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REGULATION OF TUBEROHYPOPHYSEAL
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presented by

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of the requirements for

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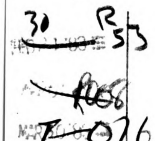

REGULATION OF TUBEROHYPOPHYSEAL DOPAMINERGIC NEURONS

By

Richard H. Alper

A DISSERTATION

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ABSTRACT

REGULATION OF TUBEROHYPOPHYSEAL DOPAMINERGIC NEURONS

By

Richard H. Alper

Radioenzymatic assays for dopamine (DA) and dihydroxyphenylalanine (DOPA) were used to compare the regulation of DA synthesis in the posterior pituitary, median eminence and striatum, brain regions which contain terminals of the tuberohypophyseal, tuberoinfundibular and nigrostriatal DA systems, respectively. DOPA accumulation after decarboxylase inhibition was used as an in vivo estimate of DA synthesis and neuronal activity.

The DA concentrations in the posterior pituitary, median eminence and striatum of rats were unaltered following five days of water deprivation or 2% NaCl drinking water. DOPA accumulation in the posterior pituitary, but not in the median eminence or striatum, was increased following three days of dehydration. The plasma sodium concentration and hematocrit were increased with 12-24 hours dehydration. DOPA accumulation in the posterior pituitary and the plasma sodium concentration, but not the hematocrit, were increased following five days of saline drinking. The data suggest that DA synthesis in the posterior pituitary is selectively increased following extended periods of hypernatremia.

DOPA accumulation in the posterior pituitary was increased 24 hours after the administration of hypertonic saline or mannitol when rats were not permitted access to water. DOPA accumulation had returned to control 36 and 48 hours after saline, but increased again at 72 hours. These data suggest that tuberohypophyseal DA neurons are latently activated by consequences of marked intracellular dehydration induced by hyperosmotic stimuli.

Three days of dehydration consistently increased DA synthesis in the posterior pituitary and the plasma sodium concentration. Both returned to control within 1-3 hours of rehydration; the hematocrit returned much slower. This suggests that increased tuberohypophyseal dopaminergic activity following dehydration is rapidly reversible and parallels plasma or cellular osmolality, but not plasma volume.

In summary, only those DA neurons terminating in the posterior pituitary are regulated by intracellular osmolality. The tuberohypophyseal DA neurons are slow to be activated, but their increased activity can be rapidly restored to control values by rehydration. The significance of the dehydration-induced activation of tuberohypophyseal DA neurons cannot be ascertained until the functional role of these neurons is understood.

to the memory of

Dorothy Eligator Alper

August 8, 1920-August 18, 1976

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INTRODUCTION

Histofluorescent and biochemical studies have revealed several distinct dopaminergic neuronal systems in the rat brain (reviewed by Lindvall and Björklund, 1978; Moore and Bloom, 1978). The nigrostriatal dopamine (DA) system was the first to be studied in great detail. It constitutes the major ascending dopaminergic fiber tract in the brain (see Figure 1). The tuberoinfundibular DA system has been studied extensively using histofluorescent techniques. Biochemical analysis has become possible only recently with the development of sensitive microanalytical techniques capable of measuring the nanogram quantities of DA and norepinephrine (NE) which are present in the median eminence, which contains terminals of the tuberoinfundibular DA neurons. The tuberoinfundibular dopaminergic pathway is known to be involved in the regulation of hormone secretion from the anterior pituitary gland (Weiner and Ganong, 1978). Mechanisms by which the activity of both the nigrostriatal and tuberoinfundibular DA neurons are regulated have been reviewed recently (Moore and Wuerthele, 1979).

A third group of DA neurons depicted in Figure 1 comprise the tuberohypophyseal system. Very little is known about this pathway even though it was revealed by early histofluorescent (Björklund, 1968; Björklund et al., 1970; Smith and Fink, 1972) and electron

Figure 1. Schematic diagram of the distribution of the dopaminergic nerves represented in mid-sagital views of the rat brain. The major ascending DA systems are presented in the upper figure: cp, caudate-putamen (striatum); ML, mesolimbic DA system; na, nucleus accumbens; NS, nigrostriatal DA system; ot, olfactory tubercle; sn, substantia nigra. The hypothalamic DA systems are shown in greater detail in the lower figure: AP, anterior pituitary; ar, arcuate nucleus; HP, hypophyseal portal system; NIL, neuro-intermediate lobe of the pituitary (posterior pituitary); pv, periventricular hypothalamic nucleus; TI, tuberoinfundibular DA system; TH, tuberohypophyseal DA system. Modified from Björklund et al. (1973), Moore and Wuerthele (1979), Moore and Bloom (1978) and Ungerstedt (1971).

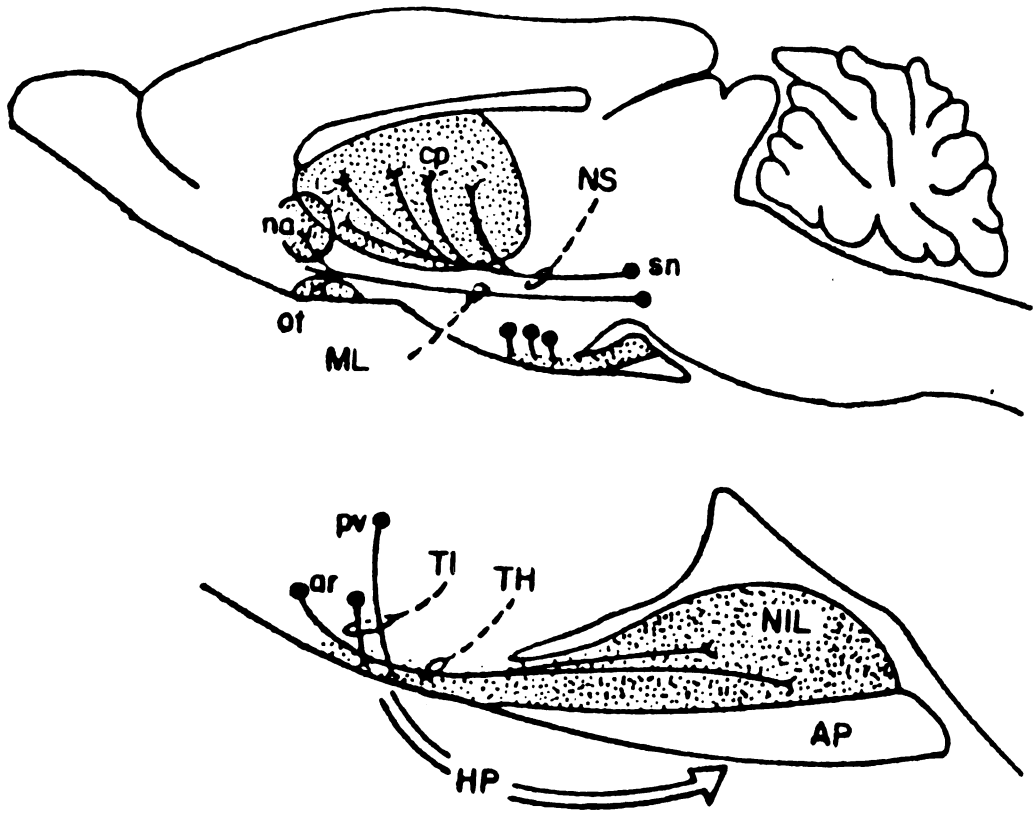

Figure 1

microscopic (Baumgarten et al., 1972) analyses. One reason is that sensitive analytical procedures required to measure the catecholamines contained in the posterior pituitary (the combined neural and intermediate lobes) have only recently been developed. A second reason is that no function has been ascribed to DA in the posterior pituitary.

The present studies represent the first experiments designed specifically to characterize factors regulating the activity of the tuberohypophyseal DA neurons. The regulation of DA synthesis in the terminals of the tuberohypophyseal neurons was compared and contrasted to the regulation of DA synthesis in the terminals of the tuberoinfundibular and nigrostriatal DA neurons. The nigrostriatal system was included in these studies primarily because most of the information on the relationship between neuronal activity and DA turnover has been ascertained from studies conducted on this system. The tuberoinfundibular DA system was included in these studies to determine if DA synthesis in the terminals of the anatomically related tuberoinfundibular and tuberohypophyseal DA systems is regulated by similar mechanisms.

I. Neuroanatomy

The initial description of the monoamine-containing neurons in the the posterior pituitary was by Björklund (1968). Several other subsequent reports using histofluorescent techniques (Björklund et al., 1970, 1973; Smith and Fink, 1972; Tilders et al., 1979) or electron microscopy (Baumgarten et al., 1972) have appeared in the literature. Although these neuroanatomical techniques provide invaluable information

they have several serious deficiencies, particularly when used to study the catecholaminergic innervation of the posterior pituitary. First, great variability in the fluorescent patterns observed throughout the posterior pituitary was reported in the initial studies (Björklund, 1968; Björklund et al., 1970). Björklund and coworkers (1973) partially overcame this obstacle by administering α-methyl NE to their rats prior to sacrifice. This treatment enhanced the fluorescent intensity of all catecholaminergic (both dopaminergic and noradrenergic) neurons. A second methodological problem, one that could not be overcome, was the inability of histofluorescent techniques to clearly distinguish between DA and NE; both amines are present in the posterior pituitary. The investigators (Baumgarten et al., 1972; Björklund et al., 1970) felt that the histofluorescence remaining in the posterior pituitary following bilateral removal of the superior cervical ganglia represented only DA-containing neurons. The NE in the posterior pituitary was postulated to be contained in terminals of peripheral sympathetic nerves. Holzbauer et al. (1980b) have used biochemical assays to demonstrate that approximately 50% of the noradrenergic innervation of the posterior pituitary originates in the superior cervical ganglia. This has been subsequently confirmed (see Results I.A). The histofluorescent descriptions of the dopaminergic innervation of the posterior pituitary may not be completely accurate due to the interference of NE. In addition, newer anatomical techniques (e.g., horseradish peroxidase and immunocytochemistry) have not been applied successfully to study the tuberohypophyseal DA system.

Therefore, the anatomical descriptions that follow are based solely on results of histofluorescent and electron microscopic studies.

The tuberohypophyseal DA neurons may actually be two distinguishable systems (Björklund et al., 1973). One component originates from a small group of cells in the most rostral regions of the arcuate nucleus (area A_{12}; Dahlström and Fuxe, 1964). These neurons innervate the entire intermediate lobe of the pituitary after passing through the median eminence and infundibular stalk. A second group of DA perikarya lies immediately caudal to this first group. These neurons also pass through the median eminence but innervate the neural lobe. As pointed out by Björklund et al. (1974), one cell in the rostral arcuate nucleus may have terminals in the median eminence as well as the neural and intermediate lobes of the pituitary. For this reason, the arbitrary assignation of the names tuberohypophyseal and tuberoinfundibular DA systems projecting to localized sites in the posterior pituitary and median eminence, respectively, may be misleading.

The catecholaminergic innervation throughout the median eminence-pituitary region of the rat appears to be a continuum of terminals (Figure 2). There is no clear anatomical demarcation between the median eminence, the infundibular stem and the posterior pituitary. In the present studies the tuberohypophyseal DA system will be considered as one fiber tract (in deference to Björklund et al., 1973) terminating in the posterior pituitary. The tuberoinfundibular DA system, terminating in the median eminence, will be considered as another (Cuello et al., 1973; see Materials and Methods II for detailed description).

Figure 2. Schematic representation of the catecholaminergic innervation of the median eminence-pituitary region of the rat as demonstrated by histofluorescent microscopy. Abbreviations: VIII, third ventricle; NL, neural lobe of the pituitary; PI, pars intermedia (intermediate lobe of the pituitary); PD, pars distalis (anterior pituitary). Taken from Björklund et al. (1973).

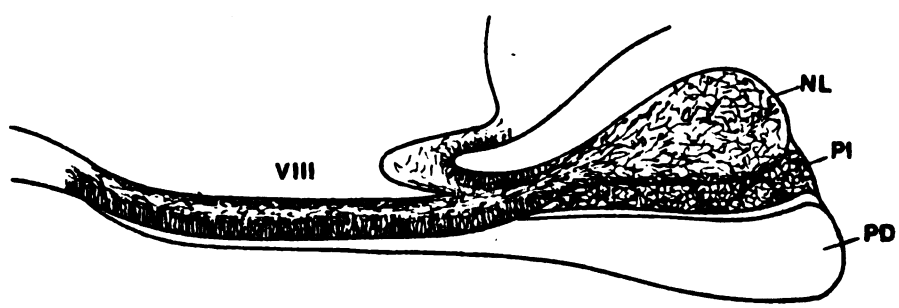

Figure 2

Histofluorescent analysis has demonstrated that the cell bodies of the tuberohypophyseal DA neurons are contained within the medial basal hypothalamus (Tilders et al., 1979). The axons are predominantly of a fine varicose type which form a plexus between the endocrine cells of the intermediate lobe (Björklund et al., 1970; Tilders et al., 1979). In the intermediate lobe the DA neurons may make direct contact on hormone secreting cells. These hormone secreting cells probably contain α-melanocyte stimulating hormone (MSH). DA most likely acts to tonically inhibit MSH secretion from the pituitary (for review see Tilders and Smelik, 1977). In the neural lobe the dopaminergic neurons have been reported to approximate (80-120 Å), but not to make true synaptic contacts with (no membrane thickenings) neurosecretory axons and pituicytes (Baumgarten et al., 1972). Tilders et al. (1979) observed a plexus of DA terminals in the neural lobe (particularly the rostral aspects) around the neurohypophyseal capillary beds.

An unusual feature of the nerve fibers in the posterior pituitary is the occurrence of large axonal swellings (greater than 2 μ in diameter) filled with typical DA fluorescence (Baumgarten et al., 1972; Björklund, 1968). It was suggested that this fluorescence represents spontaneously degenerating DA axons and that tuberohypophyseal DA fibers are undergoing continuous reorganization through degeneration-regeneration cycles. The significance of this is unclear, however.

The DA neurons which terminate in the median eminence are part of a large, diverse tuberoinfundibular neuronal system. The cell bodies of these tuberoinfundibular tracts reside primarily in the medial basal hypothalamus. This has been demonstrated with a variety of techniques, including Golgi methods (Bodoky and Rethelyi, 1977), surgical isolation (Halasz et al., 1965), histofluorescent methods (Björklund et al., 1973) and microiontophoresis of horseradish peroxidase into the median eminence (Lechan et al., 1980).

The anatomical distribution of the tuberoinfundibular DA neurons in the rat has been the subject of several recent reviews (Lindvall and Björklund, 1978; Moore and Wuerthele, 1979; Moore and Bloom, 1978). Histofluorescent studies have used selective mechanical and electrolytic lesions (Björklund et al., 1973, 1974) to suggest that the cell bodies of the tuberoinfundibular DA system lie in the arcuate and anterior periventricular hypothalamic nuclei (A_{12}; Dahlström and Fuxe, 1964). These dopaminergic perikarya are not densely packed and constitute only a small fraction of the cells found in the arcuate nucleus (Bodoky and Rethelyi, 1977; Fuxe and Hökfelt, 1967; Renaud et al., 1978).

One report suggests that some DA neurons terminating in the median eminence originate in the ventral tegmental area (Kizer et al., 1976b). There is, however, much histochemical (Jonsson et al., 1972; Löfström et al., 1976) and biochemical (Brownstein et al., 1976; Gallardo et al., 1978; Gudelsky et al., 1978) evidence demonstrating that the DA concentration of the median eminence is not reduced following complete hypothalamic deafferentation.

The majority of the tuberoinfundibular DA neurons have short axons projecting in a dorsoventral orientation from all parts of the arcuate nucleus. These neurons terminate in corresponding parts of the external layer of the median eminence (Björklund et al., 1973; Smith and Fink, 1972). In the external layer, the terminals are packed very densely in close proximity to the capillaries of the hypothalamo-hypophyseal portal system (Fuxe and Hökfelt, 1967). DA is released from these nerve terminals in the median eminence and transported by the hypophyseal portal circulation to tonically inhibit prolactin secretion from the anterior pituitary gland (Gibbs and Neill, 1978; Weiner and Ganong, 1978).

The tuberoinfundibular DA nerves also terminate in the lateral palisade zone of the median eminence. Here the DA-containing neurons are in close proximity to neurons containing luteinizing hormone releasing hormone (gonadotropin releasing hormone; Hökfelt et al., 1976; Sladek et al., 1978) and tanycytes (Sladek and Sladek, 1978).

The precise topographical projections of the nigrostriatal DA system have been recently reviewed (Moore and Wuerthele, 1979; Moore and Bloom, 1978). A brief description of this major ascending dopaminergic pathway is provided because the relationships between the electrical activity (i.e., firing rate) and biochemical activity (i.e., DA synthesis and turnover) in dopaminergic systems has been obtained from studies on the nigrostriatal neurons.

The axons forming the nigrostriatal DA pathway arise from cells mainly in the pars compacta of the substantia nigra (areas A_8 and A_9 of the rat brain; Dahlström and Fuxe, 1964), although a few may

originate in the adjacent ventral tegmental area (A_{10}; Dahlstrom and Fuxe, 1964). The DA cell bodies in the substantia nigra are very densely packed, comprising approximately 80% of the total cells in this region (Andén et al., 1966). The fine unmyelinated DA axons (Lindvall and Björklund, 1974) ascend from the substantia nigra through the medial tegmentum to traverse the lateral hypothalamic areas and the internal capsule (Ungerstedt, 1971; Moore et al., 1971). Some fibers terminate in the globus pallidus but most distribute throughout the striatum (the caudate-putamen). The axons undergo massive collateralization in the striatum forming synaptic-like contacts with small striatal dendrites (Hattori et al., 1973; McGeer et al., 1975). The nigrostriatal DA projection has a highly specific and well organized topography (Carpenter and Peter, 1972; Moore et al., 1971).

II. Functions of Dopaminergic Pathways

The anatomical descriptions of the tuberohypophyseal, tuberoinfundibular and nigrostriatal DA fiber tracts provided above document the diverse regions of the brain receiving dopaminergic innervation. DA would be expected to perform a variety of functions with such a widespread distribution throughout the central nervous system. Some of the functions postulated for DA in these diverse neuronal networks will be discussed briefly.

The presence of DA in the posterior pituitary gland of a variety of mammals has been known for more than a decade. Fuxe and Hökfelt (1967) proposed that catecholamines probably influenced the release of

hormones from the posterior pituitary. Recent technicological advances such as immunocytochemistry, radioimmunoassay and high pressure liquid chromatography provide sensitive analytical techniques for studying the various peptides and hormones of the posterior pituitary. However, an unqualified function for DA in the posterior pituitary has yet to be elucidated.

There is now substantial evidence for the tonic dopaminergic inhibition of MSH secretion from the intermediate lobe of the pituitary (reviewed by Tilders and Smelik, 1977). DA antagonists (haloperidol, pimozide and sulpiride) rapidly and markedly increase, whereas DA agonists (bromocriptine and apomorphine) decrease the serum MSH concentration in rats (Penny et al., 1979; Penny and Thody, 1978; Usategui, 1976). These dopaminergic agents appear to act on DA receptors in the intermediate lobe and not in higher brain centers. DA, in a concentration dependent manner, inhibits the in vitro release of MSH from isolated posterior pituitaries (Bower et al., 1974; Tilders et al., 1979). The addition of potassium into the incubation medium also inhibits MSH secretion in vitro, this results from potassium-induced DA release (Tilders et al., 1979). These data imply that the tuberohypophyseal DA neurons inhibit MSH secretion. Nevertheless, other biogenic amines and polypeptides may also be involved in the regulation of MSH release from the posterior pituitary (Tilders and Smelik, 1977).

The evidence for dopaminergic mediation or modulation of other posterior pituitary hormones is not as convincing (Brown et al., 1979). Complicating factors are that DA can influence hormone release from

two sites. The first is at the magnocellular neurosecretory neurons located primarily in the supraoptic and paraventrciular hypothalamic nuclei. The second site where DA could alter the release of posterior pituitary hormones is at the nerve terminals in the neurohypophysis.

Some examples of contradictory reports will be provided. The addition of DA to isolated neural lobes may stimulate (Bridges et al., 1976; Negro-Vilar, 1979) or not alter (Hisada et al., 1977) arginine vasopressin (AVP; antidiuretic hormone) secretion. Alternatively, in vivo studies have reported that DA inhibits (Givant and Sulman, 1976) or does not affect (Kendler et al., 1978) the release of AVP into the blood. To date, the role of tuberohypophyseal DA neurons on tonic AVP secretion is uncertain.

It is possible that the tuberohypophyseal DA neurons do not tonically regulate AVP secretion but are involved as modulators during stimulation of the hypothalamo-neurohypophyseal system. For example, in a recent abstract Robinson et al. (1981) demonstrated that the DA agonist bromocriptine augmented AVP secretion in humans administered hypertonic saline intravenously. This study needs to be confirmed.

The data relating DA to oxytocin release is also contradictory. DA inhibits oxytocin release from hypothalamo-neurohypophyseal system explants (Seybold et al., 1978), stimulates oxytocin release from isolated neural lobes (Bridges et al., 1976), but may not be involved in the release of oxytocin in vivo (Fuchs et al., 1981; Russell et al., 1981).

The effects of DA on other posterior pituitary functions have not been studied. However, some hypothetical alternatives are included in Section III of the Discussion.

The tuberoinfundibular DA neurons terminate very near the capillary loops of the hypothalamo-hypophyseal portal system which invaginate the ventral surface of the median eminence. Thus, DA released into the portal blood may be transported directly to the anterior pituitary gland to regulate hormone secretion. Consequently, sufficient quantities of DA have been found in the hypophyseal portal blood to tonically inhibit the release of prolactin from the anterior pituitary (Gibbs and Neill, 1978). The DA concentration in the portal blood is significantly greater than that found in the systemic circulation (Ben-Jonathan et al., 1977). It is currently believed that the principle function of the tuberoinfundibular DA neurons is in the tonic regulation of prolactin (and possibly luteinizing hormone) secretion from the anterior pituitary (for review see Weiner and Ganong, 1978).

