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The Synthesis and Metabolism of 5-Hydroxytryptamine in Discrete Regions of the Rat Brain During Various Pharmacological, Environmental and Endocrinological Manipulations

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THE SYNTHESIS AND METABOLISM OF 5-HYDROXYTRYPTAMINE IN DISCRETE REGIONS OF THE RAT BRAIN DURING VARIOUS PHARMACOLOGICAL, ENVIRONMENTAL AND ENDOCRINOLOGICAL MANIPULATIONS

By

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ABSTRACT

The Synthesis and Metabolism of 5-Hydroxytryptamine in Discrete Regions of the Rat Brain During Various Pharmacological, Environmental and Endocrinological Manipulations

by

Craig Alan Johnston

A simple, sensitive and rapid method using high performance liquid chromatography (HPLC) coupled with electrochemical detection was developed for the concurrent measurement of picogram quantities of 5-hydroxytryptamine (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptophan (5-HTP) in the median eminence (ME), medial preoptic nucleus (MPO), striatum (ST), suprachiasmatic nucleus (SCN) and arcuate nucleus (AN) of a single rat brain. 5-HTP in these brain areas prior to decarboxylase inhibition is essentially zero. NSD 1015, an inhibitor of decarboxylase, caused a linear accumulation of 5-HTP in these brain areas for at least 30 minutes. In the present study the concentration of 5-HIAA was taken as an index of 5-HT metabolism, and the rate of 5-HTP accumulation as an index of 5-HT synthesis.

Pargyline increased 5-HT and decreased 5-HIAA concentrations and probenecid increased 5-HIAA in all brain regions. Reserpine increased 5-HT synthesis and metabolism at 2 and 24 hr and chlorimipramine or fluoxetine decreased 5-HT synthesis and metabolism in the selected brain regions. L-Tryptophan increased 5-HT synthesis and metabolism in the

SCN, MPO, AN and ST. Morphine increased 5-HT synthesis and metabolism in the MPO, AN and SCN through an opiate-receptor mediated mechanism.

Restraint stress increased serum prolactin and 5-HT synthesis in the MPO and SCN.

5-HT synthesis and metabolism was increased in the ME of lactating rats. Suckling increased serum prolactin and 5-HT synthesis and metabolism in the MPO.

Early in pregnancy 5-HT synthesis was increased in the AN and SCN at a time coincident with the nocturnal surge of prolactin. The diurnal surge of prolactin was associated with an increase and decrease in 5-HT synthesis in the AN and SCN, respectively. Later in pregnancy, when the nocturnal surge does not occur, 5-HT synthesis was no longer stimulated in the AN but was in the SCN. Serum prolactin and 5-HT synthesis and metabolism in the SCN increased and 5-HT synthesis in the ME decreased on the afternoon of proestrus.

to the greatest gift of my life, my lovely wife, Sharon;

and to our Lord, Jesus Christ

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INTRODUCTION

5-Hydroxytryptamine (5-HT, serotonin) neuronal systems are believed to play a role in a wide variety of functions in the brain. A partial list of these functions includes regulation of sleep-wake cycles, sexual and feeding behaviors, locomotion, aggression, body temperature, nociception, memory and cardiovascular systems. 5-HT neurons projecting to the hypothalamus appear to be involved in regulating the release of several pituitary hormones (see reviews: Wilson, 1974; Knowles and Vollrath, 1974; McCann and Ojeda, 1976, 1979; Müller et al., 1977; Weiner and Ganong, 1978; Sawyer, 1979). It has been suggested that 5-HT is a neurotransmitter involved in the stimulation of prolactin release from the anterior pituitary. This conclusion has been based primarily on results obtained from experiments using 5-HT precursors and putative 5-HT receptor antagonists. When added in vitro to pituitary glands of normal rats 5-HT is without an effect on prolactin secretion (Birge et al., 1970; Lamberts and MacLeod, 1978) suggesting that the site of interaction of 5-HT with the secretion of prolactin occurs at the level of neural circuits located upstream to the pituitary lactotrophs. However, exactly how, when and where activation of 5-HT neurons controls physiological fluctuations in prolactin secretion is still a very controversial subject. Much of the literature on this subject is difficult

to interpret because it is based largely on lesion studies, gross neurochemical analyses and pharmacological studies using drugs that are systemically administered. Furthermore, different investigators often test endocrine effects of 5-HT under different, not necessarily comparable experimental conditions. Pharmacological studies are limited in their usefulness because of two additional reasons. First, drugs affecting 5-HT transmission in one area of the brain often do so elsewhere in the brain and can thus cause simultaneous changes in many systems that may affect pituitary secretion either directly or indirectly. Secondly, nearly all drugs which are currently thought to interact with 5-HT neuronal systems lack specificity. For example, high doses ($\geq$100 mg/kg) of the 5-HT precursor, 5-hydroxytryptophan (5-HTP), can enter catecholamine terminals, be decarboxylated to form 5-HT and displace endogenous catecholamines (Ng et al., 1972). p-Chlorophenylalanine (PCPA) inhibits 5-HT biosynthesis but also affects the metabolism of catecholamines by competing with the uptake of tyrosine (the precursor for dopamine (DA) and norepinephrine) into catecholaminergic neurons (Wurtman, 1974). Furthermore, many of the putative 5-HT receptor antagonists may not be active in the central nervous system, and many exert agonistic and antagonistic effects at other aminergic receptors (Lamberts and MacLeod, 1978; Besser et al., 1980). Even some of the seemingly more specific 5-HT receptor antagonists, such as metergoline or methiotepin, are specific for 5-HT receptors only in a very narrow concentration range.

An important first piece of evidence for the involvement of 5-HT neurons in a particular behavioral, pharmacological or endocrinological

event can be obtained by measuring 5-HT neuronal activity during the event. 5-HT neuronal activity in the whole brain and large brain regions has been examined throughout several experimental paradigms (for examples see reviews: Wilson, 1974; Knowles and Vollrath, 1974; McCann and Ojeda, 1976, 1979; Müller et al., 1977; Weiner and Ganong, 1978; Sawyer, 1979). However, whether results from experiments examining this activity in large areas of the brain will identify functionally important changes occurring in discrete brain regions is unknown.

Relatively few reports of studies designed specifically to characterize 5-HT neuronal activity in discrete hypothalamic nuclei exist. This is primarily due to technical problems associated with the relatively poor histochemical procedures for this amine (Parent, 1981), and the lack of sensitivity of analytical techniques for analyzing 5-HT, 5-HTP, and its major metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA). The recent development of sensitive radioenzymatic (Tappaz and Pujol, 1980) and high performance liquid chromatographic (HPLC) (Krstulovic and Matzura, 1979; Meek and Lofstrandh, 1976) assays for 5-HTP and assays using HPLC in combination with electrochemical detection (Loullis et al., 1979) or radioimmunoassays (Delaage and Puizillout, 1981) for measuring 5-HT and 5-HIAA has made it possible to employ biochemical techniques to estimate 5-HT neuronal activity in areas such as the striatum, hypothalamus and mediobasal hypothalamus. No technique permitted the concurrent measurement of 5-HT, 5-HIAA and 5-HTP in a sample of discrete hypothalamic nuclei such as the suprachiasmatic or medial preoptic nucleus from a single rat brain. Therefore, one of the primary objectives of the present study was to develop an assay capable of

detecting 5-HT, 5-HIAA and 5-HTP in discrete hypothalamic nuclei of the rat brain that had been implicated in the regulation of prolactin secretion. The capability to measure 5-HT neuronal activity in discrete hypothalamic nuclei generates more questions than it answers initially. Much evidence already exists to suggest that 5-HT neurons not only may be involved in the regulatory control of the secretion of several hormones but also that 5-HT neuronal systems may functionally interact with several other neurotransmitter/neuropeptide/neurohormonal systems (Meites et al., 1963; Weight and Salmoiraghi, 1968; Nicoll, 1971; Meites, 1973; Ford et al., 1974; Knowles and Vollrath, 1974; Wilson, 1974; MacLeod, 1976; McCann and Ojeda, 1976, 1979; Bruni et al., 1977; Müller et al., 1977; Baumgarten et al., 1978; Weiner and Ganong, 1978; Holaday and Loh, 1979; Iwamoto and Way, 1979; Meites et al., 1979; Sawyer, 1979; Koenig et al., 1980; Moore and Johnston, 1982). Thus, the measurement of neuronal activity in these 5-HT systems only supplies the means to begin to examine the functional roles and interactions of 5-HT neurons in the brain. In addition it would be advantageous if the method could also measure DA and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). Because steady-state conditions as well as drug effects on the dopaminergic neuronal systems in discrete brain areas have been more fully characterized than on 5-HT neuronal systems, the measurement of DA and DOPAC concentrations served as a check on pharmacological effects as well as providing further verification that the regions examined were indeed the areas that had been selected to be investigated. Such a verification proved very important

because no information on the concentration of 5-HIAA or 5-HTP accumulation in some of the hypothalamic nuclei was available.

I. Justification for Selection of Regions

The discrete hypothalamic regions chosen for the present analysis included the suprachiasmatic nucleus, the medial preoptic nucleus, the arcuate nucleus and the median eminence. In addition, a sample of the striatum was also taken as an example of an extrahypothalamic region. All of these regions contain substantial quantities of 5-HT as well as the rate-limiting enzyme in 5-HT synthesis, tryptophan hydroxylase (Saavedra et al., 1974; Brownstein et al., 1976a).

The suprachiasmatic nucleus (SCN) has been shown (mostly via lesion studies) to be essential for entrainment of many types of circadian rhythms including the prolactin surges associated with the estrous cycle at the time of ovulation, and the diurnal and nocturnal prolactin surges observed in pregnancy and pseudopregnancy (Brown-Grant and Raisman, 1977; Bethea and Neill, 1979, 1980; Dunn et al., 1980; Yogev and Terkel, 1980). This nucleus also appears to be an important modulator or relay station of preoptic efferents projecting to the mediobasal hypothalamus, and is believed to exert inhibitory control on prolactin secretion (Kimura and Kawakami, 1978). The SCN receives some of its innervation from the tuberal hypothalamus and sends efferents through the ventral periventricular and arcuate nuclei into the ventral tuberal area to terminate throughout the periventricular and arcuate nuclei as well as the internal and subependymal median eminence (Moore, 1979). Furthermore, 5-HT concentrations in the SCN decrease following acute immobilization stress for up to 3 hours (Palkovits et al., 1976).

The medial preoptic nucleus (MPO) is both anatomically and functionally connected with the arcuate nucleus (Whitehead and Ruf, 1974; Renaud and Martin, 1975). Axons project from cell bodies in the MPO to innervate the external layer of the median eminence (Terasawa and Sawyer, 1969; Dyer and Saphier, 1981). Stimulation of the MPO causes ovulation, increases plasma concentrations of prolactin and electrical activity in the arcuate nucleus, and inhibits neuronal activity in the median eminence. Lesioning the MPO induces pseudopregnancy which is characterized by diurnal and nocturnal prolactin surges (Freeman and Banks, 1980). The MPO not only appears to contain neurons which are inhibitory to the nocturnal surge of prolactin but that are also stimulatory to the diurnal surge (Freeman and Banks, 1980). A bilateral lesion of the MPO blocks ovulation and the associated surges of luteinizing hormone and prolactin without affecting the follicle stimulating hormonal surge (Terasawa et al., 1980; Kimura and Kawakami, 1978).

The arcuate nucleus (AN) contains the cell bodies of several different neurons which project to the tuber cinareum, including the tuberoinfundibular dopamine (TIDA) neurons (Björklund and Nobin, 1973) which functionally act to tonically inhibit the secretion of prolactin from the pituitary (MacLeod, 1976). It has been suggested that if 5-HT neurons inhibit TIDA neurons; one possible site for this interaction is in the AN. In addition, recent studies have revealed the existence of 5-HT containing cell bodies in the arcuate nucleus (Smith and Kapers, 1975; Kent and Sladek, 1978).

The median eminence (ME) represents the final common neural pathway to the anterior pituitary. It contains nerve terminals of several

tuberoinfundibular neuronal systems including those of the TIDA neurons and possibly those thought to contain a presently unidentified prolactin releasing factor. The ME not only receives projections from the AN, but also from the SCN and MPO (Terasawa and Sawyer, 1969; Moore, 1979; Dyer and Saphier, 1981). Finally, the ME is a storehouse of putative neurotransmitter substances and hormones which regulate anterior pituitary hormonal release (Palkovits, 1980).

The striatum (ST) was selected as the extrahypothalamic control region for several reasons. The ST contains well defined innervations of both DA- and 5-HT-containing neurons (Dahlström and Fuxe, 1964; Azmitia and Segal, 1978; Ungerstedt, 1971). The ST is primarily innervated by 5-HT neurons originating in the dorsal raphé nucleus (Dray, 1981; Parent, 1981), with a minimal projection from the median raphé nucleus (Conrad et al., 1974; Miller et al., 1975; Azmitia and Segal, 1978; Jacobs et al., 1978; Parent, 1981). Lastly, aminergic neuronal responses in the ST to several pharmacological, environmental and endocrine paradigms have been frequently studied and provide a large amount of background information for comparison (Yarbrough et al., 1971, 1973; Carlsson et al., 1972a; Gudelsky and Moore, 1976; Kueng et al., 1976; Mena et al., 1976; Palkovits et al., 1976; Karoum et al., 1977; Demarest and Moore, 1979a,b; Marco and Meek, 1979; Umezu and Moore, 1979; Alper et al., 1980; Moore et al., 1980a,b; Demarest et al., 1981a,b).

II. Neuroanatomy of 5-HT Neurons

Current knowledge of the anatomy of 5-HT neurons is derived primarily from the results of innumerable investigations in the rat (see

review by Steinbusch et al., 1981). This knowledge stems primarily from results of studies using the Falck and Hillarp fluorescence histochemical method (Falck et al., 1962) which permits visualization of 5-HT at the cellular level. Other techniques that have added valuable knowledge to our present understanding of 5-HT neuroanatomy include the use of tritiated 5-HT uptake as a marker for 5-HT neurons (Descarries et al., 1975) and the use of immunohistochemical techniques to visualize tryptophan hydroxylase (Pickel et al., 1976, 1977) and 5-HT (Steinbusch et al., 1978; Hökfelt et al., 1979).

The distribution of 5-HT neurons in the brain has been extensively reviewed by Azmitia (1978). The anatomy of this distribution to areas of interest in the present study is shown schematically in Figure 1. Initially, Dahlström and Fuxe (1964) demonstrated that, in the rat, most 5-HT nerve cell bodies are located in the brain stem raphé. Originally, 9 major groups of 5-HT-containing cells were identified and designated B_1-B_9. Perikarya in at least four and probably five of these nuclear groups project to the forebrain (McGeer et al., 1978; Parent et al., 1981). The most medial ascending 5-HT pathway arises primarily from cell groups B_7 and B_8 although some fibers come from cell groups B_5 and B_6 and project ventrally through the decussation of the superior cerebellar peduncles before passing rostrally within the medial forebrain bundle (MFB) to terminate in several hypothalamic, preoptic and septal areas. A second, slightly more laterally located pathway also arises from cell bodies located mainly in the midbrain raphé of groups B_7 and B_8 with small contributions possibly being contributed from cell groups B_5 and B_6. Fibers of this 5-HT ascending system also follow the MFB and

Figure 1. Schematic horizontal section (a) of rat brain at the level of the Medial Forebrain Bundle and frontal sections at (B) the level of the anterior commissure and (c) 2500μ caudal to the anterior commissure (modified from Saavedra et al., 1974 and Palkovits et al., 1980). Abbreviations: A, amygdala; AC, anterior commissure; AN, arcuate nucleus; CI, internal capsule; F, fornix; MB, mammillary body; ME, median eminence; MFB, medial forebrain bundle; NDM, dorsomedial nucleus; NHA, anterior hypothalamic nucleus; NIST, interstitial nucleus of the striae terminalis; NPO, preoptic nucleus (l = lateral, m = medial, and p = periventricular); NVM, ventromedial nucleus; oc, optic chiasm; OT, optic nerve; PM, premammillary nuclei; RN, raphé nuclei (individual cell groups B5-9); S, preoptic suprachiasmatic nucleus; SCN, suprachiasmatic nucleus; st, striae terminalis; ST, striatum.

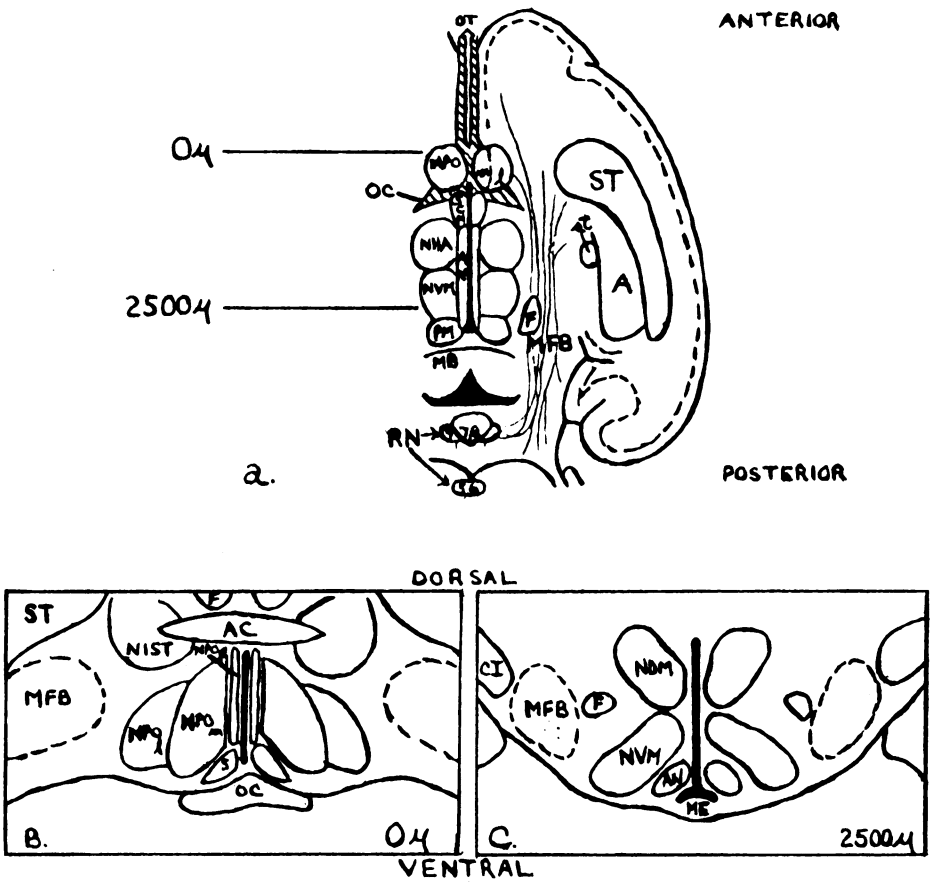

ANTERIOR
OT
0μ
2500μ
OC
ST
A
MFB
MB
RN
a.
POSTERIOR
DORSAL
ST
AC
NIST
MFB
OC
B.
0μ
MFB
NDM
NVM
C.
2500μ
VENTRAL

Figure 1

then sweep dorsally along the cingulate gyrus and laterally to terminate in the hippocampus branching along the way to innervate all cortical areas. A third system originates from cell group B_9 with some contribution from B_7 and B_8 also possible and ascends slightly lateral to the MFB to terminate primarily in the corpus striatum. 5-HT neurons innervating the SCN originate in the rostral part of the medial (B_8) and dorsal (B_7 and B_6) raphé (Héry et al., 1978). 5-HT innervation to the MPO primarily originates in the median raphé nucleus (Azmitia and Segal, 1978). 5-HT fibers destined to terminate in the tuberoinfundibular area form a compact bundle in the mesencephalon, just above the interpeduncular nucleus, prior to entering the hypothalamus (Palkovits et al., 1977). Taking advantage of the specific 5-HT uptake system (Shaskan and Snyder, 1970), Calas et al. (1974) identified ^{3}H-labelled 5-HT-containing neurons distributed throughout the ME, although they were more abundant in the external layer.

Although 5-HT neurons terminating in the ME and other intrahypothalamic areas are generally considered to originate in the dorsal midbrain raphé, the results of some experiments indicate that there may also be another source. The relatively high concentrations of 5-HT and tryptophan hydroxylase found in the ME following lesions to the raphé or surgical isolation of the medial basal hypothalamus (Brownstein et al., 1976b; Palkovits et al., 1977) suggest the presence of 5-HT neurons in the latter brain region. Neuronal perikarya in the arcuate nucleus of the rat hypothalamus which contain yellow histofluorescence possessing spectral characteristics consistent with that of a 5-HT fluorophor have been identified using microspectrofluorometric and histopharmacological techniques (Kent and Sladek, 1978). Beaudet and Descarries

(1979) have demonstrated possible 5-HT perikarya in the pars ventralis of the dorsomedial hypothalamic nucleus utilizing radioautographic techniques following intraventricular perfusion with tritiated 5-HT. 5-HT autofluorescent granules have been demonstrated by Smith and Kappers (1975) in neuronal perikarya of the arcuate and ventromedial hypothalamic nuclei of rat brains. Microspectrofluorimetric techniques have also revealed the presence of 5-HT in the area of the tanycytes in the ME (Sladek and Sladek, 1978).

III. Neurochemistry of 5-HT Neurons

A schematic representation of the events thought to be involved in the synthesis, release and inactivation of 5-HT at neuronal terminals within the brain is depicted in Figure 2. Tryptophan, the essential amino acid precursor of 5-HT, is actively transported into 5-HT neurons where it is hydroxylated to form 5-hydroxytryptophan (5-HTP). This reaction is catalyzed by the rate-limiting enzyme in the synthesis of 5-HT, tryptophan hydroxylase (tryptophan-5-monooxygenase). Although the precise manner by which tryptophan hydroxylase is regulated is not completely understood, the rate of this hydroxylation *in vivo* appears to depend not only upon the kinetic characteristics and concentration of the enzyme itself, but also upon the local concentrations of its three substrates: L-tryptophan, reduced pteridine cofactor (perhaps tetrahydrobiopterin, see Hamon *et al.*, 1979) and oxygen. Under normal physiological circumstances these substances are not present in saturating concentrations (Tappaz and Pujol, 1980; Lovenberg *et al.*, 1968). Some changes in the rate of hydroxylation of tryptophan that cannot be

Figure 2. Schematic representation of a 5-hydroxytryptaminergic nerve terminal. Abbreviations: TRP.H., tryptophan hydroxylase; L-AAAD, L-aromatic amino acid decarboxylase; MAO, monoamine oxidase; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan.

