate that aminergic activity within the SCH, MPO, ME and ARN decreases on the late afternoon of pro-oestrus, since the concentrations of the amines are mostly lower than at 14.30h. This was expected, since it is generally accepted that alterations to the activity of aminergic neurones projecting to the LHRH neurones during the critical period play a major role in the generation of the preovulatory LH surge. Similar changes in unmanipulated aminergic activity have been previously reported (Rance and Barraclough, 1982).

Administration of the drugs at 12.30h was relatively ineffective at altering biogenic amine concentrations at 18.00h. This is in marked contrast to their effects on LH concentrations at this time, and on the amines earlier on the afternoon of pro-oestrus. It is possible that by this time the drugs may have been metabolised to inactive forms. However, Duromorph has potent analgesic actions up to 24 hours after administration, and can be detected in the circulation throughout this period (Johnston, 1990). Of all the drugs, Duromorph had least effect on neurotransmitter concentrations at 18.00h. It appears that the lack of effect of Duromorph, and by inference of the other opiates, cannot be attributed to breakdown over the course of the experiment. An alternative explanation is that the neural circuitry on which these drugs exert their actions is less succeptible to pharmacological manipulation at 18.00h. Such a possibilty lends support to the hypothesis that the

neural activity in the early afternoon is responsible for the generation of the surge, rather than the activity at the time of the surge.

The antagonist, naloxone, increased only NA concentrations in the ME, and 5HIAA concentrations in the ARN. These effects are similar to, but less extensive, than those recorded at 14.30h, and may represent the 'tail-end' of its actions on these systems. Duromorph and Tifluadom produced virtually no alterations of the amine concentrations. This may reflect an increase in EOP activity which masks the effects of these drugs. Such an increase would be expected at 18.00h as the tonic EOP suppression of LH would be reinstituted to curtail LH secretion on completion of the surge. Unlike its effects earlier in the day, N-allylnormetazocine increased DA activity in the SCH, 5HT activity in the SCH, ME and ARN, and NA activity in the ARN. The non-opioid properties of N-allylnormetazocine may account for such actions. As the EOP tone increases, it would mask the possible opioid actions of N-allylnormetazocine while leaving the non-opioid actions unaffected.

In summary, the relationship between the effects of the drugs on neurotransmitter concentrations at 18.00h and on LH secretion is unclear. However, it is evident that, in saline-treated animals, spontaneous aminergic activity is decreased at a time when the release of LH would be returning to the basal secretion pattern.

TREATMENT	PLASMA LH CONCENTRATION				
Dose	Saline	Naloxone	Duromorph	Tifluadom	SKF 10047
Zero	2.1±0.3 (12)				
1 mg/kg		1.1±0.2 (5)	1.0±0.1 (5)	1.3±0.2 (5)	0.7±0.1 (5)
3 mg/kg		1.1±0.1 (5)	1.9±0.6 (10)	1.2±0.2 (10)	1.0±0.1 (10)
10 mg/kg		1.6±0.1 (11)	0.8±0.1 (5)	1.1±0.1 (5)	0.9±0.1 (5)
30 mg/kg*		2.9±0.7 (5)	1.6±0.3 (10)	1.3±0.1 (5)	1.2±0.1 (10)
50 mg/kg*		3.5±1.6 (5)	1.7±0.3 (5)	1.1±0.2 (5)	1.4±0.1 (5)

Table 5(a). Plasma LH concentrations (ng/ml ± SEM) at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. The number of animals in each group is given in parentheses.

TREATMENT	PLASMA LH CONCENTRATION				
Dose	Saline	Naloxone	Duromorph	Tifluadom	SKF 10047
Zero	9.5±2.2 (14)				
1 mg/kg		5.0±1.7 (5)	15.8±4.5 (5)	7.0±4.1 (5)	10.5±3.7 (5)
3 mg/kg		5.9±2.5 (5)	5.2±1.9 (5)	1.8±0.2b(10)	5.3±2.3 (9)
10 mg/kg		23.1±7.5a(9)	5.6±4.0 (5)	2.0±0.6a (6)	14.5±7.9 (5)
30 mg/kg*		19.3±4.0 (5)	2.2±0.9a(10)	1.8±0.2a (5)	19.8±13.3(5)
50 mg/kg*		19.6±5.1 (5)	1.2±0.1a (4)	3.1±1.0a (5)	4.7±2.0 (4)

Table 5(b). Plasma LH concentrations (ng/ml ± SEM) at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$, b: $p<0.01$ compared to controls using Scheffe's Analysis of Variance and Covariance. The number of animals in each group is given in parentheses.

* The doses of Duromorph used were 40 and 60 mg/kg rather than 30 and 50 mg/kg respectively.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE SCH				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	40.1±7.2	2.4±0.5	8.3±2.0	23.1±3.3	0.368±0.069
Naloxone					
1mg/kg	47.1±9.6	6.6±2.0 **a**	23.5±4.7 **b**	44.4±5.2 **b**	0.560±0.141
3mg/kg	37.3±6.7	7.3±1.7 **b**	16.6±4.9	37.1±5.7	0.393±0.082
10mg/kg	47.7±9.4	3.0±0.4	18.2±5.6 **a**	39.4±8.1 **a**	0.452±0.064
30mg/kg	38.3±1.1	5.8±2.0 **a**	19.6±2.4 **a**	29.6±2.0	0.681±0.111
50mg/kg	46.3±9.0	4.3±0.8	11.9±2.1	15.4±3.4	0.716±0.100
Duromorph					
1mg/kg	70.9±16.4**b**	10.0±2.5 **c**	20.5±4.6 **b**	61.3±14.1**c**	0.351±0.076
3mg/kg	43.9 ±4.7	4.8±1.1	10.7±2.0	29.5±3.4	0.410±0.064
10mg/kg	28.8±5.9	10.5±2.8 **c**	17.2±6.9	25.5±12.7	1.200±0.580**b**
40mg/kg	36.1±7.1	3.8±0.6	16.7±2.7 **a**	27.6±6.7	0.730±0.093
60mg/kg	26.2±5.8	2.6±0.7	20.1±7.4 **a**	23.0±4.7	0.674±0.233
Tifluadom					
1mg/kg	48.6±11.0	8.0±1.4	23.1±4.2 **b**	64.5±20.0**b**	0.544±0.160
3mg/kg	52.4±12.1	4.7±1.4	27.2±6.5 **c**	42.1±8.1	0.720±0.209
10mg/kg	53.8±8.2	6.6±2.0	21.7±3.3 **a**	64.8±13.1**b**	0.388±0.114
30mg/kg	42.8±6.9	3.7±1.0	21.9±3.0 **a**	62.2±10.2**b**	0.367±0.037
50mg/kg	40.5±13.9	8.1±3.1	14.4±4.0	22.7±5.8	0.633±0.074
SKF 10047					
1mg/kg	42.8±9.2	7.1±2.6 **a**	20.3±5.0	46.3±10.4	0.569±0.178
3mg/kg	37.4±4.4	4.4±0.9	16.4±2.0	39.9±8.4	0.484±0.131
10mg/kg	36.1±6.9	5.9±1.5	16.0±2.4	30.1±14.4	1.140±0.148
30mg/kg	40.5±8.2	10.3±4.4 **c**	19.2±5.5	46.2±12.0	0.436±0.069
50mg/kg	26.6±6.3	4.7±0.5	13.4±4.6	23.8±4.2	0.514±0.096

Table 6. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE MPO				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	47.6±8.9	3.5±0.6	8.7±1.3	23.0±2.4	0.472±0.109
Naloxone					
1 mg/kg	108.8±34.8 **b**	10.6±2.1 **c**	21.8±6.9 **a**	40.8±15.2	0.590±0.074
3mg/kg	74.4±8.6	9.1±2.2 **b**	21.2±3.7 **a**	35.3±6.2	0.574±0.029
10 mg/kg	43.1±7.8	4.6±1.5	19.9±4.0 **a**	23.8±5.3	0.866±0.293
30 mg/kg	36.0±8.0	8.2±0.2	17.1±3.6	51.9±10.6	0.311±0.150
50 mg/kg	28.2±12.6	3.4±1.3	13.9±4.6	25.4±6.6	0.561±0.192
Duromorph					
1 mg/kg	82.5±16.2**a**	6.8±1.9 **a**	22.0±0.8 **c**	56.1±12.6**c**	0.459±0.077
3 mg/kg	76.9±10.5	4.4±1.1	19.3±3.3 **b**	39.1±5.3	0.536±0.136
10 mg/kg	91.8±29.9**a**	11.0±2.4 **c**	18.8±7.7 **a**	36.3±14.3	0.767±0.173
40 mg/kg	42.4±9.1	5.3±0.8	13.7±1.9	24.1±6.0	0.790±0.179
60 mg/kg	39.5±13.4	4.5±0.9	21.0±4.0 **b**	37.4±10.3	0.720±0.189
Tifluadom					
1 mg/kg	74.5±4.8	9.1±3.4 **a**	38.8±15.4**c**	70.8±25.7**c**	0.594±0.119
3 mg/kg	53.9±4.8	5.3±0.5	27.4±3.2 **b**	29.7±4.4	0.999±0.223
10 mg/kg	95.7±22.0**b**	11.6±2.9 **c**	34.8±7.9 **c**	51.2±7.8 **a**	0.745±0.192
30 mg/kg	51.4±10.1	5.7±0.6	20.0±3.3	33.4±6.4	0.622±0.052
50 mg/kg	53.9±25.7	5.4±1.2	19.4±3.9	28.2±5.6	0.800±0.248
SKF 10047					
1 mg/kg	56.1±1.2	4.6±0.8	19.6±2.4	28.9±10.3	1.230±0.456
3 mg/kg	69.7±11.3	7.3±1.4 **a**	26.5±6.9	20.8±2.4	1.077±0.267
10 mg/kg	61.2±14.7	9.6±2.8 **b**	22.6±9.3	26.5±6.2	0.874±0.228
30 mg/kg	42.7±5.7	6.7±0.5	13.5±2.0	26.3±2.4	0.534±0.090
50 mg/kg	37.9±9.9	5.9±1.3	15.6±2.9	28.1±4.1	0.604±0.121

Table 7. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ME				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	22.4±4.1	21.4±6.0	5.1±0.8	13.1±2.2	0.461±0.065
Naloxone					
1 mg/kg	101.4+21.0 c	57.1±19.3a	13.6+2.8 a	66.6±15.6c	0.227±0.057
3 mg/kg	72.6±7.1 c	61.1±16.7a	12.7+5.2 a	34.6±10.4b	0.220±0.093
10 mg/kg	46.2±9.8	22.5±6.5	11.9+1.8 a	20.5±1.9	0.688±0.067
30 mg/kg	66.6±16.8b	42.3±18.3	14.9+4.2 b	28.2±5.3	0.583±0.200
50 mg/kg	12.0±3.2	3.0±1.0	6.7+2.8	11.0±4.8	0.625±0.134
Duromorph					
1 mg/kg	80.2±14.3c	46.6±16.9	10.2±1.1	42.7±4.1 b	0.248±0.032
3 mg/kg	69.1±8.9 c	71.7±21.5	15.1±2.6 a	29.9±5.1	0.534±0.088
10 mg/kg	46.0±15.6	71.0±39.5	12.6±4.8	33.3±15.0	0.622±0.313
40 mg/kg	45.9±9.1 a	58.8±14.4	11.3±2.1	27.8±6.8 a	0.572±0.146
60 mg/kg	35.8±11.3	26.5±11.3	7.3±1.0	14.2±3.9	0.559±0.082
Tifluadom					
1 mg/kg	86.7±13.3c	47.1±25.2	10.5±2.4	33.6±4.3 a	0.308±0.039
3 mg/kg	64.8±11.8c	57.5±20.0	15.0±2.7 b	20.3±5.0	0.737±0.142
10 mg/kg	63.1±7.9 a	30.6±10.7	11.7±2.2	30.8±7.0	0.474±0.152
30 mg/kg	70.7±19.9b	60.7±29.2	11.8±2.6	40.2±13.9b	0.373±0.073
50 mg/kg	30.9±10.3	37.1±16.5	9.2±2.4	8.7±2.1	1.028±0.391a
SKF 10047					
1 mg/kg	71.9±14.4b	35.1±24.1	14.0±3.6	40.0±10.3	0.365±0.043
3 mg/kg	80.9±15.2c	60.1±18.5	12.5±2.3	44.6±12.8	0.436±0.114
10 mg/kg	38.0±15.0	41.3±10.2	15.4±3.8	37.3±7.6	0.464±0.186
30 mg/kg	29.8±7.8	30.4±21.3	20.5±12.7	32.3±14.6	0.570±0.121
50 mg/kg	35.2±14.3	7.8±1.4	24.1±18.0	28.1±13.1	0.755±0.368

Table 8. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ARN				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	30.1±3.3	5.8±1.8	11.3±3.4	23.1±3.6	0.478±0.106
Naloxone					
1 mg/kg	100.9±35.7 c	11.1±3.3	22.1±4.2	52.5±15.3b	0.538±0.180
3 mg/kg	43.8±7.8	8.8±1.5	19.0±6.9	26.9±8.8	0.968±0.473
10 mg/kg	50.6±15.7	5.2±1.5	12.9±2.3	20.6±3.2	0.698±0.107
30 mg/kg	48.0±10.5	5.5±1.8	16.1±4.1	38.8±5.9	0.422±0.113
50 mg/kg	23.8±4.5	5.7±1.5	15.2±1.6	18.3±4.4	1.026±0.410
Duromorph					
1 mg/kg	65.7±13.2a	5.6±1.2	19.3±4.3	54.3±20.7b	0.492±0.169
3 mg/kg	93.3±8.89c	11.3±1.1	23.6±4.3 a	30.1±6.3	0.960±0.273
10 mg/kg	50.1±7.4	6.0±1.6	14.2±4.8	27.9±14.8	0.753±0.274
40 mg/kg	55.6±17.7	4.4±1.4	13.0±2.2	18.6±4.8	0.868±0.130
60 mg/kg	52.5±12.3	8.6±2.0	21.9±3.6 a	38.2±7.6	0.628±0.096
Tifluadom					
1 mg/kg	81.9±15.7b	14.7±4.0	27.3±7.0 a	82.9±26.7b	0.409±0.149
3 mg/kg	56.5±8.6	8.9±2.9	15.2±1.7	23.1±4.0	0.747±0.125
10 mg/kg	126±41.4 c	20.8±8.9 b	36.2±15.3b	87.5±49.2b	0.494±0.053
30 mg/kg	87.8±17.2b	7.1±1.8	19.8±4.4	48.1±13.5	0.447±0.090
50 mg/kg	30.6±8.0	6.7±4.1	16.4±3.1	11.8±0.8	1.362±0.190c
SKF10047					
1 mg/kg	57.0±8.6	6.1±1.0	15.8±1.4	38.8±6.8	0.455±0.072
3 mg/kg	78.6±17.6b	10.7±2.2	23.5±7.9	25.2±8.1	0.652±0.122
10 mg/kg	44.6±21.3	6.6±1.9	7.9±1.4	20.4±6.4	0.478±0.139
30 mg/kg	67.3±13.2a	7.5±1.3	17.7±0.9	44.2±7.1	0.441±0.078
50 mg/kg	32.9±8.8	7.6±3.8	13.8±5.1	24.7±6.9	0.581±0.130

Table 9. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE SCH				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	22.1±5.5	2.6±0.7	8.7±2.6	18.9±3.1	0.447±0.081
Naloxone					
1 mg/kg	27.2±5.9	3.1±1.3	13.3±3.9	16.7±5.0	0.873±0.624
3mg/kg	32.7±4.6	4.4±1.2	10.2±1.6	19.1±6.6	0.923±0.412
10 mg/kg	51.5±13.0	5.4±0.9	19.0±4.3	33.2±8.0	0.653±0.123
30 mg/kg	26.5±7.7	4.1±0.0	10.8±6.1	17.2±4.8	0.592±0.198
50 mg/kg	26.1±7.3	4.4±1.8	11.8±2.9	17.3±3.5	0.741±0.196
Duromorph					
1 mg/kg	29.6±4.4	5.0±2.0	12.3±4.8	37.9±13.6	0.322±0.187
3 mg/kg	26.9±4.6	3.0±0.9	10.4±1.4	18.6±4.0	0.594±0.086
10 mg/kg	18.7±9.5	2.5±0.2	8.9±1.5	9.1±2.2	0.811±0.172
40 mg/kg	24.4±4.7	2.3±0.4	9.7±2.4	19.3±5.0	0.603±0.103
60 mg/kg	23.7±9.7	3.4±1.9	16.8±10.1	21.5±8.7	0.738±0.213
Tifluadom					
1 mg/kg	20.6±7.3	1.9±0.7	5.9±0.1	12.6±3.1	0.573+0.154
3 mg/kg	23.3±2.5	3.9±0.7	8.4±1.2	20.4±3.5	0.473+0.073
10 mg/kg	14.7±3.2	4.7±2.5	18.7±8.2	17.6±3.0	1.084+0.401
30 mg/kg	32.1±6.8	3.7±1.4	14.4±0.9	30.1±11.8	0.603+0.168
50 mg/kg	13.8±5.2	10.0±3.2 **c**	15.8±6.1	15.3±3.5	0.963+0.151
SKF10047					
1 mg/kg	52.0±12.0	4.5±0.9	13.5±4.1	23.5±8.3	0.623±0.156
3 mg/kg	40.5±10.7	5.6±1.8	16.2±2.1	35.1±5.2 **b**	0.524±0.089
10 mg/kg	44.2±10.8	4.1±0.8	16.8±7.0	28.9±3.3	0.608±0.289
30 mg/kg	45.5±6.2	2.7±0.9	18.4±1.4	40.0±2.2 **b**	0.466±0.057
50 mg/kg	37.0±5.4	15.4±10.6**a**	27.4±5.9 **c**	21.8±5.1	1.610±0.680**c**

Table 10. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE MPO				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	29.1±7.0	3.8+0.4	10.4±2.7	21.2±4.3	0.717+0.227
Naloxone					
1 mg/kg	71.3±13.9	6.0±1.7	8.1±2.6	22.2±1.9	0.380±0.145
3 mg/kg	53.6±12.6	5.2±1.7	14.0±2.6	20.4±7.9	0.738±0.365
10 mg/kg	46.2±9.6	6.1±1.5	15.7±2.7	30.2±4.5	0.552±0.081
30 mg/kg	30.1±9.0	2.1±1.5	5.8±2.3	14.0±5.0	0.425±0.052
50 mg/kg	43.0±16.0	4.8±1.6	10.1±3.7	22.1±7.0	0.434±0.025
Duromorph					
1 mg/kg	50.8±5.2	13.3±4.8 **c**	25.8±6.1	33.7±12.0	0.717±0.042
3 mg/kg	30.4±4.5	4.7±0.9	11.0±3.5	15.9±3.5	1.066±0.532
10 mg/kg	26.9±3.6	6.0±2.1	17.5±4.2	22.3±4.4	0.785±0.151
40 mg/kg	38.6±6.4	4.3±1.5	17.1±5.1	15.1±3.4	1.572±0.576
60 mg/kg	47.6±3.9	4.0±0.9	18.5±6.5	22.4±5.8	0.868±0.273
Tifluadom					
1 mg/kg	42.5+6.8	2.7±0.5	8.4+1.1	18.9±6.1	0.612±0.239
3 mg/kg	29.1+3.5	3.4±0.8	9.7+1.2	18.2±2.6	0.608±0.083
10 mg/kg	22.5+0.4	2.4±0.3	10.0+0.5	18.6±5.1	0.633±0.186
30 mg/kg	52.3+12.3	3.8±0.4	10.4+2.5	23.0±2.8	0.508±0.244
50 mg/kg	40.0+15.9	4.0±1.5	10.4+4.0	26.1±6.9	0.521±0.133
SKF10047					
1 mg/kg	40.5±11.9	2.4±0.6	8.8±3.1	13.6±3.9	0.811±0.262
3 mg/kg	53.1±5.7	6.6±1.8	25.6±9.8	36.9±8.8	0.728±0.172
10 mg/kg	27.8±3.5	2.3±1.2	12.7±4.8	21.3±2.7	0.579±0.179
30 mg/kg	28.3±0.3	3.8±2.1	10.6±2.8	15.4±3.2	0.812±0.262
50 mg/kg	32.4±10.2	7.9±0.0	18.9±11.2	11.8±3.3	0.530±0.131

Table 11. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ME				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	28.7+4.4	36.3+8.0	5.9±1.3	14.8±2.0	0.453±0.087
Naloxone					
1 mg/kg	35.7±5.8	27.7±8.1	12.2±3.3	19.3±2.9	0.749±0.084
3 mg/kg	66.3±11.9b	20.2±6.4	16.0±4.2	26.4±8.2	0.767±0.285
10 mg/kg	58.4±13.3a	50.3±17.1	14.6±5.0	18.2±3.9	0.604±0.119
30 mg/kg	29.6±10.4	6.6±2.4	5.3±1.9	10.4±4.1	0.658±0.200
50 mg/kg	55.3±12.9a	10.1±3.6	11.1±5.3	23.4±11.4	0.481±0.034
Duromorph					
1 mg/kg	29.8±3.4	32.9±15.1	9.2±2.9	17.5±5.1	0.587+0.165
3 mg/kg	40.2±7.8	22.7±13.4	10.8±2.7	18.0±4.0	0.733+0.206
10 mg/kg	30.3±9.2	23.5±15.4	8.3±2.5	19.1±2.8	0.458+0.136
40 mg/kg	27.8±3.9	24.5±4.8	9.5±1.8	17.7±5.0	0.569+0.234
60 mg/kg	40.6±16.3	30.2±12.9	6.6±2.9	13.9±6.2	0.811+0.402
Tifluadom					
1 mg/kg	27.0±5.7	14.2±4.3	11.8±3.2	10.2±4.8	1.000+0.000
3 mg/kg	37.0±8.1	25.5±6.3	6.4±1.0	14.9±4.1	0.600+0.128
10 mg/kg	26.4±5.9	44.2±16.3	10.7±2.6	15.4±2.3	0.732+0.192
30 mg/kg	35.2±7.0	53.4±23.3	15.2±10.6	12.6±3.4	1.050+0.551
50 mg/kg	34.4±8.8	19.5±8.4	12.6±1.9	11.8±2.6	1.267+0.434
SKF10047					
1 mg/kg	35.5±11.3	15.1±4.4	7.5±2.5	32.6±13.1	0.393±0.220
3 mg/kg	40.6±10.6	33.9±10.2	14.3±3.0 a	21.3±3.6	0.787±0.202
10 mg/kg	32.0±8.9	14.4±8.6	5.6±0.7	15.7±7.2	0.580±0.251
30 mg/kg	72.3±21.4	22.4±5.9	15.2±4.2 a	25.1±3.8	0.595±0.125
50 mg/kg	37.2±17.7	15.3±12.1	13.1±0.2	23.0±18.1	1.510±1.200

Table 12. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ARN				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	26.8±3.5	4.4±1.3	7.7±1.2	19.6±3.0	0.425±0.049
Naloxone					
1 mg/kg	31.7±6.0	4.6±1.5	17.0±3.6 **b**	21.7±4.4	0.973±0.313
3 mg/kg	43.6±10.1	7.3±2.2	11.4±2.0	21.2±5.6	0.616±0.193
10 mg/kg	34.5±7.5	7.4±2.4	15.4±3.6 **b**	13.0±2.2	1.170±0.334
30 mg/kg	42.8±6.5	2.9±0.4	10.9±1.2	36.2±16.7	0.666±0.243
50 mg/kg	30.8±5.2	4.0±1.2	8.2±1.1	17.5±3.6	0.561±0.171
Duromorph					
1 mg/kg	41.8±5.1	7.5±1.8	10.3±1.5	21.9±6.1	0.465±0.129
3 mg/kg	24.6±5.9	3.0±0.9	6.9±1.8	16.0±5.8	0.437±0.141
10 mg/kg	34.7±6.0	4.1±1.8	11.5±1.3	14.5±4.6	0.626±0.084
40 mg/kg	23.8±4.5	4.4±1.4	7.7±2.4	7.9±1.4	0.501±0.059
60 mg/kg	26.4±6.8	2.2±0.2	8.2±2.7	13.0±2.2	0.595±0.126
Tifluadom					
1 mg/kg	27.4±2.0	4.3±0.9	8.4±2.8	21.8±5.7	0.507±0.238
3 mg/kg	25.1±4.5	4.1±1.0	8.7±1.0	18.8±2.6	0.491±0.050
10 mg/kg	28.9±4.6	7.0±2.1	11.3±1.8	17.8±4.7	0.770±0.161
30 mg/kg	25.3±10.9	5.1±2.1	6.3±1.2	10.6±2.2	0.574±0.074
50 mg/kg	22.7±6.5	4.5±0.2	8.4±0.7	No Result	No Result
SKF10047					
1 mg/kg	38.6±8.3	4.6±1.4	11.0±1.2	19.1±5.8	0.934±0.371
3 mg/kg	35.6±3.1	6.1±1.3	10.4±2.4	19.3±4.6	0.781±0.168
10 mg/kg	30.8±4.7	5.3±1.8	8.9±2.8	9.8±1.2	0.948±0.301
30 mg/kg	45.6±10.2**a**	1.9±0.5	15.7±7.8	24.7±18.4	1.776±0.981**c**
50 mg/kg	18.7±5.5	6.6±3.2	10.2±2.7	8.6±1.7	1.290±0.450**a**

Table 13. Biogenic amine concentrations (ng/mg protein ± SEM) and the ratio of 5HIAA/5HT in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to controls using Scheffe's Analysis of Variance and Covariance.