The nigrostriatal DA system is primarily involved in the regulation and coordination of motor functions. Degeneration of the nigrostriatal pathway is believed to be responsible for the akinesia and rigidity associated with Parkinson's disease (Hornykiewicz, 1966, 1977). The most specific lesion found in Parkinson's disease is the loss of melanin-containing neurons in the substantia nigra. The neurochemical correlate associated with this lesion is a marked depletion of the striatal DA concentration. In animal studies the

nigrostriatal DA neurons have been postulated to mediate the stereotyped behaviors induced by DA agonists (e.g., d-amphetamine or apomorphine) but not the locomotor activation induced by these drugs (for review see Moore and Kelly, 1978).

III. Regulation of Neuronal Activity

A. Catecholamine Biochemistry

Impulse traffic in aminergic systems in the central nervous system can be studied using electrophysiological techniques. The activity of central DA and NE neurons can be estimated by recording from the cell bodies in the substantia nigra and pons-medulla, respectively. This is not a difficult task in the nigrostriatal DA system since the cell bodies in the substantia nigra are densely packed. Electrophysiological techniques have not been used to study the tuberohypophyseal DA neurons. Antidromic stimulation from the posterior pituitary to the arcuate nucleus could be used to identify cells belonging to the tuberohypophyseal DA system. The likelihood of recording either intracellularly or extracellularly from an identifiable DA perikarya in the arcuate nucleus is very small. The DA cells (particularly those projecting to the posterior pituitary and not the median eminence) constitute a very minor proportion of the total cellular population of the arcuate nucleus. Therefore, biochemical indices of DA turnover must be used to estimate dopaminergic activity in the posterior pituitary.

Most of the neurochemical events occurring in DA nerve terminals have been studied in the striatum (see review by Moore and

Wuerthele, 1979). The relationships existing between neuronal activity and the synthesis and release of DA from the nerve terminals in the posterior pituitary and median eminence are assumed to be similar to the events that have been previously described in the striatum.

A schematic representation of catecholamine synthesis is depicted in Figure 3. Tyrosine is transported into DA neurons and converted to L-dihydroxyphenylalanine (DOPA) by the rate limiting enzyme tyrosine hydroxylase. This enzyme is regulated, in part, by endproduct feedback inhibition. Therefore, decreases in the releasable pool of DA will result in increases in DA synthesis; the converse is also true. Endproduct inhibition generally maintains the concentration of DA in the nerve terminals constant despite changes in the amount of DA released. DOPA is rapidly decarboxylated to DA by aromatic L-amino acid decarboxylase (DOPA decarboxylase). In dopaminergic neurons the newly synthesized DA can be stored in synaptic vesicles or released into the synaptic cleft in response to neuronal depolarization. In noradrenergic neurons, DA is hydroxylated to form NE by the enzyme dopamine β-hydroxylase. This enzyme is contained in the synaptic vesicles of noradrenergic neurons and is absent from dopaminergic neurons. NE, like DA, is released from nerve terminals in response to action potentials.

Catecholamines are released into the synaptic cleft where they can interact with putative pre- and postsynaptic receptors. The amount of amine in the synapse is dependent on its rate of release and metabolism. High affinity uptake systems for both DA and NE are the

Figure 3. Schematic representation of catecholamine synthesis. Represented to the left of the arrows are the enzymes involved in each reaction, to the right are inhibitors of these enzymes. The dashed line connecting DA and NE indicates that dopamine β-hydroxylase is present only in noradrenergic nerve terminals. This enzyme is not found in dopaminergic neurons.

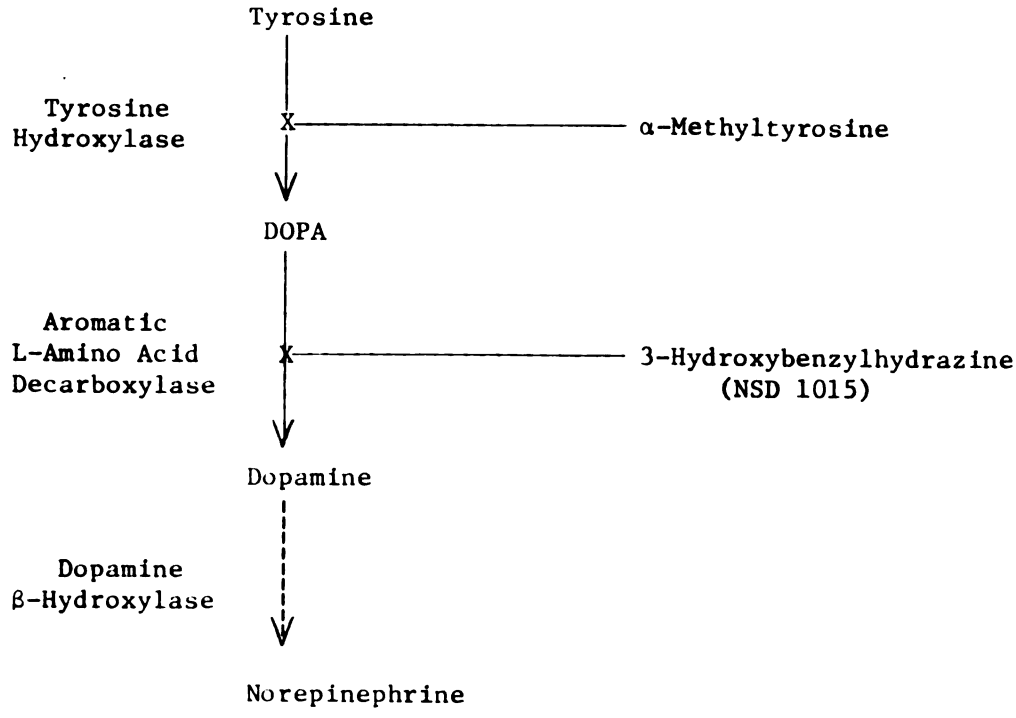
Tyrosine
Tyrosine Hydroxylase
α-Methyltyrosine
DOPA
Aromatic L-Amino Acid Decarboxylase
3-Hydroxybenzylhydrazine (NSD 1015)
Dopamine
Dopamine β-Hydroxylase
Norepinephrine

Figure 3

major routes of inactivation for these amines (exceptions do exist, see below). Following reuptake, DA and NE are exposed to mitochondrial monoamine oxidase and are deaminated to form 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethylglycol (DOPEG), respectively, in the brain. These deaminated catechols are further metabolized by catechol-o-methyltransferase (COMT) to form homovanillic acid (from DOPAC) and 3-methoxy-4-hydroxyphenylethylglycol (MHPG; from DOPEG).

In the striatum the concentration of DOPAC provides a useful index of alterations in the functional activity of the nigrostriatal DA neurons (Roth et al., 1976). That is, an increase in the striatal DOPAC concentration generally reflects an increase in nigrostriatal dopaminergic activity while a decrease in DOPAC reflects a decrease in neuronal activity. The utility of measuring DOPAC concentrations for biochemical indices of neuronal activity in various brain regions requires the presence of a high affinity DA uptake system. This system is not present in the posterior pituitary (Annunziato and Weiner, 1980; Demarest and Moore, 1979b) or the median eminence (Annunziato et al., 1980; Demarest and Moore, 1979b). An inadequate reuptake mechanism may be partially responsible for the low DOPAC concentration reported in the posterior pituitary (Annunziato and Weiner, 1980; Umezu, Alper and Moore, unpublished observations) and the median eminence (Fekete et al., 1979; Umezu and Moore, 1979). The lack of a high affinity DA uptake system and the resultant low concentration of DOPAC explain why this DA metabolite is not a good index of neuronal

activity for the tuberohypophyseal and tuberoinfundibular DA neurons (Moore et al., 1979; Umezu and Moore, 1979).

The concentration of MHPG or its sulfate metabolite has been used to estimate noradrenergic neuronal activity in the brain (Adér et al., 1978; Kohno et al., 1981; Korf et al., 1973). To date, this technique has not been employed to estimate the activity of the noradrenergic neurons terminating in the posterior pituitary or median eminence (NE is virtually undetectable in the striatum).

The events described above characterize DA biochemistry in the striatum. Most, but not all, of the same events occur in the median eminence (reviewed by Moore and Wuerthele, 1979). One of the major differences is that the dopaminergic terminals in the median eminence end in close approximation to the capillaries of hypothalamo-hypophyseal portal system. Thus, dopamine released from these neurons does not act on receptors located across a synaptic cleft. Instead, this amine is thought to be transported to the anterior pituitary to activate DA receptors located on prolactin-secreting cells. The events occurring after DA is released in the posterior pituitary are not known. Putative DA receptors have been described in the posterior pituitary (Sibley and Creese, 1980; Stefanini et al., 1980a; but the lack of a high affinity DA uptake mechanism leads one to speculate that DA may be released directly into the neurohypophyseal circulation. Thus, there would be no need for a high affinity reuptake mechanism to inactivate DA in the posterior pituitary, analogous to the median eminence.

B. Biochemical Techniques

Biochemical estimates of dopaminergic nerve activity rely on measurements of DA turnover and synthesis. The concentration of DA (and NE) in nerve terminals does not change appreciably even when impulse flow is markedly altered. It is assumed that synthesis of the amine is increased to replenish the amount released. Since the rate limiting step in catecholamine synthesis is the hydroxylation of tyrosine by tyrosine hydroxylase, *in vivo* and *in vitro* estimates of the activity of this enzyme will generally reflect catecholamine synthesis and neuronal activity.

Despite the number of assumptions that must be made to measure catecholamine turnover and synthesis (Weiner, 1974), useful information about the regulation of dopaminergic neurons has been obtained employing biochemical techniques. Two nonsteady state procedures to estimate catecholamine synthesis and turnover rates *in vivo* are the decline of catecholamine concentrations after synthesis inhibition (Brodie *et al.*, 1966; Spector *et al.*, 1965) and the rate of DOPA accumulation following decarboxylase inhibition (Carlsson *et al.*, 1972; Carlsson and Lindqvist, 1973; Demarest and Moore, 1980) (see Figure 3).

The rate of decline of both DA and NE after inhibition of tyrosine hydroxylase by α-methyltyrosine (αMT) follows first-order kinetics and is proportional to neuronal activity (Brodie *et al.*, 1966). When the activity of catecholamine neurons is increased, the rate of decline following αMT is similarly increased. This method

allows for separate estimations of both DA and NE turnover in brain regions such as the posterior pituitary and median eminence where both amines are present.

Tyrosine hydroxylase activity can be estimated *in vivo* by quantifying the rate of accumulation of DOPA following the administration of the centrally acting DOPA decarboxylase inhibitors benserazide (Ro4-4602) or 3-hydroxybenzylhydrazine (NSD 1015; see Figure 3). Roth *et al.* (1975) have demonstrated that the rate of DOPA accumulation in the striatum after the administration of a decarboxylase inhibitor is directly related to the activity of the nigrostriatal DA neurons; an increase in neuronal activity is reflected in an increase in the rate of DOPA accumulation.

Since tyrosine hydroxylase is contained in all catecholaminergic neurons, changes in enzyme activity reflect changes in DA and/or NE synthesis. This is not a problem in the striatum where the NE concentration is virtually zero. However, in the posterior pituitary and median eminence the ratio of DA to NE is about 3:1 (see Results, Tables 1-3). Kizer *et al.* (1976a) observed that *in vitro* tyrosine hydroxylase activity estimates DA synthesis, but not NE synthesis, in the median eminence. It will be demonstrated in Results Section I.B that the rate of DOPA accumulation after decarboxylase inhibition (an *in vivo* measurement of tyrosine hydroxylase activity) reflects primarily DA synthesis in the posterior pituitary and median eminence.

C. Regulatory Mechanisms

1. Receptor mediated regulation

The activity of the nigrostriatal DA neurons is altered by dopaminergic drugs, both agonists and antagonists. These drugs are believed to activate either postsynaptic DA receptors which regulate nigrostriatal activity through a long neuronal striatonigral feedback loop and/or presynaptic DA receptors (autoreceptors) located on the dopaminergic neurons (see reviews by Moore and Wuerthele, 1979; Nowycky and Roth, 1978). The location of the receptors mediating the effects of the dopaminergic agents on neuronal activity and DA synthesis is irrelevant for the purpose of this discussion.

Acute systemic injections of DA antagonists (e.g., haloperidol, chlorpromazine, spiroperidol, pimozide) increase: 1) the nigrostriatal firing rate (Bunney et al., 1973a), 2) the release of DA in the striatum (Chéramy et al., 1970), 3) the concentration of DOPAC in the striatum (Andén et al., 1964), 4) the αMT-induced decline of DA in the striatum (Gudelsky and Moore, 1977), and 5) the rate of DOPA accumulation in the striatum (Demarest and Moore, 1979a, 1980). Conversely, drugs that stimulate DA receptors (e.g., apomorphine, bromocriptine, piribedil, L-DOPA) decrease: 1) nigrostriatal firing rate (Bunney et al., 1973b), 2) striatal DOPAC concentrations (Roos, 1969), 3) the αMT-induced decline of DA in the striatum (Andén et al., 1967), and 4) the rate of DOPA accumulation in the striatum (Demarest and Moore, 1979a). That is, DA antagonists increase whereas DA agonists decrease both electrophysiological and biochemical measurements of nigrostriatal dopaminergic neuronal activity.

The tuberohypophyseal DA system appears to be regulated, in part, by receptor mediated mechanisms. Demarest and Moore (1979a) have reported that haloperidol caused a moderate increase in the rate of DOPA accumulation in the posterior pituitary (45% as compared to an increase of 400% in the striatum). Apomorphine, on the other hand, caused only a moderate decrease in the rate of DOPA accumulation in the posterior pituitary when compared to the decrease observed in the striatum. Although the tuberohypophyseal DA neurons are regulated by receptor mediated mechanisms, DA synthesis in the posterior pituitary is not quantitatively as sensitive to dopaminergic drugs as is DA synthesis in the striatum. The tuberoinfundibular DA system, on the other hand, is unresponsive to acute injections of DA agonists or antagonists (Demarest and Moore, 1979a, 1980; Fuxe and Hökfelt, 1974; Gudelsky and Moore, 1976, 1977).

Autoreceptors have been postulated to play an important role in modulating the synthesis of DA in the nigrostriatal DA system (Nowycky and Roth, 1978). The administration of γ-butyrolactone (GBL) or baclofen, both of which inhibit impulse flow in the nigrostriatal DA system, increase the concentration and rate of synthesis of DA in the striatum (Carlsson et al., 1977; Demarest and Moore, 1979a; Moore and Demarest, 1980; Walters et al., 1973). It is believed that complete cessation of impulse flow leads to the inhibition of DA released into the synaptic cleft. The presynaptic DA receptors are no longer activated leading to a disinhibition of tyrosine hydroxylase. The combination of an increase in synthesis and a decrease in release of DA causes marked elevations in the striatal DA concentration.

Both baclofen and GBL increase the concentration and the rate of synthesis of DA in the posterior pituitary but not in the median eminence (Demarest and Moore, 1979a; Moore and Demarest, 1980). Apparently, the tuberohypophyseal DA system, like the nigrostriatal DA system, is regulated, in part, by presynaptic autoreceptors.

In summary, DA synthesis in the posterior pituitary and the striatum, but not in the median eminence, is regulated by blockade and activation of DA receptors.

2. Endproduct feedback regulation

As noted in section III.A. of the Introduction, tyrosine hydroxylase is regulated by endproduct feedback inhibition. This is true in the tuberohypophyseal, tuberoinfundibular and nigrostriatal DA systems. Demarest and Moore (1979a) have reported that reserpine decreased the DA concentration and increased the rate of DOPA accumulation (i.e., tyrosine hydroxylase activity) in the posterior pituitary, median eminence and striatum. In these same regions the monoamine oxidase inhibitor nialamide increased the DA concentration and decreased the rate of DOPA accumulation. More recently, the same authors (Demarest and Moore, in press) have demonstrated that the selective Type A monoamine oxidase inhibitor clorgyline mimicked the actions of nialamide, whereas the Type B monoamine oxidase inhibitor deprenyl did not. Thus, it was suggested that the intraneuronal deamination of DA in central dopaminergic pathways is catalyzed by Type A monoamine oxidase and that tyrosine hydroxylase in all central dopaminergic systems is regulated by endproduct feedback.

3. Regulation by morphine and other opiates

The interactions of narcotic analgesics and, more recently, the opioid peptides with central catecholaminergic neuronal systems have been the subject of intensive investigation (see review by Iwamoto and Way, 1979). Briefly, it has been demonstrated that acute administration of morphine increased single unity activity in the substantia nigra (Nowycky et al., 1978), the rate of DOPA accumulation in the striatum (Alper et al., 1980; Garcia-Sevilla et al., 1978; Persson, 1979), the concentration of DOPAC in the striatum (Alper et al., 1980; Nowycky et al., 1978), the release of DA in the striatum (Chesselet et al., 1981) and the αMT-induced decline of DA in the striatum (Alper et al., 1980).

On the other hand, morphine decreases the αMT-induced decline of DA in the median eminence (Alper et al., 1980; Deyo et al., 1979), the rate of DOPA accumulation in the median eminence (Alper et al., 1980) and the release of DA into the hypophyseal portal blood (Gudelsky and Porter, 1979). In contrast, DA synthesis and turnover were not altered in the posterior pituitary following morphine administration (Alper et al., 1980).

The results of these neurochemical studies reveal that morphine stimulates the nigrostriatal DA system, inhibits the tuberoinfundibular DA system and does not influence the tuberohypophyseal DA system. None of these dopaminergic pathways are tonically regulated by the endogenous opiates since naloxone administration per se does not alter DOPA accumulation in the striatum, median eminence or posterior pituitary (Alper et al., 1980).

4. Endocrinological and related regulatory mechanisms

Hypothalamic monoaminergic mechanisms regulating the release of hormones from the anterior and posterior pituitary gland have been reviewed by many authors (see, for example, Knowles and Vollrath, 1974; Weiner and Ganong, 1978). It is now realized that hormones of the pituitary may, in turn, influence various central catecholaminergic neuronal pathways (Moore et al., 1980a,b; Moore and Wuerthele, 1979). Three endocrinological regulatory mechanisms will be discussed below.

a. Prolactin. The dopaminergic inhibition of prolactin secretion from the anterior pituitary has been discussed previously (see Introduction, Section II). It has been demonstrated using both histochemical and biochemical techniques that injections of rat or ovine prolactin systemically or into the cerebrospinal fluid (CSF) increase tuberoinfundibular dopaminergic neuronal activity 12-16 hours after the prolactin injections (Annunziato and Moore, 1978; Hökfelt and Fuxe, 1972; Wiessel et al., 1978). Prolactin has been implicated in the regulation of tuberoinfundibular but not nigrostriatal DA nerves. High doses of haloperidol latently increase DA turnover in the median eminence and not the striatum following hypophysectomy or hypothalamic deafferentation (Demarest and Moore, 1980; Gudelsky et al., 1978). Furthermore, recent data suggest that the tuberoinfundibular, but not the tuberohypophyseal, DA neurons are activated following estradiol benzoate administration (Moore et al., 1980b), intraventricular prolactin infusions (Johnston et al., 1980) and pituitary transplantation under the kidney capsule (Morgan and Herbert,

1980). All treatments are known to significantly elevate serum or CSF prolactin concentrations.

In summary, prolactin is involved in the selective regulation of tuberoinfundibular dopaminergic neuronal activity. The tuberohypophyseal and nigrostriatal DA systems are unresponsive to hyperprolactinemia.

b. α-Melanocyte stimulating hormone. DA inhibits the secretion of MSH from the intermediate lobe of the pituitary. It has been proposed, though certainly not unequivocally proven, that MSH secretion might be regulated through an inhibitory feedback loop (Kastin and Schally, 1967). One could speculate that the tuberohypophyseal DA neurons are involved in MSH autoregulation, similar to the role of the tuberoinfundibular DA system in prolactin secretion.

Lichtensteiger and coworkers (Lichtensteiger and Lienhart, 1977; Lichtensteiger and Monnet, 1978, 1979; Lichtensteiger et al., 1977) have examined the fluorescent intensity in the arcuate nucleus and substantia nigra following systemic injections of MSH. The results are quite complex but the basic interpretation put forth by the authors is that MSH increases the activity of the DA neurons in the arcuate nucleus and, to a slightly lesser degree, the activity of the nigrostriatal DA neurons. There are two serious flaws to these experiments. The first is that changes in fluorescent intensity in dopaminergic cell bodies in the substantia nigra and arcuate nucleus do not necessarily correlate with the amount of DA released from the terminals of these neurons. Secondly, the DA cell bodies in the

arcuate nucleus do not constitute a homogeneous population. The neurons project to different areas throughout the median eminence-posterior pituitary region (Björklund et al., 1973) and respond differentially to pharmacological treatments and endocrinological manipulations (Alper et al., 1980; Moore et al., 1980b).

