ANTERIOR HYPOTHALAMIC AFFERENT REGULATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONAL ACTIVITY

By

Anne Catherine Barton

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ABSTRACT

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The role of afferent connections: 1) in maintaining basal activity of tuberoinfundibular dopaminergic (TIDA) neurons, 2) in the stimulatory action of prolactin on these neurons, and 3) in the inhibitory action of restraint stress was examined. Neuronal activity was estimated by measuring the rates of synthesis and turnover, and the metabolism of DA in the terminals of TIDA neurons in the median eminence (ME). Retrochiasmatic deafferentation decreased the rates of synthesis and turnover, and the metabolism of DA in the ME of gonadally-intact female rats, whereas a knife cut 1 mm rostral to and bilateral cuts of the retrochiasmatic area had no effect. Thus, afferents originating in the caudal region of the anterior hypothalamus, in particular, the suprachiasmatic and/or paraventricular nucleus, mediate a stimulatory influence on TIDA neurons in the intact female rat. In contrast, these afferents do not regulate basal TIDA neuronal activity in male rats since retrochiasmatic deafferentation did not alter the rates of DA synthesis or turnover in the ME of male rats.

Despite a decrease in basal activity following retrochiasmatic deafferentation in the female rat, manipulations which increased prolactin levels increased TIDA neuronal activity while those which decreased prolactin levels were associated with a further decrease in TIDA neuronal activity. These findings

together with the identification of prolactin binding sites in the ME provides evidence for a direct action of prolactin on the terminals of TIDA neurons.

The deafferentation-induced decrease in TIDA neuronal activity in intact female rats may operate via a neuronal mechanism which also functions to decrease TIDA neuronal activity following ovariectomy. Both retrochiasmatic deafferentation and ovariectomy decreased DA synthesis in the ME yet both procedures together produced no further decrease than either procedure alone. This neuronal mechanism which stimulates TIDA neurons in female rats is not operational in either intact or orchidectomized male rats, possibly due to the effects of neonatal androgen exposure.

Inhibitory afferents which decrease TIDA neuronal activity and increase circulating prolactin concentrations during restraint stress also originate rostral to the mediobasal hypothalamus. Retrochiasmatic deafferentation blocked the stress-induced decrease in TIDA neuronal activity and attenuated the stress-induced increase in the secretion of prolactin. Thus, both stimulatory and inhibitory signals to the TIDA neurons originate in or pass through the anterior hypothalamus.

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LIST OF ABBREVIATIONS

AHA	anterior hypothalamic area
AR	arcuate nucleus
BROMO	bromocriptine
CA	anterior commissure
COMT	catechol-O-methyl transferase
DA	dopamine
DMN	dorsomedial nucleus
DOPA	3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
EPI	epinephrine
GBL	γ-butyrolactone
HALOPER	haloperidol
HPLC	high performance liquid chromatography
IH	incertohypothalamic
ICV	intracerebroventricular
MAO	monoamine oxidase
MBH	complete deafferentation of the mediobasal hypothalamus
αMT	α-methyltyrosine
NE	norepinephrine
NIL	neurointermediate lobe of the pituitary
NSD 1015	3-hydroxybenzylhydrazine
OC	optic chiasm
OT	optic tract
ORCHIDX	orchidectomized
OVX	ovariectomized
PEN	periventricular nucleus
PH	posterior hypothalamic nucleus

LIST OF ABBREVIATIONS (Continued)

POA	preoptic area
PVN	paraventricular nucleus
RC	retrochiasmatic deafferentation of the mediobasal hypothalamus
RCAL	deafferentation of the lateral retrochiasmatic area
rPRL	rat prolactin
SCN	suprachiasmatic nucleus
SON	supraoptic nucleus
TH	tuberohypophysial
THDA	tuberohypophysial dopaminergic
TI	tuberoinfundibular
TIDA	tuberoinfundibular dopaminergic
VMN	ventromedial nucleus

INTRODUCTION

Neuroanatomists have identified a number of distinct dopamine (DA)-containing neuronal systems within the mammalian brain. Although, most of what is known about DA neurons has been learned from studies on the nigrostriatal system, different DA neurons appear to have evolved distinctive properties that are suited to the unique requirements of their specialized functions (Moore and Wuerthele, 1979; Moore, 1987a). This is most apparent when the neurochemical properties and the responses to pharmacological and endocrinological manipulations of the different DA neurons are compared. For example, the tuberoinfundibular dopaminergic (TIDA) neurons which are located in the mediobasal hypothalamus differ from the major ascending nigrostriatal DA neurons in that the activity of TIDA neurons: (1) is modulated by prolactin, (2) is markedly different in males and females, (3) exhibits characteristic cyclical patterns during pregnancy, and (4) is inhibited by suckling and stressful manipulations (for review, see Moore and Demarest, 1982; Moore, 1987a; 1987b). Early efforts to characterize TIDA neurons focused on the ability of hormones to alter the activity of these neurons (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976; Eikenburg et al., 1977; Annunziato et al., 1978). TIDA neurons were considered to be part of a hormonal-neuronal feedback system controlling prolactin release independent of afferent neuronal influences. Results of more recent studies in pregnant, suckling and restraint stressed rats (McKay et al., 1982: Demarest et al., 1983a; 1983b; 1985a) reveal that the TIDA neurons are not only influenced by hormones but they are also

regulated by inhibitory afferent neuronal systems. The emphasis of this dissertation research has been to characterize the regulation of TIDA neuronal activity via afferent neuronal mechanisms.

A. Anatomy of Dopaminergic Neuronal Systems

The paraformaldehyde procedure of Falck-Hillarp has been used extensively to examine catecholamine-containing neuronal systems within the mammalian brain. Although this method has been extremely valuable, it requires freeze-drying, a procedure which compromises the histological quality of the tissue. Furthermore, the isoquinolone fluorescent reaction product degrades rapidly upon exposure to light causing the fluorescent label to diminish over time. The more sensitive glyoxylic acid histofluorescent technique on cryostat sections has replaced this earlier method (for review, see Björklund and Lindvall, 1978; Moore and Bloom, 1978; Lindvall and Björklund, 1983). Because of the similarities in the spectral characteristics of the catecholamines, it was difficult to distinquish DA, norepinephrine (NE) and epinephrine (EPI) neurons unless these histofluorescent studies were done in conjunction with pharmacological manipulations (i.e. inhibition of DA-β-hydroxylase) or selective lesions. Thus, the use of immunocytochemical procedures to localize catecholamine-synthesizing enzymes has made it possible to identify DA, NE and EPI containing neurons (Hökfelt et al., 1974; Swanson and Hartman, 1975; Pickel et al., 1975).

Prior to the advent of immunohistological techniques, Dahlström and Fuxe (1964) devised a numbering system to delineate various groups of catecholamine-containing neurons (Figure 1). The more caudal cells (A1-A7) in the pons medulla represent noradrenergic neurons while the rostral cells, extending from the mesencephalon (A8-A10) to the olfactory bulb (A16), are exclusively dopaminergic.

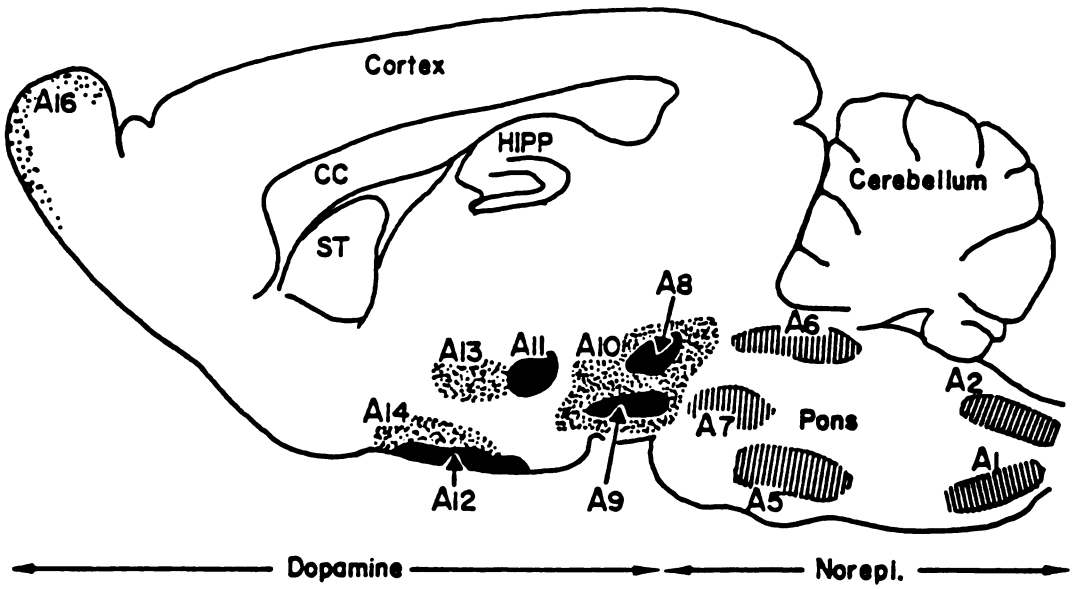

Figure 1. Schematic of a sagittal section of the rat brain depicting the location of NE (A1-A7) and DA (A8-A16) perikarya.

DA perikarya located in the pars compacta of the substantia nigra (A8-A9) and in the ventromedial mesencephalon (A10) give rise to the major ascending DA neuronal system, classified as the mesotelencephalic system (Björklund and Lindvall, 1978). These neurons are more commonly referred to as nigrostriatal, mesolimbic and mesocortical neurons which project to the caudate/putamen (striatum), nucleus accumbens and olfactory tubercle, and to various regions of the cerebral cortex, respectively.

DA cell bodies have also been identified in more rostral regions of the brain, in the olfactory bulb (A16, Halasz et al., 1977) and in four separate regions of the hypothalamus (A11-A14; Lindvall et al., 1984). Within the hypothalamus, DA neurons have been divided into two major groups, those comprising the incerto-hypothalamic (IH) DA system and those originally referred to as the tuberohypophysial system (Ungerstedt, 1971). Perikarya of the IHDA system are found in the caudal hypothalamus and zona incerta (groups A11 and A13; Fuxe et al., 1969b; Björklund and Nobin, 1973) and in the rostral periventricular hypothalamus (group A14; Björklund and Nobin, 1973). Cell bodies of the tuberohypophysial system are located in the mediobasal hypothalamus, in the arcuate and periventricular nucleus (A12). These neurons have been further subdivided on the basis of their anatomical distribution (Figure 2): tuberoinfundibular (TI) DA neurons project to the median eminence, and tuberohypophysial (TH) DA project to the neural and intermediate lobes of the pituitary.