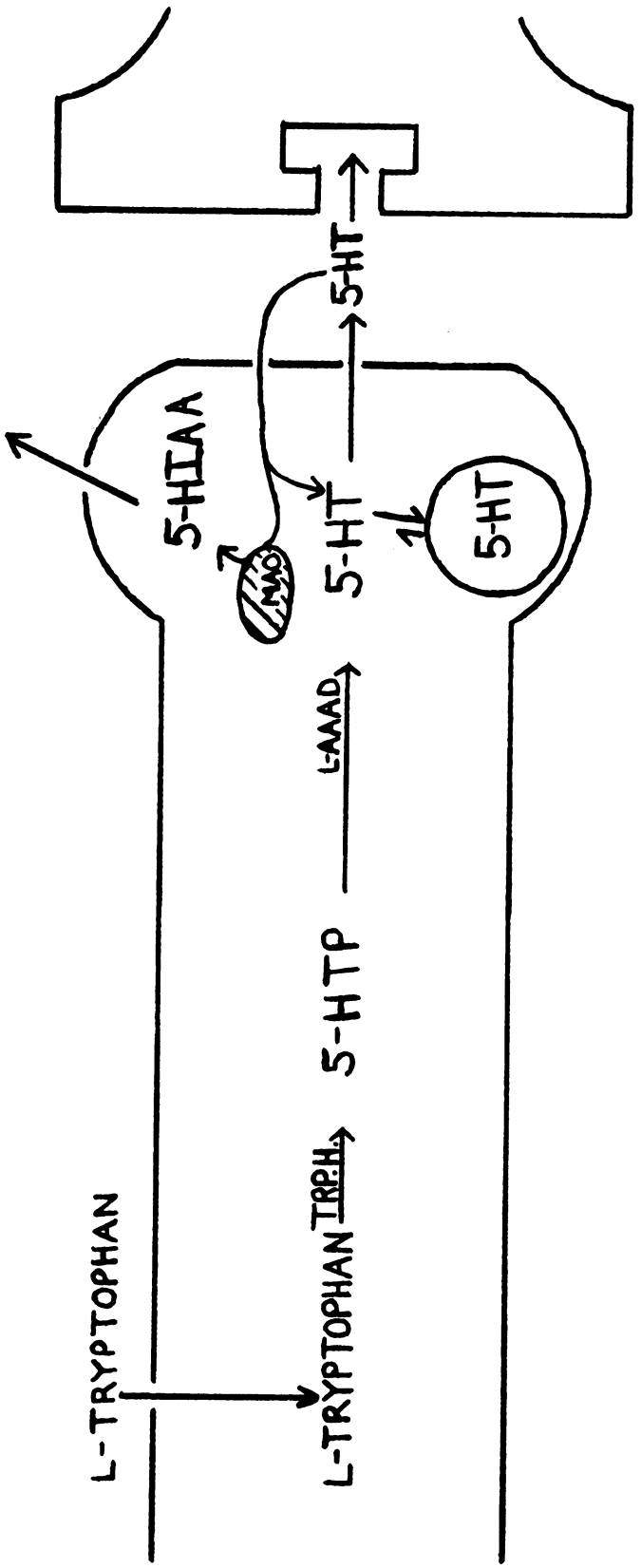

L-TRYPTOPHAN
L-TRYPTOPHAN
TRP-H
5-HTP
L-AAAD
5-HT
5-HIAA
MAO
5-HT
5-HT

Figure 2

attributed to changes in the concentration of tryptophan hydroxylase have been attributed to result from alterations in the concentration of available pteridine cofactor (Gal, 1974). Kuhn et al. (1980) suggest that the catalytic activity of tryptophan hydroxylase is dependent upon the oxidation-reduction status of -SH groups and iron binding sites, which are probably located at the catalytic (tryptophan substrate binding) site of the enzyme. The fact that the enzymatic activity of tryptophan hydroxylase (Km value for tryptophan of about 34 μM; Hamon et al., 1981) is not saturated by the concentration of tryptophan under normal physiological conditions (approximately 20 μM; Hamon et al., 1981) implies that raising or lowering brain tryptophan concentrations within its physiological range alters the saturation of the enzyme, and thus the rate of 5-HT synthesis (Fernstrom and Wurtman, 1971). Therefore, alterations in the availability of 'free' tryptophan in the brain induced by dietary, hormonal, environmental and/or pharmacological manipulations can influence brain 5-HT synthesis (Curzon et al., 1972; Curzon and Knott, 1977; Pardridge, 1977; Fernstrom and Faller, 1978).

Tryptophan is the only essential amino acid found in the plasma that is largely (80-90%) bound to albumin rather than existing in the free form (Knott and Curzon, 1972). Thus, any factor(s) influencing the binding of tryptophan to plasma albumin can alter the 'free' fraction of plasma tryptophan and, in turn, its availability for transport into the brain (Curzon and Knott, 1977).

L-Tryptophan is taken up into the brain by the same stereospecific system that transports several other large, neutral amino acids (tyrosine, phenylalanine, leucine, isoleucine and valine) into the brain

(Pardridge, 1977; Fernstrom and Faller, 1978). Therefore, tryptophan availability, and hence, the concentration in the brain can be influenced by altering the serum concentrations of any one of these competing amino acids (see discussion in Curzon and Knott, 1977).

Extracerebral metabolism of tryptophan may also regulate (at least in part) the availability of tryptophan to the brain. Liver tryptophan pyrrolase, the initial enzyme for tryptophan degradation via the kynurenine pathway, can be induced indirectly by contraceptive treatment (Nisticò and Preziosi, 1970) or directly by corticosteroids (Knox and Auerback, 1955; Nisticò and Preziosi, 1969; Scapagnini et al., 1969; Curzon and Green, 1969), tryptophan or structurally-related compounds (Sourkes and Townsend, 1955). Such an induction of tryptophan pyrrolase can effectively shunt plasma tryptophan toward the liver kynurenine pathway and away from the brain.

Finally, tryptophan availability in the brain for 5-HT formation might be altered by changes in the rate of cerebral protein synthesis as tryptophan is required as a precursor for both processes (Curzon and Knott, 1977).

There is some evidence that the rate of 5-HT synthesis may not only depend upon tryptophan availability to the brain but also upon the efficiency of the neuronal tryptophan carrier system (Hamon and Glowinski, 1974; Hamon et al., 1977) which exhibits a diurnal variation similar to the circadian rhythm in the concentration and metabolism of 5-HT that has been observed in the rat brain (Héry et al, 1974; Quay, 1968).

L-5-hydroxytryptophan synthesized in 5-HT neurons is rapidly decarboxylated to form 5-HT by a pyridoxal phosphate-dependent enzyme, L-aromatic amino acid decarboxylase (L-AAAD; possibly the same enzyme that converts L-dihydroxyphenylalanine to DA). The specific activity of L-AAAD is 70-100 times greater than tryptophan hydroxylase, indicating that the rate-limiting step for 5-HT synthesis is tryptophan hydroxylation (Peters et al., 1968).

Newly synthesized 5-HT may be stored in synaptic vesicles or released from the nerve terminals in response to nerve impulses, electrical stimulation or drugs (Müller et al., 1977). Following its release from the nerve terminal into the synaptic cleft 5-HT is free to interact with postsynaptic 5-HT receptors. Activation of these receptors is terminated when 5-HT is either metabolized by extraneuronal monoamine oxidase (MAO) (Sjoerdsma et al., 1955) or is transported back into the nerve terminal by a stereospecific active uptake mechanism (Shaskan and Snyder, 1970). Within the neuron 5-HT is oxidatively deaminated to form the intermediate metabolite, 5-hydroxyindole acetaldehyde, which undergoes immediate oxidation to form the end-product of 5-HT degradation, 5-hydroxyindole-3-acetic acid (5-HIAA). This acid metabolite is then removed from the brain by a probenecid-sensitive acid transport mechanism.

IV. Methods for Estimating 5-HT Neuronal Activity

It is difficult to obtain information on the basic processes involved in the regulation of 5-HT release in the central nervous system of mammals because simple peripheral 5-HT neuronal systems do not exist

as they do for the catecholaminergic systems. Furthermore, functional similarities between the peripheral and central 5-HT receptors do not appear to exist as they do for the catecholamines (i.e., they do not show the same sensitivity to the same drugs; Jalfre, 1974). Several types of 5-HT receptors may exist in the brain (Weight and Salmoiraghi, 1968; Tebecis and DiMaria, 1972; Bourgoin et al., 1978). Lastly, 5-HT systems in the brain appear to be organized in a more diffuse manner, in general, than central DA systems.

There appears to be a relationship between nerve impulse flow and 5-HT synthesis, release and turnover. Stimulation of 5-HT neuronal cell bodies in the raphé nuclei causes an increase in 5-HT synthesis (Eccleston et al., 1970; Shields and Eccleston, 1972; Herr et al., 1975), turnover (Andén et al., 1964; Aghajanian et al., 1967; Sheard and Aghajanian, 1968; Kostowski et al., 1969; Eccleston et al., 1969, 1970; Carlsson et al., 1972b; Shields and Eccleston, 1972), and release (Aghajanian et al., 1972; Ashkenazi et al., 1972; Bramwell and Gönye, 1976; Héry et al., 1979) in distant 5-HT nerve terminal areas. Furthermore, evidence obtained from studies in which the anterior raphé nuclei have been acutely lesioned or axons descending from the raphé through the spinal cord have been transected also supports the contention that nerve impulses play an important role in the control of 5-HT synthesis as both procedures produce a rapid decrease in the rate of tryptophan hydroxylation in distal areas (Carlsson et al., 1973; Herr and Roth, 1976). Correlations between synthesis and release of 5-HT have also been made (Héry et al., 1970, 1972, 1977; Hamon et al., 1974a,b). Finally, it appears that 5-HT may be functionally contained in two

compartments and that the newly synthesized 5-HT "pool" or compartment may be preferentially released in response to nerve stimulation (Héry et al., 1970; Sheard and Aghajanian, 1968; Shields and Eccleston, 1972).

Even though much evidence supports a correlation between nerve impulse flow and 5-HT synthesis, metabolism and release; synthesis and degradation of 5-HT in the CNS may not always be exclusively linked to 5-HT neuronal activity (Ternaux et al., 1976; Aghajanian, 1972; Héry et al., 1972). Nevertheless, changes in the rates of synthesis and metabolism of 5-HT have been used to estimate the activity of 5-HT neurons (e.g., Héry et al., 1972; Neckers and Meek, 1976). Some of the neurochemical methods that have been employed to estimate 5-HT neuronal activity include measurements of:

i) the relative concentrations of 5-HT and 5-HIAA. 5-HIAA concentrations alone, or 5-HIAA/5-HT ratios, have been related to turnover and used as an estimate of 5-HT neuronal activity in a number of physiological or pharmacological paradigms (Tagliamonte et al., 1971; Héry et al., 1972). An increase in 5-HIAA concentrations or the 5-HIAA/5-HT denotes an increase in 5-HT neuronal activity. When using this method, caution must be taken to note whether the steady-state 5-HT concentration is changing.

ii) rates of accumulation of labelled 5-HT and 5-HIAA following systemic or intracerebroventricular (icv) administration of radioactive tryptophan (Neff et al., 1971). This method depends upon the measurement of the intraneuronal precursor pool (assumed to equal the plasma precursor pool) and often

includes the assumption that there exists a single neurotransmitter pool. Another assumption that is often not evaluated carefully is that the unmeasured precursor pool does not change throughout the experiment. This assumption is critical to interpretation of data obtained with non-steady state conditions used in isotopic experiments. Finally, attempting to examine 5-HT activity in discrete areas of the rat brain such as the ME by isotopic methods is very difficult due to the size of the region, sparse 5-HT innervation, accessibility of the isotope and length of time necessary for labelled precursor to accumulate in quantities substantial enough to measure without having to pool tissues from several animals.

iii) rates of accumulation of 5-HT (Lin et al., 1969; Neff et al., 1967) or disappearance of 5-HIAA (Tozer et al., 1966) after the administration of an inhibitor of MAO such as pargyline. Assumptions included in these steady-state systems are that the synthesis of 5-HT is equal to its metabolism to 5-HIAA and that the 5-HIAA produced reflects the amount of 5-HT used functionally in the brain. Furthermore, it is assumed that the drug employed to inhibit MAO does not affect the synthesis and disposition of 5-HT itself, or the release of 5-HIAA from the neuron. Evidence has been presented that pargyline may cause an increased conversion of 5-HTP to 5-HT, and that intraneuronal 5-HT may inhibit the rate of hydroxylation of tryptophan once the amine reaches some critical concentration

(Weber, 1966; Carlsson and Lindqvist, 1973). These methods also assume that MAO is inhibited completely and that 5-HT is converted entirely to 5-HIAA.

iv) rates of accumulation of 5-HIAA after administration of the acid transport inhibitor, probenecid (Neff et al., 1967; Meek and Werdinius, 1970). This method also assumes that the drug administered does not affect the synthesis or disposition of 5-HIAA itself.

v) the activity of tryptophan hydroxylase. This has been measured in vitro following treatment of animals in vivo (Kizer et al., 1976c; Palkovits et al., 1976) or in vivo by measuring the rate of accumulation of 5-HTP after the administration of an L-AAAD inhibitor (NSD 1015; Ro4-4602). In the absence of an L-AAAD inhibitor the concentration of 5-HTP in brain is essentially zero, but it increases linearly with time once the decarboxylating enzyme is inhibited (Carlsson et al., 1972a,b). This method measures the direct product of tryptophan hydroxylation (the rate-limiting step in 5-HT synthesis) and thus the synthetic rate of tryptophan hydroxylase, without having to assume anything about the number of metabolically active 'pools' of 5-HT. Only one measurement is necessary (5-HTP concentration) so that 5-HT synthesis can be measured even if steady-state concentrations of 5-HT are changing. Limitations of this method include the fact that NSD 1015 also inhibits L-AAAD in catecholaminergic neurons which could secondarily affect 5-HT synthesis. It is also possible that

the accumulation of an intermediate substrate in an abnormal way might disturb the normal metabolic activity in the neuron.

In some cases 5-HT continues to be synthesized and metabolized to 5-HIAA even after impulse traffic in 5-HT neurons ceases and may be metabolized by MAO without first being released (Carlsson and Lindqvist, 1973). Thus, it is difficult to know what proportion of synthesized 5-HT is actually released and what proportion is merely metabolized within the neuron. Nevertheless, as already discussed, the evidence does support a relationship between 5-HT neuronal activity, 5-HT synthesis and metabolism in the brain.

In the studies to be reported two methods for examining 5-HT neuronal activity have been utilized. First, the concentrations of 5-HT and 5-HIAA and the ratio ([5-HIAA]/[5-HT]) have been studied as an estimate of metabolism. Secondly, the accumulation of 5-HTP following inhibition of L-AAAD by NSD 1015 was measured as an index of 5-HT synthesis.

Following development of a method sensitive enough to measure 5-HT, 5-HIAA and 5-HTP in the SCN, MPO, AN, ME and ST the validity of the method was tested using pharmacological manipulations. The activity of 5-HT neurons was then evaluated during several endocrinological, environmental and pharmacological paradigms in which the secretion of prolactin from the anterior pituitary is undergoing dynamic change. These included determining the effects of morphine, restraint and suckling, all of which increase the secretion of prolactin, as well as the physiological surges of prolactin that occur in the afternoon of

proestrus during the normal ovarian estrous cycle and twice daily during early pregnancy. Each of these paradigms will be introduced in their appropriate section under Results and Discussion.

STATEMENT OF PURPOSE

5-HT neuronal systems are believed to exert a modulatory role in the regulation of prolactin release from the anterior pituitary. Although 5-HT has no effect on the pituitary *per se*, indirect pharmacological evidence (mostly obtained using 5-HT agonists and antagonists) suggests that 5-HT neurons play a stimulatory role in situations where dynamic changes in prolactin secretion are occurring. An important first piece of evidence for the involvement of 5-HT neurons in the secretory control of prolactin from the anterior pituitary could be obtained by examining 5-HT neuronal activity throughout experimental paradigms where dynamic changes in prolactin secretion are occurring. Although such an examination would not provide information concerning a cause and effect relationship between 5-HT neuronal activity in particular areas of the brain and prolactin secretion, it could provide information as to what areas should be examined in the future. At the beginning of this project no single technique which would permit the quantitation of 5-HT, 5-HIAA and 5-HTP, in the ME, SCN, MPO, AN and ST of a single rat brain existed.

The objective of the studies was to:

i) Develop a method utilizing high performance liquid chromatography coupled with electrochemical detection sensitive enough

to measure 5-HT, 5-HTP and 5-HIAA in discrete regions and nuclei (ME, MPO, SCN, AN, ST) of a single rat brain.

ii) Evaluate the validity, sensitivity and capability of the method by examining the 5-HT neuronal responses occurring in these regions following the administration of various drugs with known pharmacological actions that might be expected to alter the dynamics of 5-HT neurons.

iii) Examine 5-HT synthesis and metabolism in these discrete brain regions after various pharmacological, environmental and endocrinological manipulations which alter the secretion of prolactin from the anterior pituitary.

MATERIALS AND METHODS

I. Animals and Blood Collection

Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 200-275 g were utilized in all experiments where pharmacological manipulations alone were investigated or where the effects of restraint stress on 5-hydroxytryptaminergic neuronal activity were examined. Female Long-Evans rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 225-300 g were used in all experiments where 5-hydroxtryptaminergic neuronal activity was examined during pregnancy, lactation/suckling, and throughout the day of proestrus in the ovarian cycle. All animals were maintained in air-conditioned (22±1°C) and light controlled (12 h light cycle, 07.00-19.00 h) rooms and were allowed access to food (Wayne Lab-Blox, Allied Mills, Chicago, IL) and water ad libitum.

Blood samples for the determination of serum concentrations of prolactin were collected from the trunk following decapitation. The samples were stored at 4°C for 24 h, centrifuged at 1,000 g for 30 min, and the serum was frozen at -20°C until assayed. All animals were sacrificed between 10.00 and 12.00 h unless otherwise designated.

II. Biochemical Procedures

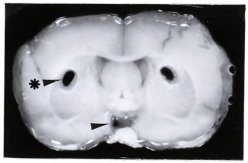
A. Dissections

Following decapitation rat brains were quickly removed from the skull and placed dorsal side down on a cold plate (Thermoelectrics

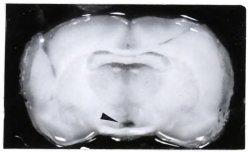
Unlimited). The median eminence was removed from the hypothalamus with the aid of a dissecting microscope and fine scissors (Cuello et al., 1973). While grasping the infundibular stalk with fine forceps cuts were made along the tuberoinfundibular sulcus on both sides to the rostral border of the median eminence. When dissected in this manner the average protein content of the whole median eminence was approximately 24 µg. The remaining brain was cut just caudal to the hypothalamus and the anterior portion of the brain was frozen on dry ice until frontal sections could be prepared using a sliding microtome fitted with a freezing stage. A modification of the procedure described by Palkovitz (1973) was used to dissect the other discrete brain areas using the atlas of König and Klippel (1967) as a guide. Sections of the appropriate thickness and location were mounted on conventional glass slides and frozen on dry ice. Examples of the 3 frontal sections and location of punches can be seen in Figure 3. The most rostral section was taken at the level of the crossing of the commissura anterior and included structures between A7020-6720 (300 µm thick). From this section a bilateral punch was taken of both the striatum (nucleus caudatus putamen) and the medial preoptic nucleus (nucleus preopticus medialis). The next section taken included structures between A6220-5720 (500 µm thick). From this section a single punch extending across the midline of the section just above the optic chiasma was taken to include the suprachiasmatic nucleus (nucleus suprachiasmaticus) bilaterally. The final section (A4920-3920; 1000 µm thick) was used to obtain a single punch containing the arcuate nucleus (nucleus arcuatus). Needles for the punches were made from stainless steel hypodermic tubing (Small

Figure 3. Photographs of three frontal sections with examples illustrating location of punches. Slices were removed according to König and Klippel (1967); rostral to caudal: A) represents a 300 µm slice (A7020-A6720) from which punches of the ST (arrow with asterisk) and MPO (arrow) were removed bilaterally. B) Represents a 500 µm slice (A6220-A5720) from which a single punch of SCN was removed (arrow). C) Represents a 1000 µm slice (A4920-A3920) from which AN was removed (arrow).

a.



B.



C.

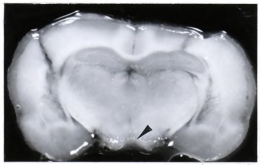


Figure 3

Parts, Inc., Miami, FL). Two punches were used. A 14 gauge tubing (0.063", i.d.) was used to construct a crescent-shaped punch to dissect the arcuate nucleus. An oblong punch was constructed from a 17 gauge (0.042", i.d.) tubing for use in dissecting the striatum, medial preoptic nucleus and the suprachiasmatic nucleus. The punch containing the arcuate nucleus may have also contained small amounts of the pars ventralis of the ventromedial nucleus. The punch for the suprachiasmatic nucleus undoubtedly contained small amounts of the tractus infundibularis and possibly a small contribution from the periventricular nucleus of the hypothalamus. Similarly, the punch containing the medial preoptic nucleus probably also contained small contributions from the periventricular preoptic and median preoptic nuclei as well as a small portion of lateral preoptic nucleus. The approximate protein contents for the bilateral striatal and medial preoptic nucleus punches as well as the single suprachiasmatic and arcuate nuclei punches are 100, 80, 40 and 110 µg, respectively. All tissue samples were homogenized directly into 30 µl of the mobile phase used for the high performance liquid chromatographic analysis described below (B). The homogenates were diluted to a total volume of 100 µl with additional mobile phase and then centrifuged for 30 sec in a Beckman microfuge. Ninety of the resulting 100 µl of supernatant was then filtered through glass wool (previously washed with 600 µl of mobile phase) in order to remove particulate matter and diluted to a final volume of 600 µl with additional mobile phase. Five hundred microliters of this final volume was then injected directly onto the chromatography column. The protein content of the homogenate pellet of each sample was analyzed as

described by Lowry et al. (1951) and all data is expressed as ng of the compound of interest per mg protein.

B. High Performance Liquid Chromatography Technique

Several neurochemical methods have been employed to measure the activity of dopaminergic and 5-hydroxytryptaminergic neurons in the rat brain. For the purposes of the present studies 5-hydroxytryptaminergic neuronal activity was estimated by measuring the ratio of metabolite to neurotransmitter (5-HIAA/5-HT), and the accumulation of the amino acid precursor of 5-HT, 5-hydroxytryptophan, following inhibition of L-aromatic amino acid decarboxylase. Until recently it had not been possible to measure 5-HT, 5-HIAA or 5-HTP in discrete brain regions primarily because of a lack of sensitivity in available analytical techniques. Development of sensitive radioenzymatic (Tappaz and Pujol, 1980) and high performance liquid chromatographic (HPLC) (Krstulovic and Matzura, 1979; Meek and Lofstrandh, 1976) assays for 5-HTP and assays using HPLC in combination with electrochemical detection for measuring 5-HT, 5-HIAA and 5-HTP (Loullis et al., 1979) have recently made it possible to employ biochemical techniques to estimate 5-HT neuronal activity in areas such as the striatum, hypothalamus and mediobasal hypothalamus. No technique allowed for the measurement of 5-HT, 5-HIAA, 5-HTP, DA and DOPAC in a sample of ME, SCN, MPO, AN or ST from a single rat brain. Thus, the development of an assay using HPLC coupled with electrochemical detection that would possess the selectivity to allow the concurrent analysis of all of these compounds without sacrificing the sensitivity required to measure them in such discrete nuclei was attempted.