Subcutaneous injections of MSH cause a dose related increase in the rate of DOPA accumulation in the posterior pituitary, but not in the median eminence or striatum (Alper and Moore, unpublished observations; Moore et al., 1980b). The effect is rapid (observed only 60-90 minutes following MSH administration) and small though statistically significant (DOPA accumulation is increased 20-25% above control). The small effect could be related to the observations of Björklund et al. (1973) on the divisions of the tuberohypophyseal DA system. MSH might regulate only the activity of the DA neurons terminating in the intermediate lobe, but not the neural lobe, of the pituitary. The neural lobe contains more DA (and probably DOPA) than the intermediate lobe (Saavedra et al., 1975). The studies by Moore et al. (1980b) analyzed the combined neural and intermediate lobes for DOPA. The data do lend some support to the hypothesis that the tuberohypophyseal DA nerves are part of an MSH feedback loop and suggest that the tuberohypophyseal DA nerves can be influenced by a factor (MSH) that has no effect on tuberoinfundibular and nigrostriatal DA neurons.

c. Dehydration. The effects of dehydration on central catecholamines have been reported in several papers (Holzbauer et al., 1978, 1980a,b; Torda et al., 1979). Torda and coworkers (1979) reported that water deprivation of 72 hours increased the DA concentration in the posterior pituitary; no other brain region was examined. Holzbauer et al. (1978, 1980b) generally, although not always, observed an increase in the DA content of the posterior pituitary following 72 hours of water deprivation plus an additional 18-96 hours access to a 2.5% NaCl drinking solution. There was no change in the DA content of the striatum or medial basal hypothalamus. In contrast to reports by Torda et al. (1979), Holzbauer et al. (1978) observed no change in the DA content of rats deprived of water for 72 hours. Furthermore, the posterior pituitary content of DA was not altered in homozygous Brattleboro rats or their heterozygous controls following 72 hours of water deprivation and then 24 hours access to 2.5% NaCl (Holzbauer et al., 1980a).

The authors of the papers cited above suggest that prolonged stimulation of the hypothalamo-neurohypophyseal system alters the functional state of the tuberohypophyseal DA neurons. Although the data on posterior pituitary DA concentrations are interesting, they are difficult to interpret. An increase in the steady state DA concentration in terminals of the tuberohypophyseal neurons could result from: 1) a decrease in the rate of release, 2) an increase in the rate of synthesis which exceeds release, or 3) a decrease in the rate of intraneuronal metabolism. Therefore, it is

impossible to precisely determine functional changes in tuberohypophyseal dopaminergic neuronal activity by measuring the concentrations of DA in the posterior pituitary. Furthermore, it was not determined if dehydration was selectively altering the tuberohypophyseal dopaminergic system or if dehydration was affecting the metabolic activity in all dopaminergic pathways in the rat brain.

PURPOSE

The synthesis of DA in the terminals of tuberohypophyseal neurons is regulated, in part, by mechanisms which are also intrinsic to the ascending nigrostriatal and mesolimbic dopaminergic neurons terminating in various forebrain regions. In particular, tyrosine hydroxylase in the posterior pituitary is regulated through endproduct feedback inhibition and receptor mediated mechanisms. Tuberohypophyseal DA neurons in contrast to the anatomically similar tuberoinfundibular DA neurons are not regulated by the anterior pituitary hormone prolactin. Reports have suggested that the functional activity of the tuberohypophyseal DA neurons might be altered during prolonged dehydration. It is unknown whether dehydration increases or decreases tuberohypophyseal dopaminergic neuronal activity. Furthermore, the mechanisms underlying the dehydration-induced effects on the dopaminergic neurons terminating in the posterior pituitary have not been characterized.

The purposes of the present studies are to: 1) demonstrate that the tuberohypophyseal DA neurons are tonically active. This will be accomplished using two in vivo biochemical estimates of DA synthesis and turnover, and 2) use a biochemical index of DA synthesis to characterize the responses of the tuberohypophyseal DA neurons to dehydration.

MATERIALS AND METHODS

I. Animals

Male and female Sprague-Dawley rats (150-250 g) were obtained from Spartan Research Animals, Haslett, MI, and maintained under 12 hour periods of light and dark (lights on from 0700 to 1900 h). Rats were housed 4 per cage and allowed free access to food and tap water for at least 3 days prior to the start of any experiment.

II. Tissue Dissections and Extraction

Rats were sacrificed by decapitation. The brain was carefully removed from the skull leaving the entire pituitary gland in the sella turcica. The pituitary gland was gently freed of connective tissue, removed from the skull and placed on a cold plate. The neural and intermediate lobes of the pituitary (the posterior pituitary) were teased from the anterior pituitary with a pair of fine forceps under a dissecting microscope.

The median eminence was removed from the base of the hypothalamus with the aid of a dissecting microscope as described previously (Cuello et al., 1973). While holding the pituitary stalk with fine forceps, cuts were made with a pair of iris scissors at the lateral and frontal borders (defined by the presence of capillary loops). The average protein content of the entire median eminence dissected as

described was approximately 30 µg. This corresponds to 0.3-0.4 mg wet weight, assuming 0.11 mg wet weight/10 µg protein, as generally observed in brain tissue. In one experiment (see Table 5 in Results) the median eminence was dissected into rostral and caudal portions. The caudal median eminence (11±2 µg protein; n=8) was that portion of the pituitary stalk remaining with the brain but not securely attached to the tuber cinereum. The rostral median eminence (20±3 µg protein; n=8) was the tissue forming the floor of the third ventricle.

The striatum (containing caudate, putamen and globus pallidus) was dissected using a modification of the method described by Glowinski and Iversen (1966). A frontal slice of brain tissue was dissected using a razor blade. The first cut was approximately 1 mm anterior to the optic chiasm. The forebrain was then discarded. A second razor cut was made through the brain just anterior to the optic chiasm. The remaining slice of tissue was approximately 1 mm thick. A small piece of striatum (3-5 mg wet weight) was dissected from this slice within the region delineated by the lateral ventricles as the internal limit and the corpus callosum as the external limit. The dissection as described included tissue from the head of the striatum exclusively.

Upon dissection, brain tissues were immediately homogenized in appropriate volumes of ice cold 0.2 N perchloric acid containing 10 mg % disodium ethylenediàmine-tetraacetic acid (EDTA). The homogenates were frozen at -15°C for analysis within two weeks; the samples were stable over this period of time. On the day of assay the homogenates were thawed on ice, centrifuged for 15 seconds in a Beckman 152 Microfuge to obtain a clear supernatant and 10 µl aliquots of the supernatants were analyzed for DA and NE or DOPA as described in Materials

and Methods Section III. The protein content of the remaining pellet was measured by the method of Lowry et al. (1951). The tissue concentrations of DA and NE were expressed as ng catecholamine/mg protein; the rate of DOPA accumulation was expressed as ng DOPA/mg protein/10 or 30 minutes (see below).

III. Radioenzymatic Assays

A. Dopamine and Norepinephrine Determinations

DA and NE were assayed simultaneously using a modification of radioenzymatic assays described previously (Moore and Phillipson, 1975; Umezu and Moore, 1979). For catecholamine determinations, the posterior pituitary, median eminence and pineal gland were homogenized in 30 µl of 0.2 N perchloric acid containing 10 mg % EDTA; the striatum was homogenized in 100 µl of this same acid solution. The assay involved incubation of the catecholamines in the presence of partially purified COMT and S-adenosyl-L-[methyl-^{3}H]methionine (^{3}H-SAM) resulting in formation of the tritiated o-methylated products of DA and NE, 3-methoxytyramine and normetanephrine, respectively. The labeled metabolites were separated from interfering o-methylated catechols and unreacted ^{3}H-SAM by extraction into an organic solvent, back extraction into acid and final separation by thin-layer chromatography. The DA and NE contents of the samples were calculated directly from standards after subtracting blank values; blanks were obtained by carrying duplicate 10 µl aliquots of the perchloric acid solution through the entire assay as described in detail below.

A 10 µl aliquot of the tissue supernatant or combined DA and NE standard (in duplicate; 0.125-2.0 ng of both amines in 0.2 N

perchloric acid containing 10 mg % EDTA) was added to a 5 ml conical centrifuge tube. The centrifuge tubes in this and all subsequent steps were kept on ice. Twenty-five microliters of freshly prepared incubation mix were then added to each tube containing standard or tissue extract. The incubation mix for a 50 tube assay consisted of 84 µl of 20 mM ethyleneglycol tetraacetic acid (EGTA), pH 7.2, 416 µl of partially purified COMT in 1 mM sodium phosphate, pH 7.0 (see Materials and Methods Section IV for details of enzyme preparation), 250 µl of ^{3}H-SAM (9-10 Ci/mmole, 250 µCi/ml; New England Nuclear Corp., Boston, MA), 84 µl of 8 mg pargyline HCl/ml 10% β-mercaptoethanol and 540 µl of 1 M Tris base containing 3 mM $MgCl_2$. The final pH of the incubation mix was 9.0-9.4, the optimum pH for this reaction (Nikodijevik et al., 1969). The COMT enzyme, ^{3}H-SAM and Tris base were stored in small volumes at -15°C; the pargyline solution was prepared fresh daily.

After addition of the incubation mix the tubes were briefly vortexed and placed in a water bath at 37°C for 60 minutes allowing the o-methylation reaction to go to completion. The tubes were removed from the bath, placed on ice and 30 µl of a mixture containing 5 volumes of 0.45 M borate buffer, pH 10.0, and 1 volume of methoxyamine carrier (5 mg/ml of both 3-methoxytyramine and normetanephrine plus 0.5 mg/ml of sodium metabisulfate in distilled water) were added; the tubes were vortexed quickly. In rapid succession, 20 µl of 1.5% tetraphenyl boron and 550 µl of toluene-isopentyl alcohol (3:2) were added; the tubes were vortexed and centrifuged. Four-hundred seventy-five microliters of the organic phase were transferred to 5 ml conical

centrifuge tubes containing 250 µl of 0.45 M borate buffer, pH 10.0. The tubes were vortexed, centrifuged and 400 µl of the organic phase were then transferred to 5 ml conical centrifuge tubes containing 40 µl of 0.1 N HCl. Again, the tubes were vortexed and centrifuged. The organic phase was aspirated and the acid phase was washed with 250 µl of water-saturated ethylacetate. The tubes were vortexed and centrifuged; the organic phase was then aspirated. Twenty-five microliters of the acid phase were spotted on thin-layer chromatography plates precoated with silica gel (LK6D, Linear K, Whatman Inc., Clifton, NJ). The spots were allowed to dry for 30 minutes at room temperature and the chromatography plates were then developed for approximately 1.5 hours until the solvent front was about three cm from the top of the plate. The developing solvent was a mixture of methylamine-ethanol-chloroform (5:18:40). The spots were visualized after spraying with phenol reagent (Folin and Ciocalteu, Harleco, Gibbstown, NJ) diluted 1:1 with distilled water. The 3-methoxytyramine spot (R_f 0.78-0.82) and the normetanephrine spot (R_f 0.50-0.55) were scraped into scintillation vials. The tritiated amines were extracted into 0.5 ml of acetic acid-ethylacetate-water (3:3:1) for 30 minutes. After addition of 10 ml of toluene-ethanol (7:3) with 0.5% 2,5-diphenyloxazole (PPO; Research Products International Corp., Elk Grove Village, IL) radioactivity was determined in a Beckman LS 100 liquid scintillation counter (20-24% efficiency).

The sensitivity of the assay, determined by the amount of DA or NE with counts twice those observed in the blank, showed slight daily variation. The background and sensitivity for DA were 70-100 cpm and 50-75 pg, respectively. For NE the corresponding values were

20-40 cpm as background, 30-50 pg for the lower limit of sensitivity. The assay was linear to at least 4 ng for both amines.

B. DOPA Determinations

DOPA was analyzed in brain tissue homogenates using a modification of radioenzymatic assays described previously (Hefti and Lichtensteiger, 1976; Demarest and Moore, 1980). For DOPA determinations, the posterior pituitary and median eminence were homogenized in 20 µl of 0.2 N perchloric acid containing 10 mg % EDTA, whereas the striatum was homogenized in 100 µl of this same solution. The homogenates were centrifuged in a Beckman 152 Microfuge to obtain clear supernatants. For radioenzymatic analysis DOPA was incubated in the presence of partially purified COMT and ^{3}H-SAM resulting in the formation of the tritiated o-methylated product of DOPA, 3-methoxytyrosine. The labeled metabolite was separated from interfering o-methylated catecholamines and unreacted ^{3}H-SAM by cation exchange chromatography, adsorption onto activated charcoal and then anion exchange chromatography. The DOPA content of each sample was calculated directly from standards after subtracting blank values. Blanks were obtained by carrying duplicate 10 µl aliquots of the perchloric acid solution through the entire assay as described in detail below.

Ten microliters of perchloric acid extracts of brain tissue from animals administered NSD 1015 (3-hydroxybenzylhydrazine dihydrochloride; Sigma Chemical Co., St. Louis, MO; see Materials and Methods Section VII.B. for details) or standard (0.125-2.0 ng DOPA) were added to 5 ml conical centrifuge tubes on ice. Twenty-five microliters of fresly prepared incubation mix were then added to each tube. The

incubation mix was identical to that for the DA/NE assay except that a DOPA decarboxylase inhibitor, o-benzylhydroxylamine (9 mg/ml 10% β-mercaptoethanol, prepared fresh daily), was substituted for pargyline. After incubation (37°C for 60 minutes) the reaction was stopped by placing the tubes on ice and adding 1.0 ml of ice-cold 0.1 M citrate buffer, pH 2. The samples were passed over cation exchange columns (15x5 mm; AG 50W-X4, H^+ form, 200-400 mesh) which had been previously prepared with 3.0 ml of 0.1 M phosphate buffer, pH 6.5, and 1.5 ml of 0.1 M citrate buffer, pH 2.0. The columns were then washed with 5.0 ml of the same citrate buffer and 0.5 ml of 0.1 M citrate buffer, pH 4.5; the radioactive 3-methoxytyrosine was eluted with 2.5 ml of 0.1 M citrate buffer, pH 4.5, and collected into 12x75 mm disposable tubes. The remaining steps were performed at room temperature. The 3-methoxytyrosine was adsorbed onto paraffin-treated activated charcoal (Asatoor and Dalgliesh, 1956) by the addition of 50 µl of a slurry consisting of approximately 2.5 g treated charcoal in 5 ml distilled water to each tube. The tubes were then vortexed and centrifuged, the aqueous supernatant was aspirated. The charcoal was washed with 2.5 ml of 0.5% acetic acid, and the 3-methoxytyrosine was eluted from the charcoal by vortexing with 1.0 ml of 5% phenol followed by centrifugation. The supernatant was transferred to test tubes (13x100 mm) containing 0.2 ml of 2 N HCl plus 1.0 ml of 0.5 M piperazine. The phenol was extracted with 2.0 ml of water-saturated ethylacetate, vortexed and centrifuged. The organic phase was aspirated and the pH of the samples was adjusted to 10.0-10.5 by the addition of 3.0 ml of 0.2 M piperazine, pH 10.5. The samples were then passed over an anion

exchange column (15x5 mm; AG 1-X2, OH^- form, 200-400 mesh) which had been prepared with 5.0 ml of 1 N NaOH, 5.0 ml of distilled water and 5.0 ml of 0.2 M piperazine, pH 10.5. After addition of the samples, the columns were washed with 5.0 ml of 0.2 M piperazine. The tritiated 3-methoxytyrosine was eluted in 3.0 ml of 0.2 M piperazine, pH 6.0, and collected directly in scintillation vials. Fifteen milliliters of ACS scintillation cocktail (Amersham/Searle Inc., Chicago, IL) were added; the radioactivity in each sample was determined by liquid scintillation spectrometry.

Similar to the radioenzymatic assay for catecholamines, the sensitivity as defined by the amount of DOPA yielding counts at least twice the blank (125-600 cpm) varied daily. The sensitivity was generally between 50-150 pg DOPA. At this sensitivity endogenous concentrations of DOPA (i.e., in animals without NSD 1015 pretreatment) were not detectable in any brain region. The assay was linear to at least 2 ng of DOPA.

To avoid interassay variation, individual brain regions from each experiment were analyzed for DA and NE or DOPA in the same assay. Furthermore, DA and NE concentrations were not determined in animals that were administered NSD 1015 as this treatment altered brain catecholamine concentrations (Demarest, Alper and Moore, unpublished observations).

IV. Preparation of Catechol-o-methyltransferase

Partially purified COMT was prepared using a modification of the procedure as described by Moore and Phillipson (1975). All procedures

were carried out at 0°-4°C. Livers (140 g) from rats fasted overnight were homogenized in a Waring Blender (5 seconds) in 500 ml of ice cold 1.1% KCl. Aliquots of this liver suspension were further homogenized in a glass homogenizer with a motor driven Teflon^{R} pestle. The homogenate was centrifuged at low speed (14,000 x g) for 10 minutes. The supernatant was transferred to ultracentrifuge tubes and spun at 95,000 x g for 60 minutes. The resulting supernatant was filtered through glass wool to remove most of the fat. Generally the procedure was stopped after this step and the KCl supernatant was refrigerated overnight. On the following day the pH was adjusted to 5.3 by adding ice-cold 1 N acetic acid. The acidified mixture was stirred for 10 minutes and then centrifuged. This and all subsequent centrifugations were carried out at 4°C at a force of 14,000 x g for 10 minutes. The supernatant was decanted and the pH adjusted to 6.8 with 0.5 M sodium phosphate buffer, pH 7.0. For every 100 ml of solution, 17.7 g of solid ultrapure ammonium sulfate (Schwarz/Mann, Orangeburg, NY) were added slowly. The suspension was stirred gently for 10 minutes and then centrifuged.

A second ammonium sulfate precipitation was performed on the supernatant fraction (16.2 g ammonium sulfate/100 ml of supernatant). Following centrifugation the supernatant was discarded and the pellet was resuspended in 40 ml of cold 45% ammonium sulfate solution (14 g ammonium sulfate/50 ml of 0.1 M sodium phosphate buffer, pH 7.0). This was stirred for 15 minutes, the precipitate was removed by centrifugation and the supernatant was again discarded. This procedure was repeated with 20 ml of 33% ammonium sulfate (5 g/25 ml of 0.1 M

sodium phosphate buffer, pH 7.0). This time, however, the supernatant fraction was saved; 4.85 g solid ammonium sulfate was added slowly while stirring. The suspension was then stirred for an additional 15 minutes. After centrifugation the resulting pellet was dissolved in 4.0 ml of 1 mM sodium phosphate buffer, pH 7.0, with 0.1 mM dithiothreitol to form the final enzyme solution. This solution was carefully placed in dialysis tubing that had been soaked for 24 hours in a 1% EDTA solution. Dialysis was overnight at 4°C against 4 liters of the 1 mM phosphate buffer, pH 7.0. The partially purified enzyme solution was divided into 250 µl aliquots and stored at -15°C. Fresh enzyme was prepared every 5 or 6 weeks; no change in activity was observed over this period of time.

V. Experimental Treatments

In general, rats were allowed 3 days to acclimate to the animal facilities prior to the start of any experiment. Throughout all treatments (except when noted) standard commercial rat chow (Wayne Lab Blox, Allied Mills, Chicago, IL) containing 0.39% sodium was available ad libitum. The initial studies involved the effects of water deprivation or the replacement of normal drinking water (tap water) with a 2% NaCl solution. When rats deprived of water for 3 days were rehydrated for short periods of time (1-6 hours) they were first placed in individual cages to insure free access to water bottles; rats rehydrated for periods longer than 6 hours were maintained 4 per cage.

Hypertonic saline (15% NaCl; 5 ml/kg, s.c.) was administered to rats in several experiments. This is a very potent dispogenic stimulus.

In several experiments the animals were allowed free access to water after the injection of 15% NaCl, but in most experiments water bottles were removed from the cages following the hypertonic saline injection.

As noted above, the animals were provided a normal rat chow. In one experiment the rats were deprived of food for 3 days and in another experiment they were provided a high sodium diet (High sodium diet, 3.15%, Ralston Purina Co., St. Louis, MO). The high sodium diet had the following composition; sodium chloride, 7.25%, casein, 21.0%; sucrose, 15.0%; solka floc, 3.0%; RP vitamin mix, 2.0%; RP mineral mix #11, 5.0%; dl-methionine, 0.15%; choline chloride, 0.2%; corn oil, 5.0%; lard, 5.0%; dextran, 36.4%. The sodium content of this food was approximately 10 times greater than the normal chow (3.15% sodium as compared to 0.39%).

VI. Plasma Analysis

Trunk blood was collected at the time of sacrifice in beakers containing 100 U heparin in 0.1 ml of 0.9% NaCl. The blood was immediately transferred to 12x75 mm test tubes and samples were taken in heparinized microcapillary tubes for triplicate hematocrit determinations. The blood was centrifuged (1100 x g, 10 minutes) at room temperature. Plasma was transferred to 10x75 mm test tubes, sealed with Parafilm^{R} and frozen at -20°C for later analysis. Plasma sodium concentrations were determined in all samples by flame photometry (Instrumentation Laboratory Inc, 343 Flame Photometer). Plasma osmolality was determined using a Wescor 5100B Vapor Pressure Osmometer.

The plasma protein concentration was estimated by the method of Lowry et al. (1951). The plasma analyses were used as determinants of plasma volume (Kutscher, 1971).

VII. Drug Treatments

A. α-Methyltyrosine-induced Decline of Dopamine and Norepinephrine

αMT inhibits tyrosine hydroxylase, the rate limiting step in catecholamine synthesis (Spector et al., 1965). DA and NE concentrations in central and peripheral neurons decline with time following the administration of αMT. This rate of decline is proportional to neuronal activity, follows first order kinetics, and can be used to calculate synthesis rates and turnover times of tissue catecholamines (Brodie et al., 1966).

Catecholamine synthesis was estimated by quantifying the decline of endogenous DA and NE concentrations after synthesis inhibition with αMT (α-methyltyrosine methylester HCl; Regis Chemical Co., Chicago, IL). Rats were injected intraperitoneally (i.p.) with 250 mg αMT free base/kg (αMT was dissolved in distilled water) 0, 30, 60 or 90 minutes prior to sacrifice (see Figure 4). DA and NE concentrations were measured using the radioenzymatic assay as described in Section III.A. above. Rate constants (and the 99% confidence intervals) for the decline of the amines were calculated by least squares regression analysis (Goldstein, 1964) as described in detail by Brodie et al. (1966). The synthesis rates were calculated as the zero time catecholamine concentration (i.e., the steady state concentration)

multiplied by the rate constant for the appropriate amine and were expressed as ng catecholamine/mg protein/hour.