Carlsson et al. (1962) and Fuxe (1964) made the initial observation of a massive supply of catecholamine fibers to the median eminence of various mammalian species. Later studies in the rat and mouse provided evidence that part of this innervation originated in the arcuate nucleus, constituting a TIDA system (Fuxe and Hökfelt, 1966; Lichtensteiger and Langemann 1966). Through the use of microspectrofluorometric analysis and stereotaxic lesions, axons of the

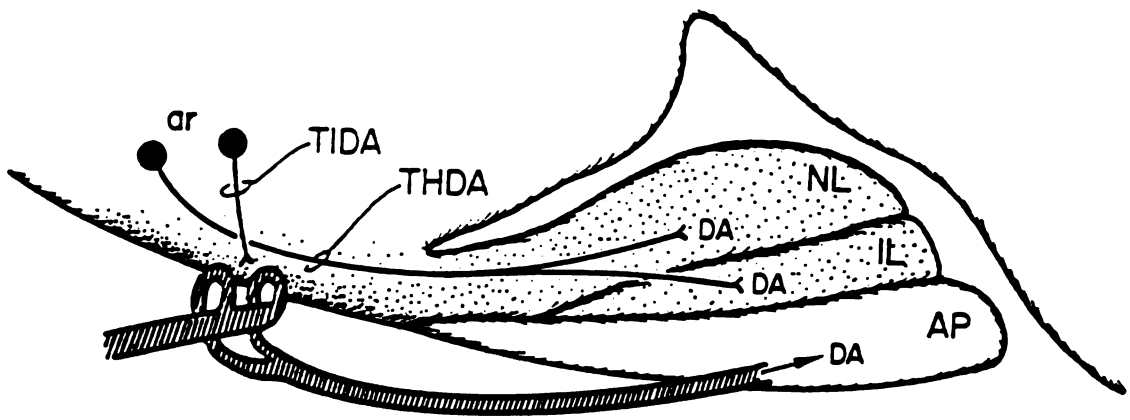

Figure 2. Schematic of a midsagittal view of the rat hypothalamus indicating the location of the tuberoinfundibular and tuberohypophysial dopaminergic neurons. AP = anterior pituitary; DA = dopamine; NL = neural lobe; IL = intermediate lobe; THDA = tuberohypophysial dopamine neurons; TIDA = tuberoinfundibular dopamine neurons.

TIDA neurons were found to arise from the arcuate and ventral periventricular nuclei, project ventrally and then turn medially into the median eminence (Björklund et al., 1970, 1973a, 1973b; Jonsson et al., 1972; Ajika and Hökfelt, 1973). In the median eminence, they make an exceedingly dense plexus of very fine varicosities that is predominantly in the external zone, but is present in the internal and ubependymal zones as well (Fuxe, 1964; Fuxe et al., 1969b; Björklund et al., 1970, 1973b, Jonsson et al., 1972; Rethelyi and Halasz, 1970; Ajika and Hökfelt, 1973, 1975).

The entire dopaminergic input to the median eminence appears to originate in the mediobasal hypothalamus, but there is an additional noradrenergic innervation which originates in the lower brainstem (Fuxe, 1964; 1965; Björklund et al., 1973a). The noradrenergic neurons terminate in the internal and subependymal layers and some have been shown to innervate the external layer of the median eminence as well (Björklund et al., 1970; Cuello et al., 1973; Löfström et al., 1976a). For example, following complete deafferentation of the mediobasal hypothalamus, a significant decrease in catecholamine fluorescence and a large number of degenerating axons were observed in the internal and subependymal zones of the median eminence with a slight decrease in catecholamine fluorescence to the medial region of the external layer. Although Kizer et al. (1976) found a significant (40%) decrease in DA content in the median eminence after large lesions of DA cells of the ventral tegmental area, this is not in agreement with a number of other investigators who, using histofluorescent (Jonsson et al., 1972; Löfström et al., 1976a) and biochemical techniques (Weiner et al., 1972; Brownstein et al., 1976b; Gallardo et al., 1976; Gudelsky et al., 1978; Barton et al., 1988) found no change in DA innervation and DA content in the median eminence after complete hypothalamic deafferentation. Overall, catecholamine-containing fibers constitute about 33% of all boutons in the lateral part of the

external layer of the median eminence and 13% in the medial part (Ajika and Hökfelt 1973). The DA terminals constitute about 10-20% of the total catecholaminergic innervation in the subependymal layer, about 50-75% in the medial part of the external layer, and more than 80% in the lateral external layer.

The anatomically-related THDA neurons also have their cell bodies in the arcuate nucleus but in the more rostral aspects of this nucleus (Figure 2; Björklund et al., 1973b). The axons of the THDA neurons project through the median eminence and infundibular stalk to terminate in the neural and intermediate lobes of the pituitary (Fuxe, 1964; Björklund et al., 1970, 1973b; Smith and Fink, 1975). Conversely, the TIDA neuronal projections connect the rostral to caudal extent of the arcuate nucleus with a corresponding part of the median eminence. The DA terminals in the median eminence do not form true synaptic connections with other tissue elements but show sites of close contact with non-aminergic neurons and ependymal cells, as well as with the precapillary space of the hypophysial portal vessels (Hökfelt, 1973; Ajika and Hökfelt, 1973). These anatomical arrangements have been taken to support the idea that DA released from the TIDA nerve terminals in the median eminence can act on neighboring peptidergic axon terminals or be released into the portal blood. For example, DA released from nerve terminals in the medial external zone of the median eminence is transported via the hypophysial portal blood to the anterior pituitary where it activates receptors on lactotrophs to inhibit the release of prolactin (MacLeod, 1976).

B. Biochemistry of Dopamine Neurons

Early or pioneering information on the neurochemical events occurring in DA nerve terminals has been obtained from studies on nigrostriatal DA neurons (Moore and Wuerthele, 1979). Tyrosine is transported into DA nerve terminals

where it is converted to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase, the enzyme that regulates this rate-limiting step in DA synthesis (Figure 3). This enzyme is regulated, in part, by an end product inhibitory process such that decreases in intraneuronal DA concentrations result in increased DA synthesis and vice versa. By this mechanism, concentrations of DA within the nerve terminal remain fairly constant despite alterations in the amount of transmitter released. DOPA, which is synthesized from tyrosine, is decarboxylated by L-aromatic amino acid decarboxylase to form DA. The newly synthesized DA can be stored in synaptic vesicles and be released in response to the arrival of nerve action potentials.

DA which is released from nigrostriatal nerve terminals can activate receptors on dendrites or cell bodies of postsynaptic neurons. In addition, DA in the synaptic cleft can activate presynaptic or autoreceptors (Carlsson, 1975). Activation of these receptors results in the inhibition of the synthesis and release of DA (Roth et al., 1975; Christiansen and Squires, 1974; Nowycky and Roth, 1978). The actions of DA at pre- and post-synaptic receptors is terminated when DA is removed from the synaptic cleft by a high affinity active uptake mechanism which transports the amine back into the presynaptic nerve terminal (Snyder and Coyle, 1969). Once inside the nerve terminal, DA can be repackaged into vesicles and be re-released in response to appropriate stimuli or it can be oxidatively deaminated to 3,4-dihydroxyphenylacetic acid (DOPAC) by mitochondrial monoamine oxidase (MAO).

Although many of the synthetic and metabolic processes are similar to nigrostriatal DA neurons, TIDA neurons differ in several important respects (see Figure 3). Synthesis of DA in TIDA neurons is regulated by end product inhibition just as in nigrostriatal neurons. This is evidenced by the fact that inhibitors of MAO increase DA concentrations and decrease the rate of DA synthesis in the

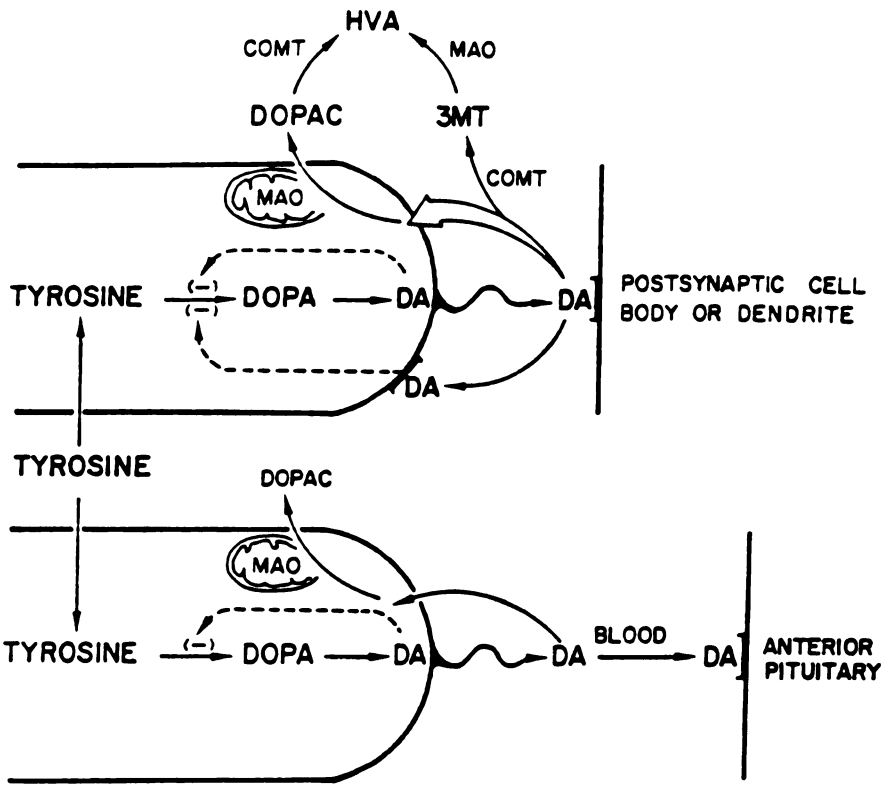

Figure 3. Schematic of a (a) nigrostriatal and (b) tuberoinfundibular DA neuron. COMT = catechol-O-methyl transferase; DA = dopamine; DOPA = 3,4-dihydroxyphenylalanine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; MAO = monoamine oxidase; 3MT = 3-methoxytyramine.

terminals of these neurons (Demarest and Moore, 1981b). Conversely, reserpine reduces DA concentrations and, thereby, increases DA synthesis in the same brain regions (Demarest and Moore, 1979a). Most TIDA neurons do not form classical synapses (Ajika and Hökfelt, 1973) but terminate adjacent to perivascular spaces in the external layer of the median eminence. DA released from these neurons enters the blood and is carried by the hypophysial portal veins to the anterior pituitary. The concentration of DOPAC, when compared to that of DA, is much lower in the median eminence than it is in other brain regions that contain DA nerve terminals (Fekete et al., 1979; Umezu and Moore, 1979; Lookingland et al., 1987b). This may be due, in part, to the fact that following release from the TI neuron, DA is quickly transported away from the terminal by the blood, thereby reducing the amount of amine that can be recaptured. In addition, the DA uptake system in terminals of TIDA neurons has a lower affinity for DA than do nigrostriatal DA terminals (Demarest and Moore, 1979b; Annunziato et al., 1980; George and vanLoon, 1982). This is probably the reason that TIDA neurons are less sensitive than other catecholaminergic neurons to the neurotoxic actions of 6-hydroxydopamine (Jonsson et al., 1972; Cuello et al., 1974; Smith et al., 1974; Demarest et al., 1980). Finally, TIDA neurons differ from nigrostriatal neurons in that they lack autoreceptors (Demarest and Moore, 1979a). Although an isolated report by Sarkar and coworkers (1983) suggests that TIDA neurons may regulate their activity via autoreceptor activation, several investigators (Fuxe and Hökfelt, 1974; Fuxe et al., 1975a; 1975b; Gudelsky and Moore, 1976; Gudelsky and Moore, 1977; Demarest and Moore, 1979a) have demonstrated that TIDA neurons do not respond acutely to DA agonists or antagonists. This has been confirmed with direct experimentation with drugs (baclofen and γ-butyrolactone, GBL) which decrease impulse flow in DA neurons. As a result, DA release ceases, the activation of autoreceptors stops and the synthesis of DA is no longer inhibited.