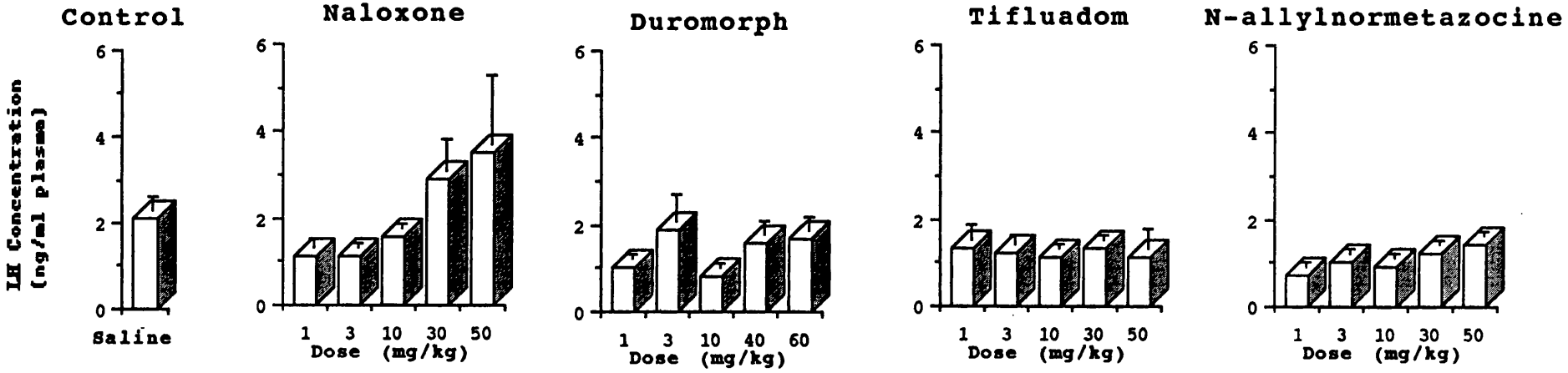

Figure 13(a). Plasma LH concentrations (±SEM) at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

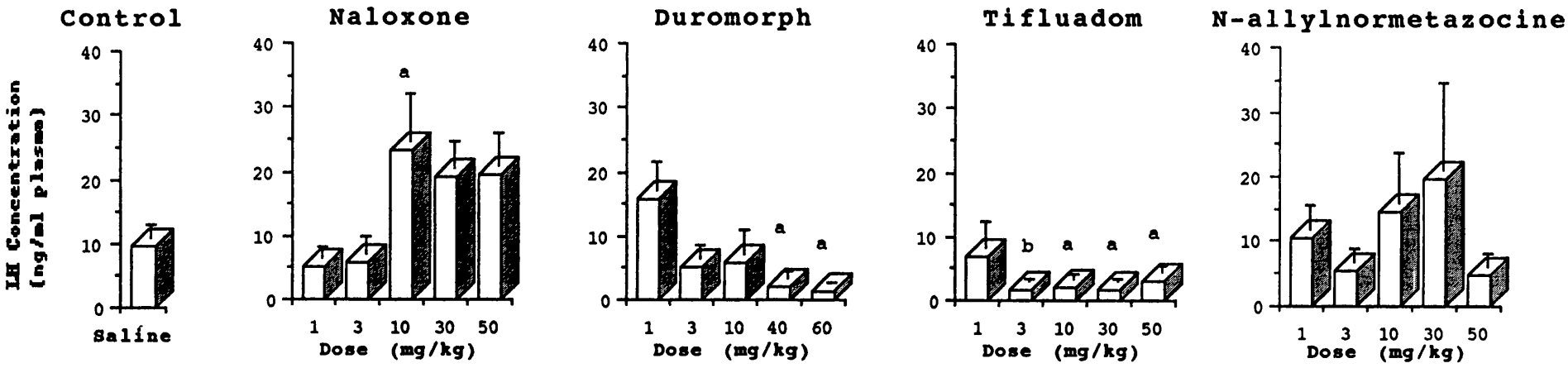

Figure 13(b). Plasma LH concentrations (±SEM) at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

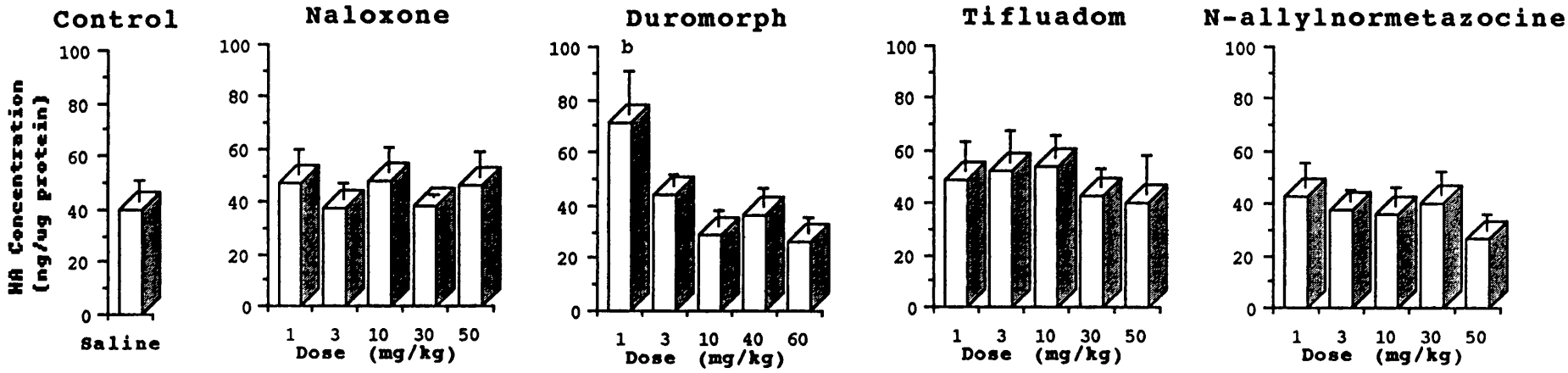

Figure 14(a). Noradrenaline concentrations (±SEM) in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

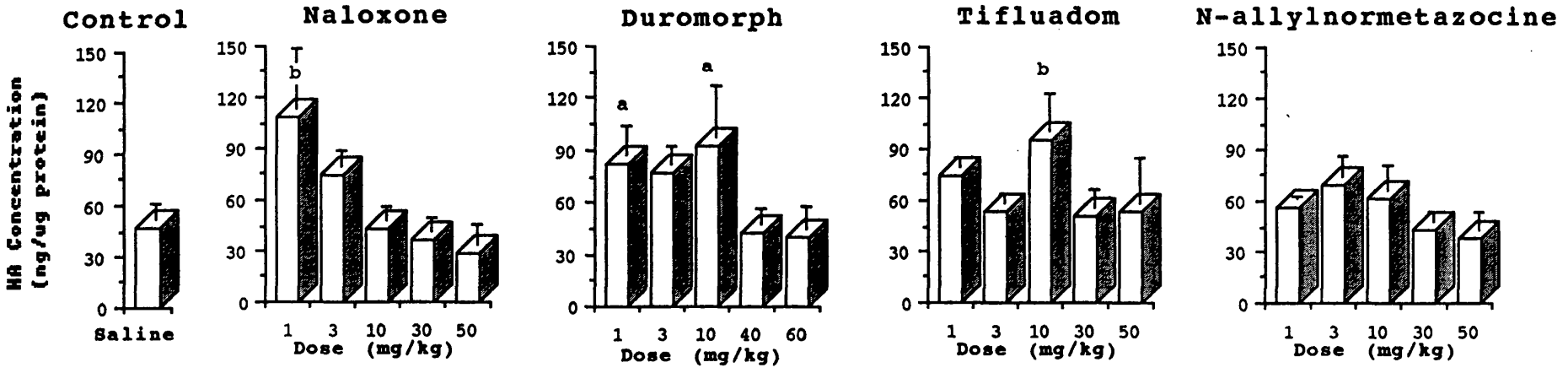

Figure 14(b). Noradrenaline concentrations (±SEM) in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

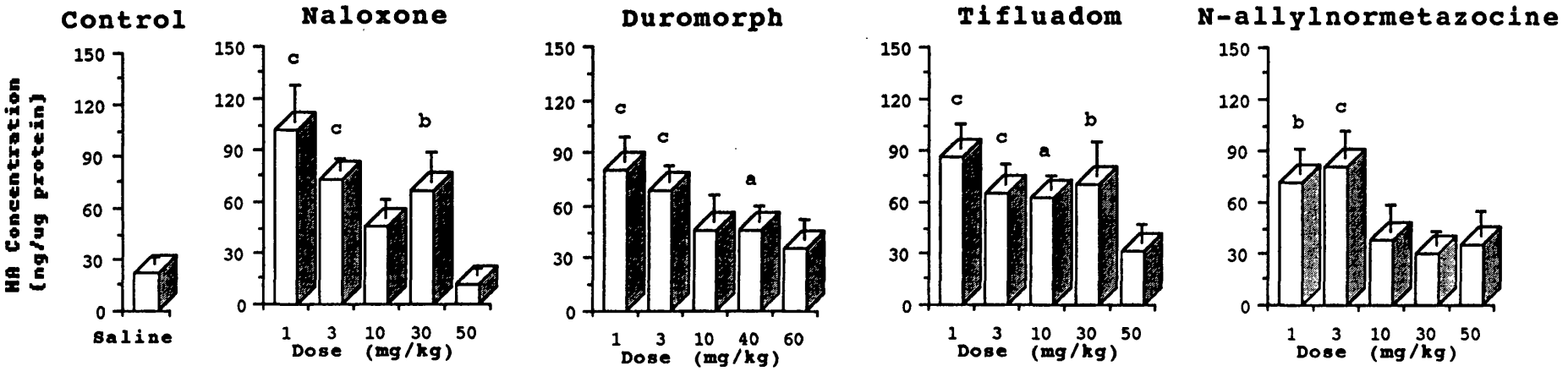

Figure 14(c). Noradrenaline concentrations (±SEM) in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

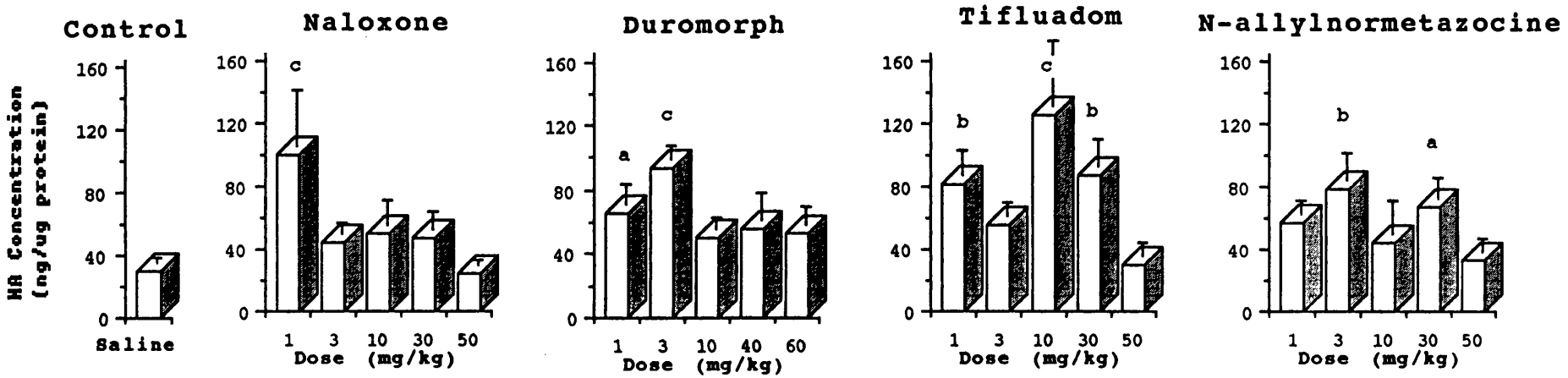

Figure 14(d). Noradrenaline concentrations (±SEM) in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

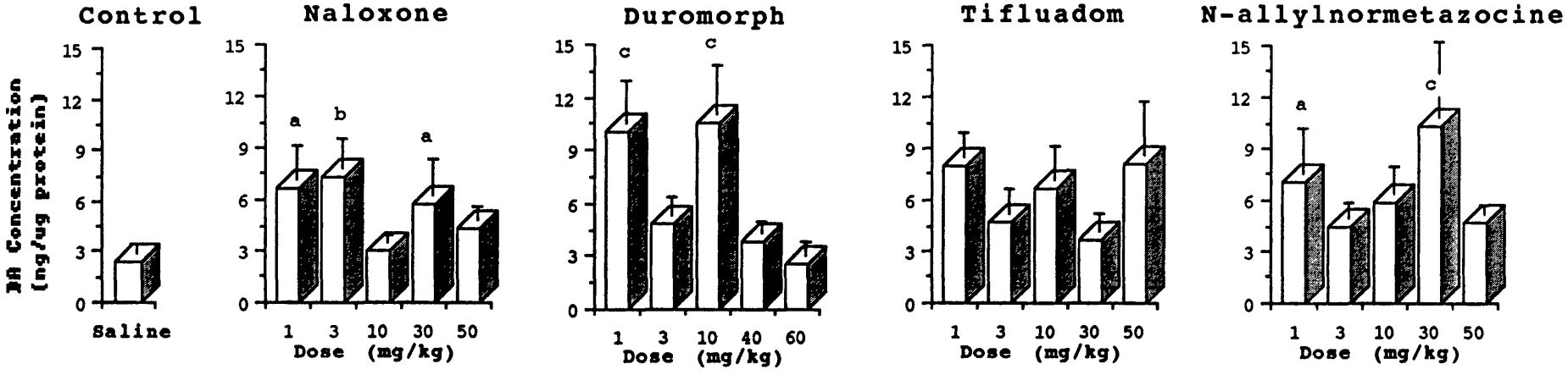

Figure 15(a). Dopamine concentrations (±SEM) in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

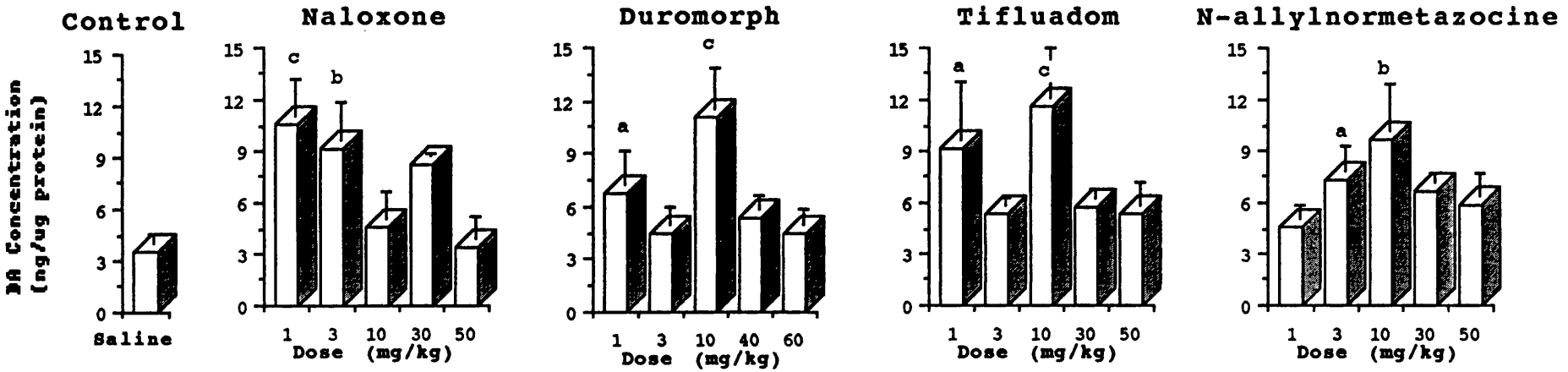

Figure 15(b). Dopamine concentrations (±SEM) in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

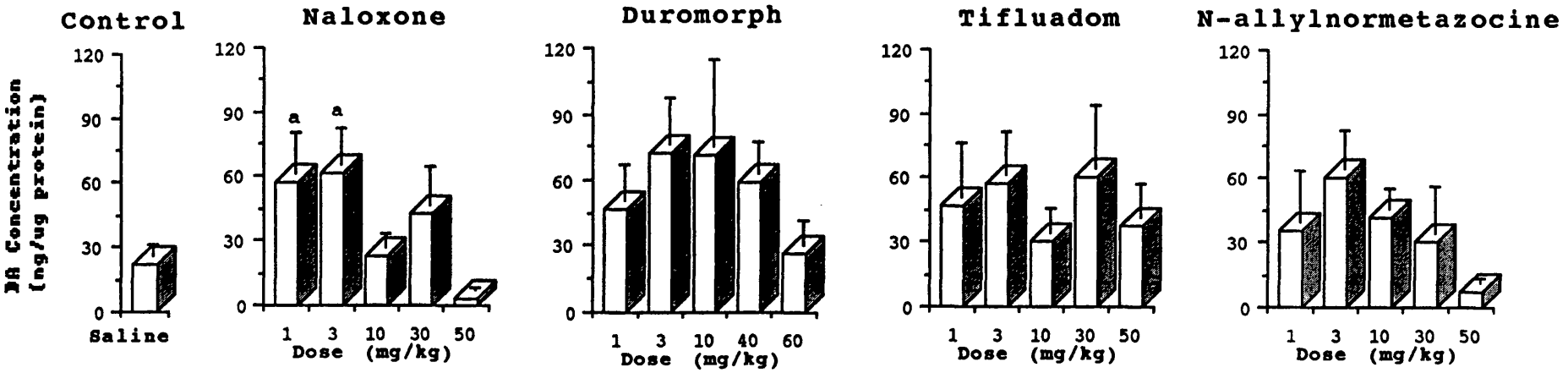

Figure 15(c). Dopamine concentrations (±SEM) in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

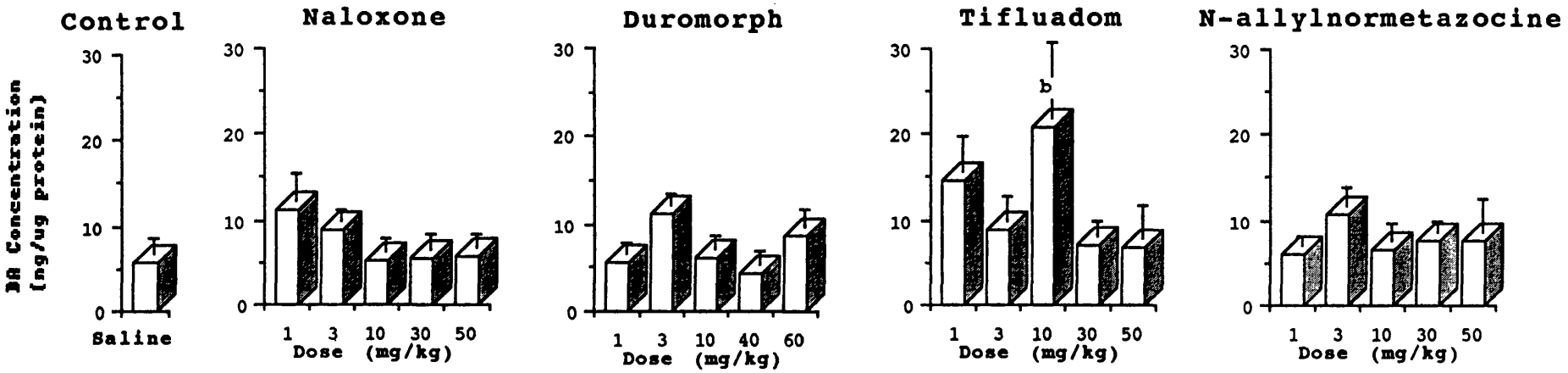

Figure 15(d). Dopamine concentrations (±SEM) in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

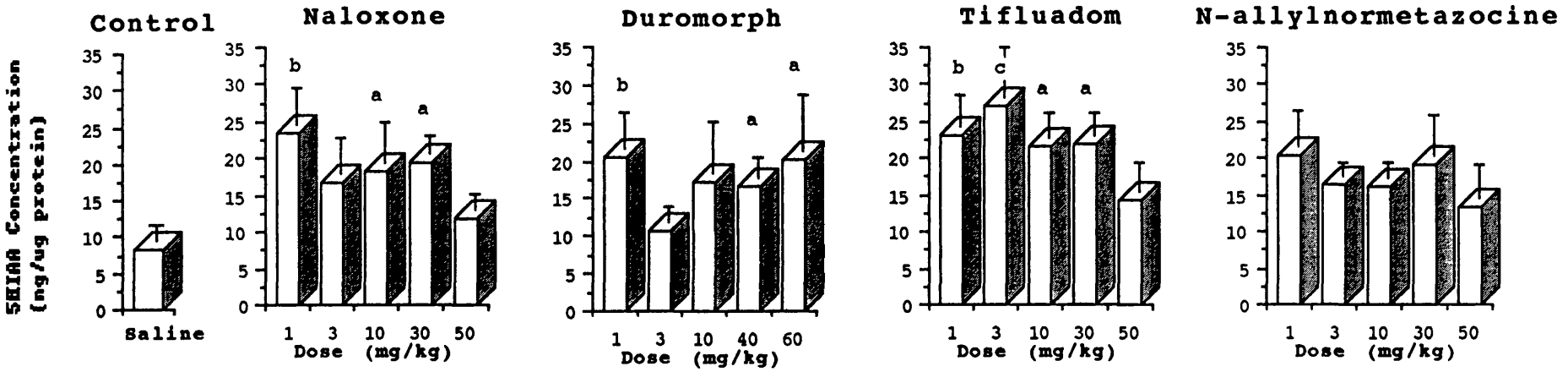

Figure 16(a). 5-Hydroxyindoleacetic Acid concentrations (±SEM) in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