B. DOPA Accumulation after NSD 1015.

The rate of DOPA accumulation in the striatum (Carlsson and Lindqvist, 1973; Demarest and Moore, 1980) and in the median eminence (Demarest and Moore, 1980) after decarboxylase inhibition is an *in vivo* estimate of tyrosine hydroxylase activity and, therefore, DA synthesis. Roth *et al*. (1976) have shown a direct correlation between changes in the firing rate of the nigrostriatal DA neurons and changes in the rate of DOPA accumulation (i.e., DA synthesis) in the striatum. These changes occur in the absence of changes in the striatal concentration of DA. It is therefore assumed that an increase in the rate of DA synthesis in the terminals of any DA neuronal system without a concomitant change in the DA concentration reflects an increase in neuronal activity.

A time course and dose-response relationship for the accumulation of DOPA following the administration of the decarboxylase inhibitor NSD 1015 have been described previously (Carlsson and Lindqvist, 1973; Demarest and Moore, 1980). When administered i.p., 100 mg NSD 1015/kg (100 mg/ml of 0.9% NaCl) caused a maximal inhibition of DOPA decarboxylase and a linear accumulation of DOPA in the striatum and median eminence for at least 30 minutes. Except when noted, NSD 1015 was administered to the rats in a dose of 100 mg/kg, i.p., 30 minutes prior to sacrifice. In 2 experiments NSD 1015 was administered intravenously (i.v.) in a dose of 25 mg/kg via the tail vein in unanesthetized, restrained rats. This dose of NSD 1015 was

found to cause a maximal rate of DOPA accumulation which increased linearly for 10-20 minutes (see Results Section I.B.2.b.). Therefore, in experiments requiring i.v. administration, 25 mg NSD 1015/kg was injected 10 minutes prior to sacrifice.

C. 6-Hydroxydopamine Treatment

The catecholaminergic neurotoxin 6-hydroxydopamine HBr (6-OHDA; Sigma Chemical Co., St. Louis, MO) was infused into the lateral cerebroventricles to cause a selective depletion of brain NE. Male rats were anesthetized with Equithesin, placed in a stereotaxic frame and implanted bilaterally with 23 gauge stainless steel cannula guides with the tips located at bregma, ±1.5 mm lateral and 2.8 mm below dura (Pellegrino and Cushman, 1968). The cannula guides were anchored to the skull by stainless steel screws and dental cement. One week after the implantation of the cannula guides, 3 µl of 6-OHDA (12.5 µg of free base) or its vehicle (0.9% NaCl containing 0.1% ascorbic acid) were infused bilaterally (1 µl/minute) three times at 48 hour intervals through 30 gauge stainless steel cannulae which extended 1 mm below the tip of the guide cannulae. The injection cannulae were connected to a motor driven Hamilton syringe with polyethylene tubing. The animals were sacrificed 48 hours after the last infusion.

D. Mannitol Infusion

Mannitol is an osmotic diuretic (Mudge, 1975). This agent can be administered in sufficient quantities to cause a large increase in plasma, glomerular filtrate and tubular fluid osmolality, resulting in a large increase in urine flow. Mannitol is used clinically

because: 1) it does not penetrate cellular membranes, 2) it is not metabolized, and 3) it is rapidly excreted by the kidney (Lazorthes and Campan, 1972). Mannitol was infused intravenously to cause a rapid cellular dehydration as a consequence of an osmotic load.

Mannitol was infused into the jugular vein of unrestrained rats (average weight of 200 grams) surgically implanted with a chronic polyvinyl jugular catheter exiting subcutaneously on the back of the animal as modified from the method described by Weeks (1962). The cannula consisted of a 30 mm length of polyvinyl tubing (0.01" inner diameter x 0.03" outer diameter) glued (Krazy Glue, Krazy Glue Inc., Chicago, IL) to a second 100 mm length of polyvinyl tubing (0.02" inner diameter x 0.06" outer diameter). Under Equithesin anesthesia the shorter piece of tubing was inserted into and tied to the jugular vein. The larger tubing was then passed subcutaneously behind the forelimb and was exited through a small incision at the base of the rat's neck. The cannula was secured to the skin with a drop of glue and was occluded with a 23 gauge needle. The patency of the cannula was tested one day prior to the experiment by injecting 0.1 ml of methohexital sodium (Brevitol^{R}, 10 mg/ml; Eli Lilly and Co., Indianapolis, IN), a short acting barbiturate. The cannula was then flushed with 0.2 ml of 0.9% NaCl containing heparin (10 U/ml) and the animal was returned to its individual cage.

The delivery system for the mannitol infusion was a glass syringe (fitted with a 23 gauge needle) driven by a Harvard infusion pump. Attached to the needle was a length of polyethylene tubing

sufficient to permit the rats free, unrestrained movement during the infusion. A short piece of 23 gauge stainless steel tubing was used to connect the polyethylene tubing to the cannula.

Infusions of hyperosmotic sorbitol or mannitol stimulate drinking (Holmes and Gregersen, 1950). If the animals are permitted access to water after the infusion of mannitol they drink sufficient volumes of water to maintain an approximately normal state of cellular hydration. Therefore, immediately following the mannitol infusion, rats were returned to cages and not provided drinking water. In all mannitol experiments the time of sacrifice was determined from the end of the infusion.

E. Vasopressin Infusions

Synthetic AVP (Sigma Chemical Co., St. Louis, MO) was continuously infused into rats for three days. The doses of AVP infused were 240 and 720 mU/rat/day. The lower dose has been reported to be the replacement dose of AVP necessary to restore normal urine volumes to Brattleboro rats deficient in AVP (G. Fink, personal communcation). The higher dose was chosen because rats deprived of water for two to three days exhibit plasma AVP concentrations 3-4-fold higher than normal (Dunn et al., 1973; Rougon-Rapuzzi et al., 1978). It was felt that the infusion of 3 times more AVP then the replacement dose might approximate circulating concentrations of AVP observed in three day dehydrated rats.

The AVP was delivered continuously for three days by means of Alzet^{R} osmotic minipumps (Alza Corp., Palo Alto, CA). Under anesthesia a small length of polyvinyl tubing (0.01" inner diameter x

0.03" outer diamater) was inserted into and tied to the jugular vein of rats weighing approximately 300 grams: sham surgery consisted of jugular ligation. The tubing was threaded subcutaneously to a minipump which was secured under the skin of the animal's back. The flow rate of the pump was 1 µl/hour. AVP was diluted in 0.9% NaCl to the appropriate concentrations to allow for delivery of either 240 or 720 mU/rat/ day.

F. Equithesin Anesthesia

All surgical procedures (ovariectomy, superior cervical ganglionectomy, lateral ventricular cannulation, jugular catheterization and minipump implantation) were performed under Equithesin anesthesia. Equithesin contains: chloral hydrate, 21.25 g; pentobarbital, 4.86 g; magnesium sulfate, 10.63 g; propylene glycol, 221.7 ml; 95% ethanol, 60.0 ml; distilled water to 500 ml. Ovariectomies were performed on female rats administered 2 ml Equithesin/kg, i.p.; all other surgery was performed on male rats administered 3 ml Equithesin/kg.

VIII. Statistical Analyses

All values presented represent the mean ± 1 standard error of the mean (S.E.). Data were initially analyzed by a one-way analysis of variance; differences between means were determined by Student-Newman-Keuls' test with the level of significance set at $p<0.01$ (Steele and Torrie, 1960).

The αMT-induced decline of DA and NE were analyzed by regression analysis (Goldstein, 1964). The lines plotted are the best fit according to this analysis, and the rate constants (k) and 99% confidence limits were computed.

RESULTS

I. Concentration and Synthesis of Catecholamines

A. Effects of Superior Cervical Ganglionectomy on Catecholamine Concentrations

Catecholamine-containing nerve terminals have been demonstrated in the median eminence-pituitary region of the rat and other mammals (for review see Björklund et al., 1974). All of the DA in the posterior pituitary appears to be contained in the terminals of tuberohypophyseal neurons originating in the arcuate nucleus. On the basis of histofluorescent analysis it was initially proposed that NE in the posterior pituitary was in neurons of central origin (Björklund, 1968) but it was later suggested that NE neurons were "exclusively of peripheral sympathetic origin" (Björklund et al., 1970).

The first study was designed to quantify the concentrations of DA and NE in the median eminence-pituitary region of the rat and to determine the contribution of the peripheral sympathetic nervous system to the noradrenergic innervation of this region. DA and NE were measured in selected brain regions seven days following bilateral superior cervical ganglionectomy (SCGx) or sham surgery, consisting of exposure but not removal of the SCG. The data are presented in Table 1. The concentrations of DA and NE in the posterior pituitary of sham-control animals are in good agreement with those reported by

TABLE 1

Effects of Superior Cervical Ganglionectomy on Catecholamine Concentrations in Selected Brain Regions

	DOPAMINE (ng/mg protein)		NOREPINEPHRINE (ng/mg protein)	
	Sham	Ganglionectomy	Sham	Ganglionectomy
Striatum	100.9±3.5	94.8±3.8	---	---
Median Eminence	112.3±7.4	111.9±9.0	52.7±4.4	41.4±5.1
Posterior Pituitary	6.3±0.3	6.8±0.3	2.7±0.3	1.7±0.3*
Pineal Gland	---	---	3.8±0.4	0.5**

Superior cervical ganglionectomy or sham surgery was performed on rats 7 days prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 10-15 rats. *, significantly different from sham-control ($p<.01$). **, the minimal concentration of NE that could be determined in a single pineal gland.

Annunziato and Weiner (1980) and by Saavedra et al. (1975), but are less than those reported by several other investigators (Holzbauer et al., 1978, 1980a,b; Morgan and Herbert, 1980; Torda et al., 1979). The amount of DA per posterior pituitary (152±10 μg protein) was 0.96±0.06 ng. SCGx did not alter the DA concentration in any region examined. The DA concentration in the pineal gland and the NE concentration in the striatum were below the sensitivity of the radioenzymatic assay and are therefore not reported. The NE content of the median eminence was slightly, but not significantly, decreased by SCGx. This may represent the residual NE found in the median eminence following hypothalamic deafferentation (Brownstein et al., 1976; Gallardo et al., 1978). However, surgical sympathectomy substantially reduced the NE content of the posterior pituitary while totally depleting the pineal gland of the amine. These results suggest that all of the NE in the pineal gland and approximately one-third of the NE in the posterior pituitary is contained in terminals of peripheral sympathetic neurons. Holzbauer et al. (1980b) have similarly reported a 50% reduction in the NE concentration and no change in the DA concentration of the posterior pituitary following SCGx. Apparently the majority of the catecholaminergic neurons terminating in the posterior pituitary is of central origin with the concentration of DA being 2- to 3-fold greater than that of NE.

B. In Vivo Estimates of Catecholamine Synthesis

There are several methods which may be applied to estimate monoamine synthesis in vivo. Each has its own advantages and disadvantages, but non-steady state methods do not yield absolute synthesis

rates (for review see Weiner, 1974). Two procedures commonly used to estimate catecholamine synthesis rates are the αMT-induced decline of DA and NE (Brodie et al., 1966) and the rate of DOPA accumulation after decarboxylase inhibition (Carlsson et al., 1972). The αMT-induced decline method was used to determine if the rate of DOPA accumulation could be employed as an in vivo estimate of DA synthesis in the median eminence and posterior pituitary. The difficulty with the latter method is that both brain regions have substantial noradrenergic innervation, and that DOPA is the amino acid precursor for both DA and NE (see Figure 3).

1. α-Methyltyrosine-induced decline of dopamine and norepinephrine

The relative rates of DA and NE synthesis in the posterior pituitary, median eminence and striatum were calculated from the rates of decline after the administration of αMT (Figure 4 and Table 2). The catecholamine concentrations presented in Table 2 represent the mean value of the zero time control (non-injected animals). The rate constant is defined as the fraction of total catecholamine lost per hour (fractional turnover rate; Brodie et al., 1966) and is a function of the slope of the corresponding line in Figure 4. The tuberohypophyseal DA neurons have a high tonic rate of activity as evidenced by a DA turnover rate of 0.53 ± 0.23 hr^{-1} in the posterior pituitary. Both the amine concentration and the rate of decline (i.e., the rate constant) were greater for DA than for NE in the posterior pituitary and median eminence. When a ratio of DA synthesis to total catecholamine synthesis was calculated for median eminence

Figure 4. The αMT-induced decline of dopamine (open circles) and norepinephrine (filled circles) in the striatum, median eminence and posterior pituitary. Animals were sacrificed 0, 30, 60 and 90 minutes after the administration of αMT (250 mg/kg, i.p.). Symbols represent means and vertical lines represent ± 1 S.E. as determined from 6-12 rats. The lines drawn were calculated by regression analysis.

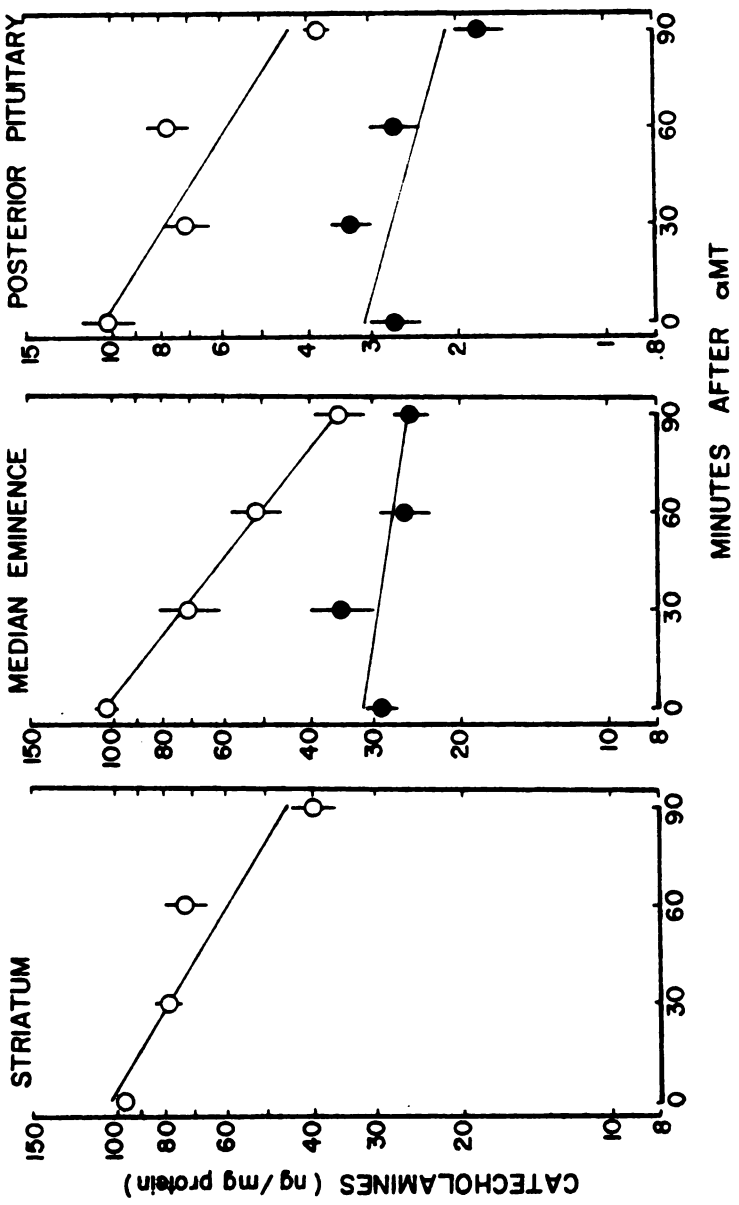
STRIATUM
MEDIAN EMINENCE
POSTERIOR PITUITARY
CATECHOLAMINES (ng/mg protein)
MINUTES AFTER αMT

Figure 4

TABLE 2

Synthesis Rates of Catecholamines in Selected Brain Regions Calculated from the αMT-induced Decline of Catecholamines

	CATECHOLAMINE CONCENTRATION (ng/mg protein)		RATE CONSTANT (k) (hr^{-1})		SYNTHESIS RATE (ng/mg protein/hr)		
	DA	NE	DA	NE	DA	NE	$\frac{DA}{DA+NE} \times 100$
Posterior Pituitary	10.3±1.3	2.7±0.3	0.53±0.23	0.26±0.23	5.5	0.7	89
Median Eminence	104.7±7.2	29.0±1.8	0.75±0.28	0.17±0.22	78.5	4.8	94
Striatum	97.4±3.3	---	0.57±0.20	---	55.5	---	100

Catecholamine concentrations represent the mean ± 1 S.E. as determined from 6 (striatum) or 12 (median eminence and posterior pituitary) rats (the zero time control in Figure 4). The rate constants represent the mean ± 99% confidence interval as determined by regression line analyses. The synthesis rate equals the rate constant X the steady-state catecholamine concentration.

and posterior pituitary it was observed that DA synthesis accounted for about 90% of the total. This suggests that under normal conditions the major portion of DOPA accumulation measured in the posterior pituitary and median eminence reflects DA synthesis.

2. DOPA accumulation after NSD 1015

a. Effects of 6-hydroxydopamine on catecholamine concentrations and DOPA accumulation. To demonstrate that noradrenergic neurons do not normally contribute substantially to the rate of DOPA accumulation after the i.p. administration of NSD 1015, studies were performed in which the concentration of NE, but not DA, was decreased in the posterior pituitary and median eminence by 6-OHDA treatment (Table 3). When infused intracerebroventricularly, 6-OHDA reduced the concentration of NE in the posterior pituitary and median eminence to 47 and 41% of control, respectively. On the other hand, neither the DA concentration nor the rate of accumulation of DOPA was altered in the posterior pituitary, median eminence or striatum by the destruction of more than 50% of the noradrenergic neurons. These results suggest that the accumulation of DOPA after the administration of a decarboxylase inhibitor is a suitable *in vivo* estimate of DA synthesis in the posterior pituitary, median eminence and striatum. Since striatal DA synthesis normally correlates with impulse traffic in the nigrostriatal DA system (Roth *et al.*, 1976), DOPA accumulation in the posterior pituitary and median eminence should be, by analogy, an index of neuronal activity in the tuberohypophyseal and tuberoinfundibular DA systems, respectively.

TABLE 3

Effects of 6-Hydroxydopamine on Catecholamine Concentrations and DOPA Accumulation in Selected Brain Regions

	NOREPINEPHRINE (ng/mg protein)		DOPAMINE (ng/mg protein)		DOPA ACCUMULATION (ng/mg protein/30 min)	
	Vehicle	6-OHDA	Vehicle	6-OHDA	Vehicle	6-OHDA
Posterior Pituitary	3.0±0.4	1.4±0.2*	8.7±0.6	8.2±0.4	1.3±0.1	1.3±0.1
Median Eminence	43.9±3.5	18.1±1.3*	98.4±4.2	96.0±6.5	9.4±0.7	8.6±0.8
Striatum	---	---	90.5±2.5	88.9±5.8	6.4±0.3	6.8±0.2

Rats received 3 bilateral intraventricular infusions of 6-OHDA (12.5 µg/3 µl) or vehicle (3 µl of 0.9% NaCl containing 0.1% ascorbic acid) at 48 hour intervals. The animals were sacrificed 48 hours after the last infusion. Rats used to determine DOPA accumulation were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 6-8 rats. *, significantly different from vehicle treated animals ($p<.01$).

b. Effects of intravenous administration of NSD 1015 on DOPA accumulation. In several studies DOPA accumulation was determined in rats deprived of water for three days and then given free access to water for various lengths of time. In the initial experiments examining 1 and 3 hours of rehydration, the DOPA content of all brain regions was extremely low and variable; many of the homogenates had no detectable DOPA. It was noted that these rats gained 12-15% of their initial body weight during the 1-3 hours of rehydration. This rapid ingestion of food and water apparently altered either the absorption and/or distribution of NSD 1015 administered i.p. To circumvent this problem, NSD 1015 was infused in the tail vein of unanesthetized, restrained rats. DOPA accumulation was measured in the striatum and posterior pituitary following graded doses and at various times after the i.v. administration of NSD 1015 (Figure 5). All doses of NSD 1015 (12.5, 25 and 50 mg/kg) caused a maximal accumulation of DOPA 30 minutes after decarboxylase inhibition. The rate of DOPA accumulation was approximately linear for 10-20 minutes. In experiments where the decarboxylase inhibitor was administered i.v., 25 mg NSD 1015/kg were administered to unanesthetized, restrained rats as a bolus injection 10 minutes prior to sacrifice.

II. Dehydration

A. Effect of Dehydration on Dopamine Concentrations

Severe dehydration was reported to increase the DA concentration in the posterior pitiutary, however the synthesis of DA was not

Figure 5. DOPA accumulation in the striatum and posterior pituitary after intravenous administration of NSD 1015 in unanesthetized, restrained rats. A) DOPA accumulation 30 minutes after the intravenous infusion of various doses of NSD 1015. B) DOPA accumulation at various times after NSD 1015 (25 mg/kg, i.v.). In panel B open circles (striatum; scale on left ordinate) and filled circles (posterior pituitary; scale on right ordinate), and columns in panel A, represent mean DOPA concentrations (ng/mg protein) ± 1 S.E. (vertical lines) as determined from 8 rats.