Several modifications of a mobile phase used originally by Felice et al. (1978) were examined for their effects on retention times for each of the compounds of interest (Nielsen and Johnston, submitted). A mobile phase that allowed separation and concurrent measurement of 5-HT, 5-HIAA, 5-HTP, DA, DOPAC and HVA in a single run of approximately 20-24 min was selected. A retention time of 20-24 minutes was required in order to resolve all of the compounds of interest in a single sample of a discrete hypothalamic nuclei. The final mobile phase consisted of a 0.1 M citrate-phosphate buffer (pH 3.6) containing 8% (v/v) methanol, 0.032% (w/v) sodium octyl sulphate (SOS) and 0.1 mM disodium ethylene-diaminetetraacetate (EDTA).

A model LC-40 (Bioanalytical Systems, West Lafayette, IN) liquid chromatography system with either a TL-3 carbon paste electrode or a TL-5 glassy carbon electrode thin-layer transducer connected to a LC-2A amperometric controller was equipped with a 30 cm x 3.9 mm, i.d. C_{18}-μBondapak reverse phase column (Waters Associates, Milford, MA). Samples and standards were injected into a 6-port valve (Rheodyne, Berkeley, CA) equipped with a 500 μl loop. Flow rate of the mobile phase was 2.0 ml/min maintaining column pressure at approximately 2000 psi. All chromatographic experiments were performed at ambient temperature in an electrically shielded room. The speed of the Omniscribe RYT B-5000-D strip chart recorder (Houston Instruments, Austin, TX) was 5 cm/10 min. The working electrode was set at +0.75 mV relative to a Ag^+/AgCl reference electrode. The electronic controller was set at 5 nA/volt and the recorder at 1.0 and 0.1 volts full scale providing 5 nA and 0.5 nA full scale chromatographs on the RYT dual pen chart recorder.

The stationary phase of the C_{18}-μBondapak column is composed of totally porous 10 μm silica particles to which N-alkyl C_{18} chains have been bonded. This type of column generally separates compounds through hydrophobic interactions and since the catecholamines are very polar molecules, they are not retained significantly by this type of stationary phase. The result is a poor resolution of the charged compounds which are often inseparable from the void volume. Thus, by itself, normal reverse phase liquid chromatography is often unusable for monoamine analysis (especially the catecholamines).

The alternative to normal reverse phase liquid chromatography is the ion-pair or "soap" chromatography modification which utilizes the same stationary phase (column) but adds, in dilute concentrations, a compound capable of forming an ion pair with the analyte(s) of interest. Because the catecholamines are cations at pH 3.6 (the pH of the mobile phase) a small quantity of sodium octyl sulphate (an ionic detergent, hence "soap" chromatography) was added to modify the properties of the C_{18} surface sufficiently for the catecholamines to be resolved.

The original theory for the resulting separation proposed the formation of an ion-pair in the mobile phase between the monoamines and the octyl sulphate groups so that they would partition into the alkyl bonded stationary phase as a hydrophobic ion pair. More recently, the theory has been advanced that the octyl sulphate partitions onto the modified silica surface to give it an anionic character and create a dynamic ion-exchange column in physical (not chemical) appearance. Positively charged analyte ions could then partition onto the stationary phase by a conventional ion exchange process resulting in their

resolution. In reality, probably both theoretical mechanisms contribute to the resulting separation.

The use of microparticulate reverse phase "soap" chromatography for analysis of monoamines is ideal in many respects. The retention of the various cationic monoamines and their amino acid precursors can be varied to suitable values simply by modifying the concentration of the ionic detergent in the mobile phase. The reverse phase column is more versatile in that under the proper conditions it can be used to separate both polar and neutral compounds. Furthermore, the reverse-phase procedure is faster, more efficient and slightly more sensitive than the earlier pellicular cation exchange catecholamine method and allows concurrent analysis of the catecholamine, DA; the indoleamine, 5-HT; the amino acid precursor of 5-HT, 5-HTP; and the acid metabolites of DA (DOPAC and 3-methoxy-4-hydroxyphenylacetic acid (HVA)) and 5-HT (5-HIAA).

Some of the principles of electrochemical detection can be better understood by examining Figure 4. At the top of Figure 4 a schematic of the TL-3 thin-layer transducer containing the carbon paste working electrode is illustrated. The eluent from the C_{18} μBondapak column flows in this diagram from left to right across the face of the working electrode (w) and then up towards the reference electrode. On the lower part of this figure an example of the oxidation process using DA as the orthodihydroxy constituent and a schematic enlargement of the thin layer cell are shown. The detector is operated in an amperometric mode which means the electrode is maintained at a constant operating

FIGURE 4. PRINCIPLES of ELECTROCHEMICAL DETECTION

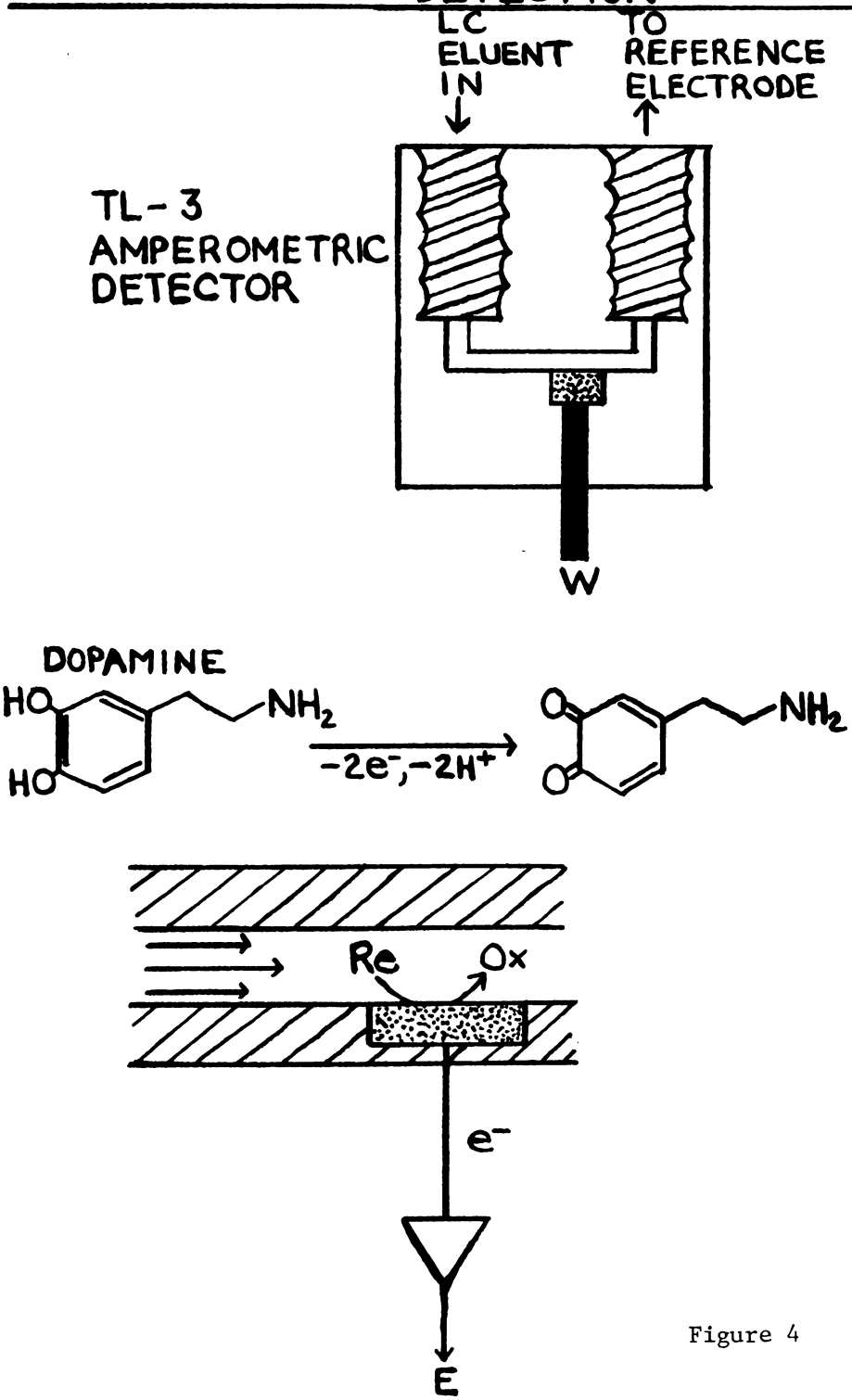


Figure 4

potential-sufficiently positive to force the orthodihydroxy group to undergo a loss of 2 electrons and 2 protons to yield an orthoquinone. The current resulting from the transfer of electrons from the analyte in solution to the electrode surface is then converted to a voltage, amplified and measured with an appropriate time-base recorder. As oxidizable bands of solute pass the detector the current rises and falls as a function of time to yield the chromatogram.

A tracing of an actual chromatogram obtained under the conditions described in this section following the injection of authentic standards of NE, DOPA, DOPAC, DA, 5-HIAA, HVA, 5-HTP and 5-HT (light peaks) and following the injection of a single median eminence sample (dark peaks) are shown in Figure 5. This example demonstrates the resolution of these compounds and the sensitivity of the assay in that it is quantitatively possible to measure DA, DOPAC, HVA, 5-HT, 5-HIAA and 5-HTP in a single median eminence (0.2-0.3 mg tissue).

The amounts of biogenic amines and their metabolites in each sample were determined by measuring the heights of the individual peaks and comparing them with the peak heights obtained from a chromatograph of a known amount of the various standards. The peak height/ng standard ratio proved to be linear over a range of 0.1-10 ng of the various injected standards. The amounts of the various amines or metabolites were expressed as ng/mg protein in all cases. Under normal working conditions the sensitivity of the method for DA, DOPAC, 5-HT, 5-HIAA and 5-HTP was 30, 30, 50, 30 and 50 picograms, respectively. Stock standard solutions of DA, DOPAC, HVA, DOPA, NE, 5-HT, 5-HIAA and 5-HTP made by dissolving each separately in the mobile phase were stored at

Figure 5. HPLC chromatograph of injection of mixture of authentic standards (light peaks) and single ME sample (dark peaks).

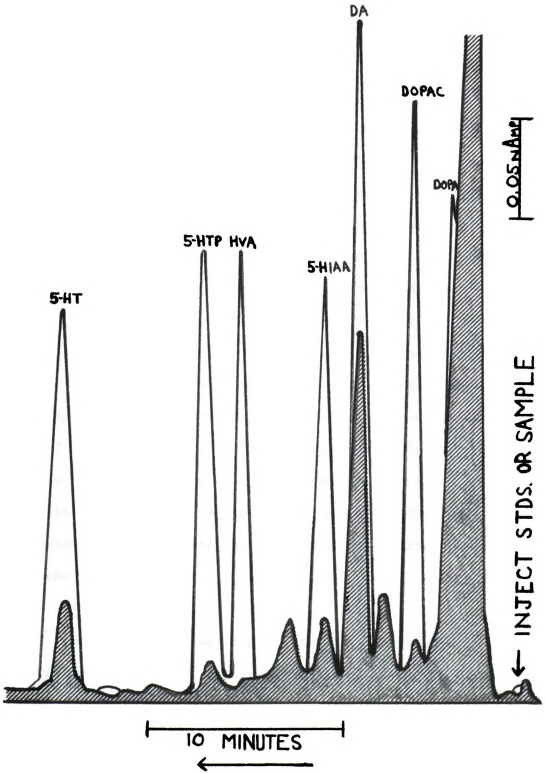

DA
DOPAC
DOPA
0.05 nAmp
5-HTP HVA
5-HIAA
5-HT
INJECT STDS. OR SAMPLE
10 MINUTES

Figure 5

-20°C for up to 1 month without a detectable decrease in peak height. A standard mix of all eight of the compounds was made from these stock solutions and injected onto the column periodically throughout the day as well as before the first sample run of the day and after the final sample run of each day.

C. Preparation of Mobile Phase

The mobile phase was prepared by adding equal volumes of 0.1 M citrate (citric acid·H_2O) and 0.1 M dibasic sodium phosphate (NA_2HPO_4, anhydrous) together. This mixture was adjusted to pH 3.6 with concentrated phosphoric acid. The solution was then filtered under vacuum through a 0.22 μm membrane filter (Millipore, Bedford, MA). Methanol (Baker resin analyzed, J.T. Baker Co., Phillipsburg, NJ) which had been previously filtered (0.5 μm teflon filters; Schleicher and Schnell, Inc., Keene, NH) was added to attain a final concentration of 8% (v/v). This solution was then gently stirred at 40°C and sodium octyl sulphate (Eastman Kodak Co., Rochester, NY) was added to achieve a final concentration of 0.032% (w/v). Finally, disodium ethylenediaminetetraacetate (EDTA) was added to attain a final concentration of 0.1 mM. Deionized water (Milli-Q Water Purification Systems; Millipore Corp., Bedford, MA) and analytical grade reagents (Mallinckrodt, Inc., Paris, KY) were used to make the mobile phase.

D. Radioimmunoassay

Prolactin was measured by double antibody radioimmunoassay using kits generously supplied by NIAMDD (Dr. A.F. Parlow; NIAMDD Rat Pituitary Hormone Distribution Program) in the laboratories of Dr. K.E.

Moore and Dr. G.D. Riegle. Results are expressed in terms of the NIAMDD-Rat Prolactin RP-2 standard.

E. Estimation of 5-HT Neuronal Activity

5-Hydroxytryptaminergic neuronal activity in the present study was estimated in one of two ways. The first method simply examines metabolism by observing the ratio of the concentration of the primary metabolite of 5-HT, 5-HIAA, to the concentration of 5-HT itself as an index of the rate of conversion of 5-HT to 5-HIAA. The concentration of 5-HIAA is believed to represent the amount of 5-HT that has been released, recaptured by the neuron and then metabolized to 5-HIAA. An increase in the concentration of 5-HIAA and thus in the ratio of the concentration of 5-HIAA to the concentration of 5-HT should reflect an increased metabolic activity in the 5-HT neuron.

The second method involves measuring the rate of accumulation of the direct precursor of 5-HT, 5-HTP, after inhibiting the decarboxylating enzyme. The tissue concentration of 5-HTP is essentially zero, but increases linearly with time (see Results) following administration of a decarboxylase inhibitor. The rate at which 5-HTP accumulates represents an *in vivo* estimate of tryptophan hydroxylase activity and thus, synthetic activity in the 5-HT neurons. 5-HTP accumulation was measured in the 30 minute period following the inhibition of L-aromatic amino acid decarboxylase (L-AAAD) with NSD 1015 (100 mg/kg, i.p.).

Although a relation between nerve impulse flow and turnover of 5-HT has been demonstrated (Carlsson *et al.*, 1972b), synthesis and degradation of 5-HT in the CNS may not be exclusively linked to 5-HT

neuronal activity. Even so, changes in the rates of synthesis and metabolism of this amine have been used to estimate 5-HT neuronal activity (e.g., Héry et al., 1972; Neckers and Meek, 1976). Indeed, 5-HIAA concentrations, alone, or 5-HIAA/5-HT ratios have been related to turnover and used to estimate activity in 5-HT neurons (Tagliamonte et al., 1971; Héry et al., 1972). Furthermore, Carlsson et al. (1972a,b) has examined the activity of tryptophan hydroxylase in vivo by measuring the accumulation of 5-HTP following the administration of an L-AAAD inhibitor and has demonstrated a linear increase in 5-HTP accumulation with time once the decarboxylating enzyme is inhibited.

F. Drugs

1. Miscellaneous Drugs:

Morphine sulphate (Mallinckrodt, St. Louis, MO) and naloxone HCl (Endolabs, Garden City, NY) were dissolved in distilled water.

Chlorimipramine (Ciba-Geigy Corp., Summit, NJ), fluoxetine HCl (Eli Lilly and Co., Indianapolis, IN), pargyline HCl (Sigma Chemical Co., St. Louis, MO) and 3-hydroxybenzylhydrazine dihydrochloride (NSD 1015, Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% saline.

L-Tryptophan (Sigma Chemical Co.) and probenecid (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ) were dissolved in 0.1 N NaOH and then neutralized with 0.1 N HCl after being diluted with distilled water.

Reserpine (S.P. Penick and Co., New York, NY) was dissolved in dilute acetic acid. Ether for anesthesia was purchased from Mallinckrodt Inc. (Paris, KY).

The routes and times of administration for each of these pharmacological agents are described in the Results section.

2. Solutions and Drugs Used in the Preparation of the Mobile Phase for the HPLC Analysis

Phosphoric acid (ortho 85%) and disodium ethylenediamine tetraacetate (EDTA) were purchased from Fisher Scientific Co. (Fairlawn, NJ); citric acid·H_2O and dibasic sodium phosphate-anhydrous from Mallinckrodt, Inc. (Paris, KY); methyl alcohol (anhydrous, Baker resin analyzed) from J.T. Baker Co. (Phillipsburg, NJ); and sodium octyl sulphate (SOS) from Eastman Kodak Co. (Rochester, NY).

3. Compounds Used as Standards for HPLC Analysis

l-Norepinephrine bitartrate (NE), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic Acid [HVA]), 5-hydroxytryptophan (5-HTP), 5-hydroxyindole-3-acetic acid (5-HIAA), 5-hydroxytryptamine creatinine sulphate complex (5-HT) and 3-hydroxytyramine HCl (DA) were all purchased from Sigma Chemical Co. (St. Louis, MO). 3,4-Dihydroxyphenyl L-alanine (L-DOPA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

G. Statistics

Differences between sample means were determined by one-way analysis of variance using the Student-Newman-Keuls' test (Steele and Torrie, 1960) except in cases where only two sample means existed. In the latter cases individual mean differences between control and treated groups were analyzed using the Student's t-test (Sokal and Rohlf, 1969).

The 0.01 level of probability was used as the minimum criterion of significance except for determination of differences between samples of serum prolactin where the 0.05 level of probability was used.

H. Specialized Methods Used in Experiments Involving Environmental and Endocrinological Manipulations

1. Restraint Stress

Male Sprague-Dawley rats were rapidly transferred from their home cages directly into an ether-saturated desiccator. Following anesthesia, the rats were restrained with tape to wire test tube racks and then placed on their backs for a period of 30 minutes prior to decapitation. Some animals were returned to their individual home cages immediately following anesthesia for 30 min prior to sacrifice to control for the effects of ether alone while control animals remained in their home cages until sacrifice.

2. Proestrous Surge

Daily vaginal smears were collected by lavage from female Long-Evans rats and the cytology present in these samples was used to monitor the estrous cycles of the individual animals. Females exhibiting a vaginal smear history of at least two consecutive 4-day estrous cycles immediately preceding the cycle in progress were sacrificed at appropriate times on the day of proestrus. All procedures occurring during the dark phase of the dark-light cycle were carried out under red light. Following decapitation the uterine horns were examined for "ballooning" characteristic of proestrus as further confirmation that the animals had continued to cycle regularly and were indeed sacrificed on proestrus.

3. 5-HTP Accumulation Time Course in Female Rats

Daily vaginal smears were collected by lavage from female Long-Evans rats and the cytology present in these samples was used to monitor the estrous cycles of the individual animals. Females exhibiting a vaginal smear history of two consecutive regular estrous cycles were injected with NSD 1015 (100 mg/kg, i.p.) on diestrus 30 min prior to sacrifice by decapitation. A postmortem vaginal smear was taken to confirm that the animal was killed on diestrous.

4. Pregnancy

The estrous cycles of female Long-Evans rats were monitored by daily vaginal lavage and only animals exhibiting two or more consecutive regular estrous cycles were used. The breeding was carried out in the laboratory of Dr. G. Riegle. Each female was placed with two fertile, sexually experienced males on the afternoon of proestrus. Successful mating and maintenance of pregnancy was confirmed by the presence of sperm in the vaginal lavage the following morning and subsequent counting of uterine implantation sites, respectively. Animals were sacrificed by decapitation at appropriate times and days throughout pregnancy.

5. Lactation/Suckling

The estrous cycles of female Long-Evans rats were monitored by daily vaginal lavage and only animals exhibiting two or more consecutive regular estrous cycles were used. The breeding was carried out in the laboratory and under the supervision of Dr. G. Riegle. Each female was placed with two fertile, sexually experienced males on the afternoon of proestrus. Successful mating and maintenance of pregnancy

was confirmed by the presence of sperm in the vaginal lavage the following morning and subsequent persistance of leucocytic vaginal cytology. All litters were equalized to eight pups on day 1 of lactation. All animals were sacrificed between 10.00 and 12.00 h on day 12 of lactation. A non-lactating diestrous control group was compared to the following two groups of lactating females: Following 4 h of pup deprivation 5-HTP accumulation and 5-HIAA/5-HT were examined in mothers where 1) the pups were returned and allowed to suckle for 30 min (suckled group) prior to sacrifice and 2) pups were not returned (non-suckled group) prior to sacrifice. All eight pups were required to suckle for the duration of the 30 min period in order for the experimental mothers to be included in the suckled group.