Thus, these drugs cause the synthesis and the concentration of DA in the terminals of nigrostriatal neurons to increase. Baclofen and GBL decrease neuronal firing on TIDA neurons as evidenced by a decrease in DA turnover (Demarest and Moore, 1979a) and DOPAC concentrations (Lookingland et al., 1987a) in the median eminence. The rate of synthesis and the concentration of DA in the terminals of TIDA neurons, however, does not increase (Gudelsky and Moore, 1976; Moore and Gudelsky, 1977; Moore et al., 1978; Demarest and Moore, 1979a; Moore and Demarest, 1980). Thus, it appears that TIDA neurons lack autoreceptor mechanisms for regulating DA synthesis.

C. Methods for Estimating TIDA Neuronal Activity

Electrophysiological techniques have been used extensively to record activity on the mesotelencephalic DA neurons, the perikarya of which are tightly packed in discrete nuclei in the ventral midbrain. The diffuse distribution of TIDA perikarya among non-DA neurons and the anatomically related THDA neurons has made it difficult to employ electrophysiological techniques to examine TIDA neuronal activity. Thus, investigators have relied on biochemical techniques to estimate activity of TIDA neurons. Neurochemical estimates are based on coupled relationships among DA synthesis, release and metabolism. For example, except when impulse traffic ceases, the concentration of DA in the terminal regions of neurons does not change. Thus, DA synthesis must keep pace with DA which is released. Little of the released DA is recaptured and subsequently metabolized to DOPAC in the terminals of the TIDA neurons (Lookingland et al., 1987a, 1987b). The amount of unreleased DA that is metabolized, however, is proportional to the amount of DA synthesized, and procedures that increase or decrease DA synthesis produce corresponding changes in DOPAC concentrations in the median eminence. Thus, changes in synthesis,

release and metabolism of DA are related so that a change in any one of the processes can be used to estimate changes in the activity of TIDA neurons.

1. Rate of dopamine synthesis

Since the synthesis of DA is regulated at the step catalyzed by tyrosine hydroxylase, estimates of TIDA neuronal activity have been based on the activity of this enzyme. Measurements of tyrosine hydroxylase activity have been made in vitro in response to a variety of endocrinological manipulations (Kizer et al., 1974; Kizer et al., 1978), but because of the limitations in sensitivity of the assay, these early studies required pooling of median eminences from two or more animals. The effects of similar endocrinological manipulations on the activity of tyrosine hydroxylase have also been examined in situ (Wang and Porter, 1986). Changes in the quantity (Porter, 1986) of enzyme, however, make it difficult to interpret changes in tyrosine hydroxylase activity. The method first introduced by Carlsson (1972) to estimate the activity of tyrosine hydroxylase in vivo by measuring the rate of accumulation of DOPA has been used extensively. This procedure involves measuring the rate of accumulation of DOPA following systemic administration of 3-hydroxybenzylhydrazine (NSD 1015), an inhibitor of L-aromatic amino acid decarboxylase. In the absence of a decarboxylase inhibitor, the concentration of DOPA in the terminal region of TIDA neurons is essentially zero, but following the administration of NSD 1015, the concentration of DOPA in the median eminence increases linearly with time at a rate that is proportional to the activity of these neurons (Demarest and Moore, 1980; Gunnet et al., 1987).

One shortcoming of the DOPA accumulation technique is that following inhibition of the decarboxylase enzyme, DOPA accumulates in both NE and DA neurons. The accumulation of DOPA in the median eminence, however, generally reflects changes in TIDA neuronal activity since the density and activity

of DA neurons in this region greatly exceed those of NE neurons (Kizer et al., 1976; Demarest et al., 1979).

2. α-Methyltyrosine-induced decline of dopamine

The advantage of the α-methyltyrosine (αMT) method is that it permits the concurrent estimation of DA and NE neuronal activities in the same brain regions. Following the administration of αMT, an inhibitor of the rate-limiting enzyme tyrosine hydroxylase, the concentrations of all catecholamines in the brain fall in an exponential manner at a rate that is proportional to the activity of catecholaminergic neurons. Fuxe and collaborators conducted the initial studies, using semiquantitative (Fuxe et al., 1969b) and subsequently more quantitative microfluorometric techniques (Löfström et al., 1976b) to estimate the decline of DA in the median eminence after αMT administration.

A problem with the use of enzyme inhibitors such as NSD 1015 and αMT for estimating TIDA neuronal activity is that by blocking DA synthesis and, thus the amount of DA released into the hypophysial blood, these procedures also increase prolactin secretion. This interferes with the concurrent measurement of TIDA neuronal activity and circulating prolactin concentrations and precludes the use of these techniques for certain neuroendocrine studies. In addition, these drugs require a minimal period of time for their action and this limits the determination of acute effects of some manipulations on TIDA neurons.

3. Release of dopamine

The release of DA from the rat median eminence has been measured in vitro (Perkins and Westfall, 1978; Wilkes and Yen, 1980; Annunziato et al., 1981; Forman and Porter, 1981) and in vivo using voltammetric electrodes implanted into the median eminence (Plotsky and Neill, 1982) and by quantifying DA concentrations in the hypophysial portal blood (Ben-Jonathan et al., 1977; Gibbs and Neill, 1978). This latter procedure has an advantage of providing a direct

measure of DA release from TIDA neurons and thus an index of impulse traffic of these neurons. One disadvantage of this technique is that it must be carried out in surgically-stressed, anesthetized animals which may alter TIDA neuronal activity (Pilotte et al., 1980).

Although small amounts of DA are found within the cells of the anterior pituitary gland, this tissue has neither DA nerve terminals nor the capacity to synthesize this amine (Saavedra et al., 1975; Dahlström and Fuxe, 1966). Thus, it has been suggested that the concentration of DA in the anterior pituitary represents released DA from TIDA neurons which has been transported via the blood and internalized in prolactin-secreting cells of the anterior pituitary (Nansel et al., 1979; Gudelsky et al., 1980). Subsequent studies, however, have revealed that changes in TIDA neuronal activity do not correlate with the anterior pituitary DA content, indicating that the latter measure is not a reliable index of impulse traffic on TIDA neurons (Demarest et al., 1984a; 1984c).

4. Concentration of dopamine metabolites

Results from early studies (Fekete et al., 1979; Umezu and Moore, 1979) suggested that DOPAC concentrations in the median eminence could not serve as an index of TIDA neuronal activity since measurable changes in DOPAC concentrations were not observed in response to various stimuli known to influence DA synthesis and turnover. Using more sensitive methods for detecting picogram quantities of amines and their metabolites, Lookingland et al. (1987a; 1987b) have demonstrated that DOPAC concentrations in the median eminence increase and decrease in response to the appropriate pharmacological and physiological stimuli. An advantage of this technique for estimating TIDA neuronal activity is that it does not require drug pretreatments and thereby allows acute neurochemical estimates of activity to be made. This procedure also permits the concurrent measurement of hormone concentrations. One drawback

is that DOPAC concentrations may vary independent of the activity of TIDA neurons if MAO activity or DA stores are interfered with. Thus, results of key experiments are generally confirmed using more than one neurochemical technique for estimating TIDA neuronal activity.

D. Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity

1. Prolactin

TIDA neurons exhibit a prominent inhibitory action on prolactin release from the anterior pituitary. In turn, prolactin plays a role in a short-loop feedback mechanism controlling its own release by activating TIDA neurons. Systemic injections of rat or ovine prolactin increase the αMT-induced decline of DA in the median eminence, but not in other DA-rich regions of the brain. This effect was first observed histochemically (Hökfelt and Fuxe, 1972) and subsequently confirmed biochemically (Gudelsky et al., 1976; Selmanoff, 1981). There is a characteristic delay of 12 to 24 hours before this effect is observed. For example, repeated systemic injections of prolactin increase the αMT-induced decline of DA in the median eminence at 26 hours but not at 10 hours after the start of the injections. Similarly, injections of ovine or rat prolactin into the cerebroventricular system increase the αMT-induced decline of DA (Annunziato and Moore, 1978), the accumulation of DOPA (Johnston et al., 1980) and the activity of tyrosine hydroxylase (Nicholson et al., 1980) in the median eminence. The concentration of DA in the hypophysial portal blood is also increased following prolactin administration (Gudelsky and Porter, 1980). These changes, however, were not seen until at least 12 to 16 hours after the injections of prolactin, at a time when the hormone had probably disappeared from the cerebroventricular system (Login and MacLeod, 1977). Thus, prolactin appears to

trigger a neuronal event which ultimately leads to a delayed activation of the TIDA neurons.

A number of endocrinological and pharmacological manipulations that result in sustained increases in serum prolactin concentrations also cause a delayed activation of TIDA neurons. Transplantation of anterior pituitaries under the renal capsule elevates serum prolactin and thus results in an increase in the αMT-induced decline of DA and the activity of tyrosine hydroxylase in the median eminence-arcuate nucleus (Olson et al., 1972; Hohn and Wuttke, 1978; Krieger and Wuttke, 1980; Morgan and Herbert, 1980; Nicholson et al., 1980). Rats with prolactin secreting tumors release increased amounts of DA into pituitary stalk blood (Cramer et al., 1979a). The DA receptor antagonist, haloperidol, increases serum prolactin concentrations by blocking the inhibitory action of DA on lactotrophs. This, in turn, stimulates TIDA neuronal activity 12 to 30 hours after injection (Gudelsky et al., 1976; Gudelsky and Moore, 1977) and after 24 hours produces an enhanced release of DA into the hypophysial portal blood (Gudelsky and Porter, 1980).