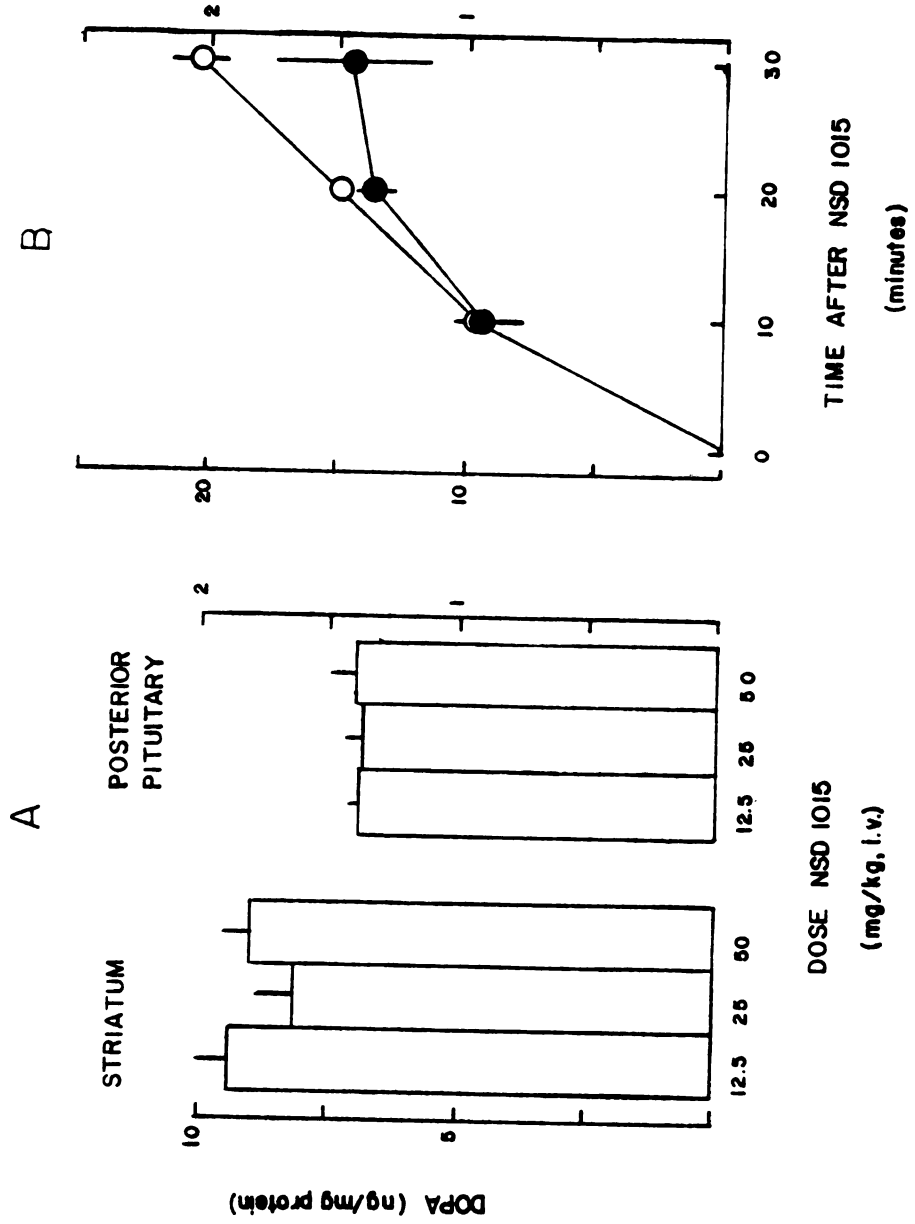
A
STRIATUM
POSTERIOR PITUITARY
DOPA (ng/mg protein)
10
5
2
1
12.5
25
50
DOSE NSD 1015 (mg/kg, i.v.)
B
20
10
2
1
0
10
20
30
TIME AFTER NSD 1015 (minutes)

Figure 5

estimated (Holzbauer et al., 1978, 1980a,b). The initial study was designed to examine a variety of dehydrating stimuli on the DA content of the posterior pituitary and to extend the experiment to other dopaminergically innervated brain regions. The results are presented in Table 4. The DA concentration of the striatum and median eminence was not altered by any treatment examined. The DA concentration of the posterior pituitary was increased only by water deprivation followed by saline drinking, similar to data previously reported (Holzbauer et al., 1978, 1980a,b).

B. Effect of Dehydration on DOPA Accumulation

1. Water deprivation in male and female rats

Pharmacological treatments and endocrinological manipulations rarely alter the catecholamine concentration of any brain region due to the coupling of neuronal activity, and thus release of amine, to the rate of catecholamine synthesis. To estimate neuronal activity the rate of DOPA accumulation after decarboxylase inhibition was measured as an index of DA synthesis in selected brain regions of male and ovariectomized rats following three days of water deprivation (Table 5). Since these experiments were not performed or assayed together a direct male:female comparison is not possible. The data reveal, however, that three days of water deprivation did not alter DOPA accumulation in the striatum of male or female rats. In males, the median eminence was divided into rostral and caudal (pituitary stalk) regions; there was no significant difference in the rates of DA synthesis in these two regions. The rate of DOPA accumulation in female median eminence appeared greater than that in the male, but in

TABLE 4

Effect of Dehydration on the Dopamine Concentration in Selected Brain Regions

	DOPAMINE (ng/mg protein)			
	Control	2% NaCl (2 days)	2% NaCl (5 days)	Water Deprivation (2 days) plus 2% NaCl (3 days)
Striatum	103.2± 4.7	105.6±4.3	116.7±5.9	116.6±3.6
Median Eminence	100.5±12.7	111.8±4.6	96.2±3.5	115.6±4.7
Posterior Pituitary	6.4± 0.8	6.9±0.6	9.0±0.8	11.0±1.1*

Rats were provided free access to food and water (control), 2% NaCl instead of normal drinking water for 2 or 5 days, or deprived of water for 2 days and then provided free access to 2% NaCl drinking water for 3 days. Values represent the mean ± 1 S.E. as determined from 6 rats. *, significantly different from control.

TABLE 5

Effect of Water Deprivation on DOPA Accumulation in Selected Brain Regions of Male and Ovariectomized Rats

	DOPA ACCUMULATION (ng/mg protein/30 minutes)			
	Male		Female	
	Control	Water-Deprived	Control	Water-Deprived
Striatum	10.1 ±0.5	11.1 ±0.7	9.6 ±0.7	11.6 ±0.9
Median Eminence	---	---	17.1 ±2.5	17.5 ±1.7
Rostral	7.6 ±1.3	7.9 ±1.0	---	---
Caudal	6.2 ±0.4	8.3 ±0.9	---	---
Posterior Pituitary	0.92±0.06	1.65±0.11*	1.09±0.11	1.60±0.13*

Male or ovariectomized rats (surgery was performed 7 days prior to dehydration) were deprived of water for 72 hours. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8-12 rats (N=4 for rostral and caudal median eminence, tissue samples from 3 rats were pooled for each determination). *, significantly different from control ($p<.01$).

neither male nor female did water deprivation alter the rate of DOPA accumulation in this brain region. On the other hand, water deprivation for 3 days significantly increased DOPA accumulation in the posterior pituitary of both males and females.

The results of this experiment reveal that water deprivation, in both male and female rats, increases the activity of the tuberohypophyseal DA neurons, but does not effect the nigrostriatal or tuberoinfundibular DA systems. It appears that some of the DA nerves terminating in the pituitary stalk (caudal median eminence) might be functionally related to those in the posterior pituitary and are stimulated by dehydration. There was often a tendency for water deprivation to cause a small but insignificant increase in DOPA accumulation in the median eminence when it was not separated into rostral and caudal portions (e.g., see Figure 6). This is presumed to be due to a small contribution of the tuberohypophyseal DA nerves to a portion of what has been arbitrarily defined as the "median eminence", for there is no clear demarcation between the median eminence and the posterior pituitary. Although a statistical comparison is not possible due to the nature of the experiments, there appears to be a sexual dimorphism in the normal rate of DOPA accumulation in the median eminence, but not in the striatum or posterior pituitary. The higher rate of DA synthesis in the median eminence of females has been confirmed in a detailed series of experiments (Demarest et al., 1981).

2. Food deprivation

Rats which are deprived of water exhibit a reduction of food intake. The data depicted in Figure 6 reveal that three days of

Figure 6. Effect of water or food deprivation on DOPA accumulation in selected brain regions. Rats were provided free access to food and water (control; C), food but not water (water-deprived; WD) or water but not food (food-deprived; FD) for 72 hours. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Columns represent mean ± 1 S.E. (vertical lines) as determined from 8 rats and are expressed as percent of control. Control values (ng DOPA/mg protein/30 min) were: striatum, 11.3±1.1; median eminence, 4.1±0.4; posterior pituitary, 0.57±0.04. *, significantly different from control ($p<.01$).

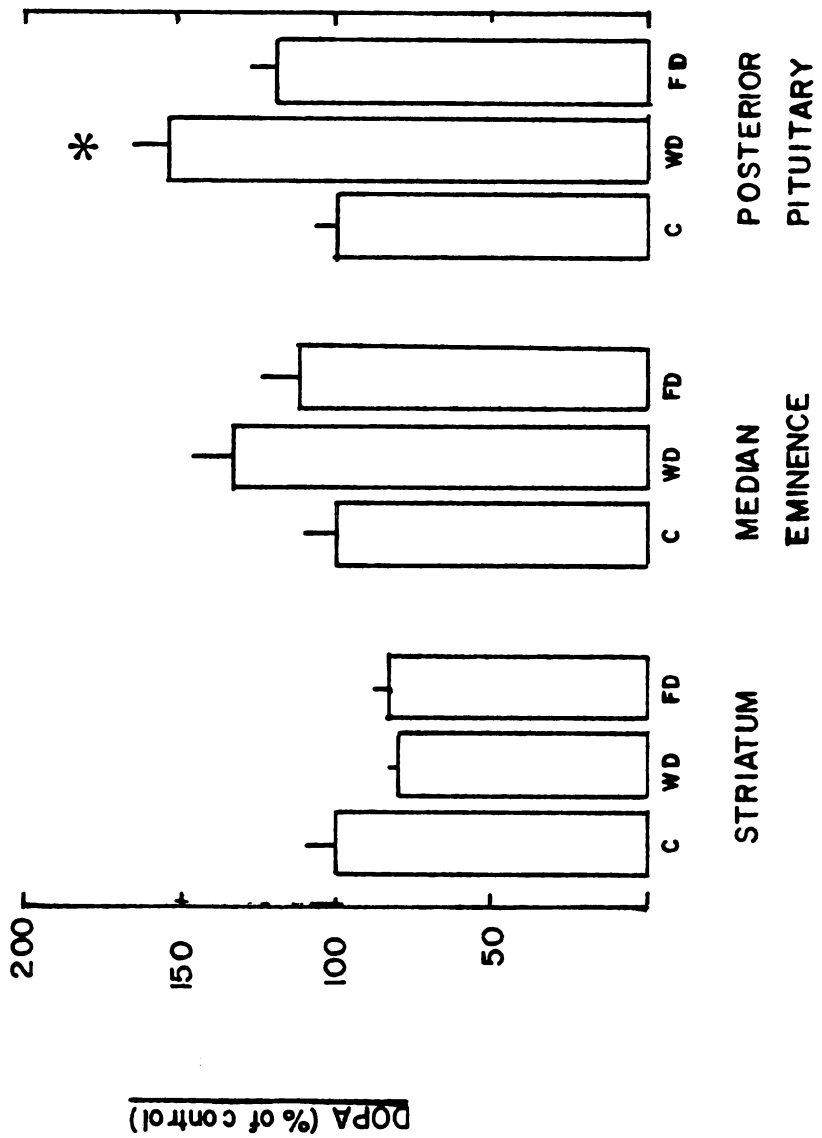
DOPA (% of control)
200
150
100
50
C
WD
FD
STRIATUM
MEDIAN EMINENCE
POSTERIOR PITUITARY
*

Figure 6

water deprivation, but not three days of food deprivation, selectively increased DOPA accumulation in the posterior pituitary. That is, water deprivation per se, and not just a reduction in body weight due to decreased food intake (control = 269±5 g; water-deprived = 193±4 g; food-deprived = 211±3 g) is involved in activating the tuberohypophyseal DA neurons.

3. Time course of water deprivation

To determine changes occurring throughout the first 72 hours of water deprivation, catecholamine concentrations and DOPA accumulation in selected brain regions, and the plasma sodium concentration and the hematocrit were measured in rats sacrificed at 24 hour intervals. DA and NE were measured in rats not receiving NSD 1015 (Table 6), whereas DOPA accumulation and the plasma parameters were determined in rats administered NSD 1015 (Table 7). Seventy-two hours of water deprivation did not alter the DA or NE concentration of any brain region examined. DOPA accumulation remained constant in the striatum and median eminence throughout the duration of the experiment. However, the rate of DOPA accumulation in the posterior pituitary progressively increased, attaining statistical difference from control following 72 hours of water deprivation. The plasma sodium concentration and the hematocrit also steadily increased, reaching statistical significance by 24 hours of dehydration.

These data clearly demonstrate that water deprivation increases DA synthesis only in the posterior pituitary. The increase in synthesis occurs at times when the DA concentration is unaltered.

TABLE 6

Effects of Water Deprivation on Dopamine and Norepinephrine Concentrations in Selected Brain Regions

	Hours Water Deprivation			
	0	24	48	72
DOPAMINE (ng/mg protein)				
Striatum	82.5±3.3	89.7±5.9	75.9± 7.2	99.9±6.2
Median Eminence	104.8±8.0	109.0±6.6	108.9±10.4	85.2±4.3
Posterior Pituitary	6.0±0.3	5.7±0.5	5.8± 0.4	5.6±0.4
NOREPINEPHRINE (ng/mg protein)				
Median Eminence	42.8±3.7	38.8±3.2	40.7± 3.3	29.6±3.6
Posterior Pituitary	2.5±0.2	3.0±0.2	2.1± 0.2	2.3±0.2

Rats were provided access to food and water (0 hours water deprivation) or were sacrificed after various periods of water deprivation. Values represent the mean ± 1 S.E. as determined from 8 rats.

TABLE 7

Effects of Water Deprivation on DOPA Accumulation in Selected Brain Regions, and on Plasma Sodium and Hematocrit

	Hours Water Deprivation			
	0	24	48	72
DOPA ACCUMULATION (ng/mg protein/30 min)				
Striatum	8.9 ±0.7	9.4 ±0.5	10.3 ±0.6	10.6 ±0.7
Median Eminence	10.1 ±0.9	8.9 ±0.7	9.5 ±0.9	9.8 ±0.9
Posterior Pituitary	1.29±0.25	1.57±0.09	2.13±0.29	2.42±0.13*
PLASMA Na^+ (mEq/l)	141.6 ±0.6	146.1 ±1.0*	149.4 ±0.6*	154.9 ±1.6*
HEMATOCRIT (% Packed Cells)	42.3 ±0.4	46.4 ±0.5*	49.5 ±0.5*	52.5 ±0.4*

Rats were provided free access to food and water (0 hours water deprivation) or were sacrificed after various periods of water deprivation. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 7-10 rats. *, significantly different from 0 time control ($p<.01$).

This suggests that in rats deprived of water for three days the synthesis and release of DA is increased selectively in the terminals of the tuberohypophyseal neurons. The effect on this dopaminergic system is preceded by many hours by an increase in the plasma sodium concentration and the hematocrit.

In all subsequent experiments data are presented for DOPA accumulation only in the posterior pituitary since only this brain region appears to be influenced by dehydration. Nevertheless, in all experiments the striatum and median eminence were dissected and analyzed for DOPA, but as none of the treatments altered the rate of DOPA accumulation in these regions the data were not presented.

4. Comparison of water deprivation and saline drinking

Water deprivation causes both intracellular dehydration and a fluid volume deficit (hypovolemia) while saline drinking is considered to cause only intracellular dehydration. An experiment was designed to examine the effects of five days of water deprivation or 2% NaCl substituted for normal drinking water on catecholamine concentrations, DOPA accumulation and several estimates of fluid balance (Table 8). Neither treatment altered the catecholamine concentrations while both water deprivation and saline drinking increased the rate of DA synthesis in the posterior pituitary. The plasma sodium concentration was markedly increased by both stimuli. On the other hand, only water deprivation induced a large decrease in the plasma volume as evidenced by the increased hematocrit and plasma protein concentration. These data suggest that long-term intracellular dehydration

TABLE 8

Effects of Water Deprivation or Saline Drinking on Catecholamine Concentrations and DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium, Hematocrit and Plasma Protein

	Control	Water Deprivation	2% NaCl
CATECHOLAMINE CONCENTRATION (ng/mg protein)			
Dopamine	5.87±0.33	6.50±0.35	7.05±0.71
Norepinephrine	2.01±0.16	1.76±0.15	1.89±0.17
DOPA ACCUMULATION (ng/mg protein/30 min)	0.89±0.07	2.69±0.24*	2.08±0.32*
PLASMA Na^+ (mEq/l)	140.1 ±0.6	161.1 ±1.3*	179.6 ±2.3*
HEMATOCRIT (% Packed Cells)	46.8 ±0.6	59.4 ±0.6*	47.4 ±0.6
PLASMA PROTEIN (g/100 ml)	6.69±0.17	8.07±0.22*	7.11±0.05

Control rats were provided free access to food and water. The treatments were 5 days of either water deprivation or 2% NaCl substituted for normal drinking water. DOPA accumulation and fluid balance parameters were determined in rats administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

can increase the activity of the tuberohypophyseal DA neurons and hypovolemia is not required for this effect.

5. Hypertonic saline injections

The changes in salt and water balance induced by water deprivation are slow in onset; the hematocrit is increased at 12 hours but it takes approximately 24 hours of water deprivation to increase the plasma sodium concentration (Table 9). In order to cause a rapid hypernatremia without a change in plasma volume, 15% NaCl (5 ml/kg) was injected subcutaneously and the rats were not allowed access to water following the saline injection. The results of several experiments have been combined and are shown in Table 10. The plasma sodium concentration and plasma osmolality were rapidly and markedly increased, and slowly returned towards control. The hematocrit was slightly decreased 30 minutes after the saline injection by the redistribution of intracellular fluid into the plasma, but no increase was seen in the hematocrit for as long as 4 hours after injection. There was no significant effect on DOPA accumulation in the posterior pituitary from 30-240 minutes after the hypertonic saline injection (Table 11).

The results of a longer time course of the effects of a hypertonic saline injection on DOPA accumulation and fluid balance parameters are shown in Figure 7. The plasma sodium concentration and the hematocrit appeared to increase in a manner similar to water deprivation without the saline injection (compare Figure 5 to Tables 7 and 9). The sole exception was that the plasma sodium was slightly elevated 12 hours after the hypertonic saline injection but

TABLE 9

Effects of Water Deprivation on Plasma Sodium and Hematocrit

	Hours Water Deprivation				
	0	12	24	36	48
PLASMA Na^+ (mEq/l)	135.5±0.7	138.4±1.1	139.8±1.3*	141.0±1.0*	141.5±0.8*
HEMATOCRIT (% Packed Cells)	48.4±0.6	51.6±0.4*	53.3±0.5*	54.2±0.3*	56.7±0.5*

Rats were provided free access to food and water (0 hours water deprivation) or were sacrificed after various periods of water deprivation. Values represent the mean ± 1 S.E. as determined from 7-8 rats. *, significantly different from 0 time control ($p<.01$).

TABLE 10

Effects of Hypertonic Saline Injection on Plasma Sodium, Plasma Osmolality and Hematocrit

	Time After 15% NaCl (minutes)					
	0	30	60	120	180	240
PLASMA Na^+ (mEq/l)	143.8±0.5	154.1±0.6*	155.4±0.5*	151.8±0.4*	150.3±0.4*	148.4±0.7*
HEMATOCRIT (% Packed Cells)	46.8±0.3	44.9±0.5*	46.4±0.3	46.9±0.4	47.7±0.3	47.0±0.4
PLASMA OSMOLALITY (mOsm/kg)	299±1	---	317±1*	310±2*	306±1*	---

Rats were injected with 15% NaCl (5 ml/kg, s.c.), returned to their cages without access to water and sacrificed at various times thereafter. Values represent the mean ± 1 S.E. as determined from 8-24 rats. *, significantly different from control ($p<.01$).

TABLE 11

Effect of Hypertonic Saline Injection on DOPA Accumulation in the Posterior Pituitary

	Time After 15% NaCl (minutes)					
	0	30	60	120	180	240
DOPA ACCUMULATION (ng/mg protein/30 min)						
Experiment 1	0.91±0.11	1.07±0.09	---	1.30±0.11	---	1.04±0.06
Experiment 2	0.68±0.05	---	1.01±0.08	1.17±0.09	1.09±0.13	---
Experiment 3	1.26±0.19	---	1.01±0.10	1.39±0.15	1.51±0.11	1.54±0.17

In three separate experiments rats were injected with 15% NaCl (5 ml/kg, s.c.), returned to their cages without access to water and were sacrificed at various times thereafter. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 7-8 rats.

Figure 7. Effect of hypertonic saline injection on DOPA accumulation in the posterior pituitary, and on plasma sodium concentration and hematocrit. Rats were injected with 15% NaCl (5 ml/kg, s.c.), returned to their cages without access to water and sacrificed at various times thereafter. All animals were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Columns represent the mean and vertical lines represent 1 S.E. as determined from 8 rats. *, significantly different from 0 time control ($p<.01$).