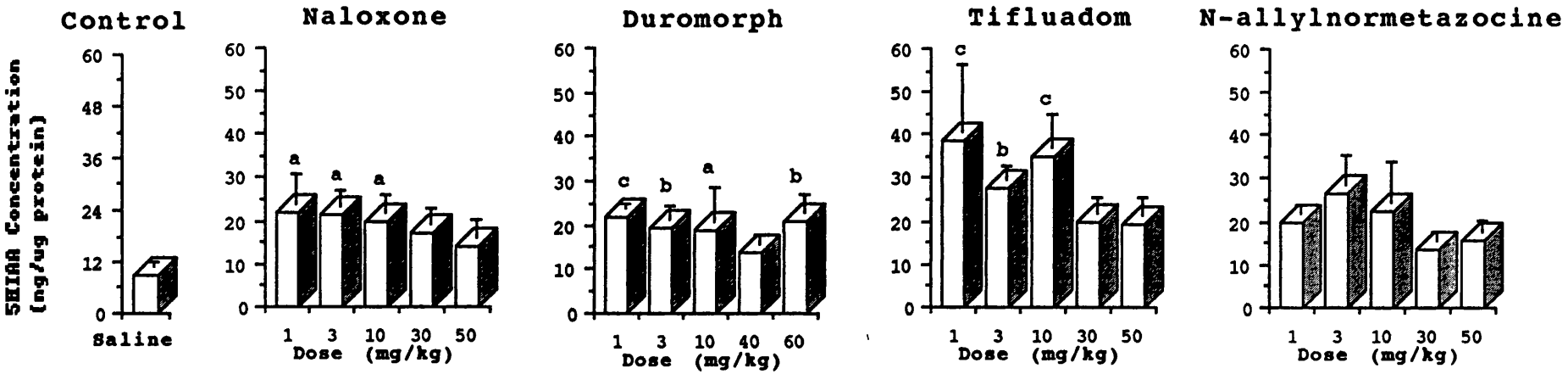

Figure 16(b). 5-Hydroxyindoleacetic Acid concentrations (±SEM) in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

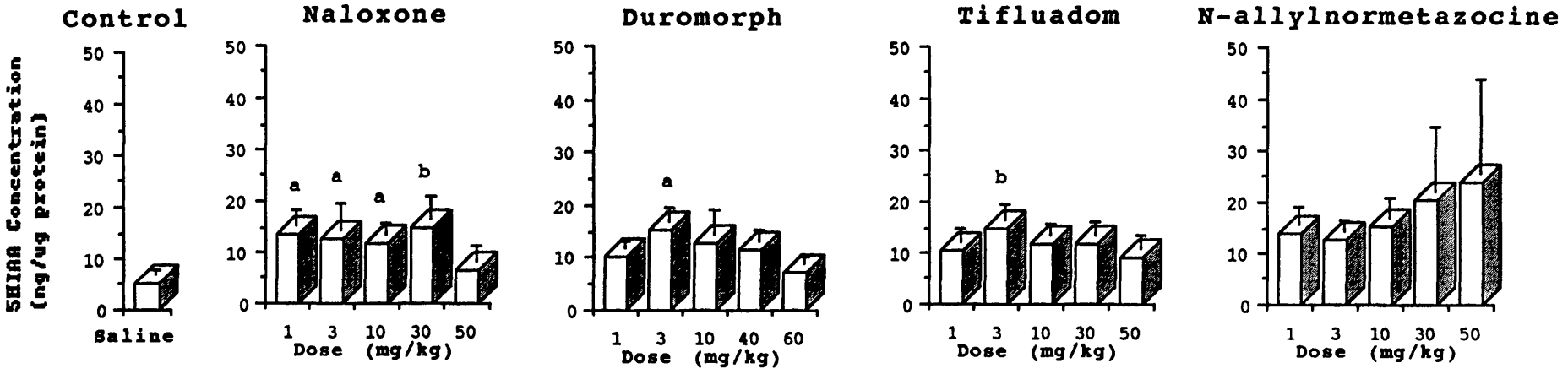

Figure 16(c). 5-Hydroxyindoleacetic Acid concentrations (±SEM) in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

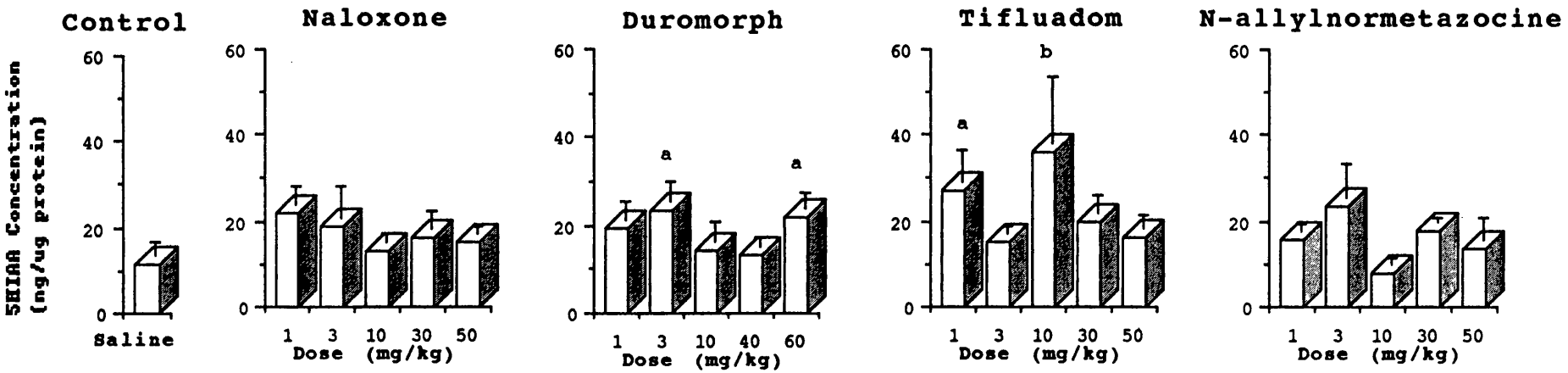

Figure 16(d). 5-Hydroxyindoleacetic Acid concentrations (±SEM) in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

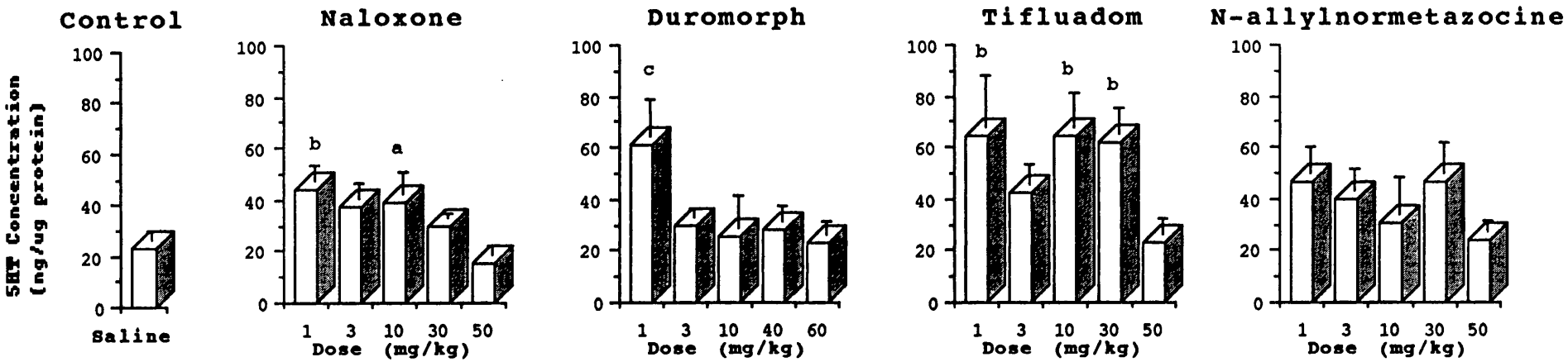

Figure 17(a). Serotonin concentrations (±SEM) in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

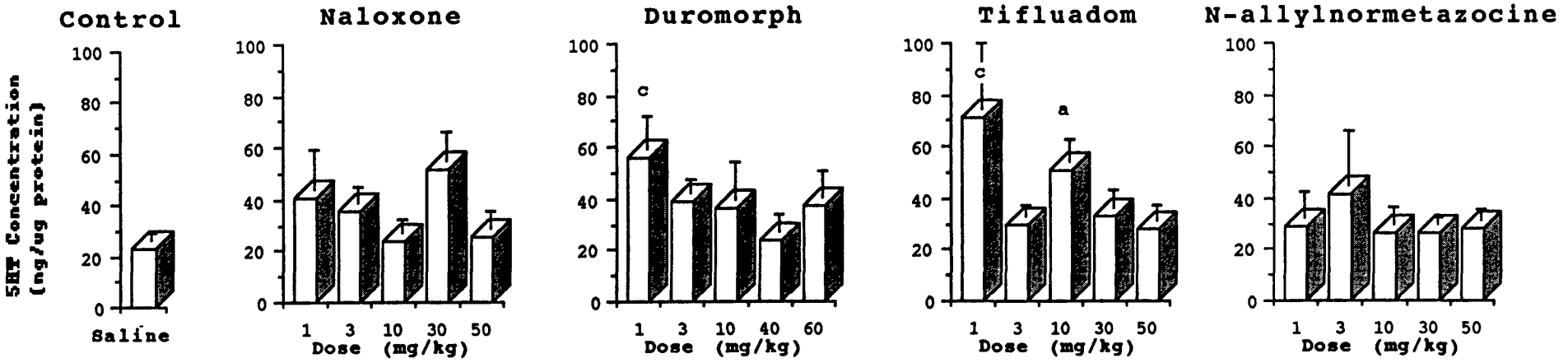

Figure 17(b). Serotonin concentrations (±SEM) in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

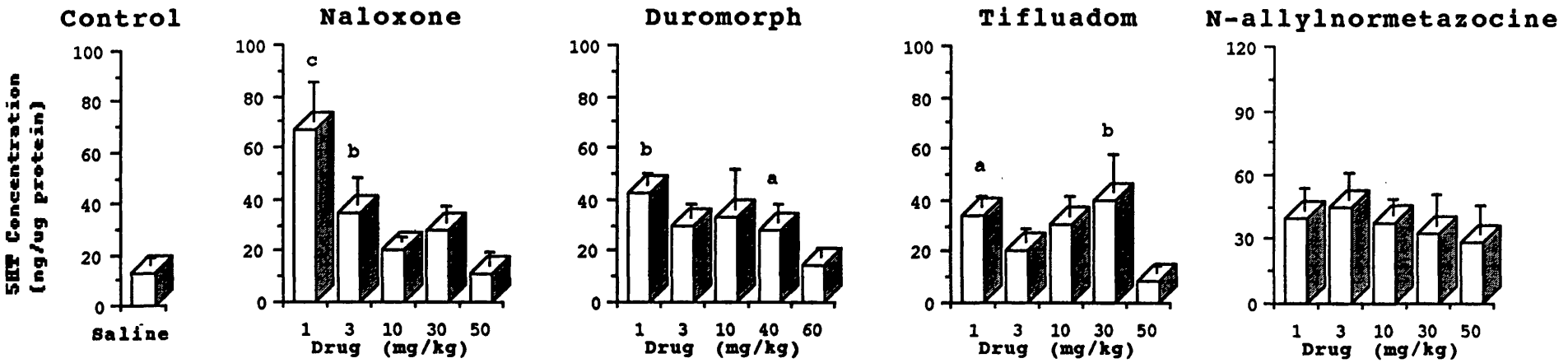

Figure 17(c). Serotonin concentrations (±SEM) in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

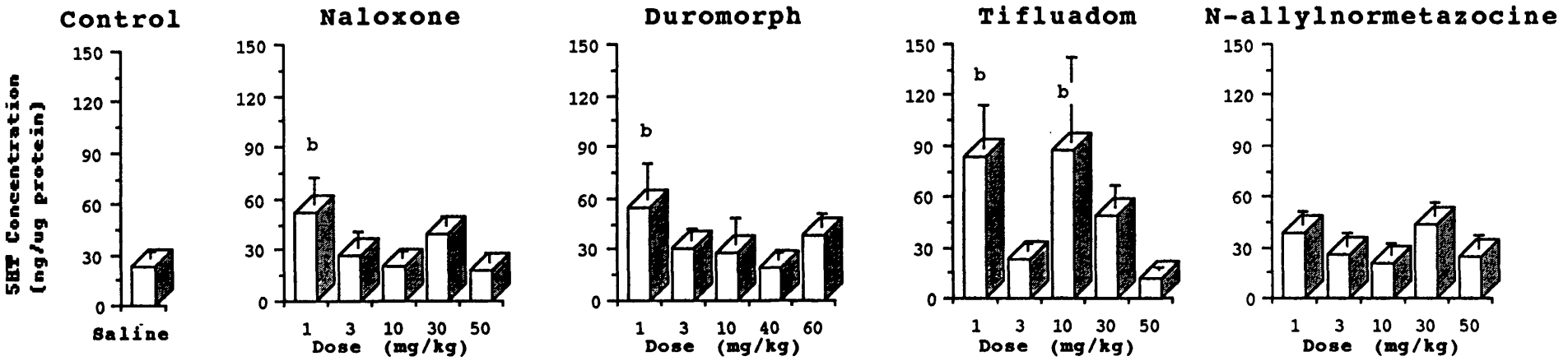

Figure 17(d). Serotonin concentrations (±SEM) in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

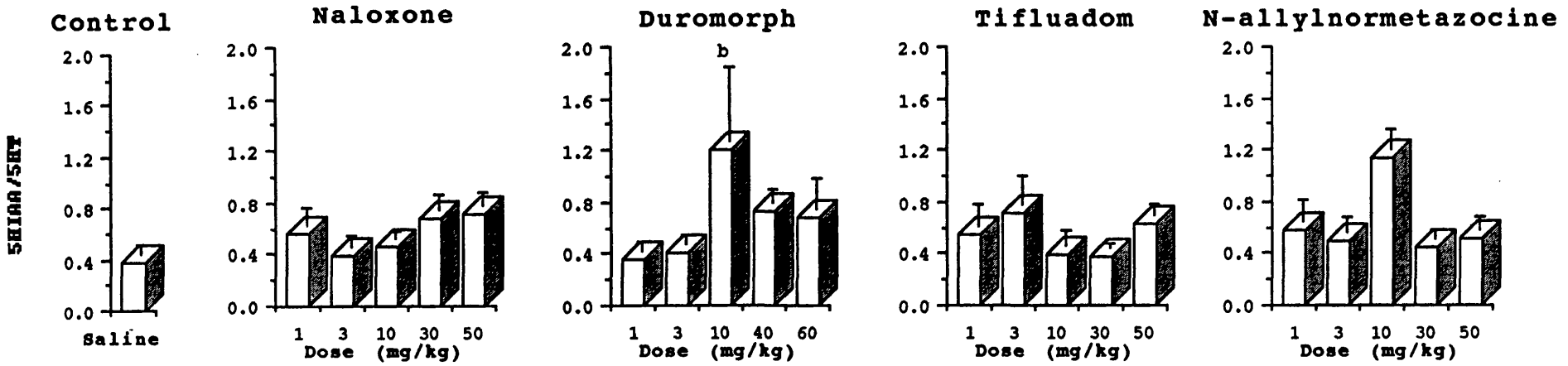

Figure 18(a). Ratio of 5HIAA/5HT (±SEM) in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

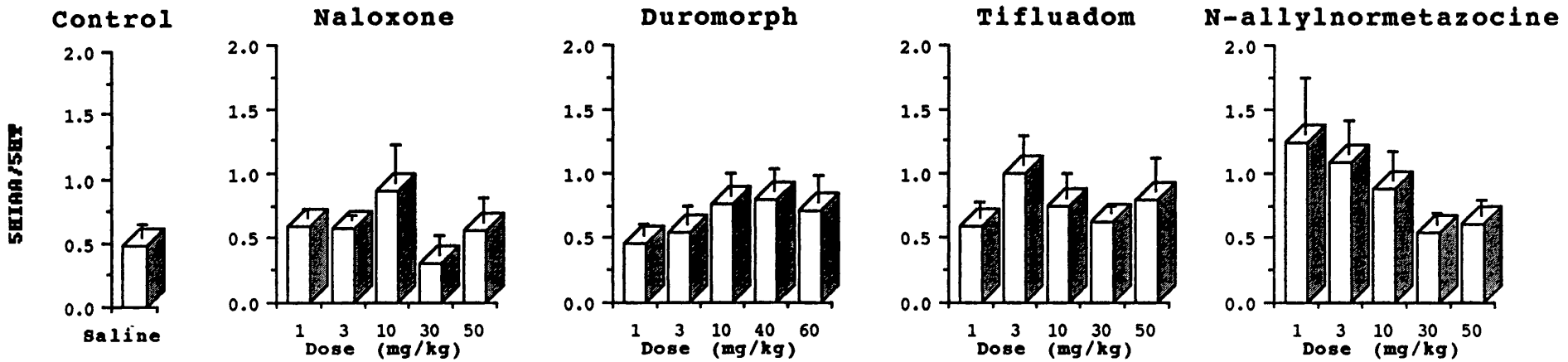

Figure 18(b). Ratio of 5HIAA/5HT (±SEM) in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

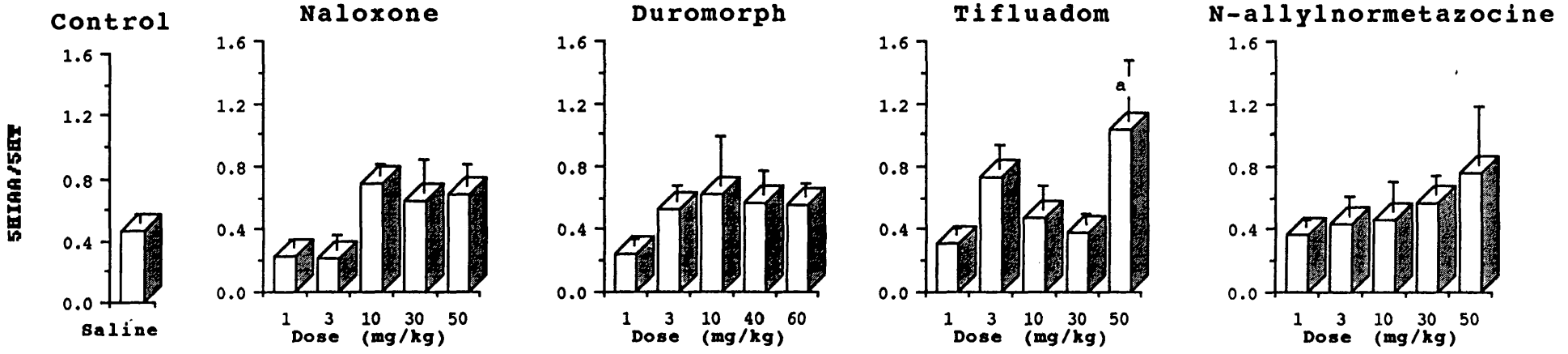

Figure 18(c). Ratio of 5HIAA/5HT (±SEM) in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

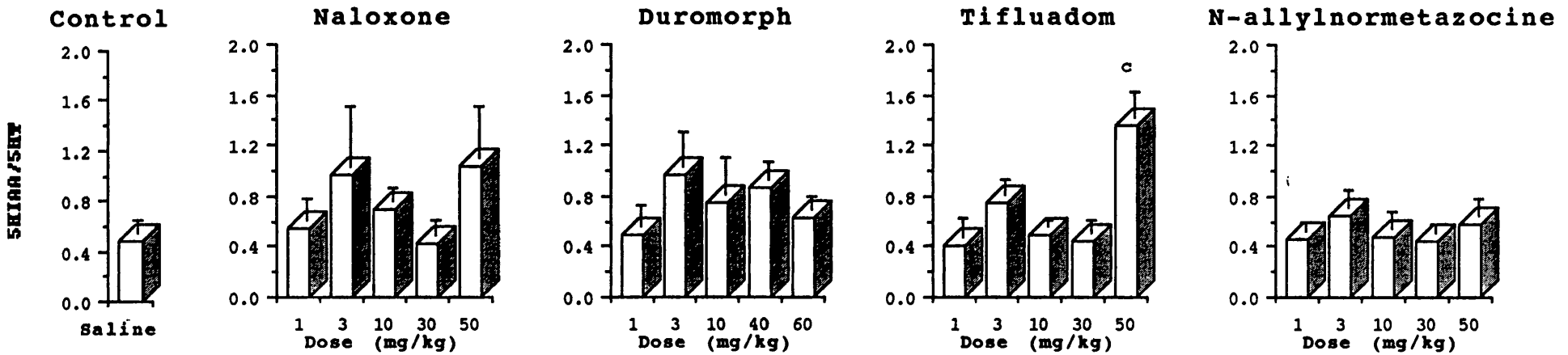

Figure 18(d). Ratio of 5HIAA/5HT (±SEM) in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

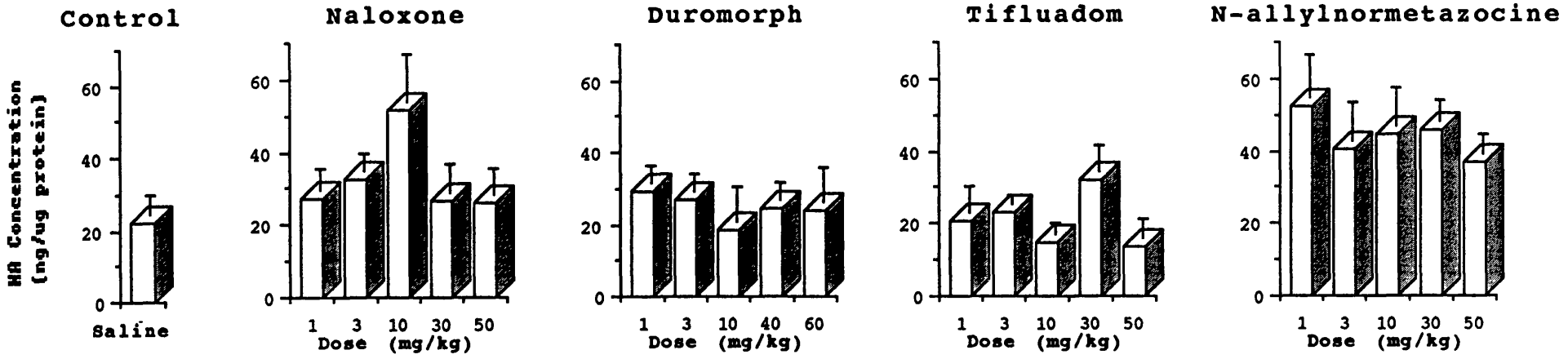

Figure 19(a). Noradrenaline Concentrations (±SEM) in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

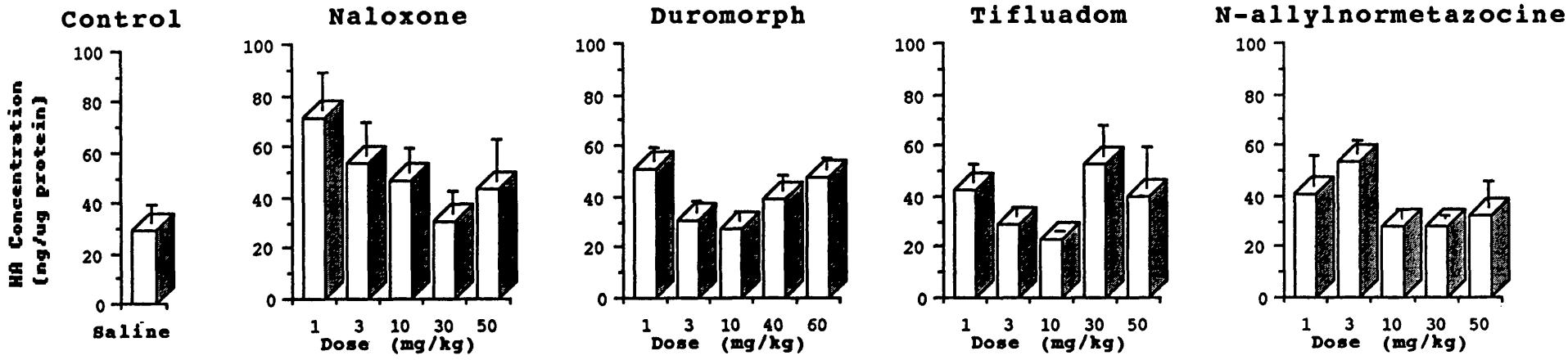

Figure 19(b). Noradrenaline Concentrations (±SEM) in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