The mechanism of the delayed activation of TIDA neurons by prolactin is not well understood, but studies with cycloheximide reveal that this inhibitor of protein synthesis prevents the ability of prolactin administered into the lateral ventricle to increase DA synthesis in the median eminence (Johnston et al., 1980). This has prompted the suggestion that prolactin may induce the synthesis of a protein, possibly tyrosine hydroxylase, in the somas of the TIDA neurons and the latent period is due to the time required for this newly synthesized enzyme to be transported down TIDA axons to their terminals in the median eminence. Consistent with this proposal is the finding that prolactin increases maximal activity of tyrosine hydroxylase (Nicholson et al., 1980). Gonzalez and Porter (1988), have later found no change in the quantity of tyrosine hydroxylase in the

median eminence in response to hyperprolactinemia. Thus, the mechanism of the prolactin-induced activation of TIDA neurons remains to be elucidated.

The actions of prolactin on the TIDA neurons consist of two separate components. Results from in vivo experiments (as described above) suggest that prolactin stimulates TIDA neurons in a sluggish manner. On the other hand, in vitro experiments demonstrate that prolactin causes a prompt increase in DA synthesis and release from tissue fragments of mediobasal hypothalamus (Forman and Porter, 1981; Perkins and Westfall, 1978). The conflicting results appears to relate to the fact that with in vivo, but not with in vitro, experiments TIDA neurons are constantly exposed to endogenous prolactin which may tonically stimulate them and thereby mask the acute effects of the exogenous hormone. The problem is exacerbated by the fact that pharmacological manipulations needed to make biochemical measurements of TIDA neuronal activity inhibit DA synthesis and thereby remove the tonic inhibitory control of this amine on prolactin secretion. Thus, in vivo rates of DA synthesis and turnover are actually values obtained in the presence of high circulating concentrations of prolactin. This confounding variable can be avoided by pretreating animals with a DA agonist (ie. bromocriptine; Demarest et al., 1984d). Bromocriptine does not influence the activity of TIDA neurons directly (Demarest and Moore, 1979a) but activates DA receptors on lactotrophs thereby reducing the secretion of prolactin. As a result of reduced circulating levels of prolactin, there is a reduction in TIDA neuronal activity (Demarest et al., 1984d). Systemic and icv administration of exogenous prolactin to a bromocriptine-pretreated rat increases TIDA neuronal activity as early as 4 hours later. Thus, there is both a rapid "tonic" component and a delayed "induction" component to the prolactin-induced activation of TIDA neurons (Demarest et al., 1984d; 1986).

There are marked sexual differences in the basal rate of activity of TIDA neurons and in the responses of these neurons to prolactin. Basal activity refers to that level of TIDA neuronal activity observed in a gonadally-intact, unmanipulated female or male rat. The higher basal rate of activity of TIDA neurons in female rats is evidenced by a higher rate of DA synthesis and turnover in the median eminence (Demarest and Moore, 1981a; Gunnet et al., 1986). Since there is no difference in the steady state concentrations of DA in the median eminence of male and female rats, the differences in rates of synthesis and turnover of DA in this region are not a consequence of a greater density of DA neurons, but rather to increased TIDA neuronal activity in the female. This is consistent with the report that DA content in portal blood is several fold higher in the female than in the male rat (Ben-Jonathan et al., 1977; Gudelsky and Porter, 1981). The greater basal activity of TIDA neurons in the female is due, in part, to a greater sensitivity of these neurons to the stimulatory action of prolactin (Demarest and Moore, 1981a). For example, TIDA neuronal activity is reduced if serum levels of prolactin are reduced by hypophysectomy or by pretreatment with bromocriptine and this effect is much more pronounced in the female rat (Demarest and Moore, 1981a; Demarest et al., 1984d). In fact, bromocriptine reduces the rate of DA synthesis in the median eminence of female rats to values equivalent to those in male rats and this effect can be prevented by concurrent injections of prolactin. Dose response curves to icv-administered prolactin reveal that the magnitude of the stimulation of TIDA neurons is greater and the dose of prolactin needed to produce this stimulation is lower in the female (Demarest and Moore, 1981a). Thus, the higher level of basal activity or normal resting tone of TIDA neurons in the female rat results, in part, from a greater sensitivity to and, consequently, a continuous or "tonic" activation by prolactin.

2. Gonadal steroids

The sexual difference in TIDA neuronal activity also appears to be due, in part, to both androgen-induced changes in neuronal differentiation during development and to differences in the hormonal environment in the adult. For example, the higher rate of DA synthesis in the median eminence of the adult female can be reduced to values similar to those in an adult male if the female is administered testosterone on day 5 after birth (Demarest et al., 1981). Conversely, higher female-like values for DA synthesis are observed in adult males in which androgenization of the brain is prevented by castration on day 1 after birth (Demarest et al., 1981). Thus, testosterone secreted during the neonatal period alters the basal rate of activity of TIDA neurons. In addition, the adult gonadal steroid hormonal milieu of both sexes affect TIDA neuronal activity. In female rats, the TIDA neurons are influenced by estrogen such that removal of the ovaries results in a decrease in DA turnover in the median eminence (Gunnet et al., 1986). A stimulatory effect of estrogen is in agreement with a number of published reports. Repeated systemic injections of estrogen to adult female and male rats increase the αMT-induced decline in catecholamine histofluorescence (Löfström et al., 1977) and increase the rate of DA synthesis and turnover in the median eminence (Fuxe et al., 1969a; Wiesel et al., 1978; Eikenburg et al., 1977; Demarest and Moore, 1980; Crowley, 1982). Acute estrogen treatment also enhances DA release into the hypophysial portal blood (Gudelsky et al., 1981). Since the former effects were not observed in hypophysectomized rats (Eikenburg et al., 1977; Demarest and Moore, 1980), it has been postulated that the estrogen-induced activation of TIDA neurons is not due to a direct action of this hormone, but rather to its ability to increase circulating concentrations of prolactin.

A complex pattern of effects on TIDA neurons is observed if rats are exposed to estrogens for longer periods (more than 2 weeks). These include a

decrease in the concentration, synthesis and turnover of DA in the median eminence and a reduced responsiveness of TIDA neurons to prolactin (Casaneuva et al., 1982; Demarest et al., 1984b; Terry et al., 1985). These effects of estrogens may be due to a direct effect of the hormone or to enlargement and possible tumor development in the anterior pituitary which can compress TIDA neurons (Riegle et al., 1985). Most of the effects of chronic estrogen treatment on TIDA neurons are reversible (Demarest et al., 1984b) but there are some reports that long term exposure to this hormone damages TIDA neurons (Sarkar et al., 1982).

Removal of the source of estrogen is believed to be responsible for the decrease in TIDA neuronal activity following ovariectomy. Although the source of progesterone is also removed, this gonadal steroid has no effect on catecholamine turnover in the median eminence (Fuxe et al., 1969a). Although Cramer et al. (1979b) reported an enhanced release of DA into pituitary stalk blood of ovariectomized-adrenalectomized rats administered progesterone, Wang and Porter (1986) reported a decrease in the quantitiy of tyrosine hydroxylase in the median eminence following progesterone pretreatment.

In contrast to the ovariectomy-induced decrease in TIDA neuronal activity, castration in the male rat increases the rate of DA synthesis and turnover in the median eminence (Kizer et al., 1978; Gunnet et al., 1986). Thus, TIDA neurons appear to be tonically inhibited by testosterone. This inhibitory influence of testosterone is supported by the observation that this hormone inhibits the activity of tyrosine hydroxylase (Brawer et al., 1986; Nakahara et al., 1976). In addition, the enhanced rate of DA turnover in the median eminence of orchidectomized rats can be reversed by testosterone replacement (Gunnet et al., 1986). The ability of testosterone to reduce TIDA neuronal activity is independent of any changes in prolactin secretion (Nazian and Mahesh, 1979). Thus, the

hormonal environment to which the TIDA neurons are exposed in neonatal and adult life influence and, in part, are responsible for a sexual difference in the basal rate of activity of these neurons.

3. Afferent neuronal influences

Inhibitory afferent systems decrease TIDA neuronal activity during physiological states that occur exclusively in the female. For example, during the first half of pregnancy in the rat, there are daily nocturnal and diurnal surges of prolactin (Butcher et al., 1972) which are important for the initiation and maintenance of luteal function (Smith et al., 1975). TIDA neurons exhibit a semi-circadian pattern of activity during early pregnancy which occurs out of phase with the surges of prolactin (McKay et al., 1982). Thus, the increased serum prolactin concentrations occur at times when TIDA neuronal activity is low. A similar pattern of TIDA neuronal activity and serum prolactin levels is seen in rats made pseudopregnant by electrical stimulation of the uterine cervix (Freeman et al., 1974; Voogt and Carr, 1981; McKay et al., 1982). During lactation, increases in serum prolactin concentrations in response to suckling are due, in part, to a reduced release of DA into the hypophysial portal blood (Ben-Jonathan et al., 1977). Suckling causes an immediate reduction in the rate of synthesis and turnover of DA in the median eminence (Demarest et al., 1983a; Selmanoff and Wise, 1981) and this decrease may facilitate the surge of prolactin.

Various types of stressful manipulations are also potent stimulators of prolactin secretion in both female and male rats (Ajika et al., 1972; Krulich et al., 1974; Riegle and Meites, 1976). The increase in serum prolactin induced by acute restraint stress in the female rat is associated with a prompt reduction in the rates of synthesis and turnover of DA in the median eminence (Demarest et al., 1985a). Restraint stress has been reported to decrease the αMT-induced decline in DA fluorescence (Fuxe et al., 1983) and have no effect on DA synthesis in the

median eminence of male rats (Demarest et al., 1985b). This latter effect has been suggested to result from the inhibitory influence of testosterone in the adult male, since a stress-induced decrease in DA synthesis in the median eminence was observed in male rats following castration (Demarest et al., 1985b). Thus, inhibitory neuronal mechanisms decrease TIDA neuronal activity during pregnancy, pseudopregnancy, suckling and restraint stress in the female rat. The decrease in TIDA neuronal activity may contribute to the increase in prolactin secretion associated with these physiological states.

Several investigators have studied the importance of neural connections to the mediobasal hypothalamus in mediating the prolactin response to various stimuli. Deafferentation of neuronal afferents from anterior hypothalamus to mediobasal hypothalamus was found to significantly attenuate the increase in prolactin produced by restraint stress (Mioduszewski and Critchlow, 1981; Kawakami and Higuchi, 1981; Kaler et al., 1984) ether stress (Mioduszewski and Critchlow, 1981) heat stress (Siegel et al., 1979) and pseudopregnancy induced by cervical stimulation (Freeman et al., 1974). Blockade of these prolactin surges by deafferentation suggests that afferent neuronal pathways from the anterior hypothalamus may project to and inhibit TIDA neuronal activity and thereby increase prolactin secretion.