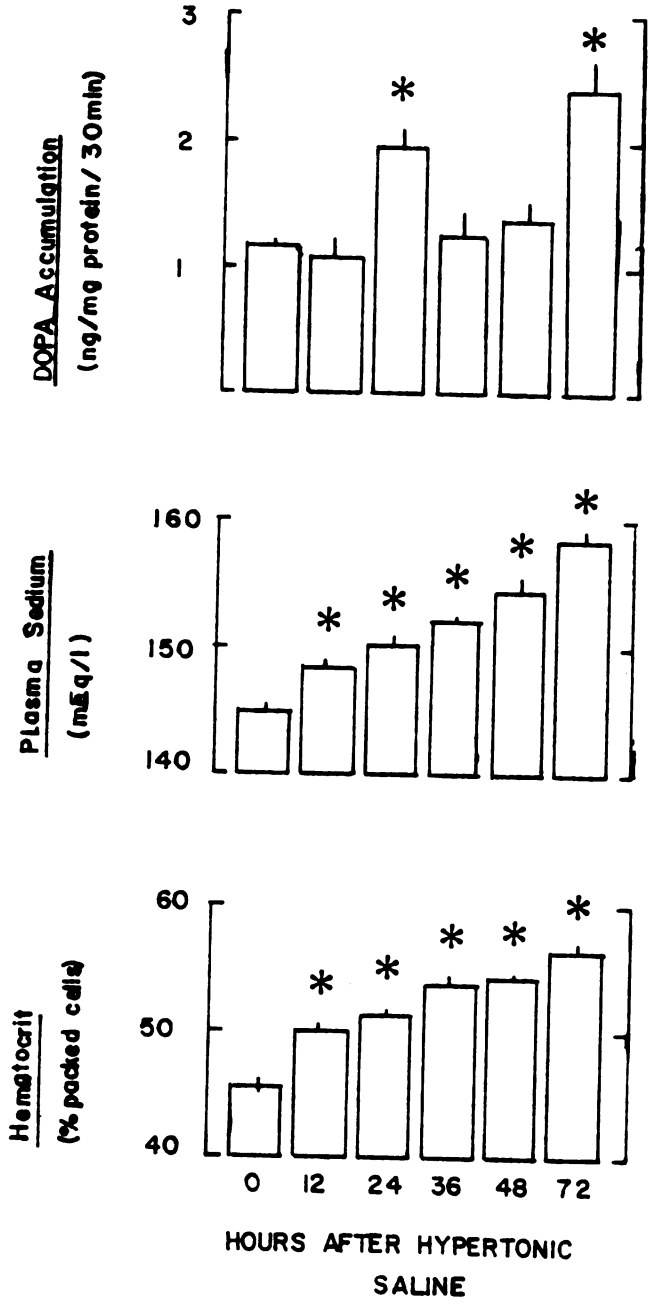
DOPA Accumulation
(ng/mg protein/ 30min)
3
2
1
Plasma Sodium
(mEq/l)
160
150
140
Hematocrit
(% packed cells)
60
50
40
0
12
24
36
48
72
HOURS AFTER HYPERTONIC SALINE

Figure 7

was not elevated following 12 hours of water deprivation. What is not apparent in Figure 5 is that the plasma sodium concentration in the animals administered 15% NaCl was elevated approximately 10 mEq/l for 1-2 hours immediately following the injection (again see Table 10). The effect of rapidly induced cellular dehydration (i.e., hypertonic saline injection) on DA synthesis and neuronal activity in the posterior pituitary was significantly different from water deprivation; 24 hours following the saline injection an increase in DOPA accumulation was observed. This increase was not sustained, for at 36 and 48 hours after the hypertonic saline injection DOPA accumulation had returned to control, only to be increased again at 72 hours by the continuing water deprivation.

An experiment was designed to compare DOPA accumulation, plasma sodium and hematocrit in rats following 24 hours of water deprivation or a hypertonic saline injection (Table 12). The plasma sodium concentration and the hematocrit in the two treated groups were elevated to a similar extent at the time of sacrifice. DOPA accumulation in the posterior pituitary was increased only in those rats which received a subcutaneous injection of 15% NaCl 24 hours prior to sacrifice.

In rats which were injected with 15% NaCl and permitted free access to water, there was a rapid (within 30 minutes) increase in the plasma sodium concentration and decrease in the hematocrit (Table 13). In direct contrast to rats administered 15% NaCl and then water-deprived, the rate of DOPA accumulation in the posterior pituitary, the plasma sodium concentration and the hematocrit in those

TABLE 12

Effects of Hypertonic Saline Injection on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

	Control	Water-deprived (24 h)	15% NaCl (24 h)
DOPA ACCUMULATION (ng/mg protein/30 min)	0.64±0.08	0.83±0.12	1.30±0.13**
PLASMA Na^+ (mEq/l)	137.9 ±0.5	143.9 ±0.6*	144.1 ±0.7*
HEMATOCRIT (% Packed Cells)	44.6 ±0.5	49.6 ±0.6*	50.7 ±0.5*

Rats were provided free access to food and water (control), were water-deprived for 24 hours or were injected with 15% NaCl (5 ml/kg, s.c.), returned to their cages without access to water and sacrificed 24 hours later. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$). **, significantly different from both control and water-deprived ($p<.01$).

TABLE 13

Effects of Hypertonic Saline Injection on Plasma Sodium and Hematocrit in Rats Allowed Free Access to Water

	Time After 15% NaCl (minutes)			
	0	30	60	120
PLASMA Na^+ (mEq/l)	139.3±0.7	151.1±0.7*	149.5±0.4*	144.7±1.7*
HEMATOCRIT (% Packed Cells)	49.0±0.8	44.0±1.0*	43.8±0.4*	43.3±1.7*

Rats were injected with 15% NaCl (5 ml/kg, s.c.) and then provided free access to water. Orbital sinus blood was sampled from 4 rats prior to injection (0 time) and 60 minutes after injection or from 5 rats at 30 and 120 minutes after injection. Orbital sinus puncture was performed on unanesthetized rats. Values represent the mean ± 1 S.E. *, significantly different from 0 time control ($p<.01$).

animals permitted free access to water for the 24 hours following the hypertonic saline injection were not increased at the time of sacrifice (Table 14).

6. Mannitol infusion

Experiments were designed to determine if marked acute increases in plasma osmolality with only slight changes in the plasma sodium concentration, could alter DOPA accumulation in the posterior pituitary.

a. 1.5 ml of 20% mannitol. Depicted in Tables 15 and 16 are results summarizing the effects of 1.5 ml of 20% mannitol infused intravenously (7 minutes) into 200 g rats. This mannitol dose represents an osmotic load of approximately 1.7 mOsm/200 g rat (the 15% NaCl injections used in the previous experiments delivered approximately 5.2 mOsm/200 g rat). Mannitol infusion did not significantly alter the plasma sodium concentration, the plasma osmolality or the hematocrit for up to 4 hours (Table 15). The increase in plasma osmolality of 7 mOsm/kg observed 30 minutes after the infusion was short of statistical significance due primarily to the small number of animals and was much less than the elevation observed following 15% NaCl (see Table 10).

The plasma sodium concentration and hematocrit increased progressively from 12-48 hours after the mannitol infusion (compare to water deprivation, Table 9, and hypertonic saline injection, Figure 7). DOPA accumulation in the posterior pituitary was not increased following the mannitol infusion; the slightly increased

TABLE 14

Effects of Hypertonic Saline Injection on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

	Control	15% NaCl (24 hours)	
		Access to Water	No Access to Water
DOPA ACCUMULATION (ng/mg protein/30 min)	0.66±0.09	0.71±0.10	1.05±0.09*
PLASMA Na^+ (mEq/l)	140.6 ±0.5	140.1 ±0.3	144.6 ±0.6*
HEMATOCRIT (% Packed Cells)	46.0 ±0.6	46.2 ±0.7	52.2 ±0.6*

Rats were provided free access to food and water (condrol) or were injected with 15% NaCl (5 ml/kg, s.c.). One group of hypertonic saline injected rats was provided free access to water for the following 24 hours while another group was water-deprived for the 24 hours following the hypertonic saline injection. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 10 rats. *, significantly different from control ($p<.01$).

TABLE 15

Acute Effects of Hypertonic Mannitol Infusion on Plasma Sodium, Plasma Osmolality and Hematocrit

	Time After 20% Mannitol (minutes)				
	0	30	60	120	240
PLASMA Na^+ (mEq/l)	142.0±0.8	138.8±0.2	142.8±1.0	141.3±0.5	142.6±0.8
PLASMA OSMOLALITY (mOsm/kg)	294±3	301±1	295±2	298±1	300±2
HEMATOCRIT (% Packed Cells)	41.8±0.6	41.8±0.2	41.2±1.0	40.8±0.6	43.0±0.3

Rats were infused with 20% mannitol (1.5 ml in 0.9% NaCl/rat/7 minutes), returned to their cages without access to water and sacrificed at various times thereafter. Values represent the mean ± 1 S.E. as determined from 4-5 rats.

TABLE 16

Effects of Mannitol Infusion on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

	Time After Mannitol (hours)				
	0	12	24	36	48
DOPA ACCUMULATION (ng/mg protein/30 min)	1.28±0.07	1.17±0.12	1.74±0.14	1.47±0.08	1.82±0.15
PLASMA Na^+ (mEq/l)	140.5 ±0.7	142.9 ±0.8	146.0 ±1.1*	147.5 ±0.7*	150.0 ±1.0*
HEMATOCRIT (%Packed Cells)	43.3 ±0.4	46.4 ±0.6*	47.8 ±0.8*	47.9 ±0.6*	50.9 ±0.5*

Rats were infused with 20% mannitol (1.5 ml in 0.9% NaCl/rat/7 minutes), returned to their cages without access to water and sacrificed at various times thereafter. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

values at 24 and 48 hours (but not 36 hours) were short of statistical significance ($p>0.01$).

b. 5.0 ml of 20% mannitol. To compare similar osmotic stimuli, rats were infused with 5.0 ml of 20% mannitol (5.5 mOsm/rat/8 minutes) or were injected subcutaneously with 15% NaCl (5.2 mOsm/rat). Also included in this experiment were 8 rats administered 15% NaCl and permitted free access to water 4 hours after the hypertonic saline injection. This was to determine if the elevation in plasma osmolality observed within the first 4 hours following the saline injection (see Table 10) triggered a 24 hour delayed increase in DOPA accumulation in the posterior pituitary (see Tables 12 and 14, and Figure 7).

Acutely, mannitol markedly increased plasma osmolality and moderately increased plasma sodium concentrations (Table 17). A slight increase in hematocrit was also observed. The hypertonic saline injection caused a large elevation in plasma osmolality; this was attributable to a marked hypernatremia. In this experiment 15% NaCl administration did not alter the hematocrit (compare to the slight hypervolemia in Table 10).

DOPA accumulation in the posterior pituitary, the plasma sodium concentration and osmolality, and the hematocrit were increased 24 hours after mannitol and hypertonic saline (Table 18). Also, the effects of the hypertonic saline injection were prevented by providing the rats access to water 4 hours after saline administration.

TABLE 17

Acute Effects of Hypertonic Saline and Mannitol on Plasma Sodium, Plasma Osmolality and Hematocrit

	Control	20% Mannitol		15% NaCl	
		30 minutes	60 minutes	30 minutes	60 minutes
PLASMA Na^+ (mEq/l)	140.0±0.7	141.9±0.8	147.0±0.9*	152.0±1.1*	149.9±0.7*
PLASMA OSMOLALITY (mOsm/kg)	285±1	316±2*	309±3*	307±1*	300±1*
HEMATOCRIT (% Packed Cells)	39.5±0.6	43.0±0.4*	43.8±1.0*	39.1±0.5	41.4±0.4

Rats were administered either 20% mannitol (5 ml in 0.9% NaCl/rat/8 minutes) or 15% NaCl (5 ml/kg, s.c.), returned to their cages without access to water and sacrificed at various times thereafter. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

TABLE 18

Effects of Hypertonic Saline and Mannitol on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium, Plasma Osmolality and Hematocrit

	Control	20% Mannitol	15% NaCl	15% NaCl plus Water
DOPA ACCUMULATION (ng/mg protein/30 min)	0.85±0.08	1.43±0.11*	1.23±0.07*	0.84±0.06
PLASMA Na^+ (mEq/l)	138.5±0.6	145.6±0.6*	144.4±0.6*	138.6±0.4
PLASMA OSMOLALITY (mOsm/kg)	286±1	299±1*	298±2*	286±1
HEMATOCRIT (% Packed Cells)	42.1±5	46.9±0.6*	48.9±0.7*	42.8±0.6

Rats were administered either 15% NaCl (5 ml/kg, s.c.) or 20% mannitol (5.0 ml in 0.9% NaCl/rat/8 minutes), returned to their cages without access to water and sacrificed at various times thereafter. One group of rats (15% NaCl plus water) was given access to water 4 hours after the saline injection until sacrifice. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

Tuberohypophyseal dopaminergic activity is not regulated by marked hyperosmolality per se. The acute effects of hypertonic saline injections on plasma sodium concentrations and osmolality are similar whether water is constantly available or not. However, DOPA accumulation in the posterior pituitary is increased only if the rats are dehydrated from the time of injection until the time of sacrifice. Apparently, the rapid marked elevation of plasma osmolality does not immediately trigger events which latently increase DA synthesis in the posterior pituitary.

C. Effect of High Sodium Diet on DOPA Accumulation

Rats fed a palatable high sodium diet (10 times the sodium content of normal rat chow) for up to seven days had normal rates of DOPA accumulation in the posterior pituitary, plasma sodium concentration, and hematocrit (Table 19). These animals apparently compensated for the high sodium intake by drinking larger volumes of water and excreting the excess sodium. This enabled maintenance of normal plasma sodium concentrations and hematocrit values.

D. Summary

The results presented in Section II suggest a direct relationship bewteen intracellular dehydration and DA synthesis in the posterior pituitary. DOPA accumulation is increased in the posterior pituitary 24 hours after the onset of intracellular dehydration induced either by hypertonic saline or mannitol injections. The saline-induced effect is only short-lived and can be prevented by allowing the injected animals access to water.

TABLE 19

Effects of High Sodium Diet on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

	Control	Days on High Sodium Diet 3	5	7
DOPA ACCUMULATION (ng/mg protein/30 min)	0.70±0.12	0.80±0.11	0.74±0.10	0.93±0.05
PLASMA Na^+ (mEq/l)	136.4 ±0.7	135.4 ±1.2	134.8 ±0.9	134.6 ±1.1
HEMATOCRIT (% Packed Cells)	47.9 ±0.5	48.6 ±0.4	48.5 ±0.5	46.1 ±0.4

Rats were fed a high sodium diet (3.15% Na^+ as compared to 0.39% Na^+ in normal rat chow; control) for various lengths of time. All animals were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats.

Small changes in plasma osmolality stimulate thirst and AVP secretion (1-2%; Dunn et al., 1973). Rats with access to water following hypertonic saline injections imbibe large volumes of water in an attempt to maintain normal cellular osmolality, dilute the elevated plasma sodium concentration and aid in the excretion of excess sodium. Rats administered a hyperosmotic stimulus and then subjected to water deprivation draw intracellular fluid into the extracellular fluid compartments. These animals will exhibit a cellular hyperosmolality which is apparently involved in stimulating tuberohypophyseal dopaminergic neuronal activity. Thus, DA synthesis in the terminals of the tuberohypophyseal neurons appears to be regulated, in part, by cellular dehydration and not directly by plasma osmolality.

III. Rehydration

From data presented in the previous sections it appears that either intracellular dehydration and/or an increase in plasma osmolality cause a delayed increase in DA synthesis in the posterior pituitary. This effect is not dependent on changes in plasma volume. The next series of experiments were to determine if the dehydration-induced effects were reversible upon rehydration. Studies were conducted to determine the time course of this reversal and the importance of alterations in fluid balance parameters during rehydration on the rate of DA synthesis in the posterior pituitary.

A. Effect of Saline and Water Rehydration on DOPA Accumulation

Water deprivation of three days is required to consistently increase DOPA accumulation in the posterior pituitary. The initial

experiment was designed to examine the effect of two days rehydration with either 2% NaCl or tap water on rats deprived of water for three days (Table 20). As shown previously, three and five days of water deprivation increased DOPA accumulation in the posterior pituitary, the plasma sodium concentration, the hematocrit and the plasma protein concentration. Five days of saline drinking also increased DOPA accumulation and the plasma sodium concentration, but did not alter either estimate of plasma volume. When rats were deprived of water for three days and then given access to 2% NaCl for two days, both the hematocrit and plasma protein concentration returned to control, but DOPA accumulation and the plasma sodium concentration remained elevated. In contrast, two days of rehydration with tap water restored all measured parameters to control values.

These data demonstrate two significant points. First, in dehydrated rats restoration of normal plasma volume without a concomitant change in the plasma sodium concentration by rehydration with 2% NaCl is not sufficient to return activity of the tuberohypophyseal DA neurons to normal. Second, although three days of dehydration are required to consistently increase DA synthesis in the posterior pituitary, this effect can be reversed within two days of rehydration.

B. Time Course of Water Rehydration on DOPA Accumulation

In rats deprived of water for three days the rate of DOPA accumulation in the posterior pituitary, the plasma sodium concentration and hematocrit are all increased. The time course of the reversal of these effects following access to drinking water was determined

TABLE 20

Effects of Dehyration and Rehydration on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium, Hematocrit and Plasma Protein

	Control	Water-deprived (3 Days)	Water-deprived (5 Days)	2% NaCl (5 Days)	Water-deprived (3 Days)	
					Plus Water (2 Days)	Plus 2% NaCl (2 Days)
DOPA ACCUMULATION (ng/mg protein/30 min)	0.71±0.06	1.37±0.09*	1.34±0.09*	1.72±0.23*	1.10±0.10	1.75±0.10*
PLASMA Na^+ (mEq/1)	128.0±2.2	140.6±1.9*	141.8±2.4*	153.1±4.8*	126.0±3.1	152.8±4.4*
HEMATOCRIT (% Packed Cells)	44.8±0.4	54.8±0.5*	57.4±0.8*	56.3±0.7	44.8±0.6	45.8±1.4
PLASMA PROTEIN (g/100 ml)	6.06±0.11	7.26±0.12*	6.86±0.10*	6.45±0.16	6.03±0.10	6.53±0.27

Rats were dehydrated by water deprivation (3 or 5 days) or given 2% NaCl to drink for 5 days. Other rats were water deprived for 3 days and then given water or 2% NaCl to drink for 2 days. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<0.01$).

in three separate experiments; the results are summarized in Tables 21, 22 and 23. Three days of water deprivation increased the plasma sodium concentration, the hematocrit and the rate of DOPA accumulation in the posterior pituitary in all 3 experiments; this occurred whether NSD 1015 was administered intraperitoneally (Table 21) or intravenously (Tables 22 and 23). DOPA accumulation could not be determined in rats given access to water for 1 hour after the i.p. administration of NSD 1015 (see Results Section I.B.2.b.), but within 24 hours of rehydration DOPA accumulation had returned to control (Table 21). In this experiment the plasma sodium concentration was restored to normal values with 1 hour of rehydration; the hematocrit remained slightly elevated for as long as 48 hours. When shorter periods of rehydration were examined (Tables 22 and 23) it was observed that DOPA accumulation and the plasma sodium concentration were returned to control with 1-3 hours of rehydration while, during the period of the experiment, the hematocirt slowly approached but did not return to normal values.

The data demonstrate that DOPA accumulation in the posterior pituitary of dehydrated rats is restored to control by rehydration in a similar temporal pattern as the restoration of the plasma sodium concentration. This may be interpreted to mean that the deactivation (as well as the activation) of the tuberohypophyseal system is dependent on intracellular electrolyte concentrations since the plasma sodium concentration generally reflects plasma osmolality and the degree of cellular hydration (Leaf, 1962a,b). The reversal of the plasma volume deficit is not a major regulatory factor in the restoration of normal tuberohypophyseal neuronal activity in rehydration.

TABLE 21

Effects of Water Deprivation and Subsequent Rehydration on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

			Hours Rehydration		
	Control	Water-deprived	1	24	48
DOPA ACCUMULATION (ng/mg protein/30 min)	1.15±0.11	1.78±0.07*	---	1.05±0.02	1.20±0.19
PLASMA Na^+ (mEq/l)	133.5 ±0.6	145.0 ±1.3*	133.0±0.5	130.7 ±0.8	130.9 ±0.6
HEMATOCRIT (% Packed Cells)	40.7 ±0.6	51.2 ±0.7*	49.8±0.6*	42.1 ±0.7*	42.6 ±0.5*

Rats provided free access to food and water (control) or were deprived of water for 3 days. Groups of rats were rehydrated for 1, 24 or 48 hours following 3 days of water deprivation. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

TABLE 22

Effects of Water Deprivation and Subsequent Rehydration on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

	Control	Water-Deprived	Hours Rehydration			
			3	6	9	12
DOPA ACCUMULATION (ng/mg protein/10 min)	1.25±0.10	2.13±0.15*	1.59±0.08	1.40±0.08	1.48±0.07	1.35±0.15
PLASMA Na^+ (mEq/l)	144.0 ±0.5	155.4 ±0.9*	142.2 ±0.8	141.6 ±0.5	141.6 ±0.4	142.7 ±0.6
HEMATOCRIT (% Packed Cells)	41.5 ±0.5	51.4 ±0.8*	49.3 ±0.6*	46.7 ±0.4*	47.9 ±0.2*	46.6 ±1.0*

Rats were provided free access to food and water (control) or were deprived of water for 3 days. Groups of rats were rehydrated for 3, 6, 9 or 12 hours following 3 days of water deprivation. All rats were administered NSD 1015 (25 mg/kg, i.v.) 10 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 5-10 rats. *, significantly different from control ($p<.01$).

TABLE 23

Effects of Water Deprivation and Subsequent Rehydration on DOPA Accumulation in the Posterior Pituitary, and on Plasma Sodium and Hematocrit

			Hours Rehydration		
	Control	Water-deprived	1	3	6
DOPA ACCUMULATION (ng/mg protein/10 min)	0.65±0.04	1.45±0.18*	1.25±0.07*	1.07±0.08	0.82±0.08
PLASMA Na^+ (mEq/l)	142.0 ±0.5	154.7 ±1.0*	145.0±1.1*	138.6 ±0.5*	139.7 ±0.5
HEMATOCRIT (% Packed Cells)	42.3 ±0.5	54.8 ±0.6*	51.8±0.7*	52.3 ±0.5*	47.9 ±0.5*

Rats were provided free access to food and water (control) or were deprived of water for 3 days. Groups of rats were rehydrated for 1, 3 or 6 hours following 3 days of water deprivation. All rats were administered NSD 1015 (25 mg/kg, i.v.) 10 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 7-10 rats. *, significantly different from control ($p<.01$).

Although both DOPA accumulation in the posterior pituitary and the plasma sodium concentration are restored to control values at about the same time, a direct cause-and-effect relationship can be hypothesized from, but is not proven by, the data presented.