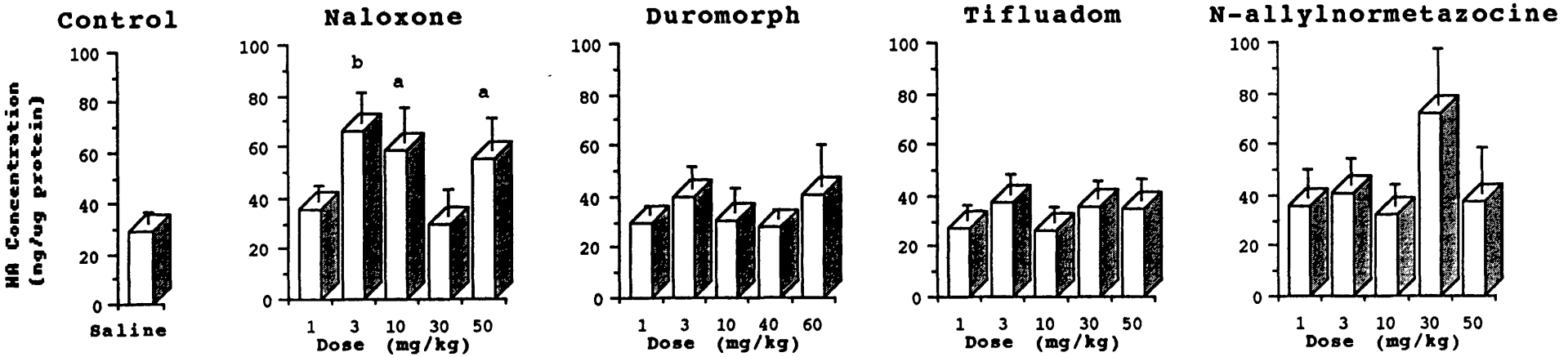

Figure 19(c). Noradrenaline Concentrations (±SEM) in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

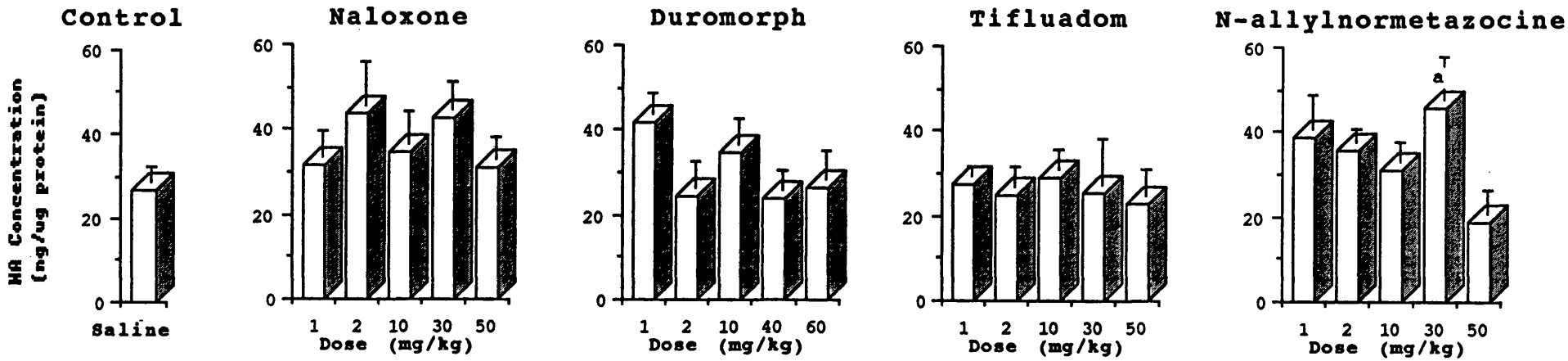

Figure 19(d). Noradrenaline Concentrations (±SEM) in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

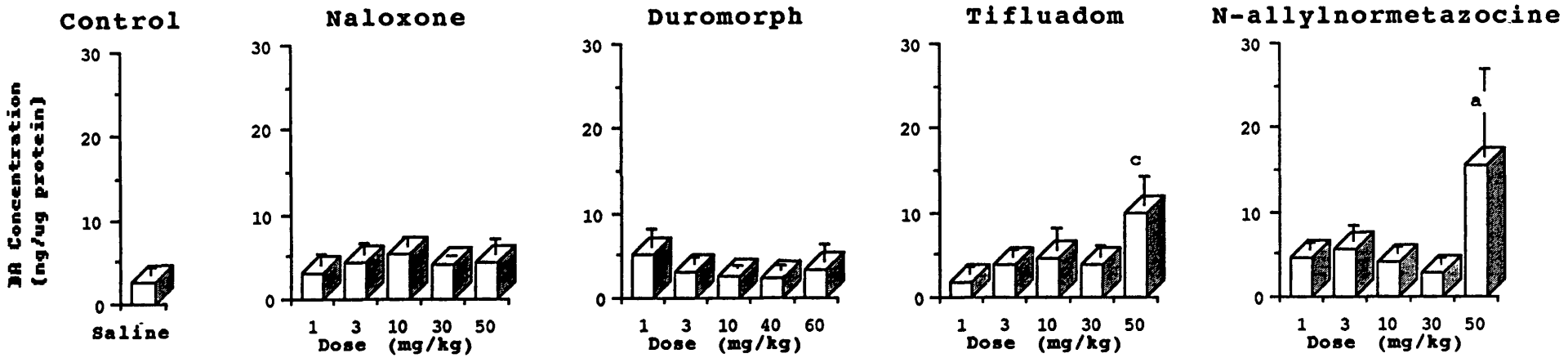

Figure 20(a). Dopamine Concentrations (±SEM) in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

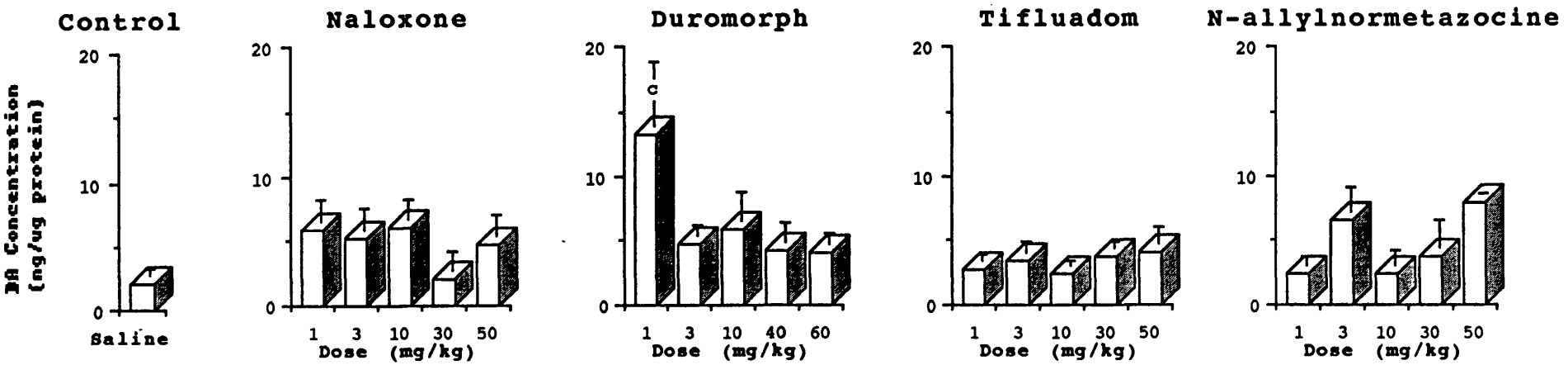

Figure 20(b). Dopamine Concentrations (±SEM) in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

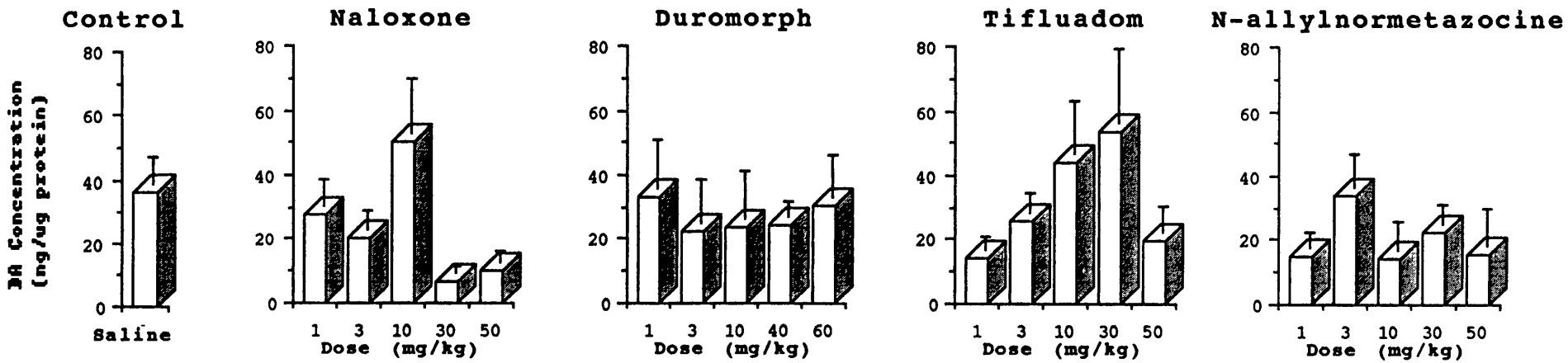

Figure 20(c). Dopamine Concentrations (±SEM) in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

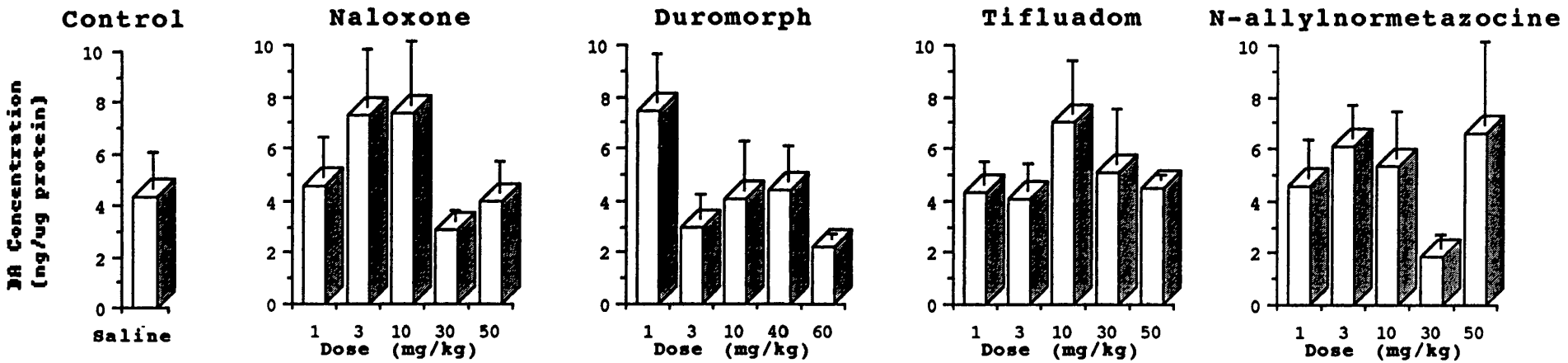

Figure 20(d). Dopamine Concentrations (±SEM) in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

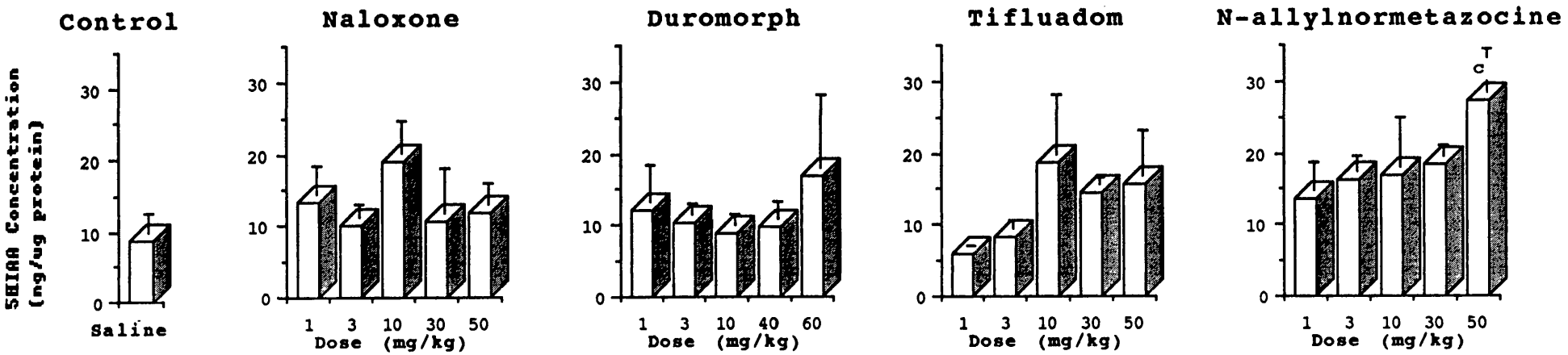

Figure 21(a). 5-Hydroxyindoleacetic Acid Concentrations (±SEM) in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

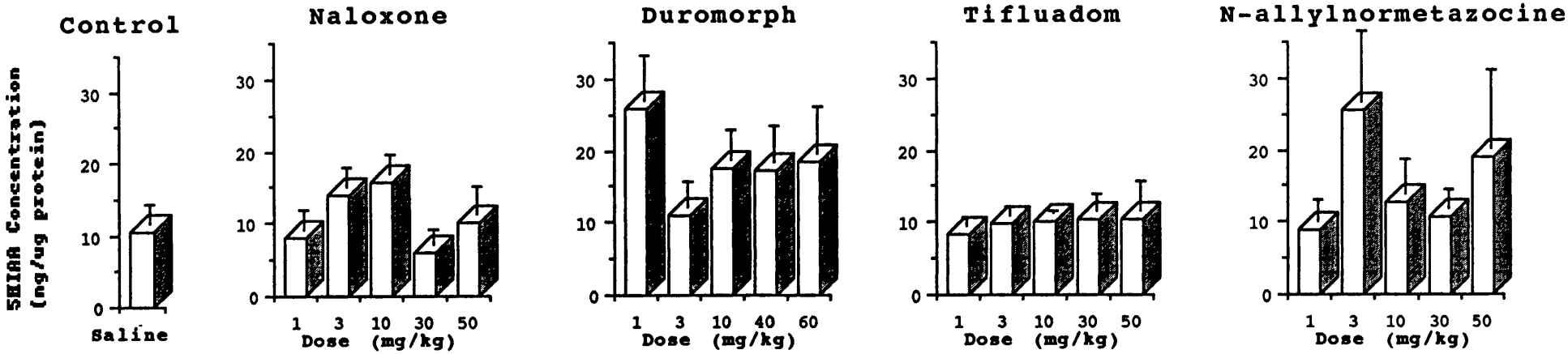

Figure 21(b). 5-Hydroxyindoleacetic Acid Concentrations (±SEM) in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

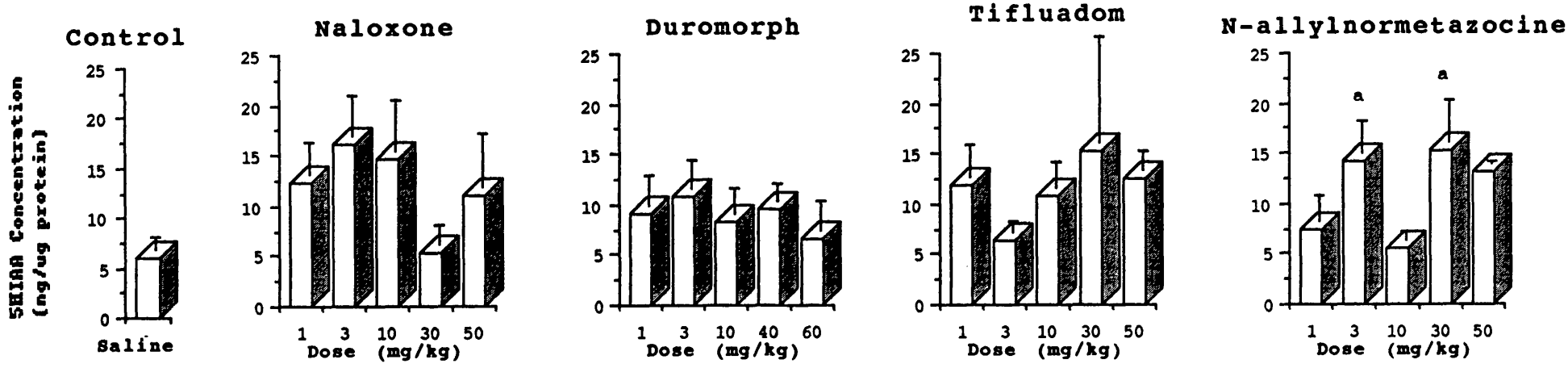

Figure 21(c). 5-Hydroxyindoleacetic Acid Concentrations (±SEM) in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

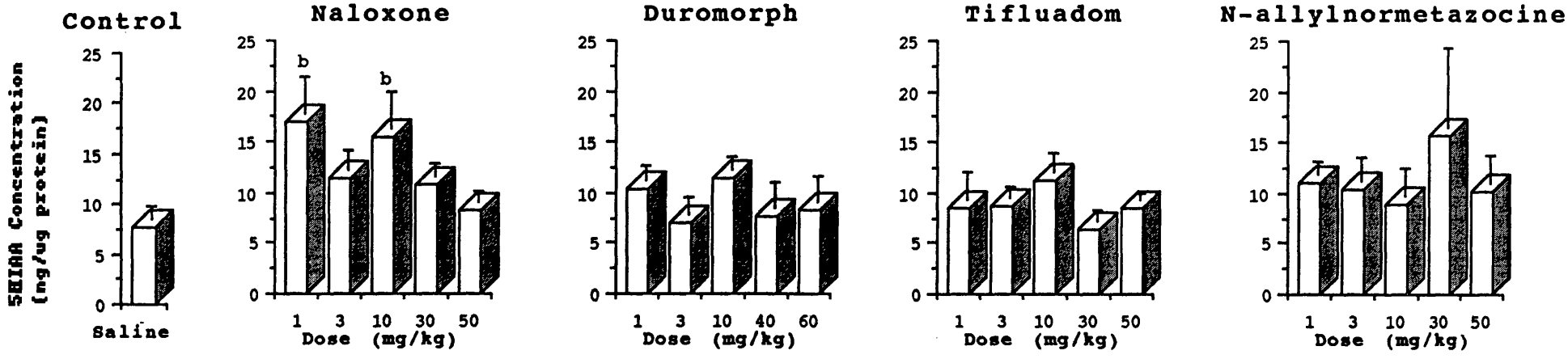

Figure 21(d). 5-Hydroxyindoleacetic Acid Concentrations (±SEM) in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

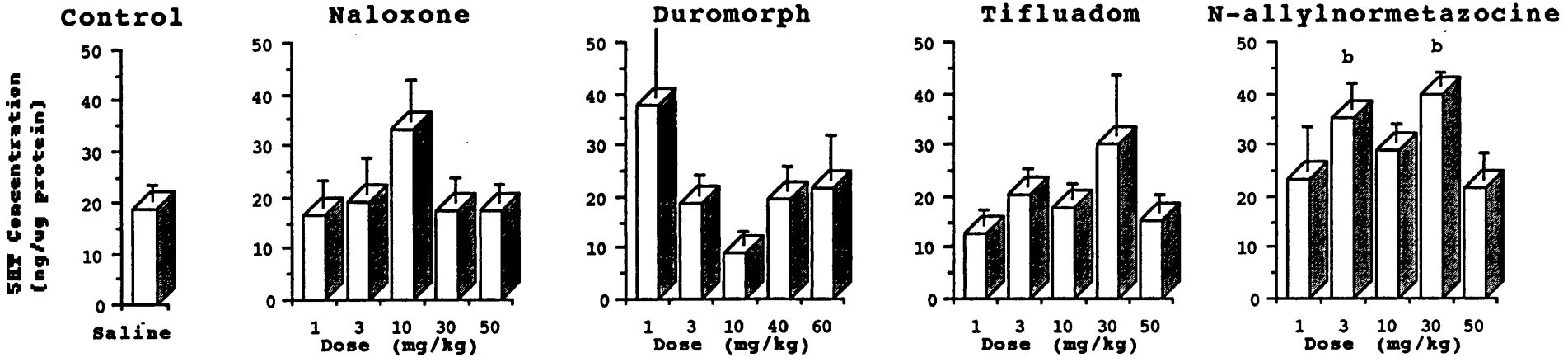

Figure 22(a). Serotonin Concentrations (±SEM) in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

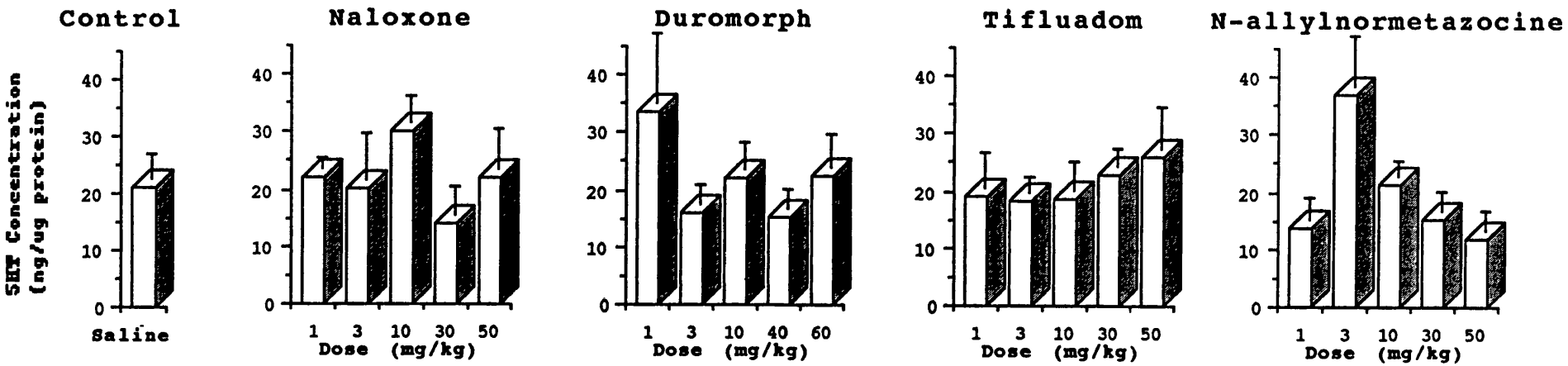

Figure 22(b). Serotonin Concentrations (±SEM) in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