STATEMENT OF PURPOSE

Little is known about stimulatory afferent projections to TIDA neurons and whether afferent input mediates the prolactin-induced activation of these neurons. Hypothalamic receptors for prolactin have been identified in the rabbit (DiCarlo and Muccioli, 1981) but not in the rat hypothalamus (Dube et al., 1980; Walsh et al., 1978; DiCarlo et al., 1983). It is, therefore, uncertain whether prolactin alters TIDA neuronal activity directly or via afferent input originating within or outside of the hypothalamus. In addition, the role of afferent neurons in the regulation of basal TIDA neuronal activity has yet to be determined. Complete deafferentation of the mediobasal hypothalamus does not alter DA content (Weiner et al., 1972) or turnover (Gudelsky et al., 1978) in the median eminence of male rats. Since there are major sex differences in the basal rate of activity on TIDA neurons it would be of interest to determine the effects of deafferentation in the female rat. Thus, the objective of the present studies was to examine the role of afferent neuronal input to the TIDA neurons: (1) in maintaining basal activity on these neurons, (2) in mediating the stimulatory action of prolactin, and (3) in mediating the inhibitory influence of restraint stress. The role of afferent neuronal regulation of basal TIDA neuronal activity was investigated in intact and castrated rats to determine the influence of the gonadal steroids. The origin of afferent input to the TIDA neurons was investigated through the use of deafferentation and lesion techniques.

MATERIALS AND METHODS

A. Animals

Female and male Long Evans rats were obtained from Charles River Breeding Labs (Wilmington, MA) and Blue Spruce Farms, Inc. (Altamont, NY) weighing 200-250 gm. Animals were housed 4 per cage in a temperature- (21 ± 1°C) and light-controlled (lights on from 7:00 am to 7:00 pm) room with food and tap water available ad libitum. Animals used in restraint stress paradigms were housed 2 per cage 3 days prior to experimentation. This eliminates a "stress effect" which occurs frequently when animals are removed sequentially from their cages and can be demonstrated by an increase in circulating prolactin concentrations in the third and fourth animal decapitated.

B. Drugs

Rat prolactin (kindly supplied by Dr. S. Raiti of the National Hormone and Pituitary Program, University of Maryland, through a contract program of NIDDK) was dissolved in 0.9% saline and administered by intracerebroventricular (icv) injection. The DA antagonist, haloperidol (McNeil Laboratories, Fort Washington, PA), was dissolved in 0.3% tartaric acid while the DA agonist, bromocriptine (Sandoz Pharmaceuticals, E. Hanover, NJ), was dissolved in a few drops of glacial acetic acid and brought to volume with 0.1% ascorbic acid. The aromatic L-amino acid decarboxylase inhibitor, NSD 1015 (3-hydroxybenzylhydrazine; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline.

D,L-alpha-methyl tyrosine methyl ester dihydrochloride (αMT; Sigma Chemical Co., St. Louis, MO) was brought to volume with distilled water just prior to use.

C. Surgical procedures

1. Anesthesia

Surgical procedures (deafferentations, electrolytic lesions, electrical stimulation and icv cannulation), performed with the aid of a rat stereotaxic instrument (David Kopf Instruments, Tujunga, CA), were carried out in animals anesthetized with ketamine (Vetalar^{R}; Parke Davis, Morris Plains, NJ) and xylazine (Rompum^{R}; Mobax Corp., Shawnee, KS). Ketamine (100 mg/ml) and xylazine (100 mg/ml) were diluted in distilled water to 88 mg/ml and 20 mg/ml respectively. On the day of surgery, equal parts of the two solutions were mixed and injected at a dose of 44 mg/kg ketamine/10 mg/kg xylazine. This anesthesia was chosen as it does not interfere with the estrous cycle, an effect reported to occur with barbiturate anesthesia (Kaneko, 1980).

Ovariectomies and orchidectomies were performed under ether anesthesia. Animals were initially placed in a chamber saturated with ether vapors. During the subsequent removal of the gonads, anesthesia was maintained with an ether nose cone.

2. Deafferentation

i. Knife construction

Complete and retrochiasmatic deafferentations of the mediobasal hypothalamus and anterior hypothalamic deafferentations were performed using a modified Halasz knife (height, 2.0 mm; radius, 1.5 mm; Halasz and Pupp, 1965). The knife was constructed from stainless steel wire (SWX-023, diameter, 0.023 inches, Small parts Inc., Miami, Fla.). A 5 cm piece of wire was ground at the tip to form a double-edged blade, the uniformity of which was examined under

a dissecting microscope. The 3.5 mm knife edge was then bent to the above dimensions. The shaft of the knife was placed into a length of 20 gauge stainless steel tubing (HTX-20, outer diameter, 0.035 inches, wall diameter, 0.0045 inches). The remaining wire not encased in the tubing was bent to form a handle and placed into a stereotaxic micromanipulator.

Bilateral deafferentation of the retrochiasmatic area was performed using two knives constructed from SWX-023 stainless steel wire, a modified method of Palkovits (1982). Straight double edged blades were ground from two lengths of wire which were secured together in a parallel arrangement so that their tips were 1.8 mm apart.

ii. Deafferentation procedures

Animals were placed in a stereotaxic frame with the incisor bar set 8 mm below the intraaural line and prepared for deafferentation by exposing the skull. The approximate anatomical locations of the complete and retrochiasmatic deafferentation of the mediobasal hypothalamus are illustrated in Figure 4. For complete deafferentation, the modified Halasz knife was lowered at the midline, 1.5 mm posterior to bregma, with the vertical blade in the rostral position. The knife tip was lowered to the base of the brain to the caudal border of the optic chiasm. The knife was turned 90° to the left, then the knife was moved posterior 2 mm and rotated 180°. The knife was then moved anterior 2 mm and rotated 90° back to the point of origin. This procedure was repeated twice and the knife was withdrawn in the same manner as it was lowered. The effectiveness of the complete deafferentation was determined postmortem by measuring NE concentrations in the median eminence (Table 1). Noradrenergic neurons in the brain stem provide 50 to 60% of the innervation to the hypothalamus (Dahlström and Fuxe, 1964; Fuxe 1964; 1965) while approximately 40% is intrinsic and remains in the median eminence after complete deafferentation.

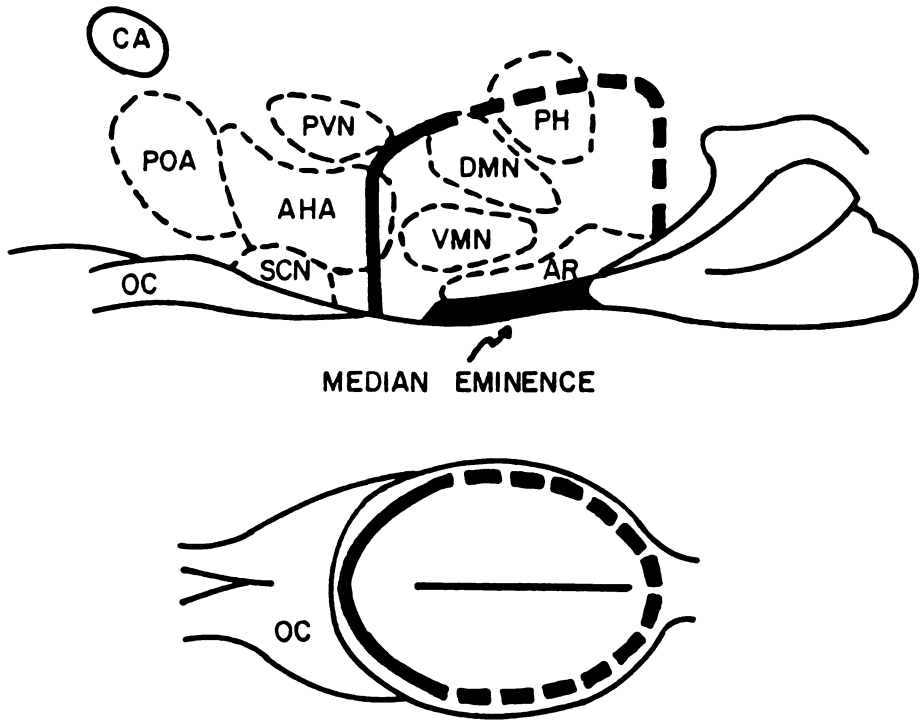

Figure 4. Schematic of sagittal and ventral views of the rat brain illustrating the complete and retrochiasmatic deafferentation of the mediobasal hypothalamus. The bold solid line represents the site of retrochiasmatic deafferentation, while the bold solid line and the bold broken line represent the complete deafferentation. AHA = anterior hypothalamic area; AR = arcuate nucleus; CA = anterior commissure; DMN = dorsomedial nucleus; OC = optic chiasm; PH = posterior hypothalamic nucleus; POA = preoptic area; PVN = paraventricular nucleus; SCN = suprachiasmatic nucleus; VMN = ventromedial nucleus.

TABLE 1

The Effect of Complete Deafferentation of the Mediobasal Hypothalamus (MBH) on NE and DA Content in the Median Eminence of Female Rats

	ng/mg protein	
	NE	DA
Sham	19.5 ± 1.9	110 ± 4.8
MBH	6.3 ± 1.0*	109 ± 5.5

Values represent the means ± 1 SE of 4-8 determinations. *Value significantly different from respective sham control rats, $p < 0.05$.

Thus, norepinephrine concentrations are significantly reduced in the median eminence while DA concentrations remain unaltered after complete deafferentation of the mediobasal hypothalamus. This observation is consistent with previous reports (Weiner et al., 1972; Brownstein et al., 1976b; Gudelsky et al., 1978).

Retrochiasmatic deafferentation was produced by lowering the knife (as with complete deafferentation) to the base of the brain until resistance was met at the ventral surface of the skull. The knife was turned 90° to the left and right two times to produce a semicircular cut and the knife was then removed in the same manner as it was lowered (Halasz and Gorski, 1967).

Anterior hypothalamic deafferentations were performed similar to retrochiasmatic deafferentation. Rather than a semicircular cut made 1.5 mm posterior to bregma, anterior hypothalamic deafferentations were made 1.0 and 0.5 mm posterior to bregma, at bregma, and 0.5 and 1.0 mm anterior to bregma (a total of 5 different anterior hypothalamic knife cuts). The most anterior cut was made at the rostral border of the preoptic area, the most anterior part of the hypothalamus.

Bilateral deafferentation of the lateral retrochiasmatic area, the anatomical location of which is illustrated in Figure 5, was performed as previously described (Palkovits, 1982) with some minor modifications. The bilateral knife assembly was centered over the midline with the tip of each knife approximately 900 μm lateral to the midline. The knives were lowered, 1.5 mm posterior to bregma, to the ventral surface of the brain where they were manipulated 1.0 mm anterior and 1.0 mm posterior and withdrawn in the same manner as they were lowered.