IV. Vasopressin

A. Effect of Continuous Vasopressin Infusion on DOPA Accumulation

Tanaka et al. (1977) reported that the intraventricular infusion of AVP increased the αMT-induced decline of DA in the median eminence and striatum (DA turnover in the posterior pituitary was not reported). Dehydration, however, increased the plasma AVP concentration but did not alter the concentrations of AVP found in the CSF (Mens et al., 1980). An experiment was designed to examine the effect of systemic AVP administration on the activity of central dopaminergic neuronal systems.

Two doses of synthetic AVP were infused intravenously at a rate of 24 µl/day using subcutaneously implanted Alzet^{R} osmotic minipumps. The doses infused were 240 mU/rat/day as a physiological replacement dose and 720 mU/rat/day to approximate plasma AVP concentrations observed following 2-3 days of dehydration. The results are summarized in Table 24.

Neither dose of AVP altered the rate of DOPA accumulation in the posterior pituitary, median eminence or striatum. The plasma sodium concentration, as expected, was slightly decreased by the higher dose of AVP (Chan, 1971; Smith et al., 1979) whereas the hematocrit was unaltered by either dose of AVP.

TABLE 24

Effects of Continuous Vasopressin Infusion on DOPA Accumulation in Selected Brain Regions, and on Plasma Sodium and Hematocrit

	Treatment		
	Sham	240 mU/day	720 mU/day
DOPA ACCUMULATION (ng/mg protein/30 min)			
Posterior Pituitary	0.64±0.05	0.75±0.08	0.69±0.04
Median Eminence	8.1 ±0.8	8.8 ±0.9	8.8 ±2.2
Striatum	8.0 ±0.7	10.7 ±0.7	9.6 ±0.7
PLASMA Na^+ (mEq/l)	142.9 ±0.7	141.5±0.8	138.4±0.7*
HEMATOCRIT (% Packed Cells)	47.6 ±0.8	47.1±0.6	47.4±0.4

Synthetic AVP was infused intravenously for 3 days using subcutaneously implanted osmotic mini-pumps. The sham-control animals were anesthetized and the jugular vein was ligated 3 days prior to sacrifice. All rats were administered NSD 1015 (100 mg/kg, i.p.) 30 minutes prior to sacrifice. Values represent the mean ± 1 S.E. as determined from 8 rats. *, significantly different from control ($p<.01$).

Infusions of AVP (Tanaka et al., 1977) or an antibody to AVP (Versteeg et al., 1979) directly into the CSF may alter DA turnover in the tuberoinfundibular and nigrostriatal neurons. However, the systemic infusion of AVP is without effect on DOPA accumulation in any region examined. These data are of interest because: 1) the CSF AVP concentration does not appear to be elevated concomitantly with the plasma AVP concentration. This has been demonstrated directly by measurement of the peptide (Mens et al., 1980) and indirectly by measurement of neurochemical events (i.e., DA synthesis and turnover) induced by AVP. 2) DA synthesis in the posterior pituitary is not regulated by exogenous AVP infusions. This does not prove, however, that the rate of DOPA accumulation in the posterior pituitary is not stimulated by endogenously released AVP. The plasma AVP concentrations following dehydration and minipump implantation may not be comparable.

DISCUSSION

I. Concentration and Synthesis of Catecholamines

DA in the posterior pituitary gland of the rat is contained in nerve terminals of central origin, the cell bodies are most likely located in the arcuate nucleus (Björklund et al., 1970; Tilders et al., 1979). The turnover rate of DA in the tuberohypophyseal neurons is similar to that in the nigrostriatal neurons. Since DA in the posterior pituitary is being continually synthesized, the steady state concentration remains constant and the intraneuronal metabolism to DOPAC is low, it must be concluded that this amine is being continually released from the terminals of the tuberohypophyseal neurons. Once released DA can activate putative DA receptors (Sibley and Creese, 1980; Stefanini et al., 1980a) which may (Ahn et al., 1979) or may not (Stefanini et al., 1980b) be linked to adenylate cyclase. The postsynaptic DA receptors may be located on neurosecretory axons, pituicytes or vascular smooth muscle. Alternatively, DA released from the terminals of the tuberohypophyseal neurons can act as a neurohormone following its release into the capillary blood flowing from the neurohypophysis.

DA synthesis in the posterior pituitary has been estimated in vivo using two biochemical techniques. All non-steady state methods

employed to estimate catecholamine synthesis and turnover have inherent deficiencies yet it is agreed that all methods yield relative synthetic rates (Weiner, 1974). With proper experimental design, the effects of pharmacological and physiological treatments on DA synthesis provide good biochemical indices of neuronal activity in dopaminergic systems.

The major advantage of the αMT technique over most others presently in use is that both DA and NE synthesis rates can be determined following tyrosine hydroxylase inhibition. The disadvantages, however, are numerous. To accurately estimate catecholamine synthesis rates, 4 time points after αMT (from 0-90 minutes) are preferable. Eight rats are required for each time and all treatments must be compared to their appropriate control. Therefore, 64 rats are required to determine the effect of just one treatment on DA and NE synthesis. Time and economic constraints limit the number of experiments that can be done employing this technique. The high dose of αMT required to completely inhibit tyrosine hydroxylase and the length of time of synthesis inhibition used experimentally probably cause effects secondary to the treatment being studied. Also, statistical analysis is quite difficult, particularly in the posterior pituitary. Comparisons between treatments are performed on the rate of decline (k) of the monoamines. This rate constant is the slope of the line (determined by regression analysis) of the natural logarithm of the catecholamine concentration versus time. The variability about each time point contributes to the variability of the slope (i.e., k). Ninety minutes after the administration of αMT, the amount of DA in control posterior

pituitaries is near the lower limit of the radioenzymatic assay. Therefore, the concentration of DA in the posterior pituitary will be extremely variable 60 to 90 minutes after αMT; this will be reflected in the confidence limits about the rate constant. If a treatment is expected to increase DA synthesis (i.e., cause a faster rate of decline following αMT), there generally will be an ever larger variation in the DA content of the posterior pituitary. A statistical comparison is unlikely to show significance.

Holzbauer et al. (1978) first published results of a study utilizing the αMT-induced decline of DA in the posterior pituitary. The same method was used in this thesis only to validate the rate of DOPA accumulation after decarboxylase inhibition as a second in vivo estimate of DA synthesis in the posterior pituitary and median eminence. It was reported previously that in vitro tyrosine hydroxylase activity in median eminence homogenates reflects primarily DA synthesis (Kizer et al., 1976a). The ratio of DA to NE is similar in the median eminence and posterior pituitary. Accordingly, it was felt that an in vivo measure of tyrosine hydroxylase, the rate of DOPA accumulation after the inhibition of DOPA decarboxylase, would estimate DA synthesis in both regions. Data verifying this assumption were presented in the Results Section I.B. Data were also presented demonstrating that DOPA accumulation can be measured as soon as 10 minutes after the intravenous administration of NSD 1015. This minimizes secondary effects of synthesis inhibition on the catecholaminergic systems. Since endogenous concentrations of DOPA are not detectable in brain tissue (Demarest and Moore, 1979a; 1980) no zero-time control is

required. The number of animals per experiment are reduced, allowing several treatments to be studied in each experiment and permitting easy statistical analysis.

Estimating DA synthesis in the posterior pituitary and median eminence by measuring the rate of DOPA accumulation after decarboxylase inhibition is not without its limitations. For instance, DOPA accumulation estimates total catecholamine synthesis. Under normal circumstances, however, 90% of the catecholamines being synthesized in the posterior pituitary are DA. Changes in NE synthesis following pharmacological or endocrinological manipulations may be obscured in measurements of DOPA accumulation. A second deficiency in this technique is that decreases in DOPA accumulation cannot be observed readily in the posterior pituitary. Care should be taken to maximize the sensitivity of the assay. For example, the SAM can be purified as suggested by Bauce et al. (1980) using the procedure of Glazer and Peale (1978).

When the advantages and disadvantages of the two techniques are compared, it is obvious that the rate of DOPA accumulation after decarboxylase inhibition is the more appropriate in vivo estimate of DA synthesis to characterize the regulation of the tuberohypophyseal DA system. The utility of this technique to study dopaminergic activity in the posterior pituitary has been recently demonstrated (Alper et al., 1980; Alper and Moore, 1981; Demarest and Moore, 1979a).

II. Dehydration and Rehydration

A. Delayed Activation of the Tuberohypophyseal Dopamine Neurons

The activity of the tuberohypophyseal DA neurons appears to be regulated, in part, by cellular dehydration. There is controversy about thirst, AVP release and the relative importance of sodium- versus osmoreceptors (see for example, Andersson, 1977). The data presented in Results Section II.B. clearly demonstrate that the mechanism responsible for increasing DA synthesis in the posterior pituitary is osmosensitive. The apparent delayed activation of the tuberohypophyseal DA neurons (three days of water deprivation or 24 hours following an osmotic stimulus) is difficult to explain.

This "delayed activation" of the tuberohypophyseal DA neurons may actually be an artifact of the in vivo estimate of DA synthesis employed. Although DOPA accumulation is clearly increased in the posterior pituitary many hours after the administration of mannitol or hypertonic saline, or following three days of water deprivation, no direct measure of neuronal activity or DA release was employed. A small increase in the firing rate and subsequent release of DA from the terminals of the tuberohypophyseal neurons could markedly alter physiological responses, yet not cause a measurable increase in the synthesis rate of DA in the posterior pituitary.

Assuming that the latent response of the tuberohypophyseal DA neurons is real, a simplistic view of this is that DA synthesis in the posterior pituitary can be increased only in a sluggish manner. Data presented by Demarest and Moore (1979a) do not support this possibility. The authors have demonstrated that several pharmacological

treatments (e.g., haloperidol, apomorphine, GBL) will moderately, but rapidly, alter the rate of DOPA accumulation in the posterior pituitary.

The molecular regulation of tyrosine hydroxylase may be different in response to physiological and pharmacological manipulations. Briefly, the activity of tyrosine hydroxylase may be increased through induction, as observed following reserpine treatment (Reis et al., 1974, 1975; Renaud et al., 1979), by increasing the affinity of the enzyme for its pteridine cofactor, as seen in the striatum after neuroleptic treatment (Zivkovic et al., 1974, 1975), or by increasing the specific activity of the enzyme without altering the amount of immunoprecipitable tyrosine hydroxylase or the affinity of the enzyme for substrate or cofactor, as seen in the retina following long-term exposure to light (Iuvone et al., 1979).

The pharmacologically induced acute changes in DOPA accumulation observed in the posterior pituitary (Demarest and Moore, 1979a) are probably due to an increase in the affinity of tyrosine hydroxylase for the pteridine cofactor. On the other hand, the sluggish response of in vivo tyrosine hydroxylase activity (i.e., DOPA accumulation) in the posterior pituitary to osmotic stimulation by dehydration, hypertonic saline or mannitol may involve enzyme induction. It should be noted at the outset of this discussion that induction of tyrosine hydroxylase has not been documented in the terminals of any dopaminergic fiber tract, although it has been suggested to occur in the median eminence (Johnston et al., 1980).

DA synthesis is increased selectively in the median eminence 8 to 12 hours after the elevation of the prolactin concentration in the serum or CSF (see review by Moore et al., 1980a). The sluggish response of the tuberoinfundibular DA neurons is dependent on protein synthesis (Johnston et al., 1980) and induction of tyrosine hydroxylase has been postulated to be the mechanism involved. DOPA accumulation in the median eminence is elevated for approximately thirty hours following the administration of a high dose of haloperidol (Demarest and Moore, unpublished observations) resulting from hyperprolactinemia induced by blockade of DA receptors located in the anterior pituitary gland (Gudelsky et al., 1978; Demarest and Moore, 1980). However, the serum prolactin concentration is elevated for only a few hours following haloperidol (Demarest and Moore, unpublished observations). Thus, the high serum concentration of prolactin triggers events dependent on protein synthesis (induction of tyrosine hydroxylase?) to latently increase DA turnover in the median eminence. The increased tuberoinfundibular DA activity is maintained long after the initiating stimulus has subsided.

The administration of a hyperosmotic stimulus (saline or mannitol) causes a delayed activation of the tuberohypophyseal DA system in a manner reminiscent of the prolactin-induced effect on the tuberoinfundibular DA system. Three experiments suggest that intracellular dehydration selectively regulates the tuberohypophyseal DA neurons by a mechanism other than induction of tyrosine hydroxylase. First, hypertonic saline injections induce only short-term increases

in DA synthesis in the posterior pituitary. Long-term elevation would be predicted if enzyme induction had indeed occurred. Secondly, rats permitted access to water 4 hours after the administration of hypertonic saline do not respond with the characteristic latent increase in the rate of DOPA accumulation in the posterior pituitary. The stimulus to increase tuberohypophyseal dopaminergic activity appears to occur within the first 4 hours of saline injection when a marked elevation of plasma osmolality is observed. Had tyrosine hydroxylase induction actually been initiated during the rapidly developing cellular dehydration, the subsequent availability of water would not have been expected to shut off the protein synthetic machinery, thereby inhibiting the effect of the saline injection on DOPA accumulation in the posterior pituitary. Thirdly, it is assumed that water deprivation and the acute osmotic stimuli activate tyrosine hydroxylase by the same mechanism. Three days of dehydration are required to increase DOPA accumulation in the posterior pituitary, whereas only 1-3 hours of rehydration can reverse this effect. If enzyme induction was the reason for the slow onset of increased tuberohypophyseal neuronal activity, the inactivation of tyrosine hydroxylase during rehydration would be gradual, related to the turnover time of the enzyme protein.

The arguments presented above assume that the activation and inactivation processes are mechanistically opposites. That is, if activation of tyrosine hydroxylase due to cellular dehydration requires protein synthesis, the inactivation involves protein degradation. This may be a false assumption. The delayed increase in tyrosine hydroxylase activity observed in the tuberohypophyseal DA neurons

resulting from osmotic stimulation could be due to enzyme induction. The restoration of normal enzymatic activity during rehydration could be a change in the affinity of the enzyme for substrate or cofactor. There are techniques presently available which permit the testing of this hypothesis.

Finally, the latent increase in DA synthesis in the terminals of the tuberohypophyseal neurons might result indirectly from marked cellular hyperosmolality (see below) induced rapidly by the injection of a hyperosmotic solution or slowly by water deprivation. The increase in cellular osmolality may initiate a series of events which culminate in the selective increase in DOPA accumulation in the posterior pituitary. These events could include, but are not limited to, synthesis of tyrosine hydroxylase as discussed previously, depletion of the AVP or oxytocin content of the neural lobe, or synthesis or depletion of some unknown factor which is the immediate regulator of the DA nerves terminating in the posterior pituitary. As noted previously, once the events are initiated they can be reversed by permitting the rats access to water.

B. Hyperosmolality and Tuberohypophyseal Dopaminergic Activity

Even more perplexing than the delayed activation of tyrosine hydroxylase in the posterior pituitary is the time course of the effects of the hypertonic stimuli on DOPA accumulation. There is a delayed increase (24 hours) in the activity of the tuberohypophyseal DA neurons in rats administered hyperosmotic solutions. The increase is not maintained (control values observed 36 and 48 hours after the

hypertonic saline injection), but later reappears following 72 hours of water deprivation. There obviously is not a 1:1 temporal correlation between plasma osmolality and DOPA accumulation in the posterior pituitary. No effect is observed on DOPA accumulation from 1 to 4 hours after hypertonic saline, the period of peak plasma osmolality. The marked hyperosmolality lasts only a few hours until there is a redistribution of body water (a shift from intracellular to extracellular spaces) and until the renal excretory mechanisms can compensate for the osmotic load. The homeostatic mechanisms act very rapidly; the fluid balance parameters are stabilized about 4 hours after mannitol or hypertonic saline injection.

A theoretical explanation for the effects of the hypertonic stimuli on DOPA accumulation in the posterior pituitary is outlined below. As the cellular osmolality surpasses a threshold value a series of events are triggered to increase DOPA accumulation in the posterior pituitary some 24 hours later. As the osmolality returns back through the threshold value towards normal (in the presence of continuing dehydration), the tuberohypophyseal DA system is inactivated, again with a 24 hour lag time. If this scheme is correct the osmo-receptive site must be exquisitely sensitive over a narrow range. When cellular dehydration is at least partially inhibited by providing water to rats administered 15% NaCl, the plasma sodium concentration rises rapidly and to a very similar degree as in those rats administered saline but not allowed to drink. However, DOPA accumulation in the posterior pituitary is not increased when rats are given drinking water simultaneous to, or 4 hours following, the injection of hypertonic

saline. This argues against a receptor sensitive to changes in plasma, CSF or extracellular fluid osmolality in the regulation of tuberohypophyseal DA neuronal activity. Instead it supports regulation of DA synthesis in the posterior pituitary directly or indirectly by an intracellular osmoreceptor. The location of the osmoreceptive cells is unknown.

Hypertonic stimuli may activate the tuberohypophyseal DA neurons by increasing intracellular osmolality and not by decreasing cellular volume. There is good evidence that the cerebral osmolality parallels the plasma osmolality with only a slight time delay (see Arieff et al., 1977). Changes in the plasma osmolality, therefore, closely mirror changes in the osmolality of the cells regulating DA synthesis in the posterior pituitary if it is assumed that these cells are located centrally, possibly in the tuberohypophyseal perikarya in the arcuate nucleus. The central nervous system is partially protected from large decreases in volume during sustained hyperosmolality (Arieff and Guisado, 1976; Arieff et al., 1977) and extended dehydration (Jelsma and McQueen, 1967). Both CSF volume and total brain water decrease acutely following rapid hypernatremia, but return to normal after a period of hours to days (depending on stimulus and species). The osmole content of the brain and CSF is elevated even when the water content has been restored. All of the major osmotically active constituents of the brain are in their normal concentration. The brain cells need to maintain normal volume during sustained hypernatremia and do so by generating "idiogenic osmoles" to retain water. These

idiogenic osmoles have not yet been identified. The measurement of brain water content during prolonged hyperosmolar states will not be an index of cellular osmolality in the brain. It may be suggested, therefore, that cellular osmolality and not cellular volume is the factor involved in the regulation of the tuberohypophyseal DA neurons.

C. Hypovolemia and Tuberohypophyseal Dopaminergic Activity

Water deprivation causes not only hypernatremia but it also reduces plasma volume. The results of the present study indicate that hypovolemia is not essential for the increased DA synthesis in the posterior pituitary. Saline drinking does not produce hypovolemia but does increase DOPA accumulation. Furthermore, reversal of the plasma volume deficit per se by allowing dehydrated rats access to a 2% NaCl drinking solution does not reduce the elevated rate of DA synthesis in the posterior pituitary of these rats. Plasma volume deficits were not considered to be vital to the water deprivation-induced effects on DA synthesis and were therefore omitted from the discussion above. This is not to say, though, that a rapid reduction in plasma volume (hemorrhage, for example) might not alter DA synthesis in the posterior pituitary.

Essentially, the data suggest that DA synthesis and release in the terminals of only the tuberohypophyseal neurons may parallel, and be partially regulated by, cellular osmolality. This correlation occurs with a delay of 24 to 72 hours, depending on the intensity and the nature of the stimulus.

III. Possible Functions for Tuberohypophyseal Dopamine Neurons

Synthetic, metabolic and receptive machinery for DA have been identified in the posterior pituitary. DA synthesis has been verified by the presence of tyrosine hydroxylase activity both *in vivo* (see Results Section I.B.) and *in vitro* (Saavedra *et al.*, 1975). DA is utilized at a rapid rate (see Results Section I.B. and Holzbauer *et al.*, 1978) and may be enzymatically inactivated by monoamine oxidase (Saavedra *et al.*, 1975; Demarest and Moore, in press). Although monoamine oxidase is present in both the neural and intermediate lobes, the concentration of DOPAC, the major deaminated metabolite of DA, is not a good index of DA release in the posterior pituitary (Annunziato and Weiner, 1980; Umezu and Moore, unpublished observations). DOPAC formation is largely dependent on DA reuptake into the presynaptic terminal. Nigrostriatal DA neurons have a high affinity DA uptake mechanism which is not found in tuberoinfundibular or tuberohypophyseal neurons (Annunziato *et al.*, 1980; Annunziato and Weiner, 1980; Demarest and Moore, 1979b). The lack of a high affinity DA transport system may explain why the DOPAC concentration in the posterior pituitary (Annunziato and Weiner, 1980; Umezu, Alper and Moore, unpublished observations) and the median eminence (Fekete *et al.*, 1979; Umezu and Moore, 1979) is very low.

The data presented in the body of the thesis demonstrate that the tuberohypophyseal DA system can be selectively activated. DA synthesis in the posterior pituitary is increased when there are many other dynamic events occurring in the neural and intermediate lobes of the pituitary gland. Thus, DA release may be increased in the posterior

pituitary of dehydrated rats to mediate or modulate some pituitary function. A few possibilities will be discussed below.

A. Vasopressin Release

1. Acute regulation

One of the major hormones of the posterior pituitary is AVP. Water deprivation, saline drinking, volume depletion, hyperosmotic states and a variety of other stimuli increase the secretion of AVP from the neurohypophysis (see Verney, 1947). Water deprivation and saline drinking have similar effects on the pituitary and plasma concentrations of AVP (Jones and Pickering, 1969; Mens et al., 1980). More detailed experiments have shown that small increases in plasma osmolality (1-2%) cause prompt increases in AVP secretion (Dunn et al., 1973). This is in contrast to the delayed effect of increases in plasma osmolality on DOPA accumulation in the posterior pituitary. Dunn et al. (1973) have also demonstrated that 12 hours of water deprivation significantly increase the plasma AVP concentration in rats. Again, this can be contrasted to the 72 hours of water deprivation required to consistently increase DA synthesis in the tuberohypophyseal neurons. The tuberohypophyseal DA system is obviously not involved in the acute release of AVP during osmotic stimulation or dehydration.