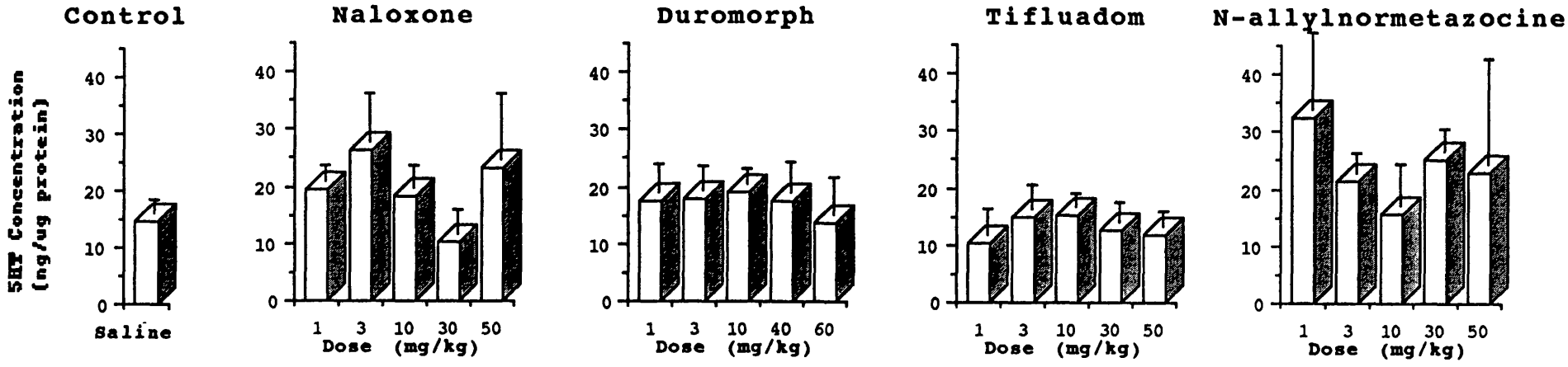

Figure 22(c). Serotonin Concentrations (±SEM) in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

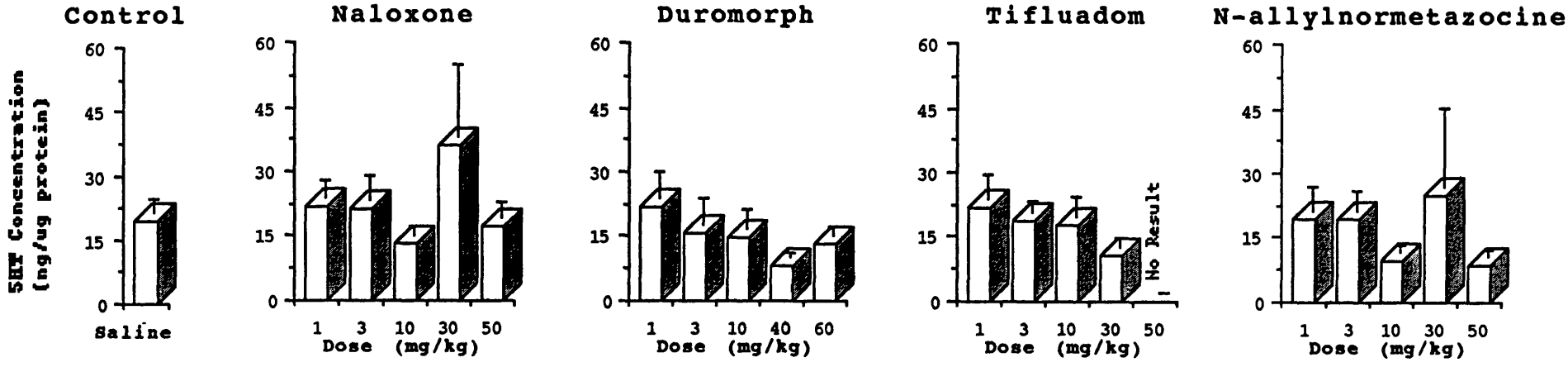

Figure 22(d). Serotonin Concentrations (±SEM) in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

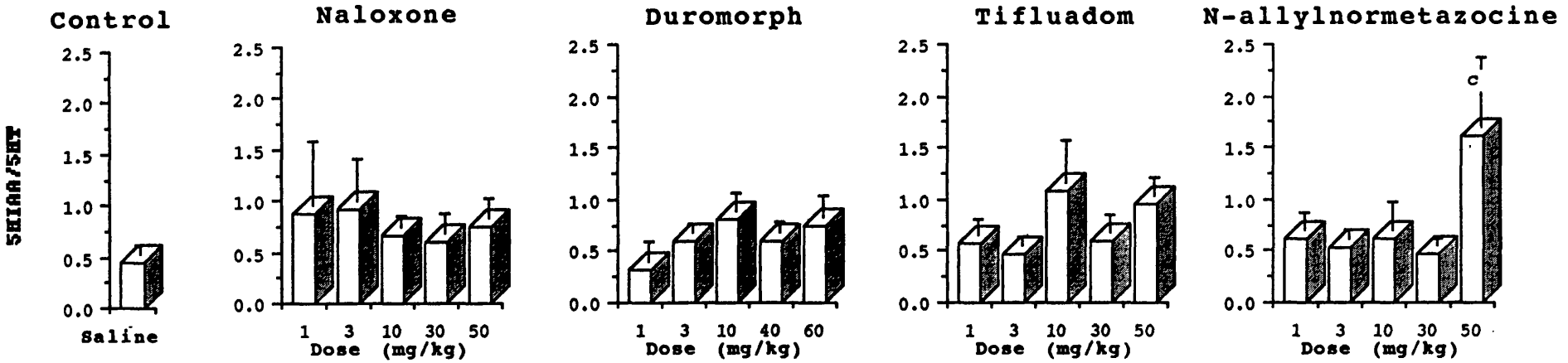

Figure 23(a). Ratio of 5HIAA/5HT (±SEM) in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

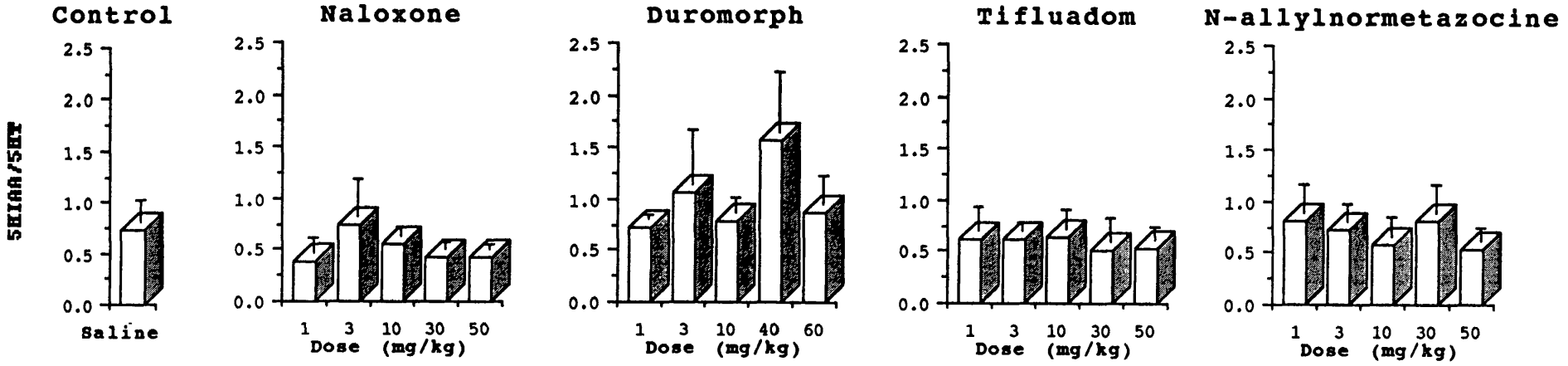

Figure 23(b). Ratio of 5HIAA/5HT (±SEM) in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

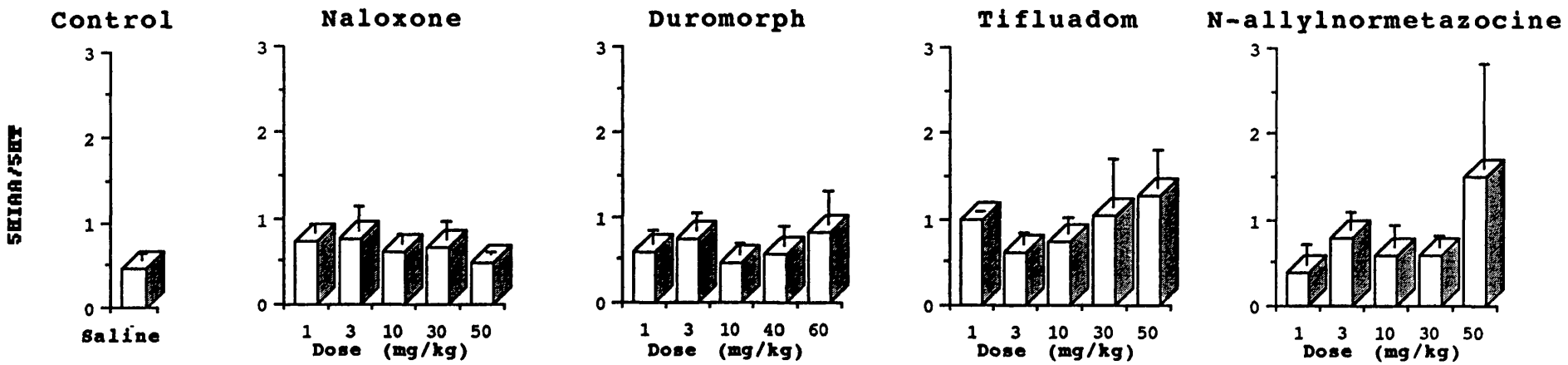

Figure 23(c). Ratio of 5HIAA/5HT (±SEM) in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

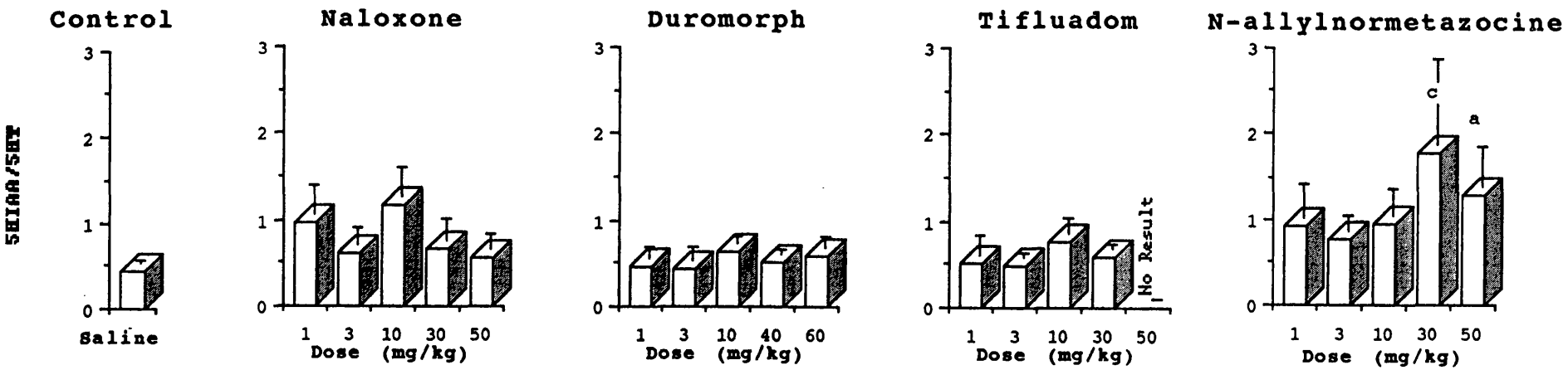

Figure 23(d). Ratio of 5HIAA/5HT (±SEM) in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals using Scheffe's Analysis of Variance and Covariance.

EXPERIMENT 2

This experiment was designed to investigate the ability of the opiate-agonists, Duromorph, Tifluadom and N-allylnormetazocine, to reverse the naloxone-induced hypersecretion of LH during the surge (demonstrated in Experiment 1).

The drug doses selected for investigation were the minima which significantly altered the magnitude of the preovulatory LH surge in Experiment 1. This was with the exception of N-allylnormetazocine, since no dose of this drug significantly affected the surge. For N-allylnormetazocine, the minimum dose which produced a non-significant reduction of the surge amplitude was used.

Furthermore, the effects of these treatments on the biogenic amine concentrations in the SCH, MPO, ME and ARN were to be investigated concomitant with their effects on the LH surge and its naloxone-enhancement. It was hoped that the central effects could be related to any effects on LH concentrations found.

Materials and Methods

Duromorph (40mg/kg), Tifluadom or N-allylnormetazocine (3mg/kg) were co-administered with the antagonist naloxone (10mg/kg) IP. in a 0.05ml/15g volume of physiological saline at 12.30h. Three groups of controls (from Experiment 1) were used for each treatment; the first administered 0.05ml/15g physiological saline (IP), the second

administered 10mg/kg Naloxone alone and the third administered the respective opiate agonist without co-administration of Naloxone. The rats were subsequently decapitated at 14.30h or 18.00h.

In this experiment the control values for saline-, naloxone-, Duromorph-, Tifluadom- and N-allylnormetazocine-treated groups are those from the animals in Experiment 1. The number of animals in each group is shown in Table 14.

Results

(1) 14.30h Results

These results are summarised in Tables 14-18 and Figures 24-29.

(a) Luteinizing Hormone

The animals treated with 3mg/kg N-allylnormetazocine concomitant with 10mg/kg Naloxone exhibited significantly raised levels of plasma LH compared to animals treated with either saline, naloxone or N-allylnormetazocine (all $p<0.01$). However, no other treatment had a significant effect on plasma LH concentrations.

(b) Noradrenaline

(i) Duromorph + Naloxone

NA concentrations in the SCH, MPO, ME and ARN of animals co-administered 40mg/kg Duromorph with 10mg/kg naloxone were not significantly different from those of saline-, naloxone- and Duromorph-treated animals.

(ii) Tifluadom + Naloxone

Animals treated with 3mg/kg Tifluadom plus 10mg/kg naloxone had NA concentrations in the SCH and ARN which did not differ significantly form saline-, Tifluadom- and naloxone-treated groups. However, in the MPO, these animals exhibited significantly lowered NA concentrations compared to saline- ($p<0.05$) and Tifluadom-treated ($p<0.05$) animals. NA concentrations in the ME of this group were significantly reduced ($p<0.01$) compared to the Tifluadom-treated group.

(iii) N-Allylnormetazocine + Naloxone

The NA concentrations in the SCH of this group were not significantly different from saline-, Naloxone- nor N-allylnormetazocine-treated groups. But, in the MPO, ME and ARN the NA concentrations of this group were significantly lower than the N-allylnormetazocine-treated group ($p<0.001$, 0.001 and 0.01 respectively).

(c) Dopamine

(i) Duromorph + Naloxone, and
(iii) N-Allylnormetazocine + Naloxone

Neither of the above two groups exhibited DA concentrations that were significantly different from those of their respective control groups.

(ii) Tifluadom + Naloxone

There were no significant differences between DA concentrations in the SCH, MPO and ME of this group and the saline-, naloxone- and Tifluadom-treated groups. DA concentrations in the ARN of this group were significantly elevated compared to both saline- and naloxone-treated groups (both $p<0.01$).

(d) 5-Hydroxyindoleacetic Acid

(i) Duromorph + Naloxone

In the SCH, this group exhibited significantly lower 5HIAA concentrations than both the naloxone- and the Duromorph-treated groups (both $p<0.05$). There were no significant differences between the 5HIAA levels of this group and any of the three control groups in either the MPO or the ARN. The 5HIAA concentrations in the ME were significantly

elevated above that of the saline- ($p<0.001$), naloxone- and Duromorph-treated (both $p<0.01$) groups.

(ii) Tifluadom + Naloxone

5HIAA concentrations in both the SCH and MPO of this group were significantly reduced in comparison to the Tifluadom-treated group only ($p<0.01$ and 0.05 respectively). Furthermore, the ME of this group showed significantly higher 5HIAA levels than in the saline-treated animals ($p<0.01$) but, there were no significant differences from any of the control groups in the ARN.

(iii) N-allylnormetazocine + Naloxone

There were no significant differences between this group and any of the control groups in the SCH, ME and ARN. In the MPO, the 5HIAA concentrations were significantly lower in these animals than in the N-allylnormetazocine-treated group ($p<0.01$).

(e) Serotonin

There were no significant differences between the 5HT levels in any of the four brain areas of the groups co-administered naloxone and their respective controls. The only exception was that the 5HT concentrations in the ME of the Duromorph + Naloxone- and the N-allylnormetazocine + Naloxone-treated groups were significantly elevated above

those of animals administered saline ($p<0.01$ and $p<0.05$ respectively).

(f) 5HIAA/5HT

There were no significant differences in the ratio of 5HIAA/5HT in any of the four areas examined under any of the treatment regimes.

18.00h Results

These results are summarised in Tables 14 and 19-22, and in Figures 24 and 30-34.

(a) Luteinizing Hormone

The plasma LH levels of all three groups administered an agonist along with naloxone were significantly lower than that of the naloxone-treated group ($p<0.001$), but not significantly different from the saline-, and their respective agonist-treated groups.

(b) Noradrenaline

(i) Duromorph + Naloxone and
(ii) Tifluadom + Naloxone

The only differences produced by the above treatments were that in the SCH and MPO they both significantly lowered NA

concentrations below those of the naloxone-treated group ($p<0.01$ and $p<0.05$ respectively).

(iii) N-allylnormetazocine + Naloxone

No significant differences were found in the SCH, ME and the ARN. In the MPO, the NA concentrations of this group were significantly reduced compared to N-allylnormetazocine-treated animals ($p<0.05$).

(c) Dopamine

No significant differences occurred in the DA concentrations of the four areas studied after any of the treatments administered.

(d) 5-Hydroxyindoleacetic Acid

(i) Duromorph + Naloxone, and
(ii) Tifluadom + Naloxone

No significant effects were found in the MPO, ME and ARN with either of these combined treatments. However, both significantly reduced 5HIAA concentrations in the SCH compared to the naloxone-treated group (both $p<0.001$).

(iii) N-allylnormetazocine + Naloxone

No significant differences resulted from this treatment.

(e) Serotonin

(i) Duromorph + Naloxone

No significant effects resulted form this treatment in the SCH, MPO nor the ARN. However, in the ME the combined treatment produced significantly raised 5HT concentrations compared to saline-, naloxone- and Duromorph-treated groups (all $p<0.001$).

(ii) Tifluadom + Naloxone

No significant effects were found.

(iii) N-allylnormetazocine + Naloxone

In the SCH, administration of the combined treatment resulted in a significant reduction of the 5HT levels compared to the naloxone- and N-allylnormetazocine-treated groups (both $p<0.05$), but no significant effects were evident in the MPO, ME nor ARN.

(f) 5HIAA/5HT

(i) Duromorph + Naloxone

No significant effects in the SCH or ME resulted from this treatment. However, the ratio of 5HIAA/5HT was

significantly reduced in the MPO by the combined treatment compared to the Duromorph-treated group ($p<0.01$), and in the ARN compared to the naloxone-treated group ($p<0.05$).

(ii) Tifluadom + Naloxone and
(iii) N-allylnormetazocine + Naloxone

In the SCH, MPO and ME, no significant effects of these treatments were found. In the ARN, both of the above treatments produced a significantly lower ratio of 5HIAA/5HT than did naloxone administration ($p<0.01$ and $p<0.001$ respectively).

Discussion

Luteinizing Hormone

Of all the treatments administered, only the combination of N-allylnormetazocine and naloxone altered plasma LH concentrations at 14.30h, causing an increase compared to the saline-, naloxone- and N-allylnormetazocine-treated groups. The marked elevation found may be due to the μ-antagonist properties of (-)N-allylnormetazocine which, in combination with the opiate antagonist properties of naloxone, may have reduced the EOP tone sufficiently to advance the surge on pro-oestrus. Such an event can result from administration of naloxone alone (Allen and Kalra, 1985). Thus, the elevated LH levels at 14.30h may represent the rising phase of an 'early' surge.

Duromorph, Tifluadom and N-allylnormetazocine all successfully reduced the naloxone-induced elevation of LH concentrations at 18.00h. This clearly indicates that μ-, κ- and σ-receptors are all involved in naloxone enhancement of the LH surge amplitude. Furthermore, reduction of naloxone-enhanced LH secretion by N-allylnormetazocine supports the existence of opioid σ-receptors within this neural network, since antagonism by naloxone is itself a test for opioid activity. The suppression of the LH surge which resulted from administration of the μ- and κ-agonists was prevented by naloxone. The results indicate that the effects of Duromorph and Tifluadom on LH secretion on the

afternoon of pro-oestrus are mediated by specific actions at their respective opioid receptors.

Aminergic Activity at 14.30h

As reported in Experiment 1, the 10mg/kg dose of naloxone did not alter NA nor DA concentrations in any of the four areas studied, but did increase 5HT activity in the SCH and MPO during the critical period, and increase the magnitude of the preovulatory LH surge. Such a finding was taken to indicate that naloxone enhancement of the surge amplitude was mediated by indoleamine, and not by CA neurones. However, it is possible that small, non-significant alterations of CA activity may be sufficient to mediate changes in LHRH secretion which can alter the LH surge amplitude.

Duromorph's ability to negate the effects of naloxone on the surge was not reflected in any alteration of CA activity, which provides further evidence that the mechanism of naloxone-enhanced LH secretion does not involve hypothalamic CA's. However, when administered concomitantly, Duromorph and naloxone increased 5HIAA concentrations in the ME above those of the agonist-treated group, suggesting that the naloxone enhancement of the LH surge involves the antagonism of μ-receptors to increase the stimulatory activity of 5HT in the ME.

As reported in Experiment 1, Duromorph increased NA concentrations in the ME and reduced the magnitude of the LH surge. Co-administration of naloxone prevented both of these actions of Duromorph. If NA is fulfilling an inhibitory role in the regulation of LHRH secretion, it would appear that the μ-inhibition of the surge is mediated by an inhibitory action of NA.

The κ-agonist increased 5HIAA concentrations in the SCH, MPO and ME, and the effect was generally antagonized by naloxone. Such an action on the activity of 5HT is inconsistent with the κ-inhibition of the surge and the antagonism of this inhibition by naloxone. However, naloxone also significantly decreased NA concentrations in the MPO and ME that had been caused to rise by the administration of Tifluadom. In light of the above, the actions of Tifluadom on circulating LH levels may be explained by mediation of its effects through an inhibitory NA pathway. It appears that this inhibitory action of NA is of greater influence on the LHRH neurones than the actions of 5HT since NA inhibition combined with 5HT stimulation results in a decreased surge.

Naloxone also increased DA concentrations in the ARN that resulted from the administration of Tifluadom. The ARN is the site of tuberoinfundibular DA perikarya. The rise in DA concentrations may reflect increased synthesis in the cell body which is often associated with increased release from the nerve terminal. The inference is that naloxone

increases the DA released from the ME and thus could decrease the PRL secretion induced by κ-agonist administration.

Naloxone decreased the NA concentrations in the MPO, ME and ARN, and the 5HIAA levels in the MPO, that resulted from the administration of the σ-agonist, N-allylnormetazocine. This finding clearly demonstrates the opioid nature of the receptors on which N-allylnormetazocine exerts its influence on NA and 5HT neurones in these areas. Thus, it appears that there may also be opioid σ-receptors which can, when activated, reduce the amplitude of the preovulatory LH surge.

To conclude, this experiment provides further evidence that naloxone enhancement of the LH surge is mediated by activation of a stimulatory input from 5HT neurones projecting to LHRH neurones. Furthermore, this effect of naloxone may be exerted primarily through μ-receptors.

The results also demonstrate that opioid suppression of the LH surge involves a heterogeneous population of μ-, κ- and σ-receptors, and that the suppression may be mediated by an inhibitory action of NA in the MPO and ME.