Sham operations consisted simply of lowering the respective knife 5 mm through the midline without rotation. Animals were allowed to

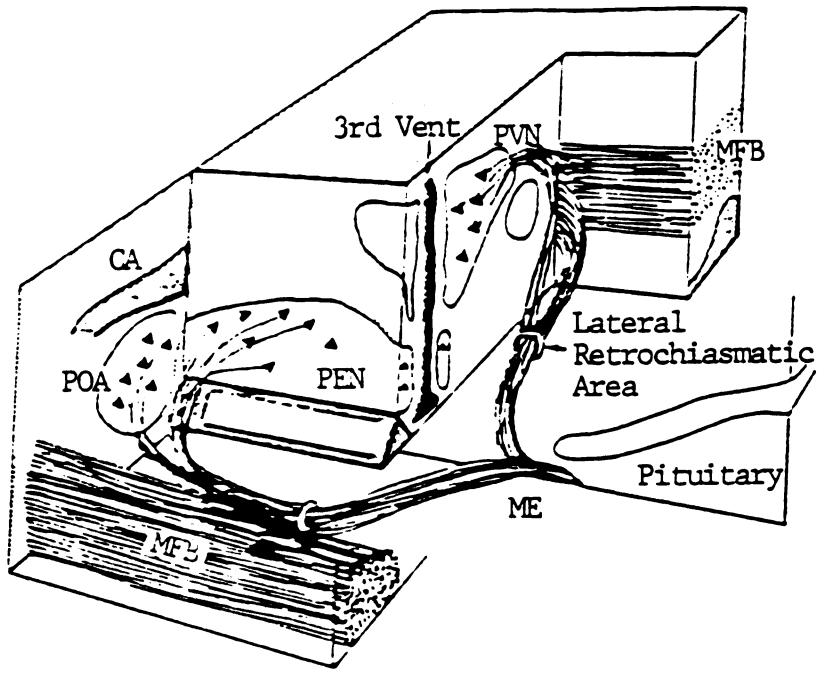

Figure 5. Schematic of the topography of the lateral retrochiasmatic area showing cell groups which project axons through it to the median eminence. CA = anterior commissure; ME = median eminence; MFB = medial forebrain bundle; PEN = periventricular nucleus; POA = preoptic area; PVN = paraventricular nucleus (Modified from Palkovits, 1982).

recover for 7 days prior to experimentation except in the case of a time course experiment where the animals were allowed to recover from 1 day up to 3 weeks after surgery. The anatomical location of the deafferentations were verified postmortem on all frozen sections of brains analyzed neurochemically. Initially, anatomical confirmation of the retrochiasmatic deafferentation was made on horizontal fixed sections of brains stained with cresyl violet. Verification of knife cut placement along with an observation that retrochiasmatic deafferentation induced a state of persistent vaginal estrus formed the inclusion criteria for acceptance of an animal into a study. The pattern of persistent vaginal cornification following retrochiasmatic deafferentation had been demonstrated previously (Halasz and Gorski, 1967; Brownstein et al., 1976a) and is depicted in Figure 6. Only those deafferentated females displaying at least 4 days of persistent vaginal estrus were used in these experiments.

Following surgery, the skull was repaired with bone wax and the scalp was closed with stainless steel wound clips. All animals received a 0.1 ml subcutaneous injection of Combiotic^{R} (200, 000 U procaine penicillin and dihydrostreptomycin per ml; Pfizer, Inc., New York, NY).

3. Electrolytic lesions

Electrolytic lesions were made in rats placed in a stereotaxic frame with the incisor bar positioned 3 mm below the intraaural line. NE-300 stainless steel electrodes (0.25 mm tip diameter and 0.5 mm exposed length; Rhodes Medical, Woodland Hills, CA) were bilaterally placed in the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA) or paraventricular nucleus (PVN). Using the atlas of König and Klippel (1963) as a reference, the electrode coordinates were approximately C 0.4 mm, L 0.2 mm, V 9.4 mm for the SCN, C 0.8 mm, L 0.75 mm, V 8.9 mm for the AHA, and C 1.4 mm, L 0.4 mm, V 8.3 mm for the PVN. The abbreviations C, L and V represent the caudal, lateral and

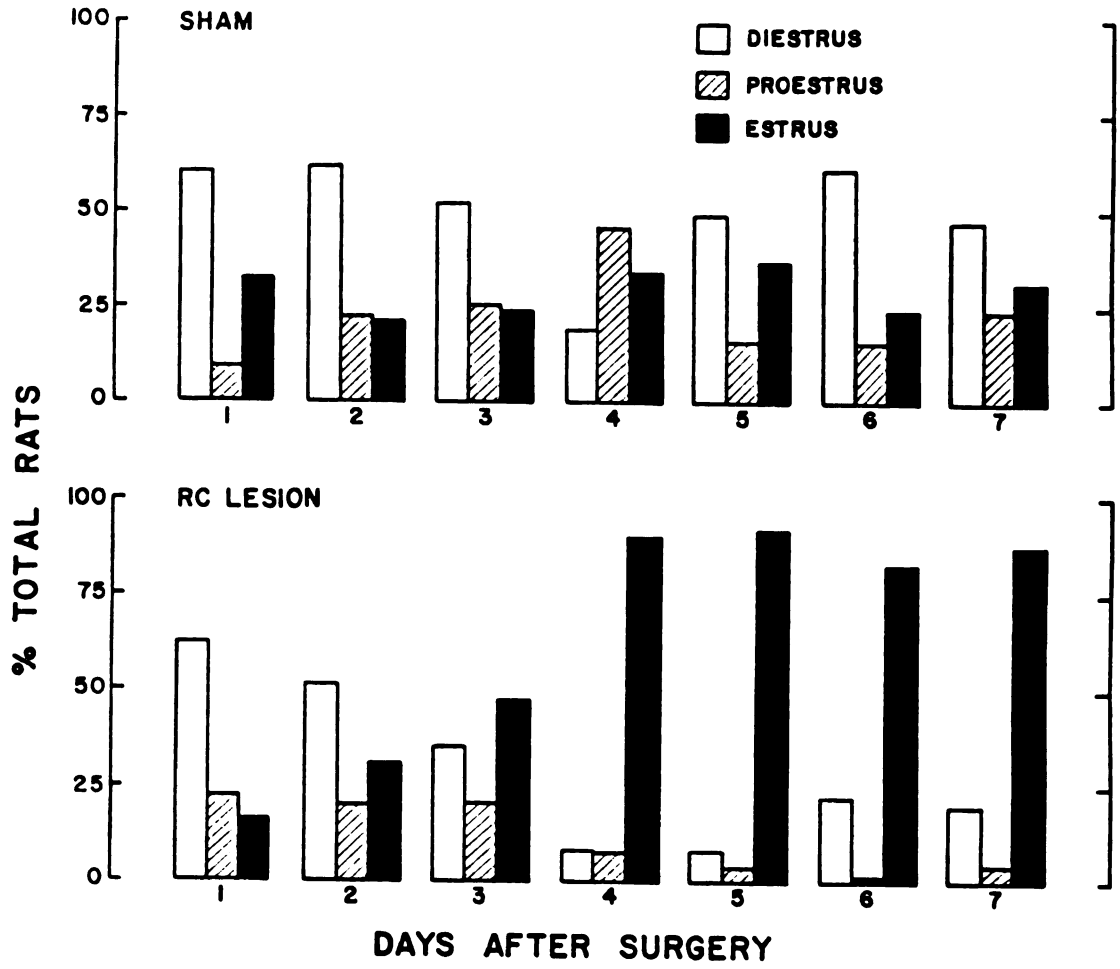

Figure 6. The effect of retrochiasmatic deafferentation (RC) on the estrous cycle of female rats. Columns represent the percent of total rats (32 sham or 32 RC) whose vaginal cytology were indicative of a given stage (diestrus, proestrus or estrus) of the estrous cycle at 1 to 7 days following sham or retrochiasmatic deafferentation.

ventral placement of the electrodes with respect to bregma and the skull. An anodal DC current of 300-500 μA intensity was supplied for 15 seconds to the electrodes by a Grass constant current unit (Grass Instrument Co., Ann Arbor, MI) which was driven by a Grass SD-9 stimulator. An initial experiment was done to determine the size of the lesion versus the amount of applied current in the PVN (Figure 7). Lesion size was measured through the use of a micrometer while viewing the brain section under a dissecting microscope. Thus, the current intensity varied depending upon the size of the region to be lesioned, i.e 300 μA for SCN, 500 μA for AHA and 400 μA for PVN. Sham lesions were made by lowering the electrodes along the same coordinates without passing current. Anatomical confirmation of lesion placement was made on frontal sections of fixed brains stained with thionin. A representative sham and electrolytic lesion of the SCN, AHA and PVN are shown in Figures 8, 9 and 10 respectively.

4. Intracerebroventricular cannulation

ICV cannulae were implanted in rats placed in a stereotaxic frame with the incisor bar set 5 mm above the intraaural line (Pellegrino and Cushman, 1968). A cannula guide consisting of a 10 mm length of stainless steel tubing (HTX-23, outer diameter 0.025 inches, wall diameter 0.006 inches, Small Parts Inc., Miami, FL) was inserted into the lateral ventricle (C 0.0, L 1.5, V 3.2 mm with respect to bregma and dura) 7 days prior to experimentation. The guide was affixed to the skull with stainless steel screws and dental cement (Repair Material, Dentsply, York, PA) and occluded with a stylet. Hormones were infused at a rate of 1 μl per minute into the lateral ventricle by replacing the stylet with a 30 gauge stainless steel injector connected with polyethylene tubing to a Hamilton syringe.