RESULTS AND DISCUSSION

I. Measurement of 5-HT, 5-HIAA and 5-HTP in Selected Regions of the Rat Brain Using HPLC with Electrochemical Detection

5-HT, 5-HIAA and the accumulation of 5-HTP following inhibition of L-AAAD have rarely been examined in the MPO, AN, SCN or ME because no method possessing the sensitivity and capability to measure these three compounds in these discrete brain regions exists. Although sensitive assays have been developed for the measurement of 5-HT, 5-HIAA and 5-HTP none of these allows the quantitation of all of these compounds in the selected discrete hypothalamic regions examined in the present studies. For example, sensitive radioenzymatic assays for the quantitation of 5-HTP (Tappaz and Pujol, 1980) and radioimmunoassays for the determination of 5-HT and 5-HIAA (Delaage and Puizillout, 1981) do not allow the examination of all of these compounds in discrete regions of a single rat brain. Determination of 5-HTP utilizing HPLC following preparative steps allows detection of approximately 500 pg 5-HTP without 5-HT or 5-HIAA (Meek and Lofstrandh, 1976; Krstulovic and Matzura, 1979). Loullis et al. (1979) measured 5-HT, 5-HIAA and 5-HTP using HPLC with electrochemical detection in larger brain regions such as the ventromedial hypothalamus but did not have the sensitivity to measure these compounds in more discrete nuclei nor the resolution to detect DA or DOPAC. Measurement of 5-HT, 5-HIAA and 5-HTP in larger brain regions such as the striatum have frequently been made. Table 1

TABLE 1

Literature Values for 5-HT, 5-HIAA and 5-HTP Accumulation in Selected Regions of the Rat Brain

Reference	Region							
	MPO	AN	ME		SCN	ST		
	5-HT	5-HT	5-HT	5-HIAA	5-HT	5-HT	5-HIAA	5-HTP
Saavedra et al., 1974	11.6	36.4	15.3		25.4			
Van de Kar et al., 1978	12.2	25.4			40.2	11.5		
Palkovits et al., 1976		11.5-17.9	12.7-18.8		15.7-31.2			
Crowley et al., 1979	7.6		10.5		11.2			
Čulman et al., 1980		7.2	7.6		8.4			
Reinhard et al., 1980			10.4	3.1		5.3[a]	3.4[a]	
Palkovits et al., 1977		9.4-16.5	12.0-21.8		18.1-27.5			
Brownstein et al., 1976b		11.0	12.7					
Neckers et al., 1979						5.4-6.0	5.3-6.6	2.0
Waldmeier and Fehr, 1978						4.6[a]	6.3[a]	
MacKenzie and Trulson, 1978						3.1[a]	4.2[a]	
Carlsson et al., 1972a								0.9 -1.0
Marco and Meek, 1979								1.2 -1.8
Garcia-Sevilla et al., 1978								0.45-0.65[a]
Tappaz and Pujol, 1980								1.8[a]

Values for 5-HT and 5-HIAA represent ng/mg protein. Values for 5-HTP represent ng/mg protein 30 min following the intraperitoneal injection of a decarboxylase inhibitor. Those values indicated by "a" were converted to ng/mg protein on the basis of 1 gm wet weight containing 110 mg protein.

contains the published values for the concentrations of 5-HT and 5-HIAA as well as the rate of accumulation of 5-HTP following inhibition of L-AAAD in the MPO, AN, ME, SCN and ST. This list is complete for published values in the MPO, AN, ME and SCN but represents only a sample of the reported values in the ST. The capability of HPLC with electrochemical detection (see section on development of method in Materials and Methods II.B) to measure 5-HT, 5-HIAA and 5-HTP in the ME, AN, SCN, MPO and ST is revealed in Table 2. Values contained in Table 2 represent the mean micrograms of protein, nanograms per sample and calculated nanograms per milligram protein of 5-HT, 5-HIAA and 5-HTP in the ME, AN, SCN, MPO and ST obtained from the first five pharmacological experiments using the newly developed method. In addition, the range of concentrations reported in the literature for 5-HT, 5-HIAA and the accumulation of 5-HTP in the selected brain regions is provided in Table 2 for direct comparison with concentrations obtained in the present study. The concentrations of 5-HT obtained using HPLC with electrochemical detection consistently fall within the range of previously reported 5-HT concentrations in all five brain areas examined. The concentration of 5-HIAA in the ST determined in the present study also falls within the range of previously reported literature values. On the other hand, the concentration of 5-HIAA in the ME is consistently 3-4 times higher than that reported in the only published measurement of ME 5-HIAA. The rate of accumulation of 5-HTP (for time-course of 5-HTP accumulation see Tables 3 and 4 as well as Figure 6) obtained for the ST using HPLC with electrochemical detection was approximately twice as high as rates previously reported in that region. The concentration of 5-HIAA in the

TABLE 2

Quantification of 5-HT, 5-HIAA and 5-HTP Concentrations in Selected Regions of Rat Brain Using High Performance Liquid Chromatography with Electrochemical Detection

	Brain Regions				
	ME	AN	SCN	MPO	ST
Protein/sample (μg)	28.1 ± 1.2	105.6 ± 4.8	46.4 ± 1.6	81.7 ± 4.0	95.7 ± 4.6
5-HT					
ng/sample	0.53± 0.04	3.0 ± 0.2	1.08± 0.07	0.96± 0.34	0.69± 0.05
ng/mg protein	17.3 ± 0.9	18.0 ± 0.8	24.1 ± 1.0	8.0 ± 0.4	7.4 ± 0.2
Published values	7.6-21.8	7.2-36.4	8.4-40.2	7.6-12.2	3.1-11.5
5-HIAA					
ng/sample	0.39± 0.03	3.7 ± 0.2	0.94± 0.08	1.16± 0.11	0.59± 0.06
ng/mg protein	12.4 ± 0.5	22.6 ± 0.8	20.6 ± 1.2	13.5 ± 0.8	6.3 ± 0.2
Published values	3.1	---	---	---	3.4-6.6
5-HTP					
ng/sample	0.13± 0.02	0.74± 0.06	0.49± 0.03	0.36± 0.04	0.38± 0.04
ng/mg protein	4.4 ± 0.5	5.6 ± 0.4	9.8 ± 0.4	4.4 ± 0.2	3.8 ± 0.3
Published values	---	---	---	---	0.4-2.0

Values represent mean ± S.E.; for 5-HT and 5-HIAA N = 25-31, for 5-HTP N = 20, and for protein N = 50-53. 5-HTP was measured 30 min after the injection of NSD 1015 (100 mg/kg, ip). Published values represent the range of concentrations (ng/mg protein) reported in the literature; see Table 1.

---, indicates no value has yet been reported in the literature.

AN, SCN and MPO as well as the rate of 5-HTP accumulation in the ME, AN, SCN and MPO obtained with the newly developed method represent the only measurements of these compounds in the selected brain regions.

The HPLC assay procedure described here represents a simple, sensitive and rapid method that allows the concurrent determination of 5-HT, 5-HIAA and 5-HTP in a single sample of ME, AN, SCN, MPO or ST for the first time. In addition, under standard operating conditions DA, DOPAC and HVA can also be resolved. The major advantages over existing methods are: 1) simple preparation of biological samples and the absence of any prepurification procedure before detection; 2) the separation and detection for all of the compounds measured occurs simultaneously and under the same assay conditions (e.g., no splitting of sample for different reaction conditions) in an 18-22 minute period; 3) the sensitivity is greater than fluorometric methods and equal to or better than radioenzymatic and gas chromatography/mass spectrometric (GC/MS) techniques; 4) the versatility is much greater in this non-isotopic method than radioenzymatic assays and the relatively low cost of establishment and maintenance of equipment compared to GC/MS or radioenzymatic techniques make it superior to these methods for routine determination of these compounds in tissue; and 5) the recoveries are essentially 100% (only loss is due to tissue extraction). It is hoped that the possibility of studying 5-HT neuronal systems at the level of discrete nuclei will aid in our understanding of those systems and their functional role(s).

II. Pharmacological Verification that HPLC with Electrochemical Detection can Detect Altered Metabolic Activity in 5-HT and DA Neurons in Selected Discrete Regions of the Rat Brain

To verify that the developed method could detect changes in 5-HT and DA neuronal activity the effects of several pharmacological agents with fairly well understood mechanisms of action were examined for their ability to alter concentrations of 5-HT, 5-HIAA (also DA and DOPAC in the case of probenecid and pargyline) and the accumulation of 5-HTP in the ST, ME, SCN, MPO and AN. The pharmacological agents included: a monoamine oxidase inhibitor, pargyline; an acid transport inhibitor, probenecid; a monoamine depletor, reserpine; 5-HT uptake inhibitors, fluoxetine and chlorimipramine; the amino acid precursor of 5-HT synthesis, tryptophan and the narcotic analgesic, morphine. In addition, the time course of 5-HTP accumulation was examined following the administration of a decarboxylase inhibitor, NSD 1015 (100 mg/kg, i.p.), in various areas of the brains of both male Sprague-Dawley and female Long-Evans rats.

A. Effect of NSD 1015 on 5-HTP Accumulation in Selected Brain Regions of Male and Female Rats

Previous studies have reported a linear accumulation of 5-HTP for the first 30 min following decarboxylase inhibition with NSD 1015 in the ST, diencephalon, cerebellum, brain stem and whole brain (Carlsson et al., 1972a,b). Results from these studies reveal that 5-HTP accumulation occurs intraneuronally, that the decarboxylating enzyme is completely inhibited and that the accumulated product is not appreciably metabolized or transported from the regions being studied.

The time course of the effect of an intraperitoneal injection of the decarboxylase inhibitor, NSD 1015 (100 mg/kg), was determined on 5-HTP accumulation in the SCN, MPO, ST, ME and AN of both male Sprague-Dawley (Table 3) and female Long-Evans rats (Table 4). 5-HTP accumulation in brain regions of both male and female rats appeared to be linear for 30 min post-NSD 1015 injection; by 1 hour 5-HTP accumulation began to level off. Figure 6 demonstrates graphically the linearity of 5-HTP accumulation in the first 30 min following decarboxylase inhibition and the subsequent tendency for the rate of accumulation to level off at 60 min in the SCN and ST of male rat brains. This effect was more apparent in the male rats except in the median eminence, which was the only region that demonstrated a strong tendency to level off in the female rats. A timepoint of thirty minutes following NSD 1015 administration was chosen to examine effects on 5-HTP accumulation in all subsequent studies. At this time the rate of 5-HTP accumulation appeared to be similar in the ST, ME and AN of both female Long-Evans and male Sprague-Dawley rats whereas the rate of 5-HTP accumulation in the SCN and MPO of female Long-Evans rat brains tended to be somewhat increased over the rates determined in the same brain regions of male Sprague-Dawley rats. These results agree and extend those of Carlsson et al. (1972a,b) who noted that the accumulation of 5-HTP in larger brain regions was linear for the first 30 min following inhibition of the decarboxylating enzyme.

B. Effect of Pargyline or Probenecid on DA, DOPAC, 5-HT and 5-HIAA Concentrations in Selected Regions of the Rat Brain

Administration of pargyline has been shown to increase the concentration of 5-HT in whole brain (Weber, 1966; Macon et al., 1971;

TABLE 3

Time Course of 5-HTP Accumulation Following NSD 1015 (100 mg/kg, ip) in Male Sprague-Dawley Rats

Region	Time After NSD 1015			
	0 min	15 min	30 min	60 min
ST	N.D.	2.44±0.27	4.05±0.79	5.02±0.21
ME	N.D.	2.41±0.28	5.76±1.59	9.19±1.73
AN	N.D.	3.22±0.46	6.82±0.73	7.82±0.63
SCN	N.D.	4.6 ±0.4	8.7 ±1.3	10.3 ±0.9
MPO	N.D.	3.55±0.65	4.38±0.66	5.59±0.28

Values represent ng 5-HTP accumulated/mg protein; mean ± S.E.; N=8.

N.D., Non-detectable.

TABLE 4

Time Course of 5-HTP Accumulation Following NSD 1015 (100 mg/kg, ip) in Female Long-Evans Rats

Region	Time After NSD 1015			
	0 min	15 min	30 min	60 min
ST	N.D.	1.70±0.24	3.54±0.32	6.23±0.59
ME	N.D.	2.61±0.22	4.71±0.56	5.98±0.54
AN	N.D.	3.4 ±0.3	7.4 ±0.8	13.2 ±1.1
SCN	N.D.	6.4 ±0.6	12.3 ±0.9	19.6 ±1.0
MPO	N.D.	3.6 ±0.4	7.0 ±0.7	12.6 ±1.2

Values represent ng 5-HTP accumulated/mg protein; mean ± S.E.; N=8.

N.D., Non-detectable.

Figure 6. Accumulation of 5-HTP in the suprachiasmatic nucleus (SCN, ○) and striatum (ST, ●) from male Sprague-Dawley rats at various times after NSD 1015 (100 mg/kg, i.p.). Dotted lines represent the projected 5-HTP accumulation if the rate for the first 30 min was maintained for 60 min. See Table 3 for S.E. of values depicted above.

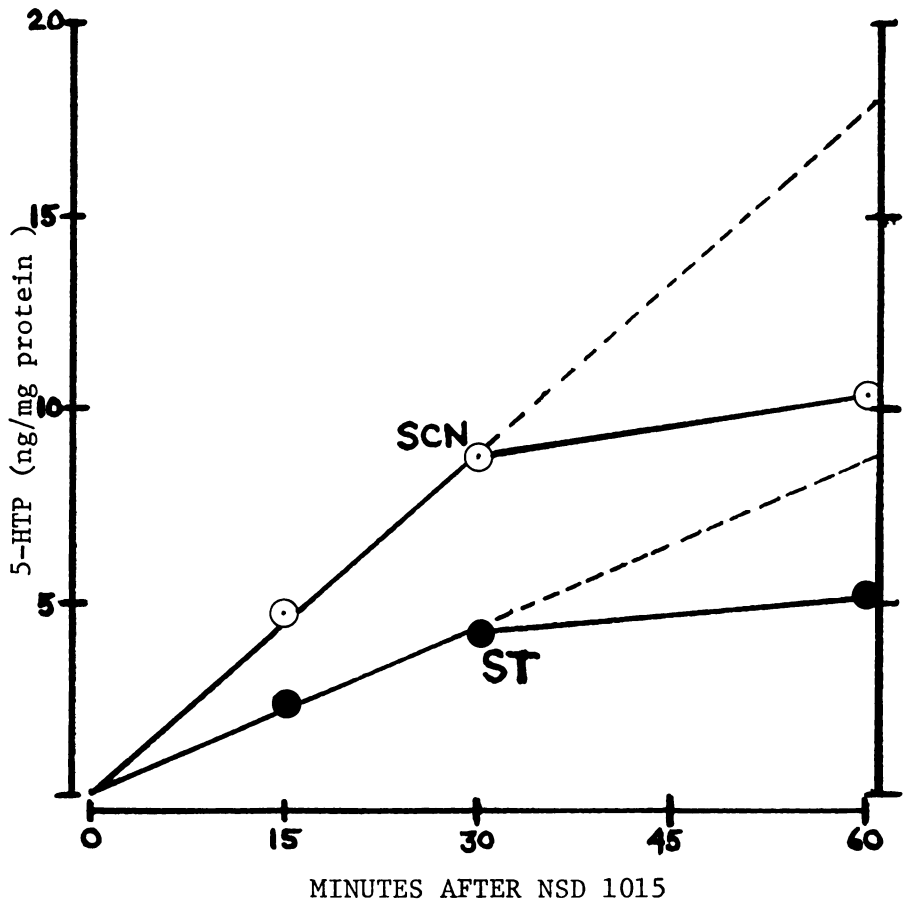

20
15
10
5
5-HTP (ng/mg protein)
SCN
ST
0
15
30
45
60
MINUTES AFTER NSD 1015

Figure 6

Maickel et al., 1974) and of DA in the ST, ME, olfactory tubercle and hypothalamus (Umezu and Moore, 1979) and to decrease whole brain 5-HIAA (Tozer et al., 1966) and striatal, olfactory tubercle, ME and hypothalamic DOPAC (Wilk et al., 1975a,b; Roth et al., 1976; Karoum et al., 1977; Umezu and Moore, 1979).

Probenecid administration has been shown to increase 5-HIAA concentrations in the brain (Neff et al., 1967; Marsden and Curzon, 1976) without affecting 5-HT concentrations; and to increase DOPAC concentrations in the whole brain, medulla, hypothalamus, midbrain, olfactory tubercle and ME but not in the ST, cerebellum, cortex and hippocampus (Wilk et al., 1975a,b; Karoum et al., 1977; Umezu and Moore, 1979).

The effects of pargyline (75 mg/kg, i.p., 60') or probenecid (200 mg/kg, i.p., 60') on the concentrations of DA, DOPAC, 5-HT and 5-HIAA were examined in selected brain regions. The results in the ST, ME and AN are shown on Table 5A while the results in the SCN and MPO are shown on Table 5B. Probenecid administration caused an increase in the concentration of 5-HIAA in all five brain regions. Probenecid did not affect the concentration of DA in any region and decreased 5-HT concentrations only in the AN. Probenecid increased DOPAC concentrations in the ME and MPO but not in the ST, AN or SCN. Pargyline treatment increased 5-HT concentrations and decreased 5-HIAA concentrations in all five brain regions. Pargyline did not significantly decrease DOPAC concentrations in the AN, MPO or SCN but did in the ST and ME. DA concentrations were increased following pargyline administration in the

TABLE 5a

Effect of Probenecid and Pargyline on DA, DOPAC, 5-HT and 5-HIAA in Selected Regions of the Rat Brain

Region	Treatment	AMINE or METABOLITE (ng/mg Protein)			
		DA	DOPAC	5-HT	5-HIAA
ST	Control	96.6± 4.4	10.3 ±0.6	8.0±0.6	6.28±0.41
	Pargyline	120.5± 6.7*	7.8 ±0.7*	11.0±0.7*	3.87±0.25*
	Probenecid	96.1± 7.2	11.2 ±1.1	8.3±0.3	8.83±0.69*
ME	Control	100.6± 6.1	9.6 ±0.6	18.4±1.0	12.7 ±1.2
	Pargyline	141.6±13.3*	7.8 ±0.4*	25.2±1.5*	9.4 ±0.6*
	Probenecid	98.1± 9.6	19.4 ±1.8*	17.7±1.7	16.1 ±0.9*
AN	Control	30.9± 1.8	1.56±0.27	18.1±1.7	22.9 ±1.6
	Pargyline	43.9± 4.0*	1.35±0.14	38.1±3.2*	12.8 ±1.6*
	Probenecid	30.0± 3.2	1.92±0.15	12.8±1.6*	36.6 ±1.9*

Pargyline (75 mg/kg, ip) or probenecid (200 mg/kg, ip) was injected 60 min before sacrifice. Values represent mean ± S.E.; N = 8. *, Value that is significantly different ($p \leq 0.01$) from control value.

TABLE 5b

Effect of Probenecid and Pargyline on DA, DOPAC, 5-HT and 5-HIAA in Selected Regions of the Rat Brain

Region	Treatment	AMINE or METABOLITE (ng/mg Protein)			
		DA	DOPAC	5-HT	5-HIAA
SCN	Control	15.1 ±1.4	4.43±0.43	23.2±2.3	17.2±0.4
	Pargyline	17.3 ±1.4	3.76±0.39	43.8±4.6*	11.8±1.4*
	Probenecid	13.9 ±1.6	5.39±0.68	23.1±2.5	25.3±1.7*
MPO	Control	4.78±0.42	1.55±0.17	7.3±0.6	15.2±1.0
	Pargyline	5.52±0.37	1.09±0.14	10.4±0.8*	8.5±1.3*
	Probenecid	3.59±0.35	2.29±0.10*	8.8±0.7	24.5±2.5*

Pargyline (75 mg/kg, ip) or probenecid (200 mg/kg, ip) was injected 60 min before sacrifice. Values represent mean ± S.E.; N = 8. *, Value that is significantly different ($p \leq 0.01$) from control value.

ST, ME and AN but not in the SCN or MPO. In general, these results fit very well with what might be predicted from the known actions of these drugs and previously reported results (Tozer et al., 1966; Weber, 1966; Macon et al., 1971; Maickel et al., 1974; Wilk et al., 1975a,b; Roth et al., 1976; Karoum et 1977; Umezu and Moore, 1979) with a couple of exceptions. First, the DA neuronal system in the SCN appeared to be very resistant to the effects of pargyline and probenecid. Secondly, the concentration of DOPAC in the AN is near to the limit of sensitivity of the assay and did not show a significant change to any treatment in this experiment. Trends towards change in AN DOPAC were consistent with effects of these drugs observed in other areas.

The primary objective of this experiment was to determine whether HPLC with electrochemical detection could detect changes in steady-state concentrations of 5-HT and 5-HIAA induced by pargyline and probenecid in the selected brain regions. 5-HTP accumulation was not measured in these experiments because NSD 1015 per se alters steady-state concentrations of 5-HT and 5-HIAA. In addition, DA and DOPAC were measured in this experiment because investigations on dopaminergic neuronal systems in the selected brain regions are much more numerous and would help confirm the location of the dissections.

5-HIAA appears to require a probenecid-sensitive acid-transport system for its removal in all five brain areas examined whereas DOPAC required such a transport system only in the ME, MPO and possibly AN (included because of the previously mentioned problem of sensitivity for detecting changes in DOPAC concentrations in the AN). The lack of probenecid on DOPAC concentrations in the ST agree with observations

by other investigators (Wilk et al., 1975a,b; Karoum et al., 1977; Umezu and Moore, 1979) but the lack of effect in the SCN has not previously been reported. The effect of pargyline on DA, 5-HT, DOPAC and 5-HIAA concentrations in the ST and ME agree with those of other investigators (Tozer et al., 1966; Weber, 1966; Macon et al., 1971; Maickel et al., 1974; Wilk et al., 1975a,b; Roth et al., 1976; Karoum et al., 1977; Umezu and Moore, 1979). The results concerning the effects of pargyline in the MPO, AN and SCN agree with the known actions of this drug. The resistance of the DA system in the SCN to change following pargyline or probenecid administration was unexpected and the reason for such a resistance remains unknown.

These results demonstrate the capability of HPLC with electrochemical detection to detect pharmacologically-induced changes in the concentration of 5-HT and 5-HIAA in discrete brain regions. Furthermore, the concentrations of DA and DOPAC and the effects of pargyline or probenecid on those concentrations agree with previous investigations and help confirm the correct location of the dissections.

C. Effect of Reserpine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Reserpine has been shown to decrease 5-HT concentrations and storage, and to increase 5-HT synthesis, 5-HIAA concentrations and tryptophan hydroxylase activity in rat brain (Tozer et al., 1966; Zivkovic et al., 1973; Sze et al., 1976; Sanders-Bush and Massari, 1977; Saner and Pletscher, 1978).

The effects of reserpine (2 mg/kg, i.p.) on the concentrations of 5-HT and 5-HIAA as well as 5-HTP accumulation were examined at 2 and

24 hours following its administration (Table 6). Reserpine disrupts the ability of neuronal storage vesicles to bind 5-HT and other putative amine neurotransmitters. Thus, over time, 5-HT concentrations decrease, 5-HIAA concentrations increase as more 5-HT is exposed to intraneuronal MAO, and 5-HTP accumulation might increase if synthesis in the neuron attempts to maintain 5-HT concentrations. As shown in Table 6, following reserpine 5-HT concentrations decreased and 5-HIAA concentrations either increased or remained at normal values at both 2 and 24 hours in all five brain regions. Therefore, the ratio of [5-HIAA]/[5-HT] was dramatically increased at both 2 and 24 hrs post-reserpine. These results suggest that 5-HT synthesis is maintained despite the loss of binding capacity of the storage vesicles. This is consistent with the 5-HTP accumulation data which show 5-HT synthesis in all five regions was increased at both 2 and 24 hrs following the administration of reserpine. Thus, both indices of 5-HT neuronal activity (5-HTP accumulation and [5-HIAA]/[5-HT]) increase in the ME, ST, SCN, MPO and AN following reserpine treatment.

These results extend those obtained by other investigators who have found that reserpine decreases 5-HT concentrations in the rat brain while the formation of 5-HIAA, 5-HT synthesis and 5-HT turnover are all increased (Tozer et al., 1966; Zivkovic et al., 1973; Sze et al., 1976; Sanders-Bush and Massari, 1977; Saner and Pletscher, 1978). Furthermore, the results using the present technique show that reserpine influences the concentration of 5-HT, 5-HIAA and the rate of accumulation of 5-HTP in discrete hypothalamic regions in a manner similar to that in larger brain areas.

TABLE 6

Effect of Reserpine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Region	Hours After Reserpine (2 mg/kg, i.p.)	5-HT[a]	5-HIAA[a]	5-HIAA/5-HT	5-HTP[b]
ST	0	6.4±0.3	4.7±0.2	0.73±0.03	3.2±0.4
	2	2.5±0.2*	7.8±0.7*	3.16±0.23*	4.0±0.3
	24	2.7±0.3*	7.8±0.8*	3.03±0.51*	4.7±1.0
ME	0	13.7±1.6	10.1±0.8	0.76±0.10	1.6±0.1
	2	6.5±1.9*	20.2±1.4*	4.56±1.07*	3.3±0.5*
	24	4.4±0.7*	12.6±1.8	3.00±0.51*	2.2±0.2*
AN	0	16.5±0.6	23.8±0.8	1.38±0.23	5.0±0.4
	2	5.2±0.6*	30.8±2.6*	5.91±0.68*	7.2±0.6*
	24	4.5±0.3*	24.5±1.8	5.44±0.61*	6.8±0.4*
SCN	0	22.1±1.6	28.0±3.0	1.29±0.18	10.6±0.7
	2	12.3±2.6*	50.4±5.9*	6.30±1.86*	16.7±0.8*
	24	5.9±0.6*	48.4±2.4*	7.47±0.34*	16.1±1.1*
MPO	0	8.6±0.8	5.5±0.7	0.66±0.10	4.5±0.4
	2	2.8±0.3*	3.6±0.4*	1.27±0.23*	7.9±0.4*
	24	1.2±0.3*	2.9±0.4*	2.52±0.66*	6.6±0.8*

[a]Each value represents 5-HT or 5-HIAA concentration (ng/mg protein; mean ± S.E.; N=6).