Aminergic Activity at 18.00h

Naloxone increased NA concentrations in the SCH and MPO, 5HIAA and 5HT concentrations in the SCH, and the ratio of

5HIAA/5HT in the ARN. The co-administration of each of the agonists returned all of these values to the same levels as in the saline-treated animals, which suggests that the actions of naloxone may still be mediated by a mixed population of receptors, involving all three subtypes.

While the 40mg/kg dose of Duromorph and the 3mg/kg dose of Tifluadom did not affect the amine concentrations compared to saline-treated control animals, they did cause several changes when co-administered with naloxone, in comparison to the levels that resulted from administration of naloxone alone. These are outlined in the paragraph above.

N-allylnormetazocine increased NA and 5HT concentrations in the SCH and MPO, and the ratio of 5HIAA/5HT in the ARN. The effects on NA in the SCH, 5HT in the MPO, and the ratio of 5HIAA/5HT in the ARN were not antagonized by naloxone, lending support to the conclusion that, as EOP tone increases, the non-opioid actions of N-allylnormetazocine are revealed. The other actions were antagonized by naloxone. Again this would point to naloxone antagonism of opiate suppression of LH release, if these transmitters retain the same action on LHRH neurones as they possess earlier on pro-oestrus.

The results suggest that the inhibition of the LHRH surge-generating system produced by these opiates may continue throughout the afternoon of pro-oestrus. If this is the case, it is probable that the LH surge in these animals is

completely abolished, rather than delayed until later on the day of pro-oestrus.

TREATMENT	PLASMA LH CONCENTRATION					
	14.30h			18.00h		
Saline	2.1±0.3		(12)	9.5±2.2		(14)
Naloxone	1.6±0.1		(11)	23.1±7.5	c	(9)
Duromorph	1.6±0.3		(10)	2.2±0.9	af	(8)
+ Naloxone	4.3±2.3		(10)	5.2±1.3	f	(5)
Tifluadom	1.2±0.2		(10)	1.8±0.2	af	(10)
+ Naloxone	3.4±1.2		(10)	4.1±1.6	f	(10)
SKF10047	1.0±0.1		(10)	5.3±2.3	f	(9)
+ Naloxone	5.8±1.5	beh	(8)	8.2±1.8	f	(9)

Table 14. Plasma LH concentrations (μg/ml±SEM) at 14.30h and 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day; a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.01$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance. The number of animals in each group is shown in parentheses.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE SCH				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	40.1±7.2	2.4±0.5	8.3±2.0	23.1±3.3	0.368±0.069
Naloxone	47.7±9.4	3.0±0.4	18.2±5.6 a	39.4±8.1	0.452±0.064
Duromorph	36.1±7.1	3.8±0.6	16.7±2.7 a	27.6±6.7	0.730±0.093
+Naloxone	19.9±3.4	2.8±0.8	7.9±1.2 dg	14.4±1.6	0.590±0.088
Tifluadom	52.4±12.1	4.7±1.4	27.2±6.5 c	42.1±8.1	0.720±0.209
+Naloxone	33.2±10.3	6.5±2.3	11.3±1.8 h	31.8±11.7	0.540±0.169
SKF 10047	37.4±4.4	4.4±0.9	16.4±2.0	39.9±8.4	0.484±0.131
+Naloxone	18.4±6.1	3.4±1.2	16.4±5.1	31.4±12.2	0.553±0.054

Table 15. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the SCH at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE MPO				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	47.6±8.9	3.5±0.6	8.7±1.3	23.0±2.4	0.472±0.109
Naloxone	43.1±7.8	4.6±1.5	19.9±4.0 a	23.8±5.3	0.866±0.293
Duromorph	42.4±9.1	5.3±0.8	13.7±1.9	24.1±6.0	0.790±0.179
+Naloxone	30.4+3.8	3.6+0.4	11.4+1.9	17.7+1.5	0.731+0.199
Tifluadom	53.9±4.8	5.3±0.5	27.4±3.2 c	29.7±4.4	0.999±0.223
+Naloxone	26.0+3.8ag	5.9+1.3	15.4+2.1 g	28.5+4.8	0.688+0.150
SKF 10047	69.7±11.3ad	7.3±1.4	26.5±6.9 c	20.8±2.4	1.077±0.267
+Naloxone	25.2+6.0 i	3.4+1.6	4.6+1.0 h	29.0	0.193

Table 16. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the MPO at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ME				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	22.4±4.1	21.4±6.0	5.1±0.8	13.1±2.2	0.461±0.065
Naloxone	46.2±9.8	22.5±6.5	11.9±1.8	20.5±1.9	0.688±0.067
Duromorph	45.9±9.1	58.8±14.4	11.3±2.1	27.8±6.8 a	0.572±0.146
+Naloxone	42.7±7.8	48.9±14.7	23.7±6.5ceh	41.4±7.5 b	0.706±0.174
Tifluadom	64.8±11.8b	57.5±20.0	15.0±2.7 a	20.3±5.0 a	0.737±0.142
+Naloxone	27.5±8.0 h	53.2±13.4	19.5±6.1 b	19.9±7.4	1.060±0.322
SKF 10047	80.9±15.2 cd	60.1±18.5	12.5±2.3	44.6±12.8 bd	0.436±0.114
+Naloxone	21.2±5.5 i	40.8±16.3	10.0±1.9	35.3±8.0 a	0.308±0.067

Table 17. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the ME at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ARN				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	30.1±3.3	5.8±1.8	11.3±3.4	23.1±3.6	0.478±0.106
Naloxone	50.6±15.7	5.2±1.5	12.9±2.3	20.6±3.2	0.698±0.107
Duromorph	55.6±17.7	4.4±1.4	13.0±2.2	18.6±4.8	0.868±0.130
+Naloxone	28.8±5.2	5.6±1.1	9.3±2.1	22.3±4.4	0.512±0.111
Tifluadom	56.5±8.6	8.9±2.9	15.2±1.7	23.1±4.0	0.747±0.125
+Naloxone	30.7±6.1	15.1±2.7be	14.6±1.6	24.5±5.1	0.670±0.081
SKF 10047	78.6±17.6b	10.7±2.2	23.5±7.9	25.2±8.1	0.652±0.122
+Naloxone	18.4±5.6 h	9.6±4.3	17.7±4.5	22.2±6.3	1.230±0.514

Table 18. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE SCH				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	22.1±5.5	2.6±0.7	8.7±2.6	18.9±3.1	0.447±0.081
Naloxone	51.5±13.0b	5.4±0.9	19.0±4.3 b	33.2±8.0 a	0.653±0.123
Duromorph	24.4±4.7 e	2.3±0.4	9.7±2.4 e	19.3±5.0 d	0.603±0.103
+Naloxone	21.5±2.8 e	3.8±1.2	11.7±1.9 f	27.8±4.3	0.457±0.088
Tifluadom	23.3±2.5 e	3.9±0.7	8.4±1.2 e	20.4±3.5 d	0.473±0.073
+Naloxone	22.7±3.2 e	3.7±0.5	6.6±0.7 f	21.4±1.8	0.311±0.023
SKF 10047	40.5±10.7a	5.6±1.8	16.2±2.1 a	35.1±5.2 b	0.524±0.089
+Naloxone	33.0±4.6	4.1±1.0	10.4±1.2	20.4±2.3dg	0.521±0.078

Table 19. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the SCH at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE MPO				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	29.1±7.0	3.8±0.9	10.4±2.7	21.2±4.3	0.717±0.227
Naloxone	46.2±9.6 a	6.1±1.5	15.7±2.7	30.2±4.5	0.552±0.081
Duromorph	38.6±6.4	4.3±1.5	17.1±5.1	15.1±3.4 d	1.572±0.576 ae
+Naloxone	23.1±3.4 d	3.7±0.5	11.7±2.1	23.4±4.6	0.524±0.068h
Tifluadom	29.1±3.5 a	3.4±0.8	9.7±1.2	18.2±2.6	0.608±0.083
+Naloxone	26.1±2.9 d	5.4±1.2	8.4±1.1	24.4±2.4	0.350±0.045
SKF 10047	53.1±5.7 b	6.6±1.8	25.6±9.8	36.9±8.8 a	0.728±0.172
+Naloxone	35.2±6.3 g	3.7±0.8	14.1±2.1	30.8±4.9	0.475±0.036

Table 20. Biogenic amine concentrations (ng/µg protein ± SEM) and the ratio of 5HIAA/5HT in the MPO at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ME				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	28.7±4.4	36.3±8.0	5.9±1.3	14.8±2.0	0.453±0.087
Naloxone	58.4±13.3	50.3±17.1	14.6±5.0	18.2±3.9	0.604±0.119
Duromorph	27.8±3.9	24.5±4.8	9.5±1.8	17.7±5.0	0.569±0.234
+Naloxone	35.3±6.9	50.1±21.1	15.3±3.1	42.9±8.3cfi	0.368±0.043
Tifluadom	37.0±8.1	25.5±6.3	6.4±1.0	14.9±4.1	0.600±0.128
+Naloxone	26.5±5.6	56.0±19.3	16.4±9.0	20.5±3.6	1.005±0.723
SKF 10047	40.6±10.6	33.9±10.2	14.3±3.0	21.3±3.6	0.787±0.202
+Naloxone	29.5±5.4	39.9±11.9	17.1±5.2	18.0±3.2	1.034±0.354

Table 21. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the ME at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

TREATMENT	BIOGENIC AMINE CONCENTRATION IN THE ARN				RATIO OF
	NA	DA	5HIAA	5HT	5HIAA/5HT
Saline	26.8±3.5	4.4±1.3	7.7±1.2	19.6±3.0	0.425±0.049
Naloxone	34.5±7.5	7.4±2.4	15.4±3.6	13.0±2.2	1.170±0.334c
Duromorph	23.8±4.5	4.4±1.4	7.7±2.4	7.9±1.4	0.501±0.059e
+Naloxone	26.0±6.2	6.6±1.7	10.4±1.5	18.1±3.1	0.541±0.091d
Tifluadom	25.1±4.5	4.1±1.0	8.7±1.0	18.8±2.6	0.491±0.050e
+Naloxone	31.4±5.0	6.7±1.6	9.6±1.9	20.5±2.9	0.490±0.095e
SKF 10047	35.6±3.1	6.1±1.3	10.4±2.4	19.3±4.6	0.781±0.168a
+Naloxone	23.7±2.7	4.1±0.8	8.3±2.3	13.3±1.8	0.467±0.084f

Table 22. Biogenic amine concentrations (ng/μg protein ± SEM) and the ratio of 5HIAA/5HT in the ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$, c: $p<0.001$ compared to saline-treated animals, d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals and g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

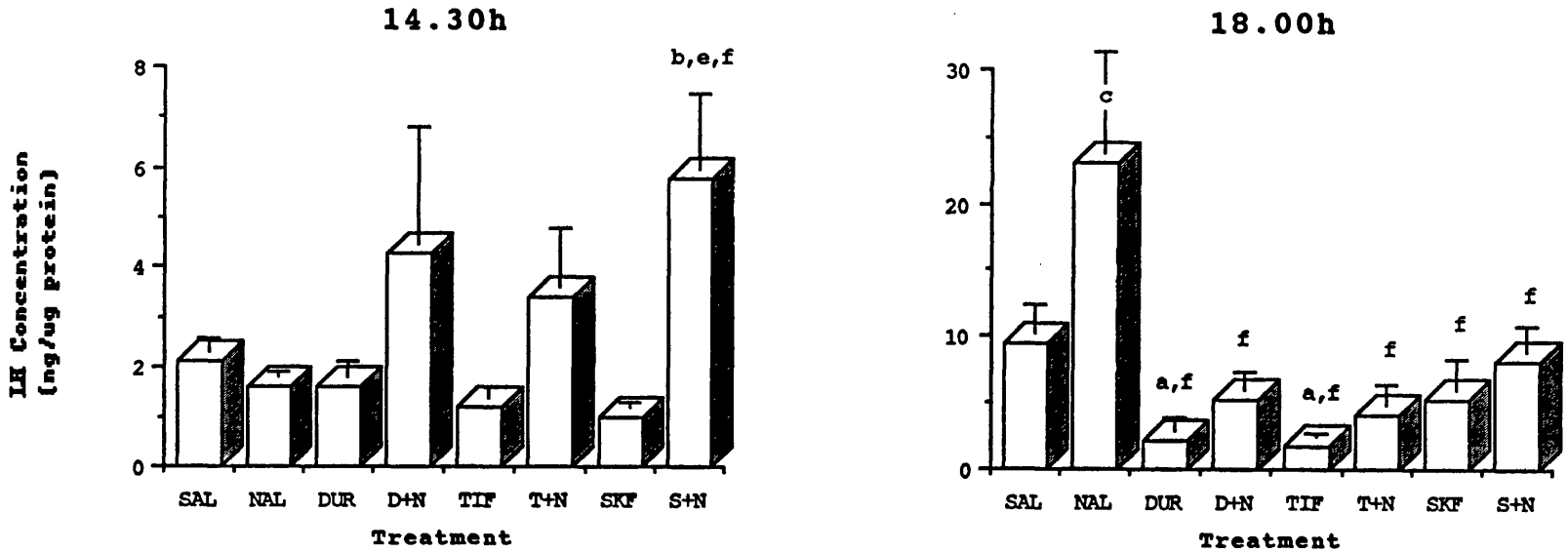

Figure 24. Plasma LH concentrations (±SEM) at 14.30h and 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate-agonist-treated animals; using *Scheffe's Analysis of Variance and Covariance.*

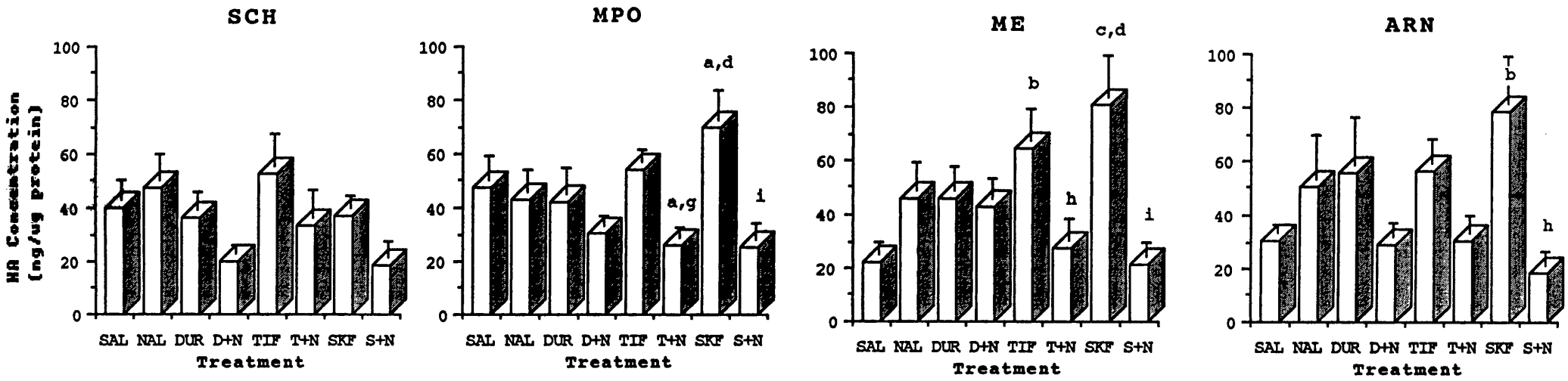

Figure 25. Noradrenaline concentrations (±SEM) in the SCH, MPO, ME and ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

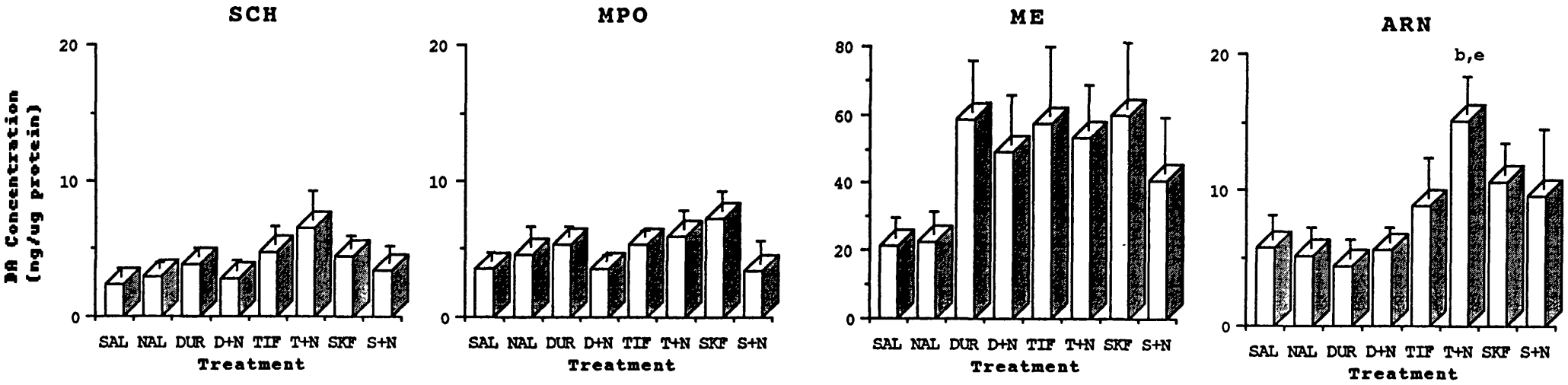

Figure 26. Dopamine concentrations (±SEM) in the SCH, MPO, ME and ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

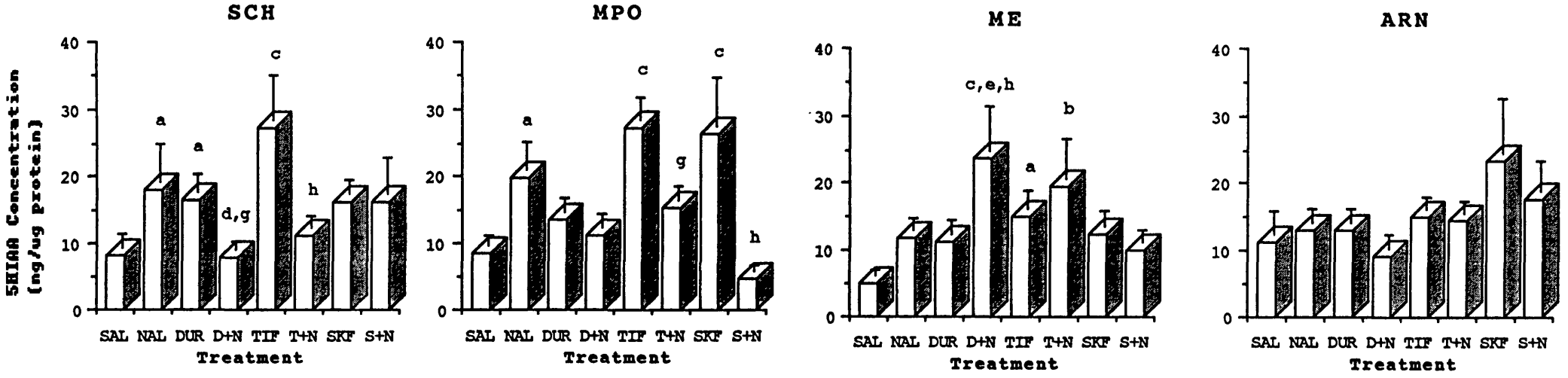

Figure 27. 5-hydroxyindoleacetic acid concentrations (±SEM) in the SCH, MPO, ME and ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

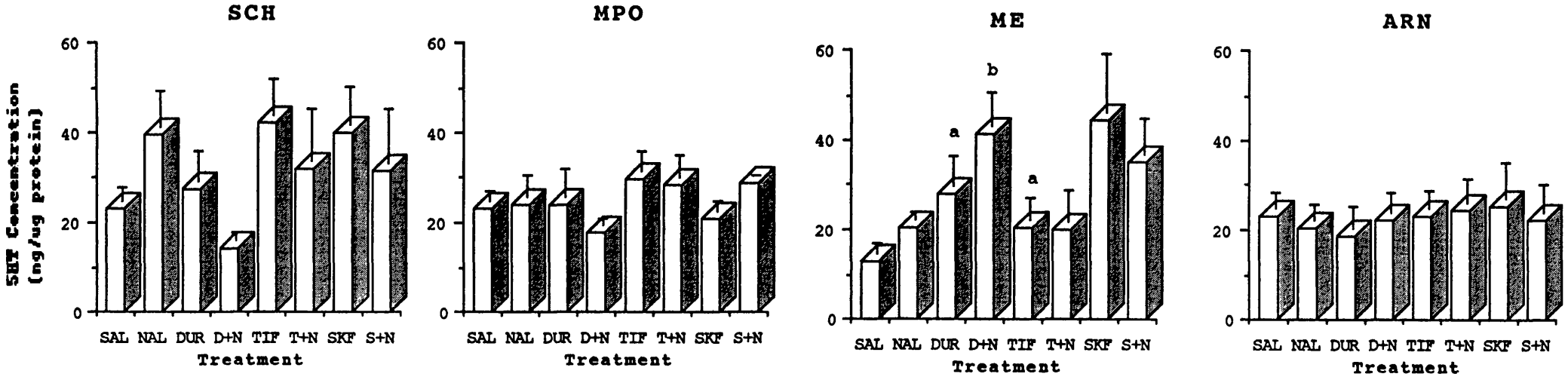

Figure 28. Serotonin concentrations (±SEM) in the SCH, MPO, ME and ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

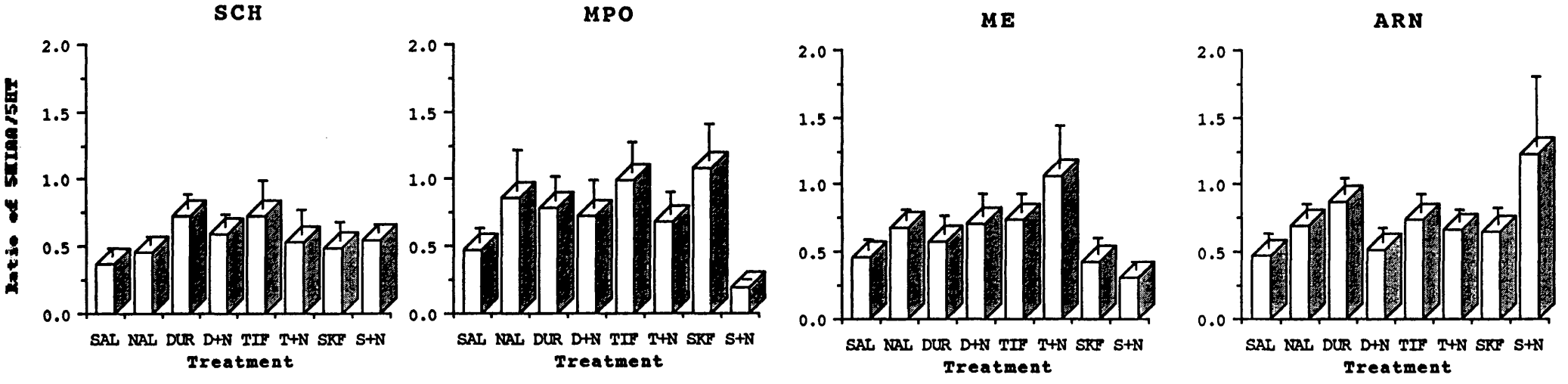

Figure 29. The ratio of 5HIAA/5HT (±SEM) in the SCH, MPO, ME and ARN at 14.30h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