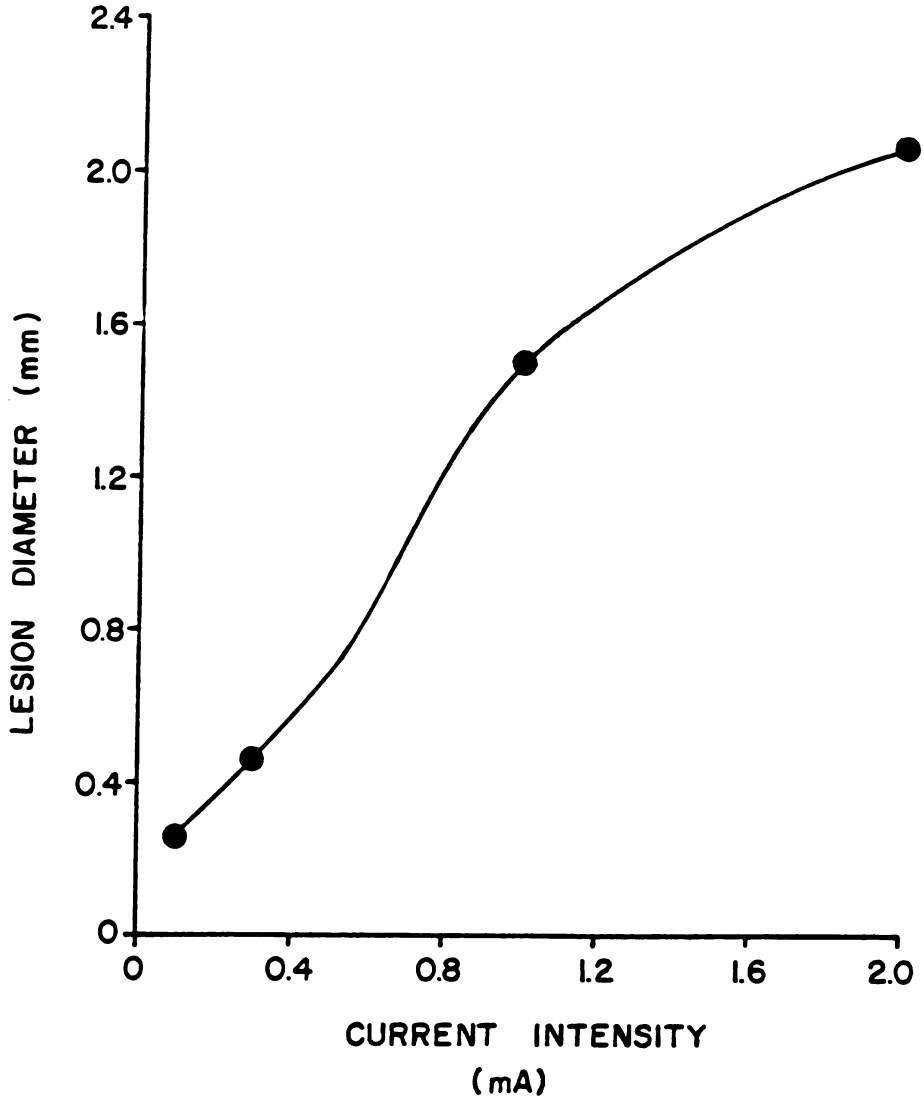

Figure 7. The effect of increasing current intensity on the size of electrolytic lesions produced with NE-300 electrodes. Values represent the means of lesion diameters (3-4 determinations) of the PVN following 0.1, 0.3, 1 and 2 mA of current applied for 15 sec.

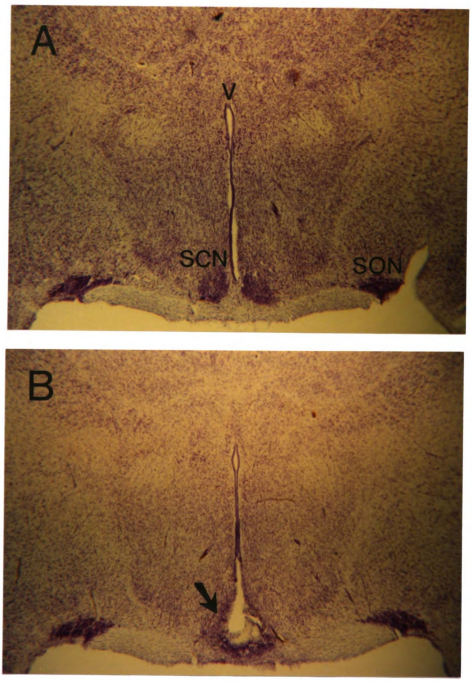

Figure 8. Frontal brain sections illustrating a sham (A) and electrolytic lesion (B) of the suprachiasmatic nucleus (SCN). SON = supraoptic nucleus; V = third ventricle.

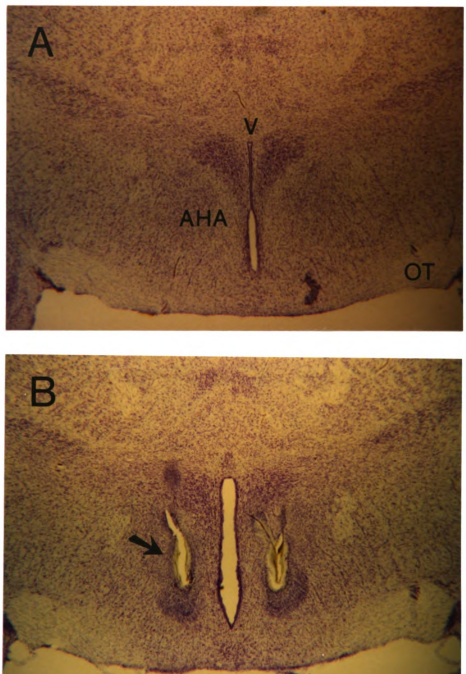

Figure 9. Frontal brain sections illustrating a sham (A) and electrolytic lesion (B) of the anterior hypothalamic area (AHA). OT = optic tract; V = third ventricle.

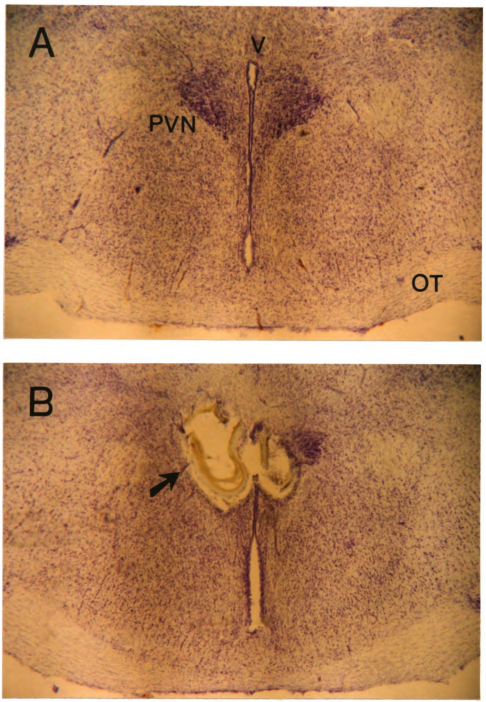

Figure 10. Frontal brain sections illustrating a sham (A) and electrolytic lesion (B) of the paraventricular nucleus (PVN). OT = optic tract; V = third ventricle.

5. Castration

Rats were anesthetized with ether and ovariectomized or orchidectomized 2 weeks prior to experimentation (1 week prior to deafferentation). A small incision was made through the abdominal wall to externalize and remove the ovaries. The muscle wall was then sutured and the incision closed with stainless steel wound clips. For removal of the testes, an incision was made in the surrounding skin and pressure was applied to the abdomen to externalize the gonads. Blood flow to the testes was tied off, the gonads were removed and the incision was closed with wound clips.

D. Restraint Stress

The stress regimen consisted of 30 minutes of restraint in which rats were lightly anesthetized with diethyl ether and then immobilized in a supine position (Demarest et al., 1985a). In studies where the effect of stress on DOPA accumulation was determined, NSD 1015 was administered immediately prior to the initiation of the 30-minute period of stress.

E. Tissue Dissection

Following the appropriate treatments, rats were decapitated and their brains were quickly removed and placed on a cold plate for dissection of the posterior pituitary and median eminence (Demarest and Moore, 1979a) or rapidly frozen over dry ice and processed using a modified method of Palkovits (1973). Consecutive 600 µm frontal sections beginning at about A9220 µm (König and Klippel, 1963) were prepared from the frozen brains and thaw mounted on a glass slide. Frozen sections were viewed through a stereodissecting microscope and selected brain regions were removed using various sizes of stainless steel tubing

by a modification (Lookingland and Moore, 1984) of the method originally described by Palkovits (1973).

Once dissected, the tissue samples were homogenized in polyethylene microsample tubes containing various volumes of cold 0.2 N perchloric acid with 10 mg% EGTA for radioenzymatic analysis. Samples were then centrifuged and the supernatants stored at -20°C for later assay. Tissue samples were placed in 0.1 M phosphate-citrate buffer (pH 2.7) with 0.1 mM EDTA, 0.035% sodium octylsulfate and 20% methanol for analysis by high performance liquid chromatography (HPLC) with electrochemical detection. Samples were then sonicated for 3 seconds (Sonicator Cell Disruptor, Heat Systems-Ultrasonics, Plainview, NY), centrifuged and the supernatants stored at -20°C. The remaining protein pellets were dissolved in 1 N sodium hydroxide and the content was determined by the method of Lowry et al. (1951).

F. Histology

In the initial studies involving retrochiasmatic deafferentation and in studies employing electrolytic lesion techniques brains were preserved in a 4% formalin solution buffered in 0.1 M phosphate (pH 7.4). Fixed tissue was sectioned (50 μm) on a freezing microtome and mounted to gelatin-coated slides. Sections were stained with either cresyl violet (cresyl escht violett, Chroma Gesellschaagt, Schmid and Co.) or thionin (Fischer Scientific Co., Fairlawn, NJ). The anatomical location of the knife cuts and electrolytic lesions was confirmed by examining the tissue under a microscope.

G. Measurement of Monoamines

1. Radioenzymatic analyses

Ten microliter aliquots of tissue supernatants were assayed for DA and NE (Umezu and Moore, 1979) and DOPA (Demarest and Moore, 1980) via radioenzymatic assay (data in Figures 11, 12, 15, 23 and 24 and Table 4) . In this procedure, DA, NE and DOPA in standards and tissue extracts were incubated in the presence of partially purified catechol-o-methyltransferase and S-adenosyl-L-[methyl-^{3}H] methionine ([^{3}H] SAM) resulting in the formation of radioactive o-methylated 3-methoxytyramine, normetanephrine and 3-methoxytyrosine respectively. The first two of these products were separated by organic extraction and thin layer chromatography and the later by cation/anion exchange chromatography. The contents of o-methylated catecholamines in tissue extracts were calculated directly from standards after subtracting blank values.

Ten microliters of perchloric acid extracts of brain tissue or standards (0.25-8 ng DA and NE, 0.125-4 ng DOPA) were added to 5 ml conical tubes on ice. Twenty-five microliters of a freshly prepared incubation mix were then added to each tube. This mixture (for 30 samples) contained: 50 µl 20 mM EGTA-sodium (pH 7.2); 125 µl catechol-o-methyltransferase prepared as described previously (Moore and Phillipson, 1975); 75 µl [^{3}H] SAM (250 µCi/ml, New England Nuclear Corp, Boston, MA); 50 µl o-benzylhydroxylamine and pargyline (18 mg/ml and 8 mg/ml in 10% mercaptoethanol); 325 µl 1 M Tris base (pH 10.2) and 75 µl of distilled water. This mixture was incubated at 37°C for 30 minutes.

For separation of DA and NE 30 µl of a solution consisting of 10 volumes of 0.45 M borate buffer and 1 volume of methoxyamine carrier (2.5 mg of each of the following compounds per ml of 0.025% sodium metabisulfite: 3-methoxytyramine HCl, normetanephrine HCl and metanephrine HCl; Sigma Chemical Co., St. Louis, MO) was added. Twenty µl of 1.5% tetraphenyl boron

and 500 µl of toluene-isopentyl alcohol (3:2) was then added in quick succession. The tubes were centrifuged and 475 µl of the organic phase was transferred to 5 ml conical tubes containing 250 µl of 0.45 M borate buffer (pH 8.0). After vortexing and centrifugation, 400 µl of the organic phase was transferred to 5 ml conical tubes containing 40 µl of 0.1 N HCl. After vortexing and centrifugation, the organic phase was aspirated and the acid phase washed with 250 µl of ethylacetate saturated with water. After aspiration of the ethylacetate, 25 µl of the acid phase was spotted on thin layer chromatography plates (LK6D, Linear K, Whatman, Clifton, NJ). The chromatographs were developed with methylamine-ethanol-chloroform (5:18:40) and visualized after spraying with Folin reagent diluted 1:1 with water. The 3-methoxytyramine and normetanephrine spots were scraped into scintillation vials and extracted with 0.5 ml of 3 M ammonium hydroxide. After the addition of 3 ml of Safety Solve (Beckman, Palo Alto, CA), radioactivity was determined in a scintillation counter.