[b]Each value represents the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N=7) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from '0 time' controls ($p<0.01$).

D. Effect of Chlorimipramine or Fluoxetine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

5-HT uptake blockers, such as fluoxetine and chlorimipramine, increase the concentration of 5-HT reaching post-synaptic receptor sites (Geyer et al., 1978; Marsden et al., 1979) and eventually produce a feedback inhibition of the 5-HT neuron. Indeed, chlorimipramine decreases 5-HT turnover in whole brain (Corrodi and Fuxe, 1969; Meek and Werdinius, 1970) and chlorimipramine and fluoxetine decrease 5-HT synthesis in the hypothalamus, hippocampus, cortex, septum and nucleus caudatus (Marco and Meek, 1979) and inhibit 5-HT unit firing (Sheard et al., 1972; Gallager and Aghajanian, 1975).

The effects of chlorimipramine (5 mg/kg, i.p., 1 hr) and fluoxetine (10 mg/kg, i.p., 1 hr, 15') on the concentrations of 5-HT and 5-HIAA as well as 5-HTP accumulation in the ME, ST, SCN, MPO and AN are shown in Table 7. 5-HT concentrations in the ME increased with both treatments and 5-HIAA concentrations in the SCN and AN decreased following fluoxetine and chlorimipramine treatment, respectively. However, the trends were strong enough towards increasing 5-HT concentrations and decreasing 5-HIAA concentrations that the ratio of [5-HIAA]/[5-HT] decreased significantly in all five brain regions. Only the ratio in the MPO following chlorimipramine and in the AN following fluoxetine did not reach statistical significance. 5-HTP accumulation in all five regions decreased following chlorimipramine treatment but only in the SCN, MPO and AN following fluoxetine. In general, 5-HT metabolism and synthesis were decreased following administration of the 5-HT uptake inhibitors, chlorimipramine and fluoxetine. This effect presumably

TABLE 7

Effect of Chlorimipramine or Fluoxetine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Region	Treatment	5-HT[a]	5-HIAA[a]	5-HIAA/5-HT	5-HTP[b]
ST	Control	5.0±0.5	3.8±0.2	0.82±0.09	3.6±0.4
	Fluoxetine	5.9±0.3	3.5±0.3	0.59±0.04*	2.8±0.4
	Chlorimipramine	5.1±0.3	3.0±0.2	0.58±0.01*	2.2±0.3*
ME	Control	13.3±2.0	8.1±0.9	0.63±0.05	4.8±0.3
	Fluoxetine	21.0±1.3*	8.1±0.5	0.38±0.02*	4.0±0.4
	Chlorimipramine	23.3±2.1*	9.0±1.2	0.39±0.04*	3.3±0.3*
AN	Control	19.4±1.1	25.4±2.3	1.31±0.11	4.5±0.3
	Fluoxetine	18.8±0.9	21.3±2.1	1.13±0.09	2.5±0.1*
	Chlorimipramine	20.8±1.2	18.8±1.9*	0.90±0.06*	2.1±0.2*
SCN	Control	20.0±0.8	31.9±2.7	1.59±0.10	10.9±0.8
	Fluoxetine	18.6±0.7	22.6±1.3*	1.23±0.09*	5.7±0.7*
	Chlorimipramine	24.1±1.5	29.5±2.7	1.21±0.04*	6.4±0.8*
MPO	Control	9.8±0.7	15.0±0.5	1.55±0.07	4.2±0.2
	Fluoxetine	12.0±0.6	13.0±0.6	1.11±0.09*	2.0±0.3*
	Chlorimipramine	9.9±0.2	14.6±0.4	1.49±0.05	2.5±0.4*

Chlorimipramine (5 mg/kg, i.p.) was injected 60 min before sacrifice and fluoxetine (10 mg/kg, i.p.) was injected 75 min before sacrifice.

[a]Each value represents 5-HT or 5-HIAA concentration (ng/mg protein; mean ± S.E.; N=7).

[b]Each value represents the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N=7) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from control ($p<0.01$).

results from a feedback inhibitory mechanism responding to the increased concentration of 5-HT in the synapse.

Although chlorimipramine and fluoxetine exerted little effect on 5-HT and 5-HIAA concentrations per se, both drugs decreased the 5-HIAA/5-HT ratio and also reduced the rate of 5-HTP accumulation. These extend findings by others that show decreased 5-HT neuronal activity following administration of chlorimipramine or fluoxetine in large brain regions (Corrodi and Fuxe, 1969; Meek and Werdinius, 1970; Sheard et al., 1972; Gallager and Aghajanian, 1975; Marco and Meek, 1979).

E. Effect of Tryptophan on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Administration of the amino acid precursor of 5-HT, tryptophan (12.5-1600 mg/kg, i.p.), increases 5-HT and 5-HIAA concentrations as well as 5-HT synthesis in the rat brain (Eccleston et al., 1965; Moir and Eccleston, 1968; Fernstrom and Wurtman, 1971; Grahame-Smith, 1971; Knott and Curzon, 1974; Ternaux et al., 1976; Wojcik et al., 1980). Mueller et al. (1976) reported that tryptophan increased 5-HT turnover in the hypothalamus.

The effects of tryptophan (25 mg/kg, i.p., 1 hr; a dose which does not raise plasma or brain tryptophan concentrations above normal nocturnal peaks, nor modify concentrations of other amino acids in the plasma; Fernstrom and Wurtman, 1971) on the concentrations of 5-HT and 5-HIAA as well as 5-HTP accumulation in the ST, ME, SCN, MPO and AN are shown in Table 8. No change in the ratio of [5-HIAA]/[5-HT] was observed in any region. This lack of change results primarily because both 5-HT and 5-HIAA concentrations increase in the ST, AN, MPO, and SCN in a

TABLE 8

Effect of Tryptophan on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Region	Treatment	5-HT[a]	5-HIAA[a]	5-HIAA/5-HT	5-HTP[b]
ST	Control	7.0±0.4	6.7±0.3	1.01±0.05	4.0±0.4
	Tryptophan	7.3±0.3	7.4±0.3	1.02±0.04	5.7±0.4*
ME	Control	20.9±2.1	13.6±1.1	0.70±0.12	5.0±0.5
	Tryptophan	33.6±3.0*	15.9±2.1	0.50±0.12	4.1±0.5
AN	Control	20.2±2.0	22.3±2.1	1.04±0.06	4.8±0.4
	Tryptophan	28.0±1.2*	34.3±1.7*	1.20±0.06	9.8±0.4*
SCN	Control	24.9±2.2	17.0±1.2	0.71±0.05	9.2±0.9
	Tryptophan	26.5±2.3	24.0±2.2*	0.98±0.15	17.9±1.2*
MPO	Control	7.6±0.4	14.9±0.5	1.97±0.07	7.2±0.7
	Tryptophan	10.8±0.5*	20.1±0.9*	1.90±0.07	16.0±1.4*

Tryptophan (25 mg/kg, i.p.) was injected 60 min before sacrifice.

[a]Each value represents 5-HT or 5-HIAA concentration (ng/mg protein; mean ± S.E.; N = 8).

[b]Each value represents the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N = 7) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from control ($p<0.01$).

similar manner. Tryptophan administration increased the synthesis of 5-HT in the ST, SCN, MPO and AN but not in the ME. Taken together these results suggest that synthesis of 5-HT is increased slightly in the ST, resulting in a slight increase in 5-HT and 5-HIAA concentrations. Tryptophan caused a large increase in the synthesis of 5-HT in the SCN, AN and MPO resulting in a large increase in 5-HIAA concentrations. In the ME, synthesis of 5-HT is not increased following tryptophan administration and yet 5-HT concentrations increase. This could result for several reasons. If release of 5-HT were inhibited an increase in 5-HT with a parallel decrease in 5-HIAA should result. 5-HT could accumulate in the ME (possibly into the tanycytes) after being formed elsewhere, thereby increasing 5-HT in the ME without being synthesized there. From the present results there is no way to determine which of these hypotheses, if any, may be responsible for the results observed in the ME. The reason for the differential effect of tryptophan on the 5-HT neuronal system in the ME provides an interesting question for future research.

The lack of effect of tryptophan administration on 5-HT synthesis in the ME has not previously been published although anoter investigator has found similar results (Alan Sved, personal communication). The effects of tryptophan administration on steady state concentrations of 5-HT and 5-HIAA generally confirmed the fact that 5-HT neuronal activity was enhanced following administration of the amino acid precursor. These results extend observations by others who have examined 5-HT neuronal activity following tryptophan administration in larger brain areas (Eccleston et al., 1965; Moir and Eccleston, 1968;

Fernstrom and Wurtman, 1971; Grahame-Smith, 1971; Knott and Curzon, 1974; Mueller et al., 1976; Ternaux et al., 1976; Wojcik et al., 1980). Furthermore, these results demonstrate a unique effect of tryptophan administration on 5-HT metabolism and synthesis in the ME.

F. Effect of Morphine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

The final pharmacological manipulation of 5-HT neuronal systems in the ST, ME, SCN, MPO and AN involved in the administration of morphine. It has long been known that morphine and other narcotic analgesics alter the secretion of hormones from the anterior pituitary. Morphine, endogenous opioid peptides and several synthetic opioid analogs alter the secretion of several hormones including prolactin, luteinizing hormone, thyroid stimulating hormone, growth hormone, corticosterone, adrenocorticotropin, antidiuretic hormone and oxytocin (Simon et al., 1975; Bruni et al., 1977; Dupont et al., 1978; VanLoon and De Souza, 1978; Holaday and Loh, 1979; Meites et al., 1979; Van Vugt and Meites, 1980; Van Vugt et al., 1981). These drugs also affect aminergic neuronal systems in the CNS (Roffman et al., 1970; Way, 1971; Yarbrough et al., 1971; Loh et al., 1973; Yarbrough et al., 1973; Ford et al., 1974; Goodlet and Sugrue, 1974; Korf et al., 1974; Sugrue, 1974; Simon et al., 1975; Ferland et al., 1977; Algeri et al., 1978; Deyo et al., 1979a; Iwamoto and Way, 1979; Alper et al., 1980). Meites et al. (1963) first suggested that morphine administration could activate prolactin secretion after observing enhanced mammary secretory activity in rats treated with this drug. Since then this hypothesis has been verified directly by several laboratories (see reviews, Bruni et

al., 1977; Meites et al., 1979; Holaday and Loh, 1979; Van Vugt and Meites, 1980). The mechanism of the morphine-induced prolactin secretion is still not completely understood. The majority of evidence suggests that the stimulatory action is not exerted directly on the anterior pituitary. For example, morphine and opioid peptides do not release prolactin when added to isolated pituitaries (Grandison and Guidotti, 1977; Rivier et al., 1977; Shaar et al., 1977; Panerai et al., 1981) and opiate agonists and antagonists which do not cross the blood-brain barrier do not affect prolactin secretion unless administered intraventricularly (Panerai et al., 1981). On the other hand, Lien et al. (1976) demonstrated that leu-enkephalin could stimulate prolactin release by acting directly on the pituitary, and Enjalbert et al. (1979) showed that although morphine did not exert a direct effect of its own it may increase prolactin secretion by interfering with the DA-induced inhibition of secretion. A CNS-mediated mechanism for the prolactin-releasing effect of morphine and opioid peptides could include a direct action on hypothalamic prolactin-inhibiting and/or -releasing factors or the involvement of brain monoamines. Two monoamines implicated in regulatory mechanisms of prolactin secretion are DA and 5-HT (Meites, 1973; Müller et al., 1977; Weiner and Ganong, 1978). It is generally believed that prolactin is under tonic inhibitory influence by DA which is released from tuberoinfundibular neurons into hypophyseal portal blood and carried to receptors on lactotrophs located in the anterior pituitary (Hökfelt and Fuxe, 1972; Meites, 1973; MacLeod, 1976; Langer et al., 1978). Indeed, the concentration of DA is higher in the pituitary stalk blood than in systemic blood (Ben-Jonathan et al.,

1977; Plotsky et al., 1978; Cramer et al., 1979; Gudelsky and Porter, 1979a) and DA receptors have been located in the anterior pituitary (Brown et al., 1976; Creese et al., 1977; Calabro and MacLeod, 1978; Cronin et al., 1978; Caron et al., 1978). Morphine and opioid peptides decrease the rate of turnover and synthesis of DA in the median eminence (Ferland et al., 1977; Deyo et al., 1979a,b; Alper et al., 1980), and reduce the concentration of DA in hypophyseal portal blood (Gudelsky and Porter, 1979b). Morphine may also influence prolactin secretion by activating 5-HT neuronal systems. For example, low doses of morphine that are ineffective in increasing serum concentrations of prolactin per se, are effective following pretreatment with fluoxetine, a 5-HT uptake blocker (Meites et al., 1979). The morphine-induced increase of serum prolactin is also attenuated by procedures which disrupt 5-HT transmission. Blocking 5-HT receptors with putative 5-HT antagonists, blocking 5-HT synthesis with p-chlorophenylalanine (a tryptophan hydroxylase inhibitor) or destroying 5-HT neurons with 5,7-dihydroxytryptamine (5,7-DHT) all reduce the increase in serum prolactin induced by morphine and opioid peptides (Koenig et al., 1979, 1980; Spampinato et al., 1979). It has also been shown that acute administration of morphine increases the turnover and synthesis of 5-HT in whole brain (Neff et al., 1967; Yarbrough et al., 1971, 1973; Goodlet and Sugrue, 1974; Pérez-Cruet et al., 1975) and in hypothalamus (Roffman et al., 1970; Haubrich and Blake, 1973). Synthetic enkephalins and β-endorphin also increase hypothalamic 5-HT turnover and synthesis (Algeri et al., 1978; Garcia-Sepilla et al., 1978; Van Loon and De Souza, 1978).

The ability of morphine to influence 5-HT neurons, tuberoinfundibular DA neurons, and serum prolactin levels may be related. Demarest and Moore (1981) demonstrated that disruption of 5-HT transmission processes with metergoline or 5,7-DHT pretreatments had no effect on basal serum prolactin concentrations or the activity of tuberoinfundibular DA neurons *per se*, indicating a lack of a tonic 5-HT regulatory influence. On the other hand, these treatments blocked the effects of morphine, suggesting that the morphine-induced depression of tuberoinfundibular DA neurons, and the consequent secretion of prolactin result from the stimulation of 5-HT neuronal systems.

To date, studies on the effects of morphine on 5-HT neurons have been performed on whole brain, or gross anatomical regions, such as the hypothalamus. The next sections discuss effects of morphine and its receptor antagonist, naloxone, on 5-HT synthesis and metabolism in discrete hypothalamic brain regions.

1. Effect of Various Doses of Morphine on 5-HT Synthesis in Selected Regions of the Rat Brain

The effects of various doses of morphine injected subcutaneously on 5-HTP accumulation in the ST, ME, MPO, SCN and AN of the rat brain are shown in Table 9. Morphine did not alter the rate of 5-HTP accumulation in the ST or ME. At a dose of 10 mg/kg, morphine significantly increased 5-HTP accumulation in the MPO, SCN and AN; 5 mg/kg of morphine also increased 5-HTP accumulation in the AN. Morphine appears to exert a biphasic dose-response curve since at the highest dose (20 mg/kg) it did not increase 5-HTP accumulation in any region. The dose of 10 mg/kg of morphine was chosen for further studies as this

TABLE 9

Effect of Various Doses of Morphine on 5-HTP Accumulation in Selected Regions of the Rat Brain

Region	Dose of Morphine (mg/kg)				
	0.0	2.5	5.0	10.0	20.0
ST	3.6±0.4	3.5±0.5	3.9±0.3	4.8±0.5	4.4±0.5
ME	4.8±0.3	5.4±0.4	5.2±0.5	5.0±0.5	4.8±0.4
AN	4.5±0.3	5.4±0.6	7.7±0.4*	6.7±0.6*	5.4±0.1
SCN	10.9±0.8	7.7±0.6	8.6±0.8	14.4±0.9*	9.6±0.6
MPO	4.2±0.2	3.9±0.4	4.4±0.3	6.1±0.2*	4.5±0.4

Morphine or its vehicle was injected subcutaneously 60 min prior to sacrifice. Values represent rate of 5-HTP accumulation (ng 5-HTP/mg protein/30 minutes; mean ± S.E.; N = 8) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from control ($p<0.01$).

dose had been previously shown to increase serum prolactin concentrations (Fanjul et al., 1981) and to decrease turnover of DA in the median eminence (Alper et al., 1980) as well as DA concentrations in pituitary stalk plasma (Gudelsky and Porter, 1979b).

The time course of a single subcutaneous injection of morphine (10 mg/kg, 1 hr) on 5-HTP accumulation in the ST, ME, MPO, SCN and AN is shown in Table 10. Morphine did not alter 5-HTP accumulation in ST or ME at any time point examined, but increased 5-HTP accumulation at 1 hour in the MPO, SCN and AN but not at 0.5 or 2.0 hours. The time of 1 hr post-morphine administration was chosen for further studies because maximal effects on 5-HTP accumulation were observed at this time. This is also the time point where increases in serum prolactin (Fanjul et al., 1981), decreases in tuberoinfundibular DA turnover (Alper et al., 1980) and decreases in pituitary stalk plasma DA concentrations (Gudelsky and Porter, 1979b) have been reported.

2. Effect of Morphine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

The effects of a single subcutaneous injection of morphine (10 mg/kg; 1 hr) on the concentrations of 5-HT and 5-HIAA as well as 5-HTP accumulation in the ST, ME, SCN, AN and MPO are summarized in Table 11. No change in 5-HT concentrations were observed in the ST, ME, SCN or MPO. A small decrease in the concentration of 5-HT was observed in the AN. Morphine caused a significant increase in 5-HIAA concentrations in the AN and MPO but not in the ST, ME or SCN. The ratio of 5-HIAA to 5-HT concentrations increased in the ST, AN and ME.

Morphine increased 5-HTP accumulation in the SCN, AN and MPO but not the ME or ST. These results suggest that 5-HT neuronal

TABLE 10

Time Course of Effect of Morphine on 5-HTP Accumulation in Selected Regions of the Rat Brain

Region	0 Hr	0.5 Hr	1.0 Hr	2.0 Hr
ST	3.6±0.4	4.6±0.5	4.8±0.5	4.3±0.6
ME	4.8±0.3	4.9±0.3	5.0±0.5	4.6±0.6
AN	4.5±0.3	4.7±0.6	6.7±0.6*	5.4±0.4
SCN	10.9±0.8	7.9±0.6	14.4±0.9*	10.4±0.7
MPO	4.2±0.2	4.1±0.4	6.1±0.2*	3.0±0.4

Morphine (10 mg/kg) was injected subcutaneously. Values represent rate of 5-HTP accumulation (ng 5-HTP/mg protein/30 minutes; mean ^{5} S.E.; N = 7) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from control ($p<0.01$).

TABLE 11

Effect of Morphine on 5-HT Metabolism and Synthesis in Selected Regions of the Rat Brain

Region	Treatment	5-HT[a]	5-HIAA[a]	5-HIAA/5-HT	5-HTP[b]
ST	Control	7.8±0.3	7.2±0.3	0.94±0.04	3.9±0.4
	Morphine	6.6±0.2	9.5±0.4	1.44±0.08*	5.0±0.5
ME	Control	14.7±0.3	12.0±0.8	0.81±0.02	5.6±0.4
	Morphine	13.9±0.6	13.8±0.4	1.15±0.05*	5.7±0.4
AN	Control	17.8±0.2	20.6±0.5	1.16±0.03	3.7±0.3
	Morphine	16.2±0.4*	24.0±0.7*	1.48±0.05*	6.2±0.2*
SCN	Control	26.0±1.4	26.3±1.9	1.01±0.03	9.2±0.4
	Morphine	25.9±1.2	28.8±3.7	1.10±0.12	12.3±0.5*
MPO	Control	7.8±0.5	15.2±0.2	2.14±0.16	3.5±0.2
	Morphine	7.7±0.4	18.6±1.4*	2.69±0.32	4.8±0.2*

Morphine (10 mg/kg) or its vehicle was injected subcutaneously 60 min prior to sacrifice.

[a]Each value represents 5-HT or 5-HIAA concentration (ng/mg protein; mean ± S.E.; N = 8).

[b]Each value represents the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N = 7) 30 min after NSD 1015 (100 mg/kg, i.p.).

*, significantly different from control ($p<0.01$).

activity is increased in the MPO, AN and possibly SCN following morphine administration.

3. Effect of Morphine and Naloxone on 5-HTP Accumulation in Selected Regions of the Rat Brain

The question of whether the morphine-induced effect on 5-HTP accumulation was an opiate receptor-mediated phenomenon was examined in another experiment. The effects of naloxone, an opiate receptor antagonist, on the ability of morphine to increase 5-HTP accumulation in the selected areas are shown in Table 12. Morphine alone significantly increased 5-HTP accumulation in SCN, AN and MPO but not in the ME or ST. Naloxone (4 mg/kg, i.p., 45') did not alter 5-HTP accumulation in any brain region studied indicating a lack of tonic opioid effect on 5-HT synthesis in these regions. However, naloxone in doses of 0.4 or 4.0 mg/kg completely blocked the ability of morphine to increase 5-HT synthesis in the SCN, AN and MPO. Thus, the ability of morphine to increase 5-HTP accumulation in the SCN, AN and MPO depends upon an opiate receptor-mediated mechanism.

Several investigators (Neff et al., 1967; Roffman et al., 1970; Yarbrough et al., 1971, 1973; Haubrich and Blake, 1973; Goodlet and Sugrue, 1974; Pérez-Cruet et al., 1975) using a great variety of techniques have demonstrated that acute injections of morphine increase 5-HT turnover and synthesis in the whole brain as well as hypothalamus of rats. Results from this present study demonstrate the ability of morphine to increase 5-HT synthesis and metabolism in discrete nuclei of the rat medial basal hypothalamic area. Specifically,

TABLE 12

Effects of Morphine and Naloxone on the Rate of 5-HT Synthesis in Selected Regions of the Rat Brain

Region	Control	Morphine	Naloxone (4)	Morphine + Naloxone (4)	Morphine + Naloxone (0.4)
ST	3.2±0.6	4.8±0.6	3.2±0.5	3.2±0.5	3.1±0.2
ME	7.0±1.3	8.9±1.8	7.3±1.8	5.7±0.5	10.0±1.1
AN	3.3±0.3	4.9±0.4*	3.5±0.3	3.6±0.4	3.8±0.3
SCN	10.8±0.5	13.8±0.4*	9.5±0.5	10.0±0.5	9.8±0.8
MPO	3.6±0.2	5.5±0.4*	3.7±0.2	3.2±0.3	3.1±0.2

Rats were injected with morphine (10 mg/kg, s.c.) or with saline vehicle 1 hr before sacrifice and/or with naloxone (0.4 or 4 mg/kg, i.p.) or saline vehicle 45 min prior to sacrifice. All animals were injected with NSD 1015 (100 mg/kg, i.p.) 30 min prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N=8).