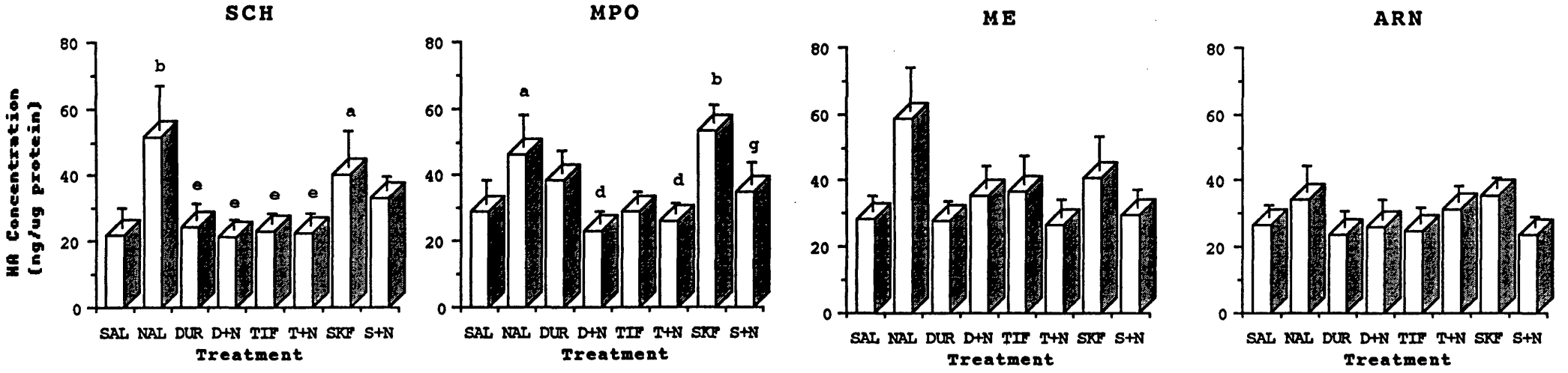

Figure 30. Noradrenaline concentrations (±SEM) in the SCH, MPO, ME and ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

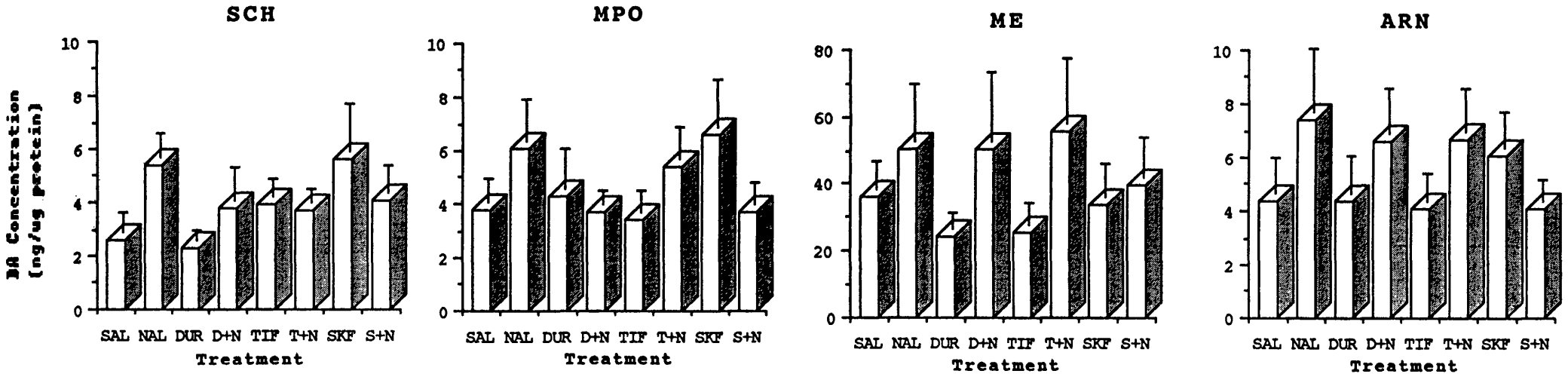

Figure 31. Dopamine concentrations (±SEM) in the SCH, MPO, ME and ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

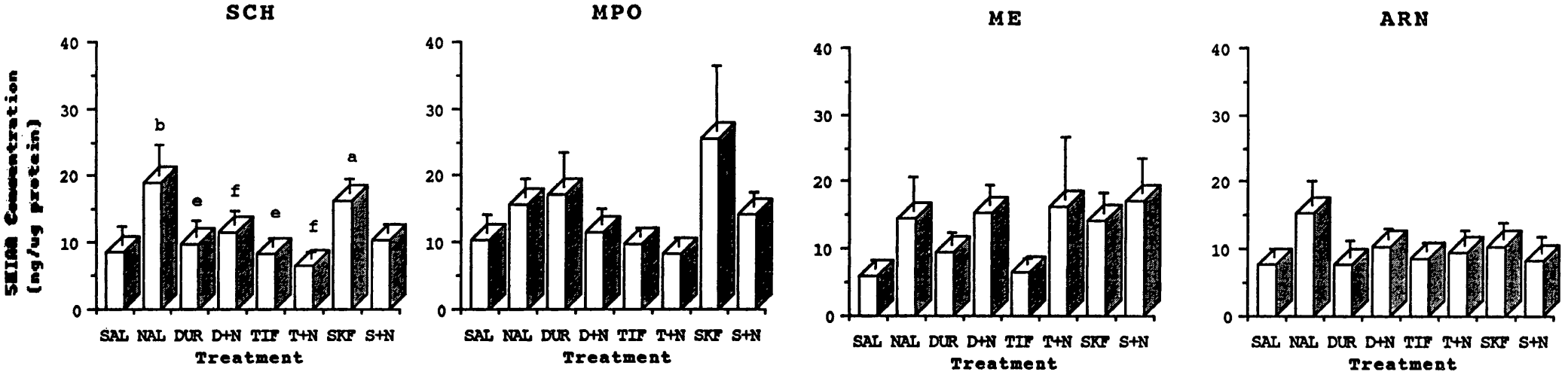

Figure 32. 5-hydroxyindoleacetic acid concentrations (±SEM) in the SCH, MPO, ME and ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

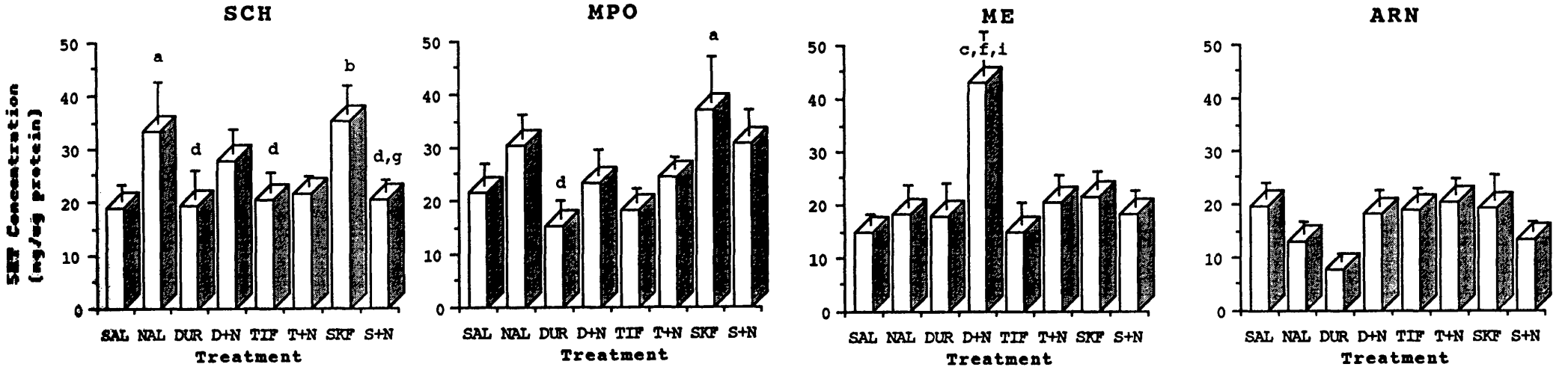

Figure 33. Serotonin concentrations (±SEM) in the SCH, MPO, ME and ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

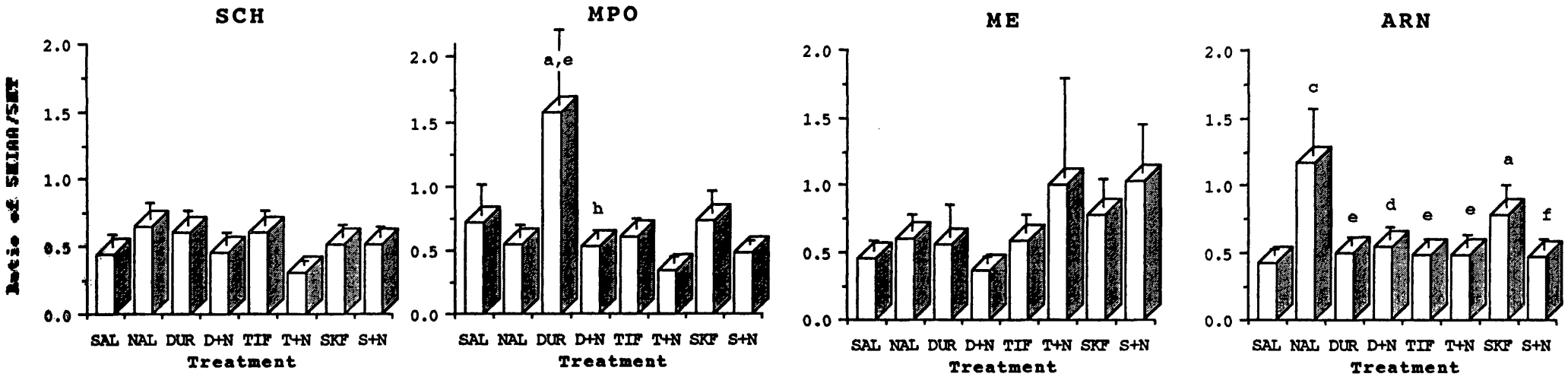

Figure 34. The ratio of 5HIAA/5HT (±SEM) in the SCH, MPO, ME and ARN at 18.00h on pro-oestrus after administration of drugs at 12.30h on the same day. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$ compared to saline-treated animals; d: $p<0.05$; e: $p<0.01$; f: $p<0.001$ compared to naloxone-treated animals; g: $p<0.05$; h: $p<0.01$; i: $p<0.001$ compared to appropriate opiate agonist-treated animals; using Scheffe's Analysis of Variance and Covariance.

EXPERIMENT 3

This experiment was undertaken as a simple method (with low morbidity and no mortality) to determine whether the LH surge was delayed by opiate agonist treatment to the day following natural pro-oestrus.

Materials and Methods

Rats were administered drugs (IP) in a 0.05ml/15g volume of physiological saline at 12.30h without anaesthesia and returned to daily vaginal smearing for at least sixteen days. The doses used were 3mg/kg for Tifluadom and N-allylnormetazocine, 40mg/kg for Duromorph and 10mg/kg for Naloxone. Control animals were injected with 0.05ml/15g physiological saline alone.

Results

These results are summarized in Table 23.

All animals in the this experiment were found to be in oestrus on the day following drug administration at 12.30h on pro-oestrus, regardless of which treatment was applied. Ninety per cent. of the rats treated with saline, naloxone and Tifluadom continued with regular four-day oestrous cycles for at least sixteen days following treatment. The corresponding figures for Duromorph and N-allylnormetazocine were 80% and 100% respectively.

Discussion

Regardless of treatment, all animals sloughed cornified vaginal epithelial cells on the day following treatment as is typical during oestrus. This was as expected as cornification results from high E_2 levels on pro-oestrus; E_2 being elevated prior to the drug administration.

No cornified cells were evident in any of the lavages performed on the second day after treatment and there was no temporal shift in the oestrous cycles of these rats. This indicates that E_2 had returned to its normal low level on the day following treatment. Thus, in those animals that had suppressed LH levels on the afternoon of pro-oestrus, the preovulatory LH surge was not delayed by 24 hours as has been reported for other pharmacological manipulations (Everett and Sawyer, 1950).

Therefore, treatments that suppressed LH release on the afternoon of pro-oestrus could have, (i) delayed the surge until later on the day of pro-oestrus, as has inhibition of catecholamine synthesis (Coen and Coombs, 1983), or (ii) completely abolished the preovulatory LH surge.

TREATMENT	% ANIMALS IN OESTRUS ON FOLLOWING DAY	% OESTRUS CYCLES UNDISTURBED	n
SALINE	100	90	10
NALOXONE (10mg/kg)	100	90	10
DUROMORPH (40mg/kg)	100	80	5
TIFLUADOM (3mg/kg)	100	90	10
SKF 10047 (3mg/kg)	100	100	10

Table 23. Percentage of animals in oestrus on the day following drug administration at 12.30h on pro-oestrus and percentage with at least four regular four day cycles following drug administration at 12.30h on pro-oestrus (n=number of animals in each group).

EXPERIMENT 4

The aims of these experiments were twofold: (i) to establish LH profiles on the afternoon and early evening of pro-oestrus for the various drug regimes, and (ii) to confirm that 14.30h is indeed towards the end of the critical period for initiation of the LH surge in these animals.

Materials and Methods

Drugs were injected (IP) in a 0.05ml/15g volume of physiological saline at 12.30h. The doses used were as in Experiment 3. The control animals were given saline alone. In both Experiments 4(a) and 4(b) only one group was used, these being the saline-treated controls; the number of animals in these two groups were 12 and 10 respectively.

(a) Xylazine + Urethane Anaesthesia at 11.00h

Initial attempts were made to anaesthetize the rats with 0.7g/kg urethane (IP) in combination with 5mg/kg xylazine (SC) as this had been reported to leave the LH surge intact (de Greef *et al*, 1987). However, these doses were found to be insufficient to induce surgical anaesthesia. Further urethane was injected until cessation of the hindlimb flexor withdrawal reflex was achieved. Blood samples were withdrawn from the rats between 14.30h to 20.00h at 30 minute intervals.

(b) Urethane Anaesthesia at 14.30h.

Animals were injected (IP) with a saturated solution of urethane in physiological saline (0.3g/100mg body weight in a volume of 0.450ml/100mg body weight) at 14.30h. Blood samples were withdrawn from 15.00h to 20.00h at 30 minute intervals.

(c) Urethane anaesthesia at 16.00h.

Animals were anaesthetized with urethane (as in Experiment 4 (b) above) at 16.00h and blood samples were taken at periods of 30 minutes from 16.30h until 20.00h. The number of animals in each group are shown in Table 25.

Results

(a) Xylazine + Urethane Anaesthesia at 11.00h.

The plasma LH concentrations at 14.30h to 20.00h (inclusive) at 30 minute intervals were 0.96±0.29, 1.04±0.36, 1.52±0.59, 1.73±1.06, 1.35±0.57, 1.23±0.67, 1.17±0.30, 1.02±0.62, 1.06±0.58, 1.11±0.46, 1.10±0.52 and 1.20±0.21 respectively; none of these values were significantly different from the control group in Experiment 1, decapitated at 14.30h.

(b) Urethane Anaesthesia at 14.30h.

These results are summarized in Table 24.

Time	PLASMA LH CONCENTRATIONS (ng/ml)									
	Animal Identification Numbers									
	442	458	459	462	463	464	465	468	469	472
15.00h	NR	NR	LOW	LOW	LOW	2.27	NR	NR	NR	NR
15.30h	NR	NR	LOW	LOW	LOW	2.15	LOW	NR	NR	NR
16.00h	LOW	NR	LOW	LOW	LOW	4.86	LOW	LOW	LOW	LOW
16.30h	LOW	NR	LOW	LOW	LOW	9.34	LOW	1.16	LOW	LOW
17.00h	LOW	LOW	LOW	LOW	LOW	NR	LOW	LOW	LOW	LOW
17.30h	LOW	NR	LOW	LOW	LOW	NR	5.13	LOW	LOW	LOW
18.00h	LOW	NR	LOW	LOW	LOW	NR	NR	1.29	LOW	LOW
18.30h	LOW	NR	LOW	LOW	LOW	NR	NR	LOW	LOW	LOW
19.00h	LOW	NR	LOW	LOW	LOW	NR	NR	LOW	1.72	LOW
19.30h	LOW	NR	LOW	LOW	LOW	NR	NR	NR	NR	LOW
20.00h	0.71	NR	LOW	LOW	LOW	NR	NR	NR	NR	LOW

Table 24. Plasma LH concentrations (ng/ml) of individual animals throughout the afternoon of pro-oestrus after injection of saline at 12.30h followed by urethane anaesthesia at 14.30h on the same day; LOW: concentration below the limit of detection (0.4ng/ml); NR: no result.

Time	Plasma LH Concentration (ng/ml)				
	Saline	Naloxone	Duromorph	Tifluadom	SKF10047
16.30h	LOW	17.4±3.4	LOW	LOW	LOW
17.00h	LOW	17.5±2.8	LOW	LOW	LOW
17.30h	LOW	20.0±2.4	LOW	LOW	LOW
18.00h	LOW	12.9±3.5	LOW	LOW	LOW
18.30h	LOW	6.5±1.3	LOW	LOW	LOW
19.00h	LOW	3.2±0.4	LOW	LOW	LOW
19.30h	LOW	1.8±0.2	LOW	LOW	LOW
20.00h	LOW	1.2±0.2	LOW	LOW	LOW
n	6	3	3	5	3

Table 25. Plasma LH concentrations (±SEM) throughout the afternoon of pro-oestrous in rats treated with opiate drugs at 12.30h and subsequently anaesthetized with saturated urethane at 16.00h on the same day. LOW=below the level of detection. n=number of animals in each group.

Of the 10 animals used, only two exhibited plasma LH concentrations on the afternoon of pro-oestrus that were elevated above baseline values.

(c) Urethane anaesthesia at 16.00h

These results are summarized in Table 25.

All animals, except those treated with naloxone, exhibited plasma LH concentrations on the afternoon of pro-oestrus that were below the level of detection of the assay. The group treated with naloxone showed maximal LH concentrations at 17.30h (20.0±2.4) which thereafter fell to minimum values by the time of the final sampling point at 20.00h (1.2±0.2).

Discussion

Xylazine + Urethane Anaesthesia at 11.00h

The failure of the combined xylazine and urethane anaesthesia to induce surgical anaesthesia in the doses previously reported may be a result of the use of different strains of rats; the de Greef group having used a hybrid strain, bred at their own institution, which exhibit 5-day oestrous cycles. It is possible that the hybrid strain may be more susceptible to anaesthesia, or their neural circuitry involved in the LH

surge more resistant to pharmacological suppression, than the Sprague-Dawley rats used in this project.

The top-up doses of urethane necessary to induce surgical anaesthesia in the animals in the current experiment were only slightly less than that which would induce the equivalent level of anaesthesia without the concurrent administration of xylazine. It is probable that the dose of urethane used completely abolished the preovulatory LH surge in these animals.

Urethane Anaesthesia at 14.30h

Eighty per cent. of the rats used in this experiment had their LH surge completely abolished by urethane administration. Urethane is known to inhibit the surge release of LH by suppression of the neural stimulus for the secretion of LH. This suggests that 14.30h is still within the critical period for the initiation of the preovulatory LH surge in early pro-oestrus. The remaining twenty per cent. of the animals in this experiment displayed plasma LH levels on the afternoon of pro-oestrus that were similar to those of unanaesthetized saline-injected animals, decapitated at 18.00h. It would appear that, in these animals, the critical period was over by 14.30h on the afternoon of pro-oestrus.

The results indicate that 14.30h is indeed toward the latter stages of the critical period in these animals. It follows that the neurotransmitter concentrations at this time, in the

areas involved in control of preovulatory secretion of LH, are a fair reflection of the central activity which stimulates the surge.

Urethane Anaesthesia at 16.00h

The failure of rats treated with saline to exhibit preovulatory surges after administration of urethane at 16.00h indicates that, in these rats, the critical period may extend to as late as this hour. No information can be gained from this experiment concerning the effects of the opiate agonists on the preovulatory LH surge, since the anaesthetic itself blocked the anticipated rise. However, it is clear from the results that naloxone not only increases the magnitude of the surge (as indicated by earlier experiments), but also temporally advances the critical period and the onset of the LH surge itself. This is evident from that fact that the plateau phase of the surge occurs from 16.30h (or earlier) until 17.30h, a period when LH levels would be anticipated to be rising in untreated animals. Indeed, the very occurrence of a surge in the naloxone-treated animals indicates that the critical period is advanced prior to the time of the urethane administration which abolished the surge in saline-treated animals.

GENERAL DISCUSSION

Site of Action of Opiates

The mode of administration of the drugs (IP) does not preclude effects on the LH concentrations mediated by actions outside the hypothalamus. The alterations of LH levels could result from actions of the drugs on:-

(i) the plasma half-life of LH,
(ii) the responsiveness of pituitary gonadotrophs to LHRH,
(iii) the axon terminals/perikarya within the hypothalamus,
(iv) the perikarya of neurones outwith the hypothalamus.

The half-life of glycoproteins, such as LH, depend upon the renal clearance and metabolic inactivation of the hormone. LH is a relatively small glycoprotein with an approximate molecular weight of 30000. This is small enough to cross the glomerular filtration barrier. However, the amount of protein lost in the urine each day is in the milligram range, and is predominantly albumin. Protein filtered in the glomerulus is reabsorbed by pinocytosis in the proximal tubule. Since virtually no protein is normally lost in the urine, it would be impossible for increases in LH concentrations to result from decreased renal clearance, but reduced levels could result from inhibition of reabsorption. For the drugs used here to have such an effect, it would require the cells of the proximal convoluted tubule to express opioid receptors. κ-binding sites have been described in the rat kidney using radioligand binding in kidney sections (Quirion *et al*, 1983). However, a recent autoradiographic study has demonstrated

that the rat kidney does not possess binding sites for μ-, κ- or δ-opioids (Dissanayake *et al*, 1991). The absence of κ-binding sites in the rat kidney is consistent with functional studies which have shown that the diuretic effects of κ-agonists are completely abolished by adrenal demedullation (Ashton *et al*, 1989). Thus, it appears that the effects of the opiate drugs used in the current investigation on plasma LH concentrations are unlikely to be a result of changes in renal clearance of the hormone.

Polypeptides are rapidly denatured in the circulation by serum proteases. This results in very short half lives of polypeptide hormones, such as LH, of only a few minutes. A change in the rate of breakdown of LH in the plasma itself could result from a change in activities and/or concentrations of proteases in the circulation. An alteration of protease activity would require binding of the drugs to the enzymes in such a manner as to alter their conformation or to block their active sites. It is unlikely that one, or all, of the drugs should have such an affinity for proteases that they could significantly alter the concentrations of LH in the plasma. To change the actual concentration of these proteases would involve an increase/decrease of synthesis and release of protein. Such events occur over a relatively long time course and so are unlikely to produce the acute effects found over the few hours of these experiments. Thus, it is proposed that the effects of these drugs reflect changes in the secretion, and not the catabolism, of LH.

As outlined above, the secretion of LH could be modified by an alteration of the signal to the pituitary gonadotrophs (i.e. secretion of LHRH) or by changes of their responsiveness to this signal.

Pituitary gonadotrophs possess opioid receptors (Simantov and Snyder, 1977), and opiates and EOPs inhibit basal and LHRH-induced secretion of LH from cultured pituitary cells (Blank *et al*, 1986; Cacicedo and Sanchez-Franco, 1986). Thus, the possibility that opiates alter circulating LH concentrations at the level of the pituitary *in vivo* cannot be entirely discounted. However, it has also been proposed that opiates do not alter LH secretion by an action on the pituitary (Bicknell, 1985) but, by effects at the hypothalamus to alter LHRH secretion into the hypophyseal portal blood system (Ching, 1983). Furthermore, a highly significant correlation between the concentrations of LHRH in hypophyseal plasma and LH in the circulation is evident after systemic administration of catecholaminergic drugs (Sarkar and Fink, 1981) indicating that these drugs exert their effects on LH via the hypothalamus. The pituitary is only sparsely populated with opioid receptors (Khachaturian *et al*, 1985) which suggests that the direct actions of opiates on the gonadotrophs may be of relatively minor importance compared to their effects within the CNS.