2. Augmentation

One possible function for the tuberohypophyseal DA neurons is to augment AVP release from the posterior pituitary. Following long-term dehydration (four days) the storage pools of AVP in the neural lobe of the rat are virtually depleted (Rougon-Rapuzzi

et al., 1978). As dehydration continues, the release of AVP from the neurohypophysis is dependent on its rate of synthesis and transport from the hypothalamus. Gainer et al. (1977) have shown that neurophysin precursors (MW 20,000) associated with AVP are transported from cells in the supraoptic nucleus into axons and nerve terminals in the neural lobe where they are processed into smaller releasable neurophysins (MW 10,000). The rate of this conversion is markedly enhanced in animals provided 2% NaCl to drink for seven days (Russell et al., 1981) allowing for the immediate release of AVP as it enters the neurohypophyseal nerve terminals. The enzymes involved in neurophysin conversion and their regulatory mechanisms have not been identified. Also, the duration of dehydration required to increase the rate of pro-hormone conversion to releasable neurophysin has not been determined; a temporal correlation with changes observed in the rate of DA synthesis in the posterior pituitary is not possible. However, the tuberohypophyseal DA system is activated when the posterior pituitary appears to be depleted of AVP. This increase in dopaminergic activity may stimulate either the axonal transport of the neurosecretory granules or the enzymatic conversion of the pro-hormone into its releasable form.

Further evidence linking DA to the stimulation of AVP secretion has appeared in two abstracts. Negro-Vilar (1979) demonstrated that AVP secretion from isolated neural lobes was increased in the presence of DA [Bridges and coworkers (1976) earlier reported that DA slightly increased the in vitro secretion of AVP from the neural lobe]. Bromocriptine (a direct acting DA agonist), administered

orally, augmented AVP release in response to osmotic stimulation in man (Robinson et al., 1981). Bromocriptine crosses the blood-brain barrier, therefore these data do not prove a pituitary site of action. Although these results have not been confirmed, a stimulatory role for the tuberohypophyseal DA neurons on AVP release is implied. The tuberohypophyseal dopaminergic pathway may not be involved in tonic regulation of hormone secretion (Kendler et al., 1978), but it may be activated to augment AVP release following prolonged stimulation of the hypothalamo-neurohypophyseal system.

3. Feedback inhibition

On the other hand, DA may function in the posterior pituitary in an AVP negative feedback loop. Bakker et al. (1975) reported that exogenously administered AVP increased the neural lobe content of the neurophysins without effecting the concentrations of AVP or oxytocin. The authors suggested that hormone release was decreased, intracellular degradation of AVP and oxytocin was increased, and the metabolism of the neurophysins was unaltered.

The tuberohypophyseal DA system has been directly implicated in the autoregulation of AVP secretion (see abstract by Ferris et al., 1979). The authors reported that low frequency stimulation of the arcuate nucleus in anesthetized rats decreased the spontaneous electrical activity in the neural lobe. This was blocked by the superfusion of pimozide (a DA antagonist) onto the neural lobe. Application of AVP on cells in the arcuate nucleus also reduced the electrical activity in the neural lobe. This was likewise blocked by superfusion of a neuroleptic onto the neurohypophysis.

Forsling and Lightman (1979), in another abstract, also proposed an inhibitory role for DA on AVP release. L-DOPA inhibited AVP secretion in man. This apparently occurred at the level of the neural lobe as pretreatment with carbidopa, a peripheral decarboxylase inhibitor, blocked the effect. It was not discussed, but L-DOPA is also converted to NE which, rather than DA, may inhibit AVP secretion.

It has been reported that the neuroleptic chlorpromazine stimulates AVP release by blocking DA receptors (Givant and Sulman, 1976). However, chlorpromazine has fairly potent anti-adrenergic and anti-cholinergic properties. Using a more specific anti-dopaminergic drug, haloperidol, Kendler et al. (1978) demonstrated that DA does not tonically regulate AVP secretion in humans.

The results presented in this thesis support a possible role for the tuberohypophyseal DA pathway in a proposed, though certainly not proven, AVP autoregulatory feedback loop. Dehydration causes progressive increases in plasma AVP concentrations (Dunn et al., 1973; Mens et al., 1980) which appear to be paralleled by similar progressive increases in the rate of DOPA accumulation in the posterior pituitary. Unfortunately, no data on plasma AVP concentrations have been reported following the hyperosmotic stimuli used experimentally in this thesis. According to the theory of Dunn et al. (1973), hypertonic saline and mannitol injections would be expected to cause rapid but short bursts of AVP secretion. The plasma AVP concentration would be directly related to the plasma osmolality. The initial rapid increase in the plasma AVP concentration could feedback through the tuberohypophyseal DA system to latently inhibit its own release. This

feedback mechanism would be inactivated as the plasma AVP concentration (reflected by the plasma osmolality) subsided. There is one serious flaw in this scheme. Three days of exogenous AVP infusion did not alter DOPA accumulation in the posterior pituitary (Table 24). However, the plasma AVP concentration in those rats administered exogenous AVP was not determined. It is unknown if either dose of AVP infused approximated the concentration of AVP in the plasma induced by three days of water deprivation or either of the hypertonic stimuli. Therefore, although weakened by these data, the hypothesis that the tuberohypophyseal DA neurons act sluggishly as feedback inhibitors of AVP secretion cannot be totally dismissed. More substantial evidence of AVP autoregulation, and then a positive correlation with DA synthesis in the posterior pituitary, is required.

4. Differential regulation of storage and releasable pools

DA may act in the posterior pituitary to differentially regulate the release of AVP from the "storage pool" (as contrasted to the "readily releasable pool"). Sachs et al. (1967) demonstrated that the initial release of AVP due to hemorrhagic hypotension was very rapid. Over time the rate of release markedly decreased and was not correlated to the AVP content of the posterior pituitary. A second hemorrhage 60 minutes after restoration of normal mean arterial pressure by reinfusion of blood stimulated AVP secretion but at a much reduced rate. The data suggest that following depletion of the readily releasable pool of AVP from the dog neurohypophysis, the release of the remaining hormone by appropriate stimuli will be at a substantially reduced rate. This observation was not related to the

pituitary content of AVP but apparently to an inhibitory mechanism located within the neural lobe. The tuberohypophyseal DA system may be that inhibitory mechanism.

Sachs et al. (1967) postulated that a factor in the neurohypophysis of anesthetized dogs inhibited AVP release 60 minutes after severe hemorrhage. Experiments have been performed demonstrating that DA synthesis in the posterior pituitary of rats is increased following water deprivation (72 hours) or hyperosmotic injections (24 hours). Any links between the studies by Sachs et al. (1967) and those reported in the body of this thesis are purely speculative since the experimental designs differ so widely. However, Sachs et al. (1967) suggest "the possibility that extensive stimulation [of AVP secretion] leads to the exhaustion of some essential transmitter substance(s) or coupling factor...". Alternatively, it can be proposed that extensive stimulation of AVP secretion leads to the activation of an inhibitory factor (i.e., DA) which will reduce the rate of AVP release in the presence of ongoing stimulation. To test this hypothesis dogs could be bled, reinfused with blood and then hemorrhaged a second time. One group of dogs would be administered a dopaminergic antagonist prior to the second hemorrhage. If DA was the inhibitory substance proposed to exist in the neural lobe, plasma AVP in the treated dogs would be expected to be elevated to higher concentrations than in non-treated dogs after the second hemorrhage.

5. Indirect regulation

Vasopressin secretion from the neurosecretory neurons into the systemic circulation requires neurovascular contacts and

adequate perfusion of the neural lobe. Changes in either of these could result in changes in plasma AVP concentrations. DA may indirectly modulate AVP secretion by quantitatively altering the neural-vascular interaction or the blood flow through the posterior pituitary.

a. Pituicyte function. Tweedle and Hatton (1980a,b) have reported that the neurosecretory nerve endings in the neurohypophysis are normally surrounded and enclosed by glial cells (pituicytes). Within 24 hours of water deprivation the number of neurosecretory axons enclosed by pituicytes is decreased. This is reversible with 24 hours of rehydration. Wittkowski and Brinkman (1974) demonstrated that three days of dehydration increased the number of neurovascular contacts and decreased the number of gliovascular contacts in the posterior pituitary. This was due to an increase in the size of the nerve terminals contacting the perivascular space, not an increase in the number of neurosecretory nerve endings. These data suggest a great plasticity in the neural lobe of the pituitary which is particularly evident when the hypothalamo-neurohypophyseal system is highly active. The net result of the events occurring between the glial processes and the neurosecretory axons is to increase the accessibility of the AVP-containing terminals to the neurohypophyseal circulation. This is due to a redistribution of glial and neuronal elements in the posterior pituitary.

Perhaps the tuberohypophyseal DA neurons are regulators of the pituicyte motility; DA could be either stimulatory or inhibitory. Hökfelt (1973) has provided data to support an inhibitory role for DA. He found that an infusion of DA into the lateral

ventricle of rats markedly decreased the number of nerve endings reaching the capillary basement membrane in the median eminence. The neurovascular contacts were disrupted by glial endfeet. DA could act similarly in the posterior pituitary as part of a hypothetical negative feedback loop. DA could reduce the number of nerve terminals freed from the pituicytes, thereby inhibiting the rate of AVP secretion. If, on the other hand, Hökfelt's observations in the median eminence are the opposite of occurrances in the posterior pituitary, DA could augment AVP secretion by stimulating the retraction of the pituicytes from the neurosecretory nerve endings and the capillaries of the neurohypophysis.

b. Blood flow. The studies of Baumgarten et al. (1972) demonstrated that catecholaminergic neurons terminated near perivascular spaces in the neural lobe of the rat pituitary. More recent studies (Tilders et al., 1979) suggested that the tuberohypophyseal DA nerves were in close proximity to the neurohypophyseal capillaries. The DA nerve terminals appear to be situated in a position to regulate blood flow through the posterior pituitary.

Blood flow to the rat posterior pituitary, but not the anterior pituitary or hypothalamus, is increased following 24 hours of water deprivation (Lichardus et al., 1977). The blood volume of the rat posterior pituitary is increased by stimuli (e.g., hemorrhage, vagal stimulation, saline drinking) associated with the release of neurohypophyseal hormones (Sooriyamoothy and Livingston, 1972). The neurohypophyseal vasodilation is mediated partially by cholinergic, but not by noradrenergic, mechanisms (Sooriyamoothy and Livingston,

1972). Increased blood flow through the posterior pituitary is not correlated to AVP secretion. Twenty-four hours of water deprivation increase neurohypophyseal blood flow in AVP deficient Brattleboro rats (Kapitola et al., 1977), whereas hemorrhage (a potent stimulus for AVP secretion) does not alter posterior pituitary blood flow in anesthetized sheep (Page et al., 1981). Nevertheless, the effect of water deprivation and saline drinking to increase blood flow through the posterior pituitary could be mediated by the tuberohypophyseal DA neurons. Administration of exogenous DA causes vasodilation in pial arteries (Edvinsson et al., 1978), renal arteries (reviewed by Goldberg, 1972) and in the superior mesenteric artery (Clark and Menninger, 1980). In all cases, specific DA receptors have been implicated. Unfortunately, the effect of DA on the neurohypophyseal vasculature has not been studied.

From the literature cited above and the data presented in the body of this thesis, an interesting scheme of events can be proposed. DA synthesis and release are increased in the posterior pituitary during dehydration, when AVP secretion would be expected to be stimulated (Dunn et al., 1973; Mens et al., 1980). Postsynaptic DA receptors in the posterior pituitary are activated and decrease the responsiveness of β-receptors (Cote et al., 1981). β-adrenergic receptors in the posterior pituitary are reportedly linked to adenylate cyclase (Stefanini et al., 1980b) which is located on the neurohypophyseal vasculature (Santolaya and Lederis, 1980). Furthermore, posterior pituitary DA receptor activation inhibits basal and

stimulated adenylate cyclase activity (Cote et al., 1981). This may cause vasodilation to sustain or increase blood flow to the posterior pituitary even when the plasma volume is severely compromised. Maximal delivery of AVP to the systemic circulation would be insured as a homeostatic mechanism to promote water reabsorption in the collecting duct of the kidney during severe dehydration.

This hypothesis is based on several inferences from various sources, but it does offer a testable function for the dopaminergic neurons terminating in the posterior pituitary. For example, if DA was acting as a vasodilatory agent, haloperidol would be expected to decrease, whereas apomorphine and piribedil would increase, blood flow to the posterior pituitary. The haloperidol-induced response may be observed only in dehydrated rats; the tuberohypophyseal DA system may have no tonic regulatory function.

B. Oxytocin Release

The second major hormone of the posterior pituitary is oxytocin. This peptide is released concomitantly with AVP during dehydration (Jones and Pickering, 1969), but is released with relative selectivity during parturition and lactation (Haldar, 1970). Specifically, oxytocin is required for milk ejection during suckling.

Dopaminergic regulation of oxytocin secretion is not very clear. DA inhibits oxytocin release from cultured hypothalamo-neurohypophyseal system explants (Seybold et al., 1978). The authors cite unpublished data suggesting that DA may stimulate oxytocin release from isolated neural lobes as had been reported previously (Bridges et al., 1976). The dopaminergic involvement in oxytocin release has also

been studied during suckling. Oxytocin release in the milk ejection reflex may be purely neurogenic without any dopaminergic regulation (Fuchs et al., 1981; Russell et al., 1981) or it may be stimulated through a dopaminergic mechanism (Clarke et al., 1979). The latter study did not localize the site of DA to the posterior pituitary.

A more direct approach to characterize the dopaminergic regulation of oxytocin secretion is to study biochemical indices of DA synthesis and turnover in the posterior pituitary. Holzbauer et al. (1978) used the αMT-induced decline of DA to suggest that DA turnover is increased in the posterior pituitary during lactation. This experiment, however, should be viewed with caution for several reasons. First, neither the number of pups suckled nor the duration of lactation was constant for all rats. Secondly, the DA concentration in the posterior pituitary was measured at only one time (30 minutes) after the administration of αMT. The most appropriate use of the αMT-induced decline technique requires several time points and comparisons of rate constants.

More recently the effect of the suckling stimulus on DOPA accumulation in the posterior pituitary has been studied (Demarest and Moore, unpublished observations; McKay et al., 1980). DOPA accumulation in the posterior pituitary was not altered following continuous suckling, 4 to 8 hours of pup deprivation, or 4 hours of pup deprivation followed by 30 minutes of suckling. These studies were performed in lactating rats twelve days post-partum and all comparisons were to diestrous females. It should also be noted that the rate of DOPA

accumulation in the posterior pituitary is identical in lactating, diestrous and male rats (Demarest et al., 1981).

Critical evaluation of the data suggest that the tuberohypophyseal DA neurons do not influence, and in turn are not influenced by, the release of oxytocin. This is in contrast to the interaction between tuberoinfundibular DA neuronal activity and the release of prolactin in lactating rats (McKay et al., 1980; Selmanoff and Wise, 1981).

C. Prolactin Release

Prolactin secretion from the anterior pituitary is under the tonic inhibitory control of DA released from the median eminence (Gibbs and Neill, 1978; Weiner and Ganong, 1978). Recently, Ben-Jonathan (1980) suggested that the tuberohypophyseal DA neurons may also be involved in the regulation of prolactin release. Little supportive evidence was presented, however. The presence of a common capillary bed uniting the anterior and posterior pituitaries (Bergland and Page, 1979), plus the anatomical juxtaposition of the dopamine nerve terminals to perivascular spaces in the posterior pituitary (Baumgarten et al., 1972; Tilders et al., 1979), make Ben-Jonathan's hypothesis tenable.

Water deprivation increases DA synthesis in the posterior pituitary, but not in the median eminence. Progressive increases in the anterior pituitary DA concentration and concomitant decreases in the plasma prolactin concentration have been observed in rats deprived of water for one to three days (Alper and Moore, in preparation).

Marshall et al. (1975), however, observed an increase in serum prolactin following dehydration. Dehydration may increase the amount of DA released directly into the neurohypophyseal capillaries or, due to the inadequate mechanisms to inactivate DA in the posterior pituitary, the excess amine being synthesized and released may overflow into the intrapituitary circulation. In either case, the amount of DA transported to the anterior pituitary will increase during dehydration. Greater quantities of DA can thus be incorporated into the prolactin-secretory granules to inhibit prolactin release from the anterior pituitary (Gudelsky et al., 1980; Nansel et al., 1979).

These data do not necessarily imply that the physiological role of the tuberohypophyseal DA system is to regulate the secretion of anterior pituitary hormones. First, the data do not prove that the increased rate of DA release in the posterior pituitary is actually causing the inhibition of prolactin release; the two events may be unrelated. Prolactin release is influenced by many biogenic amines and peptides other than DA (see review by Weiner and Ganong, 1978). Secondly, if the tuberohypophyseal DA system played an important role in regulating prolactin secretion, DA synthesis in the posterior pituitary would be expected to be regulated much like it is in the median eminence. In fact, however, DA turnover in the posterior pituitary is not increased following the infusion of prolactin into the CSF (Johnston et al., 1980) or following hyperprolactinemia induced by pituitary transplants under the kidney capsule (Morgan and Herbert, 1980), while both treatments markedly enhance DA synthesis and turnover in the median eminence.

Teleologically the dehydration-induced decrease in the plasma prolactin concentration is an interesting phenomenon. Prolactin is a well known natriuretic and anti-diuretic hormone in many species, but its role on fluid and electrolyte metabolism in mammals is not completely understood (see review by Horrobin, 1980). If prolactin is an anti-diuretic hormone in rats, plasma prolactin concentrations would be expected to increase during dehydration. Further investigations into the relationship between dehydration, the tuberohypophyseal DA system and prolactin secretion could prove extremely interesting.

SUMMARY AND CONCLUSIONS

Sensitive radioenzymatic assays for DA, NE and DOPA were employed in preliminary investigations on mechanisms regulating DA synthesis in the posterior pituitary, terminals of the tuberohypophyseal DA system. DA synthesis was also estimated in the median eminence and striatum, terminals of the tuberoinfundibular and nigrostriatal DA systems, respectively. The results of the studies will be reviewed briefly.

1) The catecholamines DA and NE appear to be synthesized, stored and released in neurons terminating in the posterior pituitary gland of the rat. Both the concentration and synthesis rate of DA are much greater than for NE. Approximately 30-35% of the NE-containing neurons of the posterior pituitary are of peripheral sympathetic origin. Even though there is a substantial concentration of NE in the posterior pituitary, the rate of DOPA accumulation following the inhibition of DOPA decarboxylase (an *in vivo* estimate of tyrosine hydroxylase activity) reflects primarily DA synthesis. Thus, DOPA accumulation can be measured in the posterior pituitary as an *in vivo* estimate of DA synthesis (and therefore neuronal activity) after the administration of the decarboxylase inhibitor NSD 1015.

2) DOPA accumulation progressively increases in the posterior pituitary of water-deprived male rats, attaining statistical significance in both male and female rats deprived of water for three days.

The increased rate of DA synthesis is observed when there is no change in the DA concentration of the posterior pituitary. Furthermore, water deprivation does not increase the DA concentration or the rate of DOPA accumulation in the terminals of the tuberoinfundibular or nigrostriatal DA systems. These results suggest that water deprivation selectively increases the synthesis and release of DA in the posterior pituitary, the terminals of the tuberohypophyseal dopaminergic neurons.

3) DA synthesis in the posterior pituitary is increased by other stimuli related to water deprivation. The activity of the tuberohypophyseal DA neurons and the plasma sodium concentration are both increased following saline drinking; indices of plasma volume are unaltered. Also, 24 hours after the administration of hypertonic saline or mannitol, DOPA accumulation in the posterior pituitary is elevated above control. This effect appears to be related (either directly or indirectly) to a rapid and marked intracellular dehydration induced by the hypertonic solutions. The effect of hypertonic saline administration on DA synthesis is blocked by providing rats free access to water. The reason for the delayed effect of osmotic stimuli on the tuberohypophyseal DA neurons is unclear.

4) The dehydration-induced increase in DA synthesis in the tuberohypophyseal neurons occurs after a long latent period. In contrast, within 3 hours of presentation of water to dehydrated rats, the rate of DOPA accumulation in the posterior pituitary is returned to control. The rapid reduction of the elevated tuberohypophyseal DA neuronal activity follows a temporal pattern similar to the reduction

in the plasma sodium concentrations, while the hematocrit is returned at a much slower rate. When water-deprived rats are provided 2% NaCl to drink, measurements of plasma volume return to control whereas the plasma sodium concentration and the rate of DA synthesis in the posterior pituitary remain elevated. These data implicate cellular osmolality and not plasma volume as a major factor associated with regulation of tuberohypophyseal DA neuronal activity.

In conclusion, DA synthesis in the posterior pituitary is moderately increased by DA antagonists and decreased by DA agonists. These effects are similar to, though quantitatively less than, the marked effects of dopaminergic drugs in the striatum. The tuberohypophyseal and nigrostriatal DA neurons have presynaptic DA receptors to regulate DA synthesis. These presynaptic autoreceptors are absent from the tuberoinfundibular DA neurons. Neither the tuberohypophyseal nor the tuberoinfundibular DA systems have a high affinity DA uptake mechanism. DA synthesis in the median eminence is latently activated by increased serum or CSF concentrations of prolactin; this is not observed in the posterior pituitary. Furthermore, the administration of morphine does not alter DA synthesis in tuberohypophyseal system, the only central dopaminergic system to behave in this manner.

The data presented in the body of this thesis demonstrate that unlike both the tuberoinfundibular and nigrostriatal DA systems, the activity of the tuberohypophyseal DA neurons is regulated, at least in part, by cellular dehydration. The pharmacological and physiological significance has yet to be determined, for the role of DA in the

posterior pituitary has not been elucidated. However, these preliminary data substantiate that the tuberohypophyseal DA system is a unique dopaminergic neuronal pathway and must be considered as such.

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