*, significantly different from vehicle-treated controls ($p<0.01$).

morphine consistently increased 5-HT synthesis in the MPO, AN and SCN via an opiate receptor-mediated mechanism. Furthermore, 5-HT metabolism to 5-HIAA also appeared to be increased in the AN and MPO, with a trend towards a similar effect in the SCN. Neither 5-HT synthesis nor metabolism were statistically significantly altered by morphine treatment in the ST or ME. However, a consistent trend toward increased 5-HT synthesis was observed in the ST. Furthermore, if 5-HT neurons inhibit the tonic inhibitory influence that the tuberoinfundibular DA neurons exert on prolactin secretion, they appear to be accomplishing this somewhere other than in the ME. The AN may be especially sensitive to the effects of morphine on 5-HT neuronal activity as 5-HT concentrations decrease and 5-HIAA concentrations as well as 5-HTP accumulation increase following its administration. Furthermore, 5-HTP accumulation is increased even at 5.0 mg/kg morphine in the AN whereas a dose of 10.0 mg/kg of morphine was required to increase 5-HT synthesis in the MPO and SCN. Data by Wilkes and Yen (1980) show that opioid peptides can decrease the efflux of DA and DOPAC from superfused rat medial basal hypothalamus suggesting that the 5-HT system necessary for this opioid-induced effect (Koenig et al., 1979; Demarest and Moore, 1981) must be located within the medial basal hypothalamus. Morphine can also increase serum prolactin concentrations in the rat following hypothalamic deafferentation (Grandison et al., 1980) which should eliminate extrahypothalamic afferent input to the tuberoinfundibular DA neurons. One must consider, therefore whether morphine might exert its neuroendocrinological effects by interacting with putative intrahypothalamic 5-HT neurons (see

Introduction - Anatomy). Dafny (1980) examined electrical neuronal activity in the medial basal hypothalamus following five incremental doses of morphine. Three basic populations of neuronal responses were identified. One population responded to morphine by increasing its firing rate as the dose of morphine increased; another responded to morphine by decreasing its firing rate as the dose of morphine increased, and the third population responded in a biphasic manner to increasing dosages of morphine (increasing its firing rate at lower doses and strong decreases in firing rate at higher doses). Unfortunately, the neurochemical identity of these groups of neurons is presently unknown.

Although the effects of morphine upon 5-HT neuronal activity are very interesting in their own right, it is not known if the population of 5-HT neurons that is involved with the morphine-induced stimulation of prolactin secretion were analyzed. Furthermore, because there are so many morphine-induced endocrine events going on at the same moment it is not obvious that changes in 5-HT neuronal activity in the hypothalamus that were recorded neurochemically are related to the release of prolactin.

These results extend those of other investigators in larger brain areas that demonstrate morphine can stimulate 5-HT neuronal activity. More precisely, the present study indicated morphine increases 5-HT synthesis and metabolism in the AN, MPO and SCN by an opiate-receptor mediated mechanism.

G. Discussion of Pharmacological Manipulations

The results of this section verified that HPLC with electrochemical detection could detect changes in 5-HT metabolism and synthesis in the ST, ME, AN, MPO and SCN induced by several pharmacological agents. Furthermore, these studies demonstrate problems associated with interpretation of results using the ratio of 5-HIAA/5-HT as an index of 5-HT neuronal activity. An illustrative example is found in the results of the tryptophan experiments (II.E., Table 8). In those results no effect on the ratio of 5-HIAA/5-HT was observed. However, both the concentration of 5-HIAA and the accumulation of 5-HTP increase following tryptophan in the AN and MPO. From a careful examination of the data it becomes apparent why this obvious increase in 5-HT synthesis and metabolism is not reflected in a change in the ratio. Both the concentration of 5-HIAA and 5-HT increase in the AN and MPO following the increase in synthesis caused by tryptophan administration. Thus, the increase in metabolism is not reflected in a change in the ratio of 5-HIAA/5-HT. Another pertinent example of the problems associated with the use of the ratio of 5-HIAA/5-HT as an index of 5-HT neuronal activity is found in the results of the 5-HT uptake inhibitor experiments (II.D., Table 7). Both chlorimipramine and fluoxetine decrease 5-HTP accumulation and the ratio of 5-HIAA/5-HT in several regions, including the ME. However, when the 5-HT and 5-HIAA data are examined individually it is apparent that the ratio of 5-HIAA/5-HT in the ME decreases because of an increase in the 5-HT concentration, not because of a decrease in the 5-HIAA concentration. This is particularly difficult to understand because the synthesis of 5-HT in the ME following 5-HT uptake inhibition

is decreased. These examples emphasize the difficulties involved in interpreting data using the ratio of 5-HIAA/5-HT as an index of 5-HT neuronal activity. Because of problems associated with the use of the ratio of 5-HIAA/5-HT such as those just cited, this index of neuronal activity has been found in the present studies to be rather insensitive and sometimes misleading. For that reason the ratio of 5-HIAA/5-HT will no longer be reported in the remaining studies although the steady state concentrations of 5-HIAA and 5-HT and effects of the various treatments on those concentrations will continue to be discussed.

III. Effect of Restraint Stress on 5-Hydroxytryptaminergic Neuronal Activity and Serum Prolactin

Pharmacological studies suggest that the stress-induced increase of serum concentrations of prolactin, ACTH and corticosteroids are associated with an activation of hypothalamic 5-HT neuronal systems. For example, putative 5-HT antagonists block (Meltzer et al., 1976) while 5-HT uptake inhibitors enhance the stress-induced release of prolactin (Krulich, 1975), ACTH and corticosteroids (Fuller et al., 1976; Fuller, 1981). Up to an eleven-fold increase in serum prolactin concentrations occurs within 5-15 minutes following application of immobilization (restraint) stress (Morgan et al., 1975; Mueller et al., 1976). Accordingly, as an important first piece of evidence for the possible involvement of 5-HT neurons in the stress-induced endocrine changes, efforts have been made to relate these changes with alterations in the activity of 5-HT neurons in the hypothalamus.

An increased concentration of 5-HT has been noted in the ME following 5 and 15 minutes of restraint (Culman et al., 1980). Palkovits et al. (1976) reported a decreased concentration of 5-HT and tryptophan hydroxylase activity in the ME after 3 hr of restraint. Although results concerning the effects of immobilization on 5-HT turnover and metabolism in whole brain (Curzon and Green, 1969; DeSchaepdryver et al., 1969; Bliss et al., 1972; Curzon et al., 1972) suggest an activation of 5-HT neuronal systems during application of restraint stress, they do not necessarily provide information on the dynamics of 5-HT neuronal activity within discrete brain nuclei. Technical problems have made it difficult to perform 5-HT turnover studies in discrete brain regions, but Mueller et al. (1976) noted an increase in 5-HT turnover in the hypothalamus following 5-15 min immobilization stress, while Morgan et al. (1975) reported no change in 5-HT turnover in the diencephalon (hypothalamus plus thalamus). Furthermore, Wuttke et al. (1977) and Baumgarten et al. (1978) found that pretreatment with 5,7-DHT which decreased hypothalamic 5-HT uptake and synthesis of 5-HT in the ME by 65-80% did not influence the increase in prolactin secretion resulting from an ether vapour stress. In this section the effects of 30 min restraint stress on 5-HT synthesis and metabolism were determined in the ME, SCN, MPO, AN and ST.

A. Effect of Restraint Stress on 5-HT Metabolism in Selected Regions of the Rat Brain

The effects of restraint stress (30 min) on the concentrations of 5-HT and 5-HIAA were examined in the ST, ME, AN, SCN and MPO in two separate experiments. The results from Experiment #1 and Experiment #2

are shown in Table 13. No effect of immobilization or ether alone on 5-HT or 5-HIAA concentrations in the ST were seen in either experiment. In Experiment #1 a significant decrease in the concentration of 5-HIAA in the ME was detected following ether alone or restraint stress and an increase in the concentration of 5-HT was observed in the MPO following restraint. None of these effects were observed in Experiment #2. However, in Experiment #2 restraint decreased the concentration of 5-HIAA and increased the concentration of 5-HT in the AN. The concentration of 5-HT in the SCN also increased following restraint in the latter experiment. Therefore, no statistically significant effects of restraint on 5-HT metabolism were consistently observed although a trend towards an increased 5-HT metabolism in the SCN was observed in both experiments.

B. Effect of Restraint Stress on 5-HT Synthesis in Selected Regions of the Rat Brain and Serum Prolactin

The effects of immobilization stress on 5-HTP accumulation in the ST, ME, AN, SCN and MPO were examined in two separate experiments; the results are shown in Table 14. 5-HTP accumulation in the ST or AN did not change following ether alone or restraint stress in either experiment. Ether treatment alone increased 5-HTP accumulation in the ME in experiment #2 but not experiment #1. 5-HTP accumulation in the SCN and MPO was increased following application of restraint stress and ether but not ether alone. This effect was observed in the SCN in both experiments but only in Experiment #1 for the MPO. Serum prolactin was not affected by ether exposure alone but increased following restraint

TABLE 13

Effect of Acute Restraint Stress on 5-HT Metabolism in Selected Regions of the Rat Brain

Region	Treatment	Experiment #1		Experiment #2	
		5-HT	5-HIAA	5-HT	5-HIAA
ST	Control	6.6±0.4	5.7±0.3	6.8±0.3	6.1±0.3
	Ether	6.7±0.6	5.5±0.5	6.6±0.3	5.6±0.4
	Ether + Restraint	6.7±0.8	5.1±0.4	6.7±0.6	5.2±0.4
ME	Control	16.2±1.6	8.9±0.4	13.6±0.6	8.3±0.6
	Ether	12.9±0.7	5.4±0.4*	16.6±0.6	9.8±0.3
	Ether + Restraint	9.3±2.6	4.8±0.3*	13.5±0.7	7.0±0.2
AN	Control	18.0±0.6	20.0±1.2	15.2±0.7	18.5±0.9
	Ether	19.2±1.1	17.7±1.0	14.4±0.6	15.1±0.6
	Ether + Restraint	18.0±1.3	16.0±1.3	19.5±0.8*	14.5±1.0*
SCN	Control	23.7±1.4	13.3±0.4	21.3±0.7	13.0±0.6
	Ether	22.8±1.4	13.8±0.8	19.3±0.4	11.2±0.2
	Ether + Restraint	27.2±1.1	16.1±0.4	26.6±1.5*	14.6±1.4
MPO	Control	7.4±0.3	12.6±0.8	7.9±0.4	12.6±0.4
	Ether	6.3±0.6	10.3±0.8	7.7±0.2	12.6±0.4
	Ether + Restraint	10.2±0.4*	14.7±0.7	8.1±0.5	13.2±1.2

Values represent 5-HT or 5-HIAA concentration (ng/mg protein; mean S.E.; $N \geq 6$) after 30 min of restraint stress, ether exposure alone or no previous treatment.

*, significantly different from control value ($p<0.01$).

TABLE 14

Effect of Acute Restraint Stress on 5-HTP Accumulation in Selected Regions of the Rat Brain

Region	Treatment	Experiment #1	Experiment #2
ST	Control	3.1±0.2	3.3±0.2
	Ether	2.9±0.2	2.9±0.2
	Ether + Restraint	2.5±0.1	2.6±0.1
ME	Control	5.9±0.3	5.0±0.3
	Ether	4.9±0.4	7.5±0.2*
	Ether + Restraint	4.6±0.3	4.4±0.3
AN	Control	5.3±0.5	3.2±0.1
	Ether	5.6±0.5	2.9±0.2
	Ether + Restraint	4.9±0.5	3.4±0.1
SCN	Control	12.5±0.7	12.7±0.4
	Ether	11.6±1.0	12.3±0.6
	Ether + Restraint	21.8±0.8*	15.5±0.2*
MPO	Control	3.2±0.4	3.1±0.2
	Ether	3.0±0.3	2.5±0.1
	Ether + Restraint	7.4±0.4*	3.8±0.2

All animals received an injection of NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 minutes; mean ± S.E.; N = 8) after 30 minutes of restraint stress, ether exposure or no previous treatment.

*, significantly different from vehicle-treated controls ($p<0.01$).

stress. Actual concentrations for control, ether and ether plus restraint serum prolactin (ng/ml serum, mean ± SE) were 15.6±2.9, 10.4± 1.1 and 54.7±17.7, respectively.

These results support the hypothesis that 5-HT neuronal activity in certain discrete nuclei of the rat brain may be stimulated by restraint stress. In particular, restraint stress appears to increase 5-HT synthesis and metabolism in the SCN and possibly MPO. Čulman et al. (1980) reported an increase in the concentration of 5-HT in the ME at 5 and 15 min following the introduction of restraint stress. The failure to observe such changes in the present study may be attributable to the fact that the present experiments were performed using a longer (30 min) duration of immobilization. Čulman et al. (1980) could no longer detect a significant change in 5-HT concentrations in the ME following 30 min of restraint. The same investigators also reported increases in 5-HT in the SCN following 5-150 min restraint without affecting 5-HT concentrations in the AN. Their findings in the SCN could have resulted from an increased synthesis of 5-HT, a decreased release of 5-HT or several other possible explanations. The present results suggest that 5-HT synthesis and metabolism increased in the SCN and possibly the MPO. What proportion of this increased synthesis and metabolism produces a functionally important increase in the release of 5-HT into the synapse and what proportion simply represents a conversion of excess 5-HT to 5-HIAA intraneuronally by MAO without ever being released is not known. However, a relation between nerve impulse flow and 5-HT synthesis and release has been demonstrated (Aghajanian et al.,

1972; Ashkenazi et al., 1972; Shields and Eccleston, 1972; Herr et al., 1975; Bramwell and Gonye, 1976; Héry et al., 1979). Mueller et al. (1976) observed an increased 5-HT turnover in the whole hypothalamus following 15 min of immobilization but did not examine hypothalamic 5-HT turnover at 30 min. In contrast, Morgan et al. (1975) could not find changes in 5-HIAA concentrations or 5-HT turnover in the diencephalon (hypothalamus plus thalamus) following 30, 60 or 90 min immobilization. The discrepancy between these findings and the present data can probably be attributed to the much larger brain area being examined by Morgan and coworkers. Certainly, the lack of effect on diencephalic 5-HT does not discount the possibility that alterations in 5-HT neuronal activity in discrete regions like the SCN and MPO are occurring.

The lack of effect of 5,7-DHT pretreatment on the increase in prolactin secretion resulting from ether vapour stress (Wuttke et al., 1977; Baumgarten et al., 1978) does not necessarily preclude the possibility that 5-HT neuronal systems in discrete areas may be playing a role in the prolactin response to restraint stress, let alone any of the other endocrine events occurring during stress. First, recovery of function does not necessarily have to be identical with the extent of 5-HT neurons present in the area if supersensitivity of the 5-HT post-synaptic receptors involved in the response has occurred. In that case, the normal hormonal secretory response could be elicited despite dramatic decreases in 5-HT innervation to the area. Furthermore, the minimum amount of 5-HT innervation required to maintain a functional hormonal response (allowing for a sufficient reserve capacity) is not known.

Wuttke et al. (1977) evaluated 5-HT integrity by measuring hypothalamic 5-HT uptake and synthesis of 5-HT in the ME following 5,7-DHT treatment and found both parameters were reduced by about 70% which possibly might not have been severe enough. Furthermore, the present results suggest 5-HT in the ME may not be involved in the endocrine responses occurring during stress. Thus, the indices of 5-HT integrity examined by Wuttke and coworkers may not have been appropriate. Baumgarten et al. (1978) did not examine the effect of 5,7-DHT pretreatment on 5-HT concentrations in the mediobasal hypothalamus or ME. Neither study examined possible non-specific effects of 5,7-DHT treatment on other neurotransmitter systems that may be involved in the regulation of endocrine responses to stress. Finally, it is not presently well understood to what extent endocrine responses to different types of stress (such as ether vapour and restraint) are mediated by the same neuronal pathways. Therefore, the lack of effect of a 5,7-DHT pretreatment on an ether vapour-induced prolactin surge does not necessarily prove that a restraint-induced prolactin surge would also be unaffected.

Although both serum prolactin and 5-HT neuronal activity in the SCN and MPO are increased following 30 min of immobilization stress, these results should not be interpreted as illustrating a cause-and-effect relationship between increases in 5-HT neuronal activity and secretion of prolactin in response to restraint stress. The data only indicate that restraint stress increases 5-HT neuronal activity in the SCN and possibly MPO. Immobilization is known to produce an activation of several components of the pituitary-adrenocortical system. In rats, increases in plasma ACTH, corticosterone and prolactin (Morgan et al.,

1975; Seggie and Brown, 1975; Kvetňanský et al., 1976) as well as a decrease in growth hormone (Seggie and Brown, 1975) have been reported following stress. Inhibition of 5-HT reuptake as well as direct stimulation of 5-HT receptors in the brain have been reported to stimulate the pituitary-adrenocortical system (Fuller et al., 1976; Meyer et al., 1978). Furthermore, 5-HT has been shown to stimulate release of corticotrophin releasing factor from isolated hypothalami (Jones et al., 1976; Buckingham and Hodges, 1977). Therefore, the increases in 5-HT neuronal activity observed in the SCN and MPO may be related to another of these endocrine responses or perhaps some event that has not yet been identified. Investigations concerning questions like these should be the subject of future studies. Interestingly, Moore and Eichler (1972) reported the SCN participates in the regulation of the circadian adrenal corticosterone rhythm. Thus, the SCN might represent a good location for the beginning of future investigations into the possible role of 5-HT in the control of plasma corticosterone responses to immobilization stress.

IV. Effects of Suckling and Pregnancy on 5-Hydroxytryptaminergic Synthesis and Metabolism in Selected Brain Regions and Serum Prolactin in the Female Rat

A. Evidence for Involvement of 5-HT Neurons in the Suckling-induced Release of Prolactin

Results of studies in the lactating rat suggest that 5-HT neuronal systems are involved in the suckling-induced release of prolactin. In fact, serotonin facilitation of prolactin responses to the

suckling reflex in lactating rats represents the most documented illustration of 5-HT involvement in a neuroendocrine reflex. The dramatic increase in serum prolactin concentrations observed 5-10 min after the onset of the suckling stimulus (Nicoll, 1971) is maintained until the suckling stimulus is removed (Kordon et al., 1973). This response can be abolished by prior blockade of 5-HT biosynthesis by p-chlorophenylalanine (PCPA) (Kordon et al., 1973) or of its receptors by methysergide (Gallo et al., 1975) and can be re-established following PCPA treatment by administering the immediate precursor for 5-HT synthesis, 5-HTP (Kordon et al., 1973; Héry et al., 1976). Transient secretory episodes of oxytocin, which are also induced by suckling, are equally inhibited following 5-HT depletion (Moss and Richard, 1979).

5-HT neuronal pathways involved in this effect probably project from the raphé nuclei to the mediobasal hypothalamus across the medial forebrain bundle because transection of this tract impairs the suckling-induced activation of neurosecretory neurons (Averill and Purves, 1966). The bundle of Schultz has also been reported to play an important role in the reflex (Beyer and Mena, 1965).

Results from experiments examining the neurotransmitter content of various brain regions during suckling also support an important role of 5-HT innervation to the hypothalamus in the regulation of prolactin secretion during lactation. Mena et al. (1976) and Coppings and McCann (1981) observed a decrease in hypothalamic 5-HT concentrations in parallel with an increase in 5-HIAA concentrations within 5 min of the suckling stimulus that was maintained as long as suckling was continued. The activity of tryptophan hydroxylase is also increased in the basal

hypothalamus by suckling (Carr and Jimenez, personal communication). In contrast, the 5-HT concentration in other brain areas remains unaffected, as does hypothalamic noradrenaline (Mena et al., 1976). The physiological significance of these neurochemical results are supported by the fact that the 5-HT response does not occur under weaning or pre-weaning conditions where the pups have been separated from their mother for a period of 24 hr rather than 8 hr or less (Mena et al., 1976). Grosvenor and Mena (1971) have reported that prolactin is no longer released by suckling under similar conditions. Furthermore, Rowland et al. (1978a) reported that administration of p-chloroamphetamine (PCA), a serotonin neurotoxin, into the third ventricle depleted hypothalamic 5-HT by 43% and resulted in a 98% incidence of pup mortality. In contrast, PCA infusion into the lateral ventricle depleted hypothalamic 5-HT by 23% and resulted in a 48% incidence of pup mortality. Coincident with the hypothalamic 5-HT depletion was a reduction in serum prolactin, and subcutaneous injections of prolactin into the treated females sharply reduced the number of pup deaths. From these results the authors concluded that 5-HT neurons in the hypothalamus mediate the suckling-induced release of prolactin necessary for the maintenance of lactation. These results suggest that hypothalamic 5-HT neurons are activated during suckling. Therefore, the effects of suckling on 5-HT synthesis and metabolism in the ME, AN, SCN, MPO and ST were examined in Long-Evans lactating female rats.

1. Effect of Suckling on 5-HT Metabolism in Selected Regions of the Rat Brain

5-HT metabolism in the ST, ME, SCN, MPO and AN was measured in lactating Long-Evans rats who had been 1) deprived of their

pups for 4 hr (non-suckled), 2) deprived of their pups for 4 hr and then allowed to be suckled for 30 min (suckled) and in 3) non-lactating diestrous controls. The results of this experiment are shown in Table 15. Suckling did not effect 5-HT or 5-HIAA concentrations in the ST, SCN, ME or AN. Both lactating experimental groups demonstrated tendencies toward increased 5-HT and 5-HIAA concentrations in the ME. Lactation tended to decrease 5-HT concentrations and increase 5-HIAA concentrations in the AN reaching statistical significance in the suckled group. Lactation by itself did not alter 5-HT or 5-HIAA concentrations in the MPO but the addition of a suckling stimulus caused an increase in both 5-HT and 5-HIAA. These results suggest that the state of lactation may alter 5-HT metabolism to some extent in the ME and AN. Furthermore, suckling increases 5-HT metabolism in the MPO.