It is generally accepted that the actions of EOPs, and therefore opiates, in the CNS are invariably inhibitory (North, 1979). Reports of stimulation resulting from

ionotophoretic application of EOPs are now considered to have resulted form inhibitory actions on local neurones projecting to the cells from which the responses were measured.

EOPs exert their inhibitory influence by hyperpolarizing the membrane of their target cells. μ-receptor activation increases the calcium-sensitive potassium conductance (Williams *et al*, 1982) to decrease the action potential duration (North and Williams, 1983), while κ-receptor activation decreases the voltage-dependent calcium conductance by decreasing channel open time (Werz and Macdonald, 1984; Werz and Macdonald, 1985; Macdonald and Werz, 1986). Thus EOPs decrease spontaneous electrical activity and transmitter release from their target cells in the CNS.

The central effects of the opiates occur at hypothalamic and extrahypothalamic loci. Within the hypothalamus, implantation of naloxone into the MPO, ME and ARN stimulated LH release, while implants into other nuclei failed to do so (Kalra, 1981). Furthermore, naloxone stimulates the outflow of LHRH from the MBH-POA of OVX, steroid-primed rats *in vitro* (Leadem *et al*, 1985). Thus, the effects of opiates appear to be confined to the preoptic-tuberal pathway which contains the LHRH neurones. Here, the opiates could be exerting their influence directly upon the LHRH neurones since they make functional connections in these areas (Leranth *et al*, 1986). Alternatively, or additionally, they may alter the activity

of nerve terminals impinging upon the LHRH neurones, as has been described in detail in the Introduction.

Injection of opiates into regions outwith the hypothalamus can also alter LH secretion. Microinjection of morphine into the medial and dorsal raphe nuclei, the amygdala and the periaqueductal grey all suppressed LH release (Johnson *et al*, 1982; Lakoski and Gebhart, 1982). These effects of opiates almost certainly represent actions on neurones which project, directly or indirectly, to the LHRH neurones in the preoptic-tuberal pathway. Again, these effects may be direct upon these neurones, or indirect via actions on nerve terminals located on them.

In summary, it appears that systemic administration of opiates could alter LH secretion by actions at both a CNS and pituitary level. The central actions could occur at hypothalamic and extrahypothalamic loci to alter, directly or indirectly, the secretion of LHRH from the ME. The action in the pituitary may alter the response of the gonadotrophs to the LHRH signal from the hypothalamus. This last effect may be of least physiological importance since it has yet to be demonstrated *in vivo*.

Catecholamine Concentrations and Neuronal Activity

The metabolites of both NA and DA were consistently below the level of detection of the system. It is therefore necessary

to assess the nature of the information conveyed by the concentrations of the neurotransmitters alone.

In general, transmitters are synthesized in perikarya and transported via the axon to the nerve terminal where they are stored in vesicles but, in aminergic neurones some synthesis also occurs in the nerve terminal itself (see Figure 4). Upon release, they bind to their respective receptors and/or are metabolized or subject to re-uptake. Thus they can appear both intra- and extraneuronally. The perikarya of the NA and DA neurones lie outwith the four areas in which the measurements in the present study are made (with the exception of DA in the ARN). Released NA and DA are rapidly metabolized or taken up into their neurones. It follows that the majority of the NA and DA measured in this experiment is situated within the nerve terminals of their respective neurones.

An increase in concentration in the nerve terminal could result from increased synthesis, axonal transport and/or decreased release. Secretion of transmitter itself stimulates synthesis and axonal transport. Thus, only when release outstrips supply would concentrations of the transmitter fall. Intuitively, increased concentrations are likely to reflect decreased release. However, the information recorded regarding 5HT does not support this hypothesis. The concentration of the 5HT metabolite, 5HIAA, was within detectable limits in virtually every sample measured. The production of 5HIAA directly relates to the secretion of 5HT.

In every instance where 5HT levels were significantly elevated, 5HIAA concentrations were also increased (in most cases significantly). It appears that when release is increased, so too is the replenishment process; to such a degree that elevated, rather than reduced, transmitter concentrations result. Increased transmitter concentrations may result from a direct stimulation of synthesis or by secretion-induced synthesis.

In conclusion, the evidence indicates that in the SCH, MPO, ME and ARN, concentrations of NA and DA relate directly to the activity in the nerve terminals of these neurones (except DA in the ARN).

Opiate Effects on the LH Surge

Antagonism of EOP tone by administration of naloxone increases the basal release of LH in OVX rats (Sylvester *et al*, 1982). However, conflicting results have been obtained when the antagonist is administered to pro-oestrous or to OVX, steroid-primed rats, with both increases (Sylvester *et al*, 1980; Piva *et al*, 1985; Petragalia *et al*, 1986) and no effects (Gabriel *et al*, 1986) having been reported. The latter result indicates that EOP tone is completely abolished during the LH surge. The former results demonstrate that EOP tone may be reduced but not abolished at this time. In the experiments reported here, in conscious, unrestrained rats a marked elevation of LH surge magnitude was found after administration of the higher doses of naloxone, lending

support to the hypothesis that EOP tone persists to some degree throughout the spontaneous LH surge.

The lower doses of naloxone failed to have a similar effect. This suggests that there may be a threshold below which antagonism of EOP tone will not succeed in augmenting the amplitude of the LH surge. Indeed, the LH levels that resulted from administration of the lower doses of naloxone were half those of controls. Furthermore, the lowest dose of Duromorph almost doubled the LH surge concentrations. These findings are similar to previously reported work where low doses of morphine increased LH surge levels (Pang *et al*, 1977). Although none of the effects found here was significant, the results provide qualitative evidence for a stimulatory opioid input to the LH surge-generating system. Others have demonstrated such a stimulatory activity of opioids under certain experimental conditions (Piva *et al*, 1986). As postulated earlier, an opioid autoreceptor could account for these actions, although the existence of such receptors have not been demonstrated on EOP neurones to date. Alternatively, a distinct stimulatory pathway may exist, and this possibility will be dealt with in more detail later in the Discussion.

The ability of morphine to suppress LH secretion has been demonstrated on many occasions, under various experimental conditions (see Kalra *et al*, 1989). Here, again, activation of μ-receptors has been demonstrated to have a profound

inhibitory effect on the secretion of LH on the afternoon of pro-oestrus.

There are conflicting reports concerning the involvement of κ-receptors in the suppression of LH secretion. Inhibition of LH secretion has been demonstrated by administration of specific κ-agonists (Leadem and Kalra, 1983; Marko and Romer, 1983; Leadem and Yagenova, 1987). However, κ-inhibition of LH secretion was questioned by the ability of a μ-antagonist to reverse the suppression of LH release caused by the administration of a κ-agonist (Pfeiffer *et al*, 1987). The inference from this last experiment is that the inhibition of LH secretion by κ-opioids results from the interaction of poorly-selective agonists with μ-receptors. In the present study, a marked suppression of LH release was found after administration of very low doses of the highly selective κ-agonist, Tifluadom. The suppression was prevented by administration of the antagonist naloxone which binds to both μ- and κ-receptors. Although specific antagonists of the receptor subtypes were not used, it is unlikely that an agonist, such as Tifluadom, which exhibits a selectivity of over two orders of magnitude greater for the κ-receptor over the μ-receptor, should exert its effects through the latter subtype. It is therefore proposed that the suppression of LH secretion, evident after administration of Tifluadom, results from a specific action at κ-opioid receptors.

The disparity between the data obtained here and those of Pfeiffer *et al* (1987) may result from the different

experimental models. The work showing an absence of κ-action was carried out on OVX rats while κ-suppression was found in intact rats on the afternoon of pro-oestrus. Since CNS μ-receptor populations have been shown to vary over the oestrous cycle, decreasing on the afternoon of pro-oestrus as a result of elevated circulating gonadal steroids (Limonta *et al*, 1989), it is not inconceivable that κ-receptor numbers might also fluctuate over the oestrous cycle. Thus, the differing results may reflect actions of gonadal steroids on opioid receptor numbers. It is also possible that the responsiveness of the cells on which the receptors are located varies over the oestrous cycle, as has been proposed for μ-receptors during the preovulatory LH surge (Berglund *et al*, 1988).

Very little work has been carried out to determine the possible involvement of σ-receptors in the control of LH secretion. Where this has been attempted, it has proved inconclusive (Gopalan *et al*, 1989). N-allylnormetazocine proved to have widely divergent effects on the LH surge, depending on the dose administered. None of the changes in LH concentrations were significant due to the large standard deviations found in all groups injected with N-allylnormetazocine. As pointed out earlier, this drug acts at a variety of receptors. Its effects on LH secretion are unlikely to be mediated by its action on PCP-receptors since these are relatively sparse in the hypothalamus and thought to be involved mainly in the behavioural actions of this drug (Largent *et al*, 1986). The μ-antagonist properties of N-

allylnormetazocine would be expected to increase the release of LH as did naloxone. This was seen to result from some doses of N-allylnormetazocine. However, suppression of LH secretion was evident after administration of two of the doses of N-allylnormetazocine. Having eliminated PCP- and μ-receptor mediated actions, the suppression may be ascribed to the σ-agonist properties of the drug. This conclusion is supported by the ability of naloxone to prevent the N-allylnormetazocine-induced suppression of LH levels. With the advent of the highly selective σ-agonist, ditolylguanidine (Weber *et al*, 1986), it should be possible to confirm such an action of σ-opioid receptor activation.

In conclusion, all three of the opioid receptor subtypes appear to suppress LH release when activated by exogenous opiate agonists during the preovulatory surge. The evidence in favour of this hypothesis is strongest for μ-receptors, and weakest for σ-receptors.

NA Mediation of Opiate Effects on LH

As described in the Introduction, NA is generally believed to be stimulatory to LHRH neurones in the presence of ovarian steroids but, inhibitory in their absence. Two mechanisms have been proposed to account for these actions of NA.

In the first scheme, both stimulatory and inhibitory NA inputs make direct connections to the LHRH neurones. The existence of such connections is supported by the ability of

LC stimulation to inhibit the LH surge via β-receptors (Dotti and Taleisnik, 1982 and 1984). EOPs inhibit the stimulatory NA input, but are themselves inhibited by E_2. Thus, increasing E_2 frees the stimulatory NA input from tonic EOP inhibition which over-rides the inhibitory actions of this catecholamine to elicit the preovulatory LH surge. This mechanism is summarized diagrammatically in Figure 35.

The second hypothesis involves the activation of inhibitory interneurones (GABA) to mediate the inhibitory effects of NA on LHRH neurones. Again, a second stimulatory input is under tonic inhibition of EOPs and is relaxed under the influence of E_2, resulting in the LH surge. The ability of β-adrenergic agonists to stimulate LH release (Al-Hamood *et al*, 1985) supports the existence of such an interneurone pathway since β-activation is generally held to have an inhibitory action on its target cell. Thus, β-stimulation of LH release may result from the suppression of the activity of inhibitory interneurones projecting to the LHRH neurones, rather than direct stimulation of the LHRH cells themselves.

Regardless of which of the above paradigms is active, opiate suppression of LH release would be expected to involve a decrease of NA activity in the areas containing LHRH neurones. Morphine has been shown to decrease NA turnover in the MPO concomitant with its suppression of LH release in OVX, steroid-primed rats (Akabori and Barraclough, 1986). This finding is consistent with the hypothesis that μ-

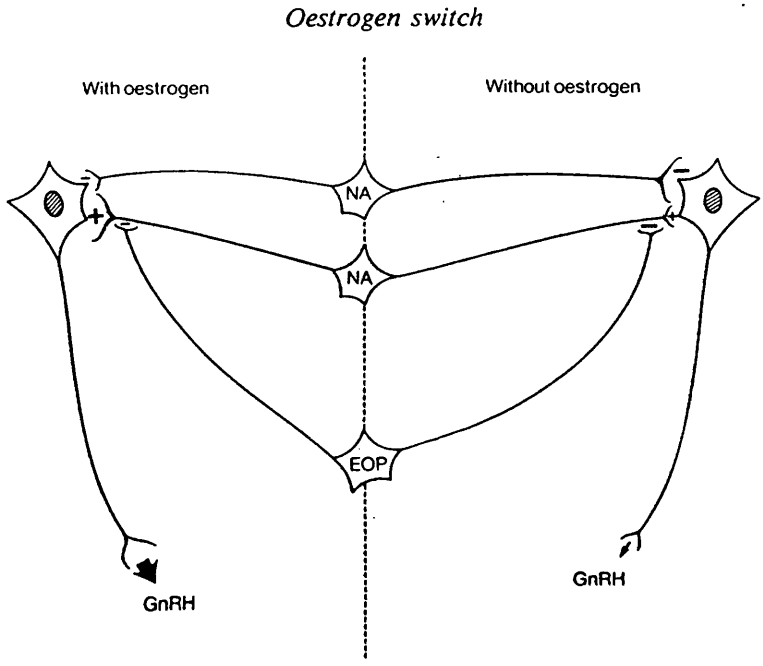

Figure 35. The 'Oestrogen Switch'; a diagrammatic representation of the different balance of the direct excitatory and inhibitory NA inputs to the LHRH-release system, brought about by the presence or absence of oestrogen. EOPs suppress the stimulatory input to the LHRH cells. The EOP neurones concentrate, and are inhibited by, oestrogen. Thus, in the presence of high levels of oestrogen, the excitatory NA input is released from tonic EOP suppression, and stimulates the preovulatory LH surge. From: Grossman and Dyer, 1989.

suppression of the LH surge is mediated by inhibition of a stimulatory NA input to LHRH neurones in the MPO.

Administration of Duromorph to pro-oestrous rats generally elevated NA activity in the areas studied. The elevation occurred most consistently in the ME, where the increased activity was reversed by naloxone, indicating that it resulted from a specific action at opioid receptors. If the action of Duromorph in the ME is responsible for the opiate's suppression of LH release, an inhibitory role for NA in the ME can be inferred. The involvement of an inhibitory action of NA is somewhat surprising because NA is generally believed to be stimulatory in the presence of E_2 (Grossman and Dyer, 1989). However, opioid receptor activation mimics the high EOP tone which exists in the absence of steroids, and the work of Dotti and Taleisnik (1982) demonstrates that an inhibitory NA input to the LHRH neurones can be activated on pro-oestrus. Thus, an inhibitory action of NA is not to be wholly unexpected. As opioid receptor activation has an inhibitory effect on the electrical activity of the cell on which it is situated (North, 1979), the stimulation of NA found here must involve the suppression of activity in an inhibitory, non-opioid interneurone.

The effects of the κ- and σ-agonists on NA activity in the ME were almost identical to those of Duromorph at doses which decreased the amplitude of the preovulatory LH surge. Tifluadom and N-allylnormetazocine also produced this effect in the MPO. Thus the inhibitory NA pathway may project to

LHRH neurones at the level of the MPO as well as the ME, and these neurones may also be activated by κ- and σ-opioid agonists.

In summary, μ-, κ- and σ-inhibition of the preovulatory LH surge may involve the activation of an inhibitory NA input to the LHRH neurones of the preoptic-tuberal pathway. This may act in concert with the more widely accepted suppression of the stimulatory NA input to the MPO caused by opiates (Grossman and Dyer, 1989).

DA Mediation of Opiate Effects on LH

DA is not considered to play a primary role in the generation of the LH surge (see Ramirez *et al*, 1984). However, manipulations of the DA systems projecting to the preoptic-tuberal pathway can alter LH secretion. As detailed in the Introduction, the effects of DA on LH release are dependent on the system involved; the tuberoinfundibular system being inhibitory in the ME and the incertohypothalamic being stimulatory in the MPO. DA turnover in the ME is decreased on pro-oestrus (Ahren *et al*, 1971), implicating a reduction of its inhibitory influence in the elevated release of LHRH during the preovulatory LH surge. However, a stimulatory action of DA prior to the surge has also been elucidated, and this was mediated by receptors pharmacologically distinct from those which cause inhibition (Sarkar and Fink, 1981). For the opiate agonist inhibition of the surge to be mediated by alterations of DA neurotransmission, one would expect an

increase in DA activity in the ME and/or a decrease in the MPO. The effects of the opiate agonists in these areas were inconsistent with their effects on the LH surge, confirming that this neurotransmitter may be of minor importance in the mediation of opiate inhibition of the surge.

The antagonist, naloxone, exhibited a more consistent pattern of effects on the DA activity, which indicated that naloxone's enhancement of the surge may be mediated, in part, by increasing the stimulatory action of DA in the MPO. However, the opiate agonists failed to alter the increased DA activity in the MPO which was caused by naloxone, but did prevent its stimulation of the LH surge. This suggests that DA mediation of the naloxone-induced increase in the LH surge amplitude may be of relatively little importance.

The results for DA are equivocal, and suggest that DA may be of little importance in the mediation of opiate modulation of the preovulatory LH surge. The feedback effects of the ovarian steroids on the two DA systems may thus be direct, or be mediated by a neural system other than EOPs.

Given that many DA cells of the tuberoinfundibular tract concentrate P (Fox *et al*, 1990), it would appear that P may act directly upon neurones of this tract to decrease their activity on the afternoon of pro-oestrus (Ahren *et al*, 1971; Kerdelhue *et al*, 1989). If so, DA in the MPO may be involved in the P-enhancement of the surge, rather than possessing an obligatory role in its initiation.

Concentration of ovarian steroids by the DA neurones of the incertohypothalamic tract has not been demonstrated. However, it has been noted that GABA and DA produce reciprocal effects on LHRH secretion in the MPO; the former being inhibitory and the latter stimulatory (Wilson *et al*, 1990). It is possible that GABA exerts its influence on LHRH neurones in this area by altering DA activity, in addition to its widely known effects on NA neurotransmission (Adler and Crowley, 1986; Herbison *et al*, 1990).

5HT Mediation of Opiate Effects on LH

The physiological role of 5HT in the regulation of LH secretion has not been definitively established. The reports of the effects of 5HT have ranged from stimulation to inhibition, and to no effect on LH release.

The inhibitory effects of 5HT may result from an action mediated by other neuronal systems, since blockade of the LH surge by stimulation of the medial raphe nucleus is dependent on GABA (Morrello and Taleisnik, 1989).

When isolated from pro-oestrous rats, the ME will release LHRH in response to 5HT (Vitale *et al*, 1985). This observation clearly establishes a facilitatory role for 5HT in the ME in the modulation of LH secretion on pro-oestrus. Further support is seen in the naturally occurring increase of 5HT activity in the ME on the afternoon of pro-oestrus

(Kerdelhue *et al*, 1989). Increased 5HT synthesis in the MPO of OVX rats in response to ovarian steroids (King and Kang, 1988) points to a stimulatory role for 5HT in this area also. Thus, opiate agonist inhibition of the LH surge should involve decreased activity of 5HT in the MPO and ME, if opiate effects on LH are mediated by 5HT neurones.

The conclusion from the current experiments is that 5HT may not play a pivotal role in mediating the suppression of the LH surge caused by the opiate agonists. However, some evidence was provided which implicated a stimulatory action of 5HT in the MPO and ME in the mediation of the naloxone-induced enhancement of the LH surge, and that this stimulatory action appeared to be via μ-receptors; an intriguing finding, since recent evidence has been provided which indicates that 5HT systems are involved in P-enhancement of the surge (King and Kang, 1988). Thus, 5HT may be involved in 'fine-tuning' the surge, rather than being essential for its initiation. The results presented here are consistent with the hypothesis that P-enhancement of the surge via stimulatory 5HT systems may involve reduced activity of EOP neurones.

As outlined earlier, increased 5HT activity in the MPO was interpreted as being representative of a stimulatory influence on LHRH neurones. The opiate agonist suppression of the LH surge in association with elevated 5HT concentrations in the MPO appears to contradict such a conclusion. In the earlier discussions, the disparity between the effects of the

agonists and antagonist on 5HT and LH was attributed to the more profound influence of NA on the activity of LHRH neurones. However, it is possible that the agonists increased 5HT activity to stimulate GABA neurones, thus inhibiting the surge (Dotti and Taleisnik, 1989), while the antagonist activated a direct stimulatory action of 5HT on LHRH neurones.

In summary, no clear evidence of 5HT involvement in opiate inhibition of the LH surge was provided by the present study. However, naloxone-induced elevation of the LH surge amplitude may involve antagonism of EOPs, principally at μ-receptors, to release a stimulatory 5HT input to the LHRH neurones from tonic EOP inhibition. It is proposed that such a reduction of EOP activity at 5HT neurones may be involved in the enhancement of the surge produced by P.

Conclusion

Morphine has long been known to have a profound inhibitory influence on the secretion of LH, including the preovulatory surge on pro-oestrus; an action mediated by μ-opioid receptors. Conflicting results have been obtained for the involvement of κ-receptors in opiate suppression of LH release, and the possible involvement of σ-receptors has not been the subject of extensive investigation. The experiments reported here provide strong evidence that a heterogeneous population of μ- and κ-receptors are involved in the central suppression of the LH surge by opiates. Furthermore, some

evidence is provided that σ-opioid receptors may also be involved in the central EOP systems which inhibit LH release.

The effects of the opiates on NA activity in the hypothalamic nuclei suggest that they may inhibit the LH surge by activating an inhibitory action of NA in the preoptic-tuberal pathway, in addition to the well-established inhibition of stimulatory NA inputs to the LHRH system. The activation of this inhibitory NA influence appears to involve a heterogeneous population of μ-, κ- and σ-opioid receptors.

The naloxone-induced enhancement of the LH surge amplitude may involve activation, principally through antagonism at μ-receptors, of a stimulatory 5HT input to LHRH neurones in the MPO and ME. There is less convincing evidence which suggests that a stimulatory DA system projecting to the MPO may also be involved in the mediation of the naloxone-enhancement of the LH surge.

RECOMMENDATIONS FOR FURTHER STUDY

In the experiments presented here, opiate suppression of the preovulatory LH surge by a mixed population of receptors has been clearly demonstrated. Furthermore, some evidence is provided which indicates that these opioid receptors may be located on monoamine neurones which project to the LHRH neurones of the preoptic-tuberal pathway. To further investigate the above findings, acute central administration of the opiates could be used. To this end, indwelling intracranial cannulae could be placed (under anaesthesia) to allow for ICV administration of drugs to conscious, unrestrained animals. Such a method of administration would provide for the deposition of drugs in predominantly hypothalamic loci, without the possible unintentional ablation of neurones within, or projecting to, the hypothalamus itself. Again, various doses would be administered at 12.30h on pro-oestrus to find the minimum amount of a particular drug which could successfully alter the magnitude of the preovulatory LH surge. This would be determined by withdrawal of serial blood samples throughout the morning and early evening of pro-oestrus to measure circulating LH levels.

To elucidate the possible involvement of hypothalamic monoamines in the mediation of the effects of the opiates, drugs would be administered as above (at the minimum effective dose), and the animals decapitated at 14.30h. The brains would be removed and the MPO and ME isolated as described earlier. The monoamine contents of these two nuclei would then be determined by HPLC-ECD. To further investigate

the opioid receptor subtypes involved, specific antagonists of each subtype could be co-administered with their respective agonists, and the effects on LH secretion, and monoamine activity in the hypothalamus, determined.

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APPENDIX

LIST OF COMMUNICATIONS

Brown, C.H. (1991). Hypothalamic control of LH secretion. Endocrinologie Colloquium, Faculteit der Geneeskunde, Erasmus University, Rotterdam, Netherlands.

Brown, C.H. and Gilmore D.P. (1990). Association between opiate effects on the magnitude of the preovulatory luteinizing hormone surge and hypothalamic noradrenaline levels in conscious unrestrained rats. J. Reprod. Fert. Abstract Series 6, pp58.

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