2. Effect of Suckling on 5-HT Synthesis in Selected Regions of the Rat Brain and on Serum Prolactin

The effects of lactation alone (non-suckled, 4 hr pup-deprived) or suckling stimulus (4 hr pup-deprived, 30' suckled) on 5-HTP accumulation in the ST, ME, SCN, MPO and AN were compared to a non-lactating diestrous control. The results from this experiment are shown in Table 16. Neither suckling nor lactation influenced 5-HTP accumulation in the ST, SCN or AN. Lactation, itself, increased 5-HT synthesis in the ME but suckling did not cause an additional effect of its own. Lactation alone did not alter 5-HTP accumulation in the MPO. However, suckling increased 5-HTP accumulation in the MPO over both the non-lactating, diestrous control and the lactating, 4 hr pup-deprived control. These results suggest an effect of lactation on 5-HT synthesis

TABLE 15

Effect of Acute Suckling on 5-HT Metabolism in Selected Regions of the Rat Brain

Region	Treatment	5-HT	5-HIAA
ST	Diestrous Control	6.5±0.7	5.3±0.5
	Non-Suckled	5.7±0.5	4.8±0.3
	Suckled	5.5±0.5	4.6±0.5
ME	Diestrous Control	18.4±1.3	12.2±1.1
	Non-Suckled	$22.3\pm2.1_{N}$	14.8±1.3
	Suckled	23.4 ± 2.2^{N}	15.7±1.5
AN	Diestrous Control	20.2±1.9	19.9±1.2
	Non-Suckled	$17.7\pm1.4_{N}$	$23.7\pm1.9_{N}$
	Suckled	15.7 ± 1.5^{N}	25.3 ± 1.6^{N}
SCN	Diestrous Control	23.2±2.1	18.1±1.7
	Non-Suckled	25.6±2.4	19.8±2.0
	Suckled	21.7±2.2	17.2±1.6
MPO	Diestrous Control	6.5±0.4	15.1±1.5
	Non-Suckled	$7.2\pm0.3_{*N}$	$15.4\pm0.8_{*}$
	Suckled	$9.8\pm1.1^{*N}$	$18.1\pm0.9^{*}$

Suckled animals were deprived of pups for 4 hr prior to re-establishment of suckling stimulus for 30 min. Non-suckled animals were deprived of pups for 4 hr prior to sacrifice.

Values represent ng/mg protein (mean ± S.E.; $N \geq 7$). *, significantly different from non-suckled control ($p<0.01$).

^{N}, significantly different from diestrous, non-lactating control ($p<0.01$).

TABLE 16

Effect of Acute Suckling on 5-HTP Accumulation in Selected Regions of the Rat Brain

Region	Treatment	5-HTP
ST	Diestrous Control	4.9±0.4
	Non-Suckled	3.8±0.2
	Suckled	3.5±0.2
ME	Diestrous Control	5.3±0.5^{N}
	Non-Suckled	8.2±0.7^{N}
	Suckled	10.2±1.1^{N}
AN	Diestrous Control	6.5±0.3
	Non-Suckled	6.4±0.3
	Suckled	6.4±0.4
SCN	Diestrous Control	10.9±0.7
	Non-Suckled	12.2±0.9
	Suckled	11.0±0.6
MPO	Diestrous Control	4.9±0.3
	Non-Suckled	4.7±0.3^{*N}
	Suckled	7.3±0.8

Suckled animals were deprived of pups for 4 hr prior to re-establishment of suckling stimulus for 30 min. Non-Suckled animals were deprived of pups for 4 hr prior to sacrifice.

All animals received an injection of NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 min; mean ± S.E.; N = 8).

^{N}, significantly different from diestrous, non-lactating control ($p<0.01$).

*, significantly different from non-suckled control ($p<0.01$).

in the ME and that suckling increases 5-HT synthesis in the MPO. Serum prolactin concentrations in lactating rats (8.3±1.2 ng/ml) was not significantly different from that in diestrous controls, but suckling elicited an increase in serum prolactin (98.4±12.3 ng/ml).

These results support the large amount of evidence that certain 5-HT neuronal systems may be activated during suckling. In particular, 5-HT and 5-HIAA concentrations as well as 5-HTP accumulation are all increased in the MPO, suggesting that 5-HT neuronal activity in this nucleus is greatly affected by suckling. 5-HT synthesis and, to a lesser degree, 5-HT and 5-HIAA concentrations are increased in the ME during lactation whether the suckling stimulus is present or not. These results extend observations by others which indicate that 5-HT neuronal activity in the hypothalamus is stimulated during suckling (Mena et al., 1976; Carr and Jimenez, 1981; Coppings and McCann, 1981). It is possible that 5-HT neurons in the MPO may be part of a neuronal system that contributes in some way to the decreased DA turnover in the ME that is observed during suckling (Selmanoff and Wise, 1981). For example, 5-HT neurons could be indirectly controlling prolactin release via effects on the tuberoinfundibular DA neurons. It is also tempting to suggest that 5-HT may be involved in the inhibition of ovulation that occurs during lactation. Evidence suggests that 5-HT may inhibit gonadotropin secretion (O'Steen, 1965; Kordon et al., 1968; Kamberi et al., 1971). In this regard, an elevation of 5-HT neuronal activity could simultaneously facilitate prolactin secretion to suckling and antagonize LH secretion; and conversely, a decrease in 5-HT neuronal activity could possibly disinhibit tuberoinfundibular DA neurons (letting them re-establish

their tonic inhibition on prolactin secretion) and restore normal phasic patterns of FSH and LH secretion. LHRH-containing nerve cell bodies are located in the MPO and connections between the MPO and ME are known to exist so the anatomical correlates of this latter hypothesis already exist.

No causal effect between the increase in 5-HT neuronal activity in the MPO and the suckling-induced increase in prolactin secretion has been shown in the present experiment. Furthermore, suckling in the lactating rat not only produces increases in the release of prolactin but also ACTH (Grégoire, 1947; Voogt et al., 1969; Zarrow et al., 1972), growth hormone (Grosvenor, 1964; Sar and Meites, 1969), melanocyte stimulating hormone (Taleisnik and Orias, 1966; Deis and Orias, 1968), thyroid stimulating hormone (Blake, 1974; Grosvenor, 1964) and oxytocin (Cowie and Folley, 1961). Therefore, the possibility that activation of the 5-HT neuronal system in the MPO is involved with one of the other endocrine events occurring during suckling cannot be ruled out.

B. Evidence for Involvement of 5-HT Neurons in Prolactin Secretion During Pregnancy

5-HT neurons may also be involved in the regulatory processes governing prolactin and gonadotropin secretion througout pregnancy. Following copulation or cervical stimulation during the period of estrus, two daily surges of prolactin can be observed during early pregnancy/pseudopregnancy (Butcher et al., 1972; Freeman et al., 1974). One surge of prolactin occurs at the end of the light period (diurnal surge, 1700-1900 hr) while the second daily surge occurs at the end of

the dark period (nocturnal surge, 300-500 hr). These surges appear to be necessary for the initiation and maintenance of progesterone secretion from the corpus luteum in early pregnancy (Smith et al., 1975; Morishige and Rothchild, 1974). The daily diurnal surge of prolactin continues until day 8 of pregnancy whereas the nocturnal surge normally ends on day 10 of pregnancy (Yogev and Terkel, 1978). Presently, little is known about the neurochemical mechanisms governing the regulation of these surges of prolactin during pregnancy. Both surges are abolished if hypothalamic retrochiasmatic cuts are made, suggesting that information necessary for their initiation arrives at the medial hypothalamus from a rostral direction (Freeman et al., 1974). Lesions of the suprachiasmatic nuclei abolish both of the daily surges of prolactin characteristically observed in female rats during pregnancy and pseudopregnancy (Bethea and Neill, 1980; Yogev and Terkel, 1980). It appears that the medial preoptic area possesses neurons which inhibit the nocturnal surge and stimulate the diurnal surge of prolactin, whereas stimulation of the dorsomedial-ventromedial (DMN-VMN) areas of the hypothalamus initiates both surges of prolactin (Freeman and Banks, 1980; Gunnet et al., 1981). Excitation of the DMN-VMN appears to be a definite requirement for initiation of the diurnal but not the nocturnal surge of prolactin (Gunnet et al., 1981). Although some information exists concerning the pathways and areas of the brain that are necessary for initiation and/or maintenance of these surges, experiments examining the neurochemical systems involved are few. Biphasic changes in the activity of the tuberoinfundibular DA neurons and in tyrosine hydroxylase activity in the ME have been reported in pregnant and pseudopregnant

rats that correlate inversely with prolactin secretion (McKay et al., 1981; Voogt and Carr, 1981). However, few studies examining 5-HT neuronal activity throughout pregnancy and especially throughout these dynamic changes in prolactin secretion have been performed. Rowland et al. (1978b) observed no changes in 5-HT or 5-HIAA concentrations in the hypothalamus, hippocampus and cortex during selected stages of pregnancy. Thus, it is not known if 5-HT neuronal systems are involved in the daily surges of prolactin or in the alterations in tuberoinfundibular DA neuronal activity. Thus, 5-HT neuronal activity in the ST, MPO, AN and SCN and serum prolactin concentrations at various times throughout day 6 of pregnancy (a day when both surges should be occurring) were examined.

1. Serum Prolactin and 5-HT Metabolism in Selected Regions of the Rat Brain During Day 6 of Pregnancy

The profile of serum prolactin at various times on day 6 is shown in Figure 7. The nocturnal surge peaks at 300 hr and is still present at 600 hr. The diurnal surge is much smaller than the nocturnal surge and peaks at 1800-2100 hr. 5-HT metabolism was also measured in the SCN, MPO, ST and AN at various times throughout day 6 of pregnancy, including 300 and 600 hr (times when the nocturnal surge occurs), 1200 hr (a control time point when serum prolactin is low) and 1800 and 2100 hr (times when the diurnal surge of prolactin occurs). The results of this experiment are shown on Table 17. No effects on 5-HT or 5-HIAA concentrations were observed in the SCN throughout day 6 of pregnancy. In the MPO 5-HT was low at 300 hr and rose by 600 hr to levels that were maintained throughout the day whereas the concentration of 5-HIAA increased from a low at 300 hr to become significantly higher at 1200

Figure 7. Serum prolactin during day 6 of pregnancy. ^{a,b}, values with different letters are significantly different ($p<0.05$). ^{d,e}, values with different letters are significantly different ($p<0.01$).

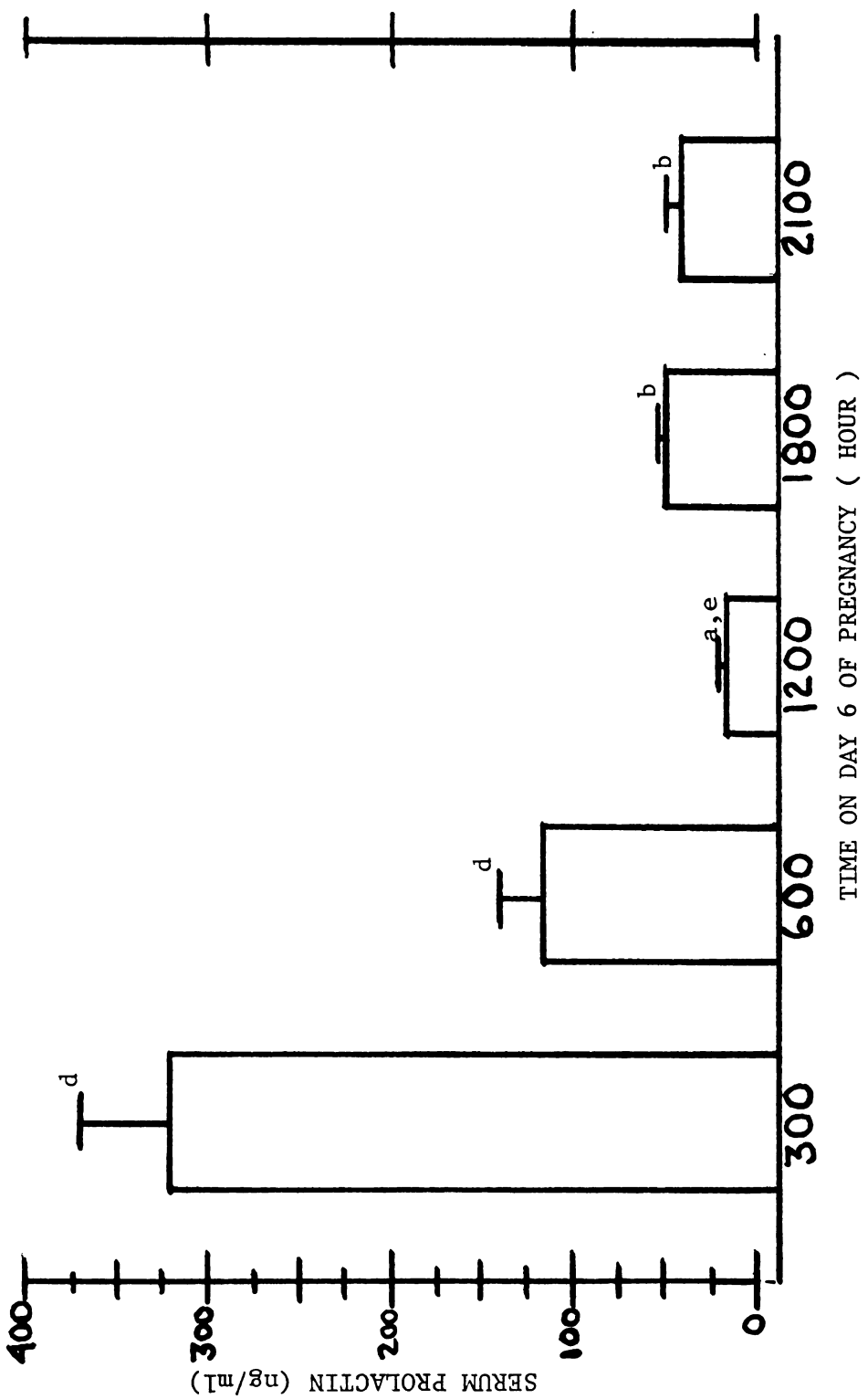

SERUM PROLACTIN (ng/ml)
400
300
200
100
0
300
600
1200
1800
2100
TIME ON DAY 6 OF PREGNANCY (HOUR)
d
d
a,e
b
b

Figure 7

TABLE 17

5-HT Metabolism in Selected Regions of the Rat Brain at Various Times of Day 6 of Pregnancy

Region	Compound	Time on Day 6 of Pregnancy				
		300 Hr	600 Hr	1200 Hr	1800 Hr	2100 Hr
ST	5-HT	9.5±0.8[a]	9.2±1.0[a]	6.3±0.5[b]	6.6±0.5[b]	5.2±1.2[b]
	5-HIAA	17.4±1.9[a]	13.9±1.0[a,b]	11.3±0.9[b]	11.8±0.9[b]	10.8±1.3[b]
AN	5-HT	24.5±2.2[a]	20.6±2.4[a,b]	16.0±1.7[b]	23.8±1.8[a]	26.0±1.8[a]
	5-HIAA	14.2±1.0[a]	11.8±1.8[a]	17.8±1.1[b]	11.8±0.7[a]	14.0±1.0[a]
SCN	5-HT	22.8±2.2	25.2±2.6	29.6±0.9	28.1±1.8	30.1±6.1
	5-HIAA	19.9±3.7	16.2±2.6	18.7±0.8	19.4±2.0	14.8±1.4
MPO	5-HT	6.0±0.8[a]	9.3±1.0[b]	12.5±1.1[b]	11.5±1.0[b]	10.4±1.0[b]
	5-HIAA	8.8±1.0[a]	9.2±0.4[a]	13.7±1.0[b]	11.0±0.9[a,b]	10.8±0.1[a]

Values represent ng/mg protein (mean ± S.E., $N \geq 7$).

[a,b]Groups without the same letter are significantly different ($p<0.01$).

hr, decreasing again by 1800 hr. In the ST, both 5-HT and 5-HIAA concentrations were higher before 1200 hr than they were afterward. 5-HT concentrations in the AN decreased from high values at 300 hr to a low at 1200 hr before increasing back to high values at 1800-2100 hr. In contrast, 5-HIAA concentrations in the AN were highest at 1200 hr with respect to any other time point examined.

2. 5-HT Synthesis in Selected Regions of the Rat Brain During Day 6 of Pregnancy

5-HTP accumulation in the ST, MPO, SCN and AN was examined at 300 hr, 600 hr, 1200 hr, 1800 hr and 2100 hr on day 6 of pregnancy. The results of this experiment are shown on Table 18. 5-HT synthesis in the ST or MPO did not change at any time on day 6 of pregnancy. 5-HTP accumulation in the SCN decreased from a high at 300 hr throughout the day to a low at 2100 hr. Thus, at the time of the nocturnal surge of prolactin, 5-HT synthesis is increased in the SCN whereas at the time of the diurnal surge (1800-2100 hr) 5-HT synthesis is decreasing. In the AN, 5-HTP accumulation is increased at 300 hr and at 1800 hr compared to the other time points examined on day 6 of pregnancy.

3. 5-HT Synthesis in Selected Regions of the Rat Brain at 4:30 and 1200 hr on Day 8 and Day 13 of Pregnancy

An experiment was performed to examine whether the changes observed in 5-HT synthesis on day 6 of pregnancy were still occurring when the prolactin surges were absent. 5-HTP accumulation in the ST, MPO, SCN and AN was examined at 430 hr (a time during the nocturnal surge of prolactin) and at 1200 hr (a time point when serum

TABLE 18

5-HTP Accumulation in Selected Regions of the Rat Brain at Various Times of Day 6 of Pregnancy

Region	Time on Day 6 of Pregnancy				
	300 Hr	600 Hr	1200 Hr	1800 Hr	2100 Hr
ST	3.8±0.1	3.8±0.3	4.2±0.3	4.2±0.2	4.6±0.2
AN	14.8±1.3[a]	7.1±0.5	6.1±0.3	10.0±0.7[b]	6.1±0.6
SCN	14.9±1.7[a]	13.0±1.2[a,b]	10.5±1.2[b]	10.3±0.9[b]	7.7±0.7[b]
MPO	7.0±0.7	6.2±0.5	5.5±0.5	6.5±0.5	7.3±0.3

All animals received an injection of NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 minutes; mean ± S.E.; N = 8).

[a,b]Groups without the same letter are significantly different ($p<0.01$).

prolactin is low) on day 8 (when the nocturnal and diurnal surges are both present) and on day 13 (when both surges of prolactin have disappeared) of pregnancy. The results of this experiment are shown on Table 19. No differences in 5-HTP accumulation in the ST or MPO were observed on day 8 or day 13. In the SCN the rate of 5-HTP accumulation at 430 on both day 8 and day 13 is significantly greater than the 1200 hr value. On the other hand, the significant increase in 5-HTP accumulation observed in the AN on day 8 of pregnancy is no longer present on day 13 when the nocturnal surge in prolactin is also absent.

These results suggest that 5-HT synthesis in the AN is increased during both the nocturnal and diurnal surges of prolactin and that 5-HT synthesis in the SCN is increased during the nocturnal surge and possibly decreased during the diurnal surge of prolactin. 5-HT metabolism, on the other hand, appears to be enhanced in the AN at 1200 hr and does not change in the SCN. This apparent discrepancy with the reports of Rowland et al. (1978b) that 5-HT metabolism does not change throughout pregnancy is probably explained by the fact that Rowland and his coworkers examined these concentrations at one time point on day 5, day 15 and day 21 of pregnancy. No differences would have been detected in the present study if we had examined one time point (for example, 1200 hr) on three different days. Also, Rowland et al. (1978b) examined effects in the whole hypothalamus. With the great variety of effects occurring in discrete areas of the hypothalamus in the present study it is not surprising that effects were not observed in the whole hypothalamus.

TABLE 19

5-HTP Accumulation in Selected Regions of the Rat Brain at 430 and 1200 hr on Day 8 and Day 13 of Pregnancy

Region	Hour of Day	Day of Pregnancy	
		Day 8	Day 13
ST	430	3.9±0.2	4.0±0.4
	1200	4.4±0.4	3.9±0.4
AN	430	11.3±0.9	5.5±0.3
	1200	6.6±0.7*	6.2±0.5
SCN	430	17.6±1.5	16.7±1.1
	1200	13.1±1.5*	13.0±1.1*
MPO	430	6.9±1.0	4.5±0.8
	1200	4.4±0.6	5.4±0.4

All animals received an injection of NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 minutes; mean ± S.E.; N = 8).

*, significantly different from 430 hour value ($p<0.01$) on same day.

When 5-HTP accumulation was examined in the AN and SCN at a time when the prolactin surges have stopped, the changes in the SCN still occur; whereas the increase in 5-HT synthesis observed at 430 hr in the AN is no longer present. These results suggest that the increase in 5-HT synthesis in the AN may be correlated to the presence of the nocturnal prolactin surge whereas the increase in 5-HT synthesis in the SCN is not. Therefore, the changes in 5-HT neuronal activity in the SCN are probably not involved in the surges of prolactin observed in early pregnancy but the changes in 5-HT neuronal activity in the AN may, indeed, play a role in this regulation.

V. 5-Hydroxytryptamine Metabolism and Synthesis in Selected Brain Regions and Serum Prolactin Throughout Proestrus in the Female Rat

5-HT neuronal systems appear to play an important role in the regulation of neuroendocrinological processes leading to ovulation. On the afternoon of proestrus at or close to the time of ovulation there occur surges of the secretion of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin (Butcher et al., 1974). Although many experiments have been performed examining catecholamine content and turnover in the ME during proestrus and relating a decrease in tuberoinfundibular DA to the observed surge of prolactin (Ahrén et al., 1971; Löfström, 1977; Crowley et al., 1978; Demarest et al., 1981; Rance et al., 1981), most work concerning the possible 5-hydroxytryptaminergic involvement in the neuroendocrinological events occurring on the afternoon of proestrus has been concerned with LH secretion. The marked increases in serum LH concentrations occurring on the afternoon

of proestrus or in estradiol-pretreated ovariectomized rats as well as ovulation can be prevented by drugs which disrupt 5-HT neurotransmission (Héry et al., 1975, 1976, 1978; Baumgarten et al., 1978; Coen and MacKinnon, 1979; Walker, 1980; Walker et al., 1981), and 5-HTP administration can temporarily reinstate the estrogen-induced surge if it is given at certain times of the day (Héry et al., 1976; Coen and MacKinnon, 1979). Administration of 5-HT agonists at certain times on the day of proestrus can elicit a surge in LH secretion (Walker, 1980). In addition, there is a correlation between the 5-HT antagonistic properties of a number of tricyclic and ergot-derivative compounds and their ability to inhibit the pre-ovulatory surges of LH (Markó and Flückiger, 1980).

Estradiol pretreatment in ovariectomized rats also induces a daily surge of prolactin and disrupts 5-HT neurotransmission, lesioning the SCN or interrupting frontal afferents to the mediobasal hypothalamus at the retrochiasmatic level can attenuate or abolish this surge (Caligaris and Taleisnik, 1974; Subramanian and Gala, 1976; Kawakami et al., 1980).

Walker (1980) observed an increased 5-HT turnover in the hypothalamus coincident with the onset of the proestrus pre-ovulatory LH and prolactin surges. Furthermore, when the LH (and probably prolactin) surge was prolonged by exposing the rats to light on the evening of proestrus, the hypothalamic 5-HT turnover remained high for an extended period. Stimulation of the MPO may be at least partially responsible for the secretion of prolactin on the afternoon of proestrus (Kimura and Kawakami, 1978). Kueng et al. (1976) reported a decrease in 5-HT concentrations in many hypothalamic, limbic and midbrain structures at

1500 hr on proestrus versus 1000 hr values. The decrease was especially evident in the lateral parts of the preoptic area. The authors interpreted this decrease as indicating a release from the 'normal' inhibitory effects of 5-HT on gonadotropin release mechanisms but also may have demonstrated an increased release and metabolism of 5-HT in these areas at 1500 hr before the surges of LH, FSH and prolactin occur. These results taken together suggest that the proestrus surges of FSH, LH and prolactin are accompanied by and may be at least partially dependent upon dynamic changes in 5-HT neuronal activity. Thus, 5-HT neuronal activity in the ST, SCN, MPO, ME and AN and serum prolactin were examined at various times on the day of proestrus in female rats.

A. Serum Prolactin and 5-HT Metabolism in Selected Brain Regions During Proestrus in Female Rats

The profile of serum prolactin at various times throughout proestrus is shown in Figure 8. Serum prolactin began to increase at 1500 hr, peaked at 1700 hr and then slowly fell throughout the rest of the evening. 5-HT metabolism in the ST, MPO, SCN, AN and ME was also studied at 800, 1200, 1500, 1700, 1900, 2100 and 2400 hr on the day of proestrus in female Long-Evans rats. The results of this experiment are shown on Table 20. 5-HIAA concentrations did not change at any time in the ST, MPO, AN or ME but increased in the SCN from a low at 800 hr to a high at 1700-1900 hr (same time that prolactin is reaching its peak) before declining again by 2100 hr. 5-HT concentrations in the ST, MPO and AN showed small differences throughout the day of proestrus. In the ME, 5-HT concentrations remain fairly constant through 1500 hr and then drop dramatically at 1700 hr (when the surge of prolactin is peaking).

Figure 8. Serum prolactin on proestrus. *, value is significantly different from 800 and 1200 hr value ($p<0.05$).

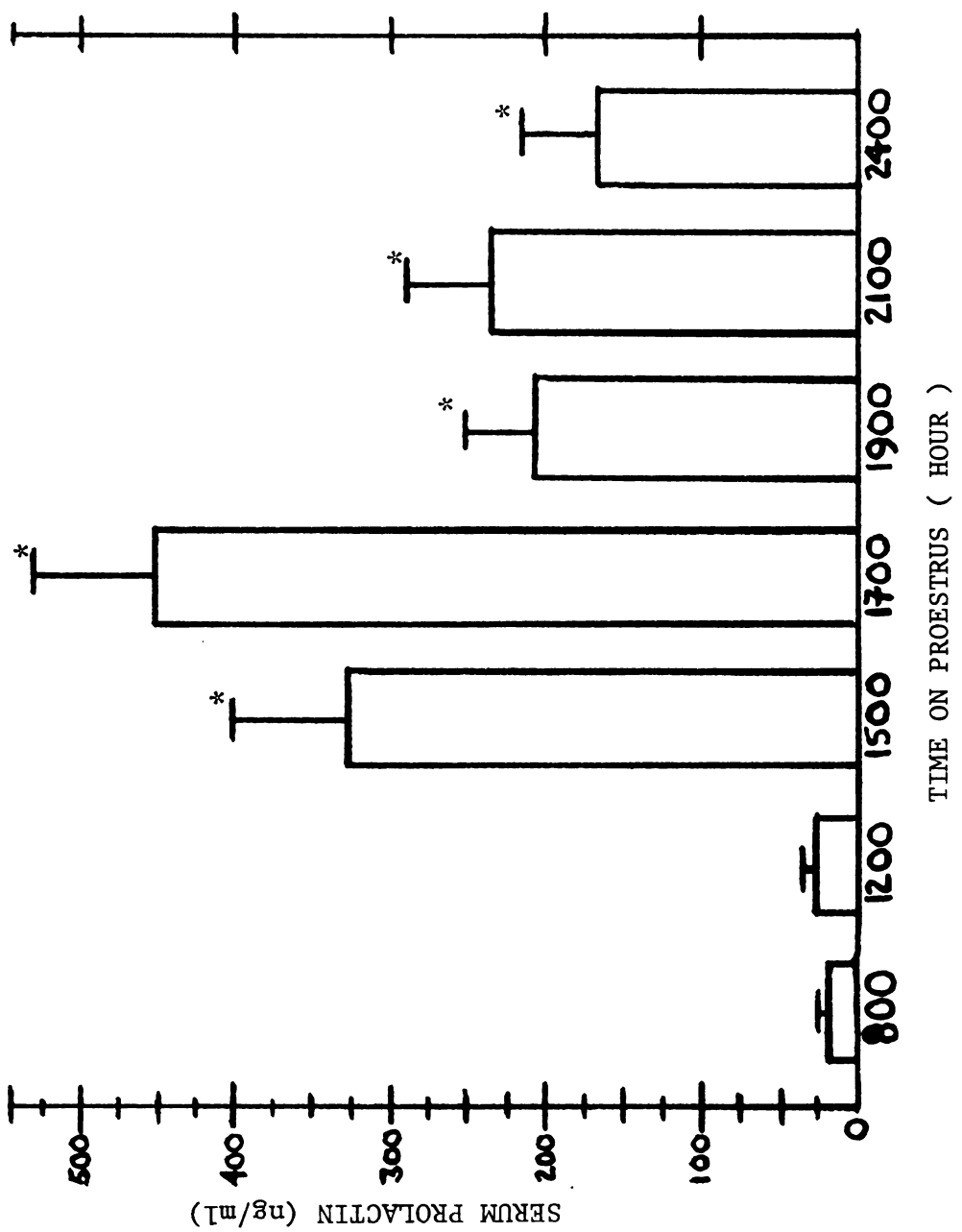

SERUM PROLACTIN (ng/ml)
500
400
300
200
100
0
800
1200
1500
1700
1900
2100
2400
*
TIME ON PROESTRUS (HOUR)

Figure 8

TABLE 20

5-HT Metabolism in Selected Regions of the Rat Brain at Various Times on Proestrus

Region	Compound	Time on Proestrus						
		800 Hr	1200 Hr	1500 Hr	1700 Hr	1900 Hr	2100 Hr	2400 Hr
ST	5-HT	8.2±0.8	6.5±0.7	6.8±0.7	6.7±0.8	6.8±1.1	5.4±0.9	7.2±1.0
	5-HIAA	15.3±1.7	12.4±1.3	13.8±1.4	12.2±0.9	13.6±0.8	12.8±1.3	14.1±1.3
ME	5-HT	13.7±3.8^{a,b}	12.1±1.0^{a}	16.1±3.2^{a,b}	9.3±1.3^{a}	17.8±1.9^{b}	14.4±2.3^{a,b}	13.1±2.0^{a,b}
	5-HIAA	8.3±1.2	6.7±0.7	7.5±0.8	5.9±0.5	7.8±0.9	7.4±1.2	6.2±0.5
AN	5-HT	21.3±2.2^{a,b}	18.4±1.9^{a}	22.5±2.4^{a,b}	23.4±2.4^{a,b}	19.6±2.0^{a,b}	25.4±2.8^{b}	23.8±2.1^{a,b}
	5-HIAA	13.6±1.5	16.4±1.5	15.3±1.8	12.8±1.1	13.1±1.3	14.2±1.5	15.0±1.6
SCN	5-HT	21.3±2.0^{a,b}	19.4±1.7^{a}	20.4±1.6^{a}	25.6±1.8^{a,b}	24.2±1.3^{a,b}	26.8±2.0^{b}	23.8±2.1^{a,b}
	5-HIAA	15.1±0.9^{a}	16.2±1.2^{a,b}	15.7±1.1^{a,b}	18.4±1.0^{a,b}	18.8±1.1^{b}	17.1±1.3^{a,b}	16.3±1.4^{a,b}
MPO	5-HT	7.1±0.5^{a,b}	8.2±1.2^{a,b}	8.7±0.5^{a}	8.4±0.4^{a}	6.4±0.4^{b}	6.9±0.5^{a,b}	7.3±0.4^{a,b}
	5-HIAA	10.3±0.8	10.9±2.1	9.7±1.0	8.4±0.5	9.6±0.4	9.0±0.4	10.1±0.3

Values represent ng/mg protein (mean ± S.E.; $N \geq 7$).

^{a,b}Groups without the same letter are significantly different ($p<0.01$).

By 1900 hr the 5-HT concentrations in the ME have returned to values observed at 1500 hr. 5-HT concentrations in the SCN parallel those of the 5-HIAA concentrations in that they start low at 800 hr and peak at 1700-2100 hr before beginning to fall. These results suggest that 5-HT metabolism in the SCN is activated at a time when the surges in prolactin, LH and FSH are occurring. On the other hand, 5-HT neuronal activity in the ME is decreased at the onset of these hormonal surges.

B. 5-HT Synthesis in Selected Brain Regions During Proestrus in Female Rats

5-HT synthesis in the ST, MPO, SCN, AN and ME as well as serum prolactin was examined at 800, 1200, 1500, 1700, 1900, 2100 and 2400 hr on the day of proestrus. The results of these experiments are shown on Table 21. 5-HTP accumulation was not statistically altered at any time during proestrus in the ST, MPO, SCN or AN. However, a definite trend towards an increase in 5-HT synthesis in the SCN occurs at the same time that the increases in 5-HT and 5-HIAA concentrations were observed. In the ME, 5-HTP accumulation decreases at 1700 hr and 2100 hr. Results from these studies suggest that neuronal activity in the SCN is increased while that in the ME is decreased at the time of the peak concentration of serum prolactin on proestrus.

The results from this section support those of other investigators which suggest that 5-HT neuronal activity is activated in the hypothalamus during the afternoon of proestrus and may be related to the preovulatory surges of LH, prolactin or FSH (Héry et al., 1975, 1976, 1978; Baumgarten et al., 1978; Coen and MacKinnon, 1979; Markó and Flückiger, 1980; Walker, 1980; Walker et al., 1981). However, the

TABLE 21

5-HTP Accumulation in Selected Regions of the Rat Brain at Various Times on Proestrus

Region	Time on Proestrus						
	800 Hr	1200 Hr	1500 Hr	1700 Hr	1900 Hr	2100 Hr	2400 Hr
ST	4.02±0.62	3.73±0.41	3.83±0.51	4.21±0.58	3.23±0.41	3.66±0.48	3.81±0.52
ME	7.39±1.10[a]	4.88±0.71[a,b]	5.91±0.81[a]	3.36±0.29[b]	4.77±0.73[a,b]	3.36±0.65[b]	5.68±0.68[a]
AN	6.02±1.50	6.51±0.94	5.44±0.80	6.58±0.92	6.42±1.27	6.27±0.67	6.74±0.54
SCN	13.4 ±1.6	13.4 ±2.3	12.3 ±2.0	15.2 ±1.3	15.8 ±0.9	15.3 ±1.7	15.3 ±1.5
MPO	5.58±0.74	5.08±0.83	5.28±0.46	4.76±0.61	5.54±0.52	4.85±0.83	4.63±0.41

All animals received an injection of NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the rate of 5-HTP accumulation (ng/mg protein/30 minutes; mean ± S.E.; N = 8).

[a,b]Groups without the same letter are significantly different ($p<0.01$).

present results do not agree with those of Kueng et al. (1976) who reported decreases in 5-HT concentrations in several hypothalamic, limbic and midbrain structures at 1500 hr when compared to 1000 hr on the day of proestrus. No 1000 hr time point was examined in the present experiment and the hypothalamic areas examined did not coincide with many areas examined by Kueng et al. (both examined MPO and AN; and Kueng et al. examined the ventral middle hypothalamus that included the ME). These workers did not observe changes in the AN or MPO. On the other hand, decreases in 5-HT concentrations in the ME (plus some extra ventro-medial hypothalamic tissue) were reported. In this regard, the present results agree quite well with those of Kueng and his co-workers except for the dramatic decrease observed by these authors in the ME-containing area at 1500 hr. At 1700 hr, however, a dramatic decrease in 5-HT and 5-HIAA concentrations as well as 5-HT synthesis in the ME was observed. An increase in 5-HT and 5-HIAA concentrations as well as a trend towards an increase in 5-HT synthesis in the SCN that began at 1700 hr and continued throughout the duration of the prolactin surge was also observed. These results suggest that 5-HT neuronal activity in the SCN increases during the beginning of the pre-ovulatory surges of prolactin, LH and FSH and is maintained throughout the prolactin surge. Whether the observed changes in 5-HT neuronal activity are related to the surges of prolactin, LH or FSH is not known and provides the basis for future investigations.

SUMMARY AND CONCLUSIONS

A simple, sensitive and rapid method utilizing HPLC coupled with electrochemical detection was developed that allows the concurrent measurement of 100 picograms or less of 5-HT, 5-HIAA and 5-HTP in the ME, MPO, ST, SCN and AN of a single rat brain. In addition, under standard operating conditions, DA, DOPAC and HVA can also be resolved. The concentrations of 5-HT, 5-HIAA and the rate of 5-HTP accumulation obtained using HPLC with electrochemical detection were similar to previously reported values in cases where previous investigations had been performed. The validity and capability of this method to quantitatively evaluate changes in 5-hydroxytryptaminergic neuronal activity in these discrete brain regions were determined by measuring the concentrations of 5-HT and 5-HIAA and the accumulation of 5-HTP before and after the administration of various pharmacological agents. The significant observations and conclusive remarks of these studies are summarized below.

A. An intraperitoneal injection of the decarboxylase inhibitor, NSD 1015, caused 5-HTP to accumulate in a linear fashion for at least thirty minutes in the SCN, ST, ME, MPO and AN of both male Sprague-Dawley and female Long-Evans rat brains. The results also indicate that 5-HTP may accumulate at a higher rate in the MPO and SCN of female Long-Evans rat brains when compared to male Sprague-Dawley rat brains.

B. The systemic administration of pargyline increased the concentration of 5-HT and decreased the concentration of 5-HIAA in the ME, ST, MPO, SCN and AN. Pargyline increased DA concentrations in the ST, ME and AN and decreased DOPAC concentrations only in the ST and ME. The systemic injection of probenecid did not markedly affect 5-HT or DA concentrations in any area examined except for the AN where 5-HT decreased. On the other hand, probenecid caused an increase in the concentration of 5-HIAA in all five brain regions and of DOPAC in the ME and MPO. These results indicate that 5-HT neuronal systems in the ST, ME, MPO, SCN and AN react similarly to probenecid or pargyline administration. The results following probenecid administration suggest that a probenecid-sensitive acid transport system is required for removal of 5-HIAA in all five of the brain regions examined but is only required for removal of DOPAC in the ME, MPO and possibly the AN (see discussion above). The results concerning DA and DOPAC concentrations and the effects of pargyline or probenecid on those concentrations helped confirm the correct location of the dissection for the various regions examined.

C. Following the intraperitoneal injection of reserpine the concentration of 5-HT decreased, 5-HIAA increased or did not change and the synthesis of 5-HT increased in all five brain areas at both two and twenty-four hours. These results demonstrate that 5-HT metabolism and synthesis increase in the ME, ST, SCN, AN and to a lesser extent, the MPO following reserpine treatment. This increased activity could result from an attempt by the 5-HT neuronal systems to compensate for the

depletion of 5-HT caused by reserpine through some feedback mechanism. These data extend observations by other investigators in larger brain regions and indicate that reserpine influences the concentration of 5-HT, 5-HIAA and the rate of accumulation of 5-HTP in discrete brain regions in a manner similar to that in larger brain areas.

D. The systemic injection of two inhibitors of 5-HT uptake, chlorimipramine or fluoxetine, did not markedly affect the concentrations of 5-HT or 5-HIAA in any region. However, the trends towards increasing 5-HT and decreasing 5-HIAA concentrations were such that the ratio of the concentration of 5-HIAA/5-HT was decreased in all five brain areas. In addition, the rate of accumulation of 5-HTP also decreased following the administration of the uptake inhibitors. Taken together, these results extend the results of others that show decreased 5-HT neuronal activity in larger brain regions following administration of chlorimipramine or fluoxetine and suggest that the administration of inhibitors of 5-HT uptake decrease both 5-HT metabolism and synthesis in the ST, ME, AN, SCN and MPO. This effect presumably results from an inhibitory feedback mechanism(s) responding to the increased concentration of 5-HT in the synaptic cleft.

E. The intraperitoneal injection of a small dose of L-tryptophan increased the concentration of 5-HT in the ME, AN and MPO and the concentration of 5-HIAA in the SCN, MPO and AN. The accumulation of 5-HTP was increased in the ST, SCN, MPO and AN but not the ME following tryptophan administration. Thus, the 5-HT system in the median eminence responds differently than the 5-HT systems studied in the other four brain regions. The increases in 5-HT and 5-HIAA in the ST, SCN,

MPO and AN apparently result from an increased synthesis of 5-HT whereas the increase in 5-HT observed in the median eminence does not. These results extend observations by others who have examined 5-HT neuronal activity in larger brain areas following tryptophan administration and indicate that tryptophan increases 5-HT neuronal activity in the ST, SCN, MPO and AN but not in the ME. Furthermore, these results demonstrate a unique effect of tryptophan administration on 5-HT metabolism and synthesis in the ME.

F. Subcutaneous injections of morphine in various doses did not affect 5-HTP accumulation in the ST or ME. A dose of 10 mg/kg of morphine increased 5-HTP accumulation in the MPO, SCN and AN. The AN appeared to be very sensitive to the stimulatory effects of morphine as a dose of 5 mg/kg also increased 5-HTP accumulation in this region. Morphine exerted a biphasic dose-response curve on 5-HT synthesis in the MPO, SCN and AN since at the highest dose (20 mg/kg) it did not cause an increase in 5-HTP accumulation in any area. Morphine (10 mg/kg) increased 5-HTP accumulation in the MPO, SCN and AN at 1 hr but not at 0.5 or 2 hr. Therefore, the stimulatory effect of morphine upon 5-HT synthesis in these three discrete brain areas is restricted to a very narrow time period as well as a narrow dose range. Steady-state concentrations of 5-HT are not greatly affected by morphine but the concentration of 5-HIAA increased in the AN and MPO following its administration. The morphine-induced increase in 5-HT synthesis in the MPO, AN and SCN involved an opiate receptor-mediated mechanism as naloxone blocked this effect. These results demonstrate a differential effect of morphine on 5-HT synthesis and metabolism in discrete brain regions.

Specifically, the present results indicate that morphine causes an increase in 5-HT synthesis and metabolism via an opiate receptor mediated mechanism in the AN, MPO and SCN but not in the ME or ST.

The results from A-F verified that HPLC with electrochemical detection could detect changes in 5-HT metabolism and synthesis in the ST, ME, AN, MPO and SCN induced by several pharmacological agents. Furthermore, careful analysis of the data indicated that although measurement of steady-state 5-HT and 5-HIAA concentrations is important for determining how 5-HT neurons are responding to various treatments, several problems exist with the interpretation of results using the ratio of the concentration of 5-HIAA/5-HT as an index of 5-HT neuronal activity. In the present experiments this index was found to be rather insensitive and in some cases, even misleading. Therefore, because of these reasons and the fact that 5-HTP accumulation is more quickly measured (a single peak) and more easily interpreted the index of the ratio of the concentrations of 5-HIAA/5-HT was no longer used in the remaining experiments. Instead, 5-HTP accumulation and the concentrations of 5-HT and 5-HIAA were measured and evaluated. In these latter experiments 5-HT metabolism and synthesis in the ST, ME, AN, MPO and SCN were evaluated throughout various environmental and endocrinological states where the secretion of prolactin from the anterior pituitary is undergoing dynamic change. The significant observations in these studies are summarized below.

G. Application of restraint stress for thirty minutes accelerates synthesis and metabolism of 5-HT in the MPO and SCN. Restraint treatment

also increased serum prolactin significantly above values obtained from animals that were non-stressed and animals that were exposed to ether treatment alone. Neither ether treatment alone or restraint stress plus ether produced any consistent statistically significant effects on 5-HT metabolism in any region. These results demonstrate the ability of restraint stress to increase serum prolactin and differentially affect 5-HT synthesis in discrete areas of the rat brain. Specifically, restraint stress caused an increased 5-HT synthesis in the SCN and possibly the MPO.

H. Acute suckling increased 5-HT synthesis and metabolism in the MPO but not in the ST, SCN, AN or ME. The state of lactation increased 5-HT synthesis and, to a lesser degree, 5-HT metabolism in the ME whether the suckling stimulus was present or not. Suckling stimulus also caused a marked increase in serum prolactin. These results support the large amount of evidence by other investigators that 5-HT neuronal systems may be activated during suckling and demonstrate the ability of suckling to differentially increase 5-HT synthesis and metabolism in the MPO without affecting the same parameters in the ST, ME, AN or SCN at a time when serum prolactin is also elevated. These results also suggest that 5-HT neuronal activity in the ME may be elevated in the lactating female rat.

I. 5-Hydroxytryptaminergic neuronal activity in the median eminence, striatum and medial preoptic nucleus was not altered on day 6 of pregnancy at 300, 600, 1200, 1800 or 2100 hr. On the other hand, 5-HT synthesis and concentrations in the AN were increased at 300 and 1800 hr

and 5-HT synthesis was increased in the SCN at 300-600 hr. In addition, synthesis of 5-HT in the SCN was decreased at 2100 hr. Serum prolactin increased at 300-600 hr (nocturnal surge) and at 1800-2100 hr (diurnal surge). When 5-HT synthesis was examined at day 13 of pregnancy (when both the nocturnal and diurnal surges are no longer occurring) the increase in 5-HTP accumulation in the AN that occurred at the time of the nocturnal surge of prolactin was no longer present suggesting that there may be a correlation between the two events. In contrast, the increase in 5-HT synthesis in the SCN that occurred at the time of the nocturnal surge of prolactin is still present on day 13 of pregnancy when the nocturnal surge of prolactin is absent. These results indicate that the changes in 5-HTP accumulation in the SCN are probably not involved with the nocturnal surge of prolactin observed in early pregnancy but the changes in 5-HTP accumulation in the AN may, indeed, play a role in this regulation. Future experiments should be designed to answer this question.

J. Serum prolactin increased at 1500 hr, peaked at 1700 hr and remained elevated above control values until 2400 hr on the afternoon of proestrus in female Long-Evans rats. 5-HT synthesis and metabolism in the SCN increased at 1700-1900 hr and 5-HT synthesis in the ME decreased at 1700 and 2100 hr. These results suggest that 5-hydroxytryptaminergic neuronal activity in the SCN is activated during the early stages of the pre-ovulatory surge of prolactin and is maintained throughout the duration of the prolactin surge. Whether this increase or the abrupt decrease in 5-HT synthesis observed in the median eminence at 1700 hr

are related to the proestrous surge of prolactin or some other event remains to be determined in future investigations.

In summary, 5-HTP accumulation proved to be the more reproducible, easier to interpret and more quickly measured (a single peak) of the two biochemical indices of 5-HT neuronal activity used. Although measuring the concentrations of 5-HT and 5-HIAA before and after treatments produced valuable information concerning changes in 5-HT metabolism in response to those treatments, the use of the ratio of the concentration of 5-HIAA/5-HT as an index of 5-HT neuronal activity can be hard to interpret, insensitive and even misleading.

The method described in this work using HPLC with electrochemical detection provides a simple, sensitive and rapid assay for the concurrent determination of 5-HT, 5-HIAA and 5-HTP in a single sample of ME, AN, SCN, MPO or ST. In addition, DA, DOPAC and HVA can also be resolved under standard operating conditions. The major advantages over existing methods include: 1) simple preparation of biological samples without an extensive prepurification procedure prior to detection; 2) the concurrent separation and detection of all of the compounds measured under the same assay conditions in an 18-22 min period; 3) a sensitivity that is equal to or better than radioenzymatic and GC/MS techniques; 4) greater versatility in the number of compounds that can possibly be measured than with radioenzymatic assays without a high cost of establishment and maintenance of equipment or isotopes as with GC/MS and radioenzymatic techniques; and 5) essentially 100% recovery (loss only due to tissue extraction).

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