For separation of DOPA, after incubation, enzymatic activity was quenched by the addition of 3 ml of 0.5 N acetic acid containing 10 µg/ml 3-methoxytyrosine. The samples were then passed over cation exchange columns (15 x 5 mm, AG 50W-X4, H+, 200-400 mesh) which were prepared with 10 ml 0.2 M ammonium acetate (pH 6.5) and 5 ml of distilled water. The columns were then washed with 5 ml 0.1 M citrate buffer and 0.5 ml 0.2 M ammonium acetate (pH 4.5). Radioactive 3-methoxytyrosine was eluted with 3 ml 0.2 M ammonium acetate (pH 4.5) into 12 x 75 glass test tubes. The samples were adjusted to pH 10-10.5 by the addition of 250 µl of 5 N sodium hydroxide and passed over a 30 x 5 mm anion exchange column (AG 1-X2, 200-400 mesh, OH- form) prepared by passing 5 ml 2 N sodium hydroxide, 5 ml distilled water, 5 ml 10% sodium chloride and 5 ml distilled water. After addition of the sample, the columns were washed with 5 ml distilled water, 5 ml 0.45 M borate buffer (pH 10.0) and 1 ml 0.45 M

borate buffer (pH 7.0). The radioactive 3-methoxytyrosine was eluted with 5 ml 0.45 M borate buffer (pH 7.0) directly into scintillation vials or into 13 x 100 glass test tubes. In some experiments, the 3-methoxytyrosine was further purified by adsorption onto paraffin-treated activated charcoal. Fifty microliters of a charcoal slurry, prepared by stirring 2.5 g treated charcoal in 5 ml water were added to each column eluate and vortexed. After centrifugation, the aqueous supernatant was aspirated and the remaining charcoal washed with 2.5 ml of 0.5% acetic acid. After centrifugation and aspiration, 3-methoxytyrosine was eluted from the charcoal with 1 ml of 5% phenol which was then transferred to a scintillation vial. Fifteen ml of Safety Solve was added and tritium was analyzed by liquid scintillation spectrometry.

2. High performance liquid chromatography

Fifty microliters of tissue supernatant was analyzed for DA, NE, DOPA and DOPAC (data in Figures 13, 14, 16-20, 25-30, and Tables 1 and 2) by injecting the sample onto a C18 reverse-phase analytical column (Bioanalytical Systems, Inc., West Lafayette, Ind.) which was protected by a precolumn cartridge filter (10 μm spheres, 4.6 mm internal diameter x 3 cm; Bioanalytical Systems). The HPLC column was coupled to an electrochemical detector (LC4A; Bioanalytical Systems) equipped with a TL-5 glassy carbon electrode set at a potential of +0.75 V relative to an Ag/AgCl reference electrode. Depending on the age of the column, the concentrations of the mobile phase constituents and the pH of the mobile phase were altered slightly to attain maximal separation of the compounds of intere t while minimizing the total retention time (Chapin et al., 1986). All separations were performed at a flow rate of approximately 0.8 ml/min and a pressure of 2500 psi. The amount of compounds in each sample was determined by comparing peak heights with those obtained from standards as reported by a

Hewlett Packard Integrator (Model 3390A). The limit of sensitivity was approximately 20 pg for the compounds measured.

H. Biochemical Estimates of Neuronal Activity

TIDA neuronal activity was estimated from measurements of the concentrations of DOPAC (Lookingland et al., 1987), the major metabolite of DA, and from the rates of synthesis and turnover of DA in the median eminence which contains the terminals of these neurons. The in vivo rate of DA synthesis was estimated by measuring DOPA accumulation 30 minutes after the administration of the decarboxylase inhibitor NSD 1015 (100 mg/kg, i.p.; Demarest and Moore, 1980). Turnover rates of DA and NE were determined by linear regression analysis of the decline of DA and NE 30 and 60 minutes following αMT administration (an inhibitor of tyrosine hydroxylase, 250 mg/kg free base, i.p.; Lookingland and Moore, 1984).

I. Radioimmunoassay

Prolactin concentrations were measured in the serum or plasma using reagents and procedures in the rat prolactin radioimmunoassay kit supplied through the NIDDK Rat Pituitary Hormone Distribution Program by Drs. S. Raiti and A.F. Parlow. These reagents include an antiserum to rat prolactin produced in rabbits, a rat prolactin reference preparation to serve as a standard and a rat prolactin antigen which was labelled with ^{125}I (Amersham, Arlington Heights, IL) using the lactoperoxidase technique (Chard, 1982). Rat serum or plasma samples were incubated at room temperature with 24-hour periods between the addition of hormone antibody, iodinated hormone and precipitating second antibody (goat anti-rabbit gamma globulin; Arnel Products, New York, NY). Twenty-four hours after addition of second antibody, the mixture was centrifuged and the precipitate

counted in a Micromedic gamma counter (Model 4/200). The confidence limits of the assay were 1 to 12 ng/ml prolactin in 50 µl of plasma or serum. The intra- and inter-assay coefficients of variation were approximately 10% and 17%, respectively.

J. Autoradiographic Analysis of Prolactin Binding Sites

1. Animals and tissue preparation

Female Sprague Dawley rats obtained from Charles River Breeding Labs weighing 200-225 gm were housed as previously described. Daily vaginal cytology was monitored to confirm the stage of the cycle and only those females displaying a vaginal smear consistent with the second day of diestrus were used in the experiments.

Animals were anesthetized with ketamine/xylazine (44 mg/kg/ 10 mg/kg, i.p.) and then perfused through the aorta with equal parts of phosphate-buffered saline and 0.32 M sucrose (pH 7.4). The brains were rapidly removed, frozen in liquid nitrogen for 10-15 seconds and stored at -70°C. The tissues were then sectioned (10 µm thickness) using a Leitz 1720 digital motorized cryostat at -16°C and thaw-mounted on supercleaned glass slides. Six serial sections of brain were taken at various levels, 500 µm apart, from 0.3 mm to 3.8 mm posterior to bregma according to the atlas of Paxinos and Watson (1982). Tissues were stored at -70°C until assay.

2. Receptor labeling

Slide mounted tissue sections were brought to room temperature and humidified for a period of 15 minutes. The incubation procedure was carried out as previously described (Dube et al., 1980; DiCarlo and Muccioli, 1981) with some modifications. The incubation contained 0.4 nM ^{125}I-ovine prolactin (custom prepared by New England Nuclear using a chloramine-T iodination procedure,

specific activity 98 μCi/μg). Ovine prolactin was chosen as the ligand since it is less sensitive to oxidative damage during iodination and is more potent at inhibiting binding of homologous hormone to its receptors than is rat prolactin (Barkey et al., 1981). A concentration of 0.4 nM ^{125}I-ovine prolactin was chosen since it represents the KD for hypothalamic prolactin receptors in rabbit (DiCarlo and Muccioli, 1981) and would thereby permit binding to the more relevant, high-affinity prolactin receptor. One experiment was run with a concentration of labeled prolactin of 0.04 nM in an attempt to decrease the amount of nonspecific binding and enhance specific binding. Iodinated prolactin was buffered in 25 mM Tris-HCl (pH 7.4) containing 10 mM $MgCl_2$, 0.1% bovine serum albumin, aprotinin (100 KIU/ml) and 0.1 mM bacitracin. To correct for nonspecific binding adjacent sections were incubated with iodinated prolactin in buffer containing μM concentrations of unlabeled prolactin. Tissue sections were preincubated in tissue buffer for 15 minutes followed by a 60 minute incubation period. After incubation, tissue sections were rinsed with 25 mM Tris-HCl buffer, dipped in deionized water, and dried rapidly under a stream of cold, dry air.

3. Autoradiography and data analysis

The dried, labeled, slide-mounted sections were exposed to ^{3}H-Ultrafilm (LKB Instruments, Gaithersburg, MD), and after 2-3 days of exposure at 4°C the autoradiographs were developed. Optical density readings were converted to dpms and subsequently to fmols using standard computer programs in the Amersham RAS computerized image analysis system. This system was initially calibrated with an ^{125}I microscale (Amersham, Arlington Heights, IL) cut to the same thickness (10 μm) as the tissue sections. The dpm concentrations which were generated for each standard were fit with a third order polynomial curve. Those brain regions containing bilateral structures were sampled and the binding quantified from both the left and right hemispheres in a minimum of 3 total

binding and 3 nonspecific binding sections. Midline brain regions (median eminence, arcuate nucleus, paraventricular nucleus and choroid plexus of the third ventricle) were sampled and the binding quantified from a minimum of 5 brain sections representing total and nonspecific binding. Values for specific binding were generated from a total of 3 brains by subtracting nonspecific binding values from corresponding total binding values. Values for specific binding, in all brain regions sampled, were tested using a one-way analysis of variance followed by the Student-Newman-Keuls test (Steel and Torrie, 1960), and differences with a probability of error of less than 5% were considered significant.

K. Statistical Analyses

Statistical analyses were conducted using the Student's t-test for comparisons between two groups. Comparisons among 3 or more groups in experiments with one variable (i.e. deafferentation) were analyzed using a one-way analysis of variance. Experiments utilizing 2 variables (i.e. drug treatment and deafferentation) were analyzed using a two-way analysis of variance, random factorial design. Between-group comparisons were made by the Student-Newman-Keuls test (Steel and Torrie, 1960). Differences with a probability of error of less than 5% were considered significant.

RESULTS

A. Role of Afferent Neuronal Input in the Regulation of Basal Tuberoinfundibular Dopaminergic Neuronal Activity in the Female Rat

1. Effect of deafferentation of the mediobasal hypothalamus on basal tuberoinfundibular dopaminergic neuronal activity

Studies were undertaken to determine the effect of complete and retrochiasmatic deafferentation on the rate of DOPA accumulation in the median eminence and neurointermediate lobe of the pituitary gland in female rats (Figure 11). A complete deafferentation of the mediobasal hypothalamus reduced the basal rate of DOPA accumulation in the median eminence with no effect in the neurointermediate lobe, the terminal region of the tuberohypophysial DA neurons. Similarly, retrochiasmatic deafferentation of the mediobasal hypothalamus, which represents the anterior portion of the complete deafferentation, selectively reduced the basal rate of DOPA accumulation in the median eminence of female rats. Retrochiasmatic deafferentation also reduced the basal rate of DA turnover in the median eminence of female rats (Figure 12) but did not alter steady-state concentrations of DA and NE and the rate of NE turnover in the median eminence. In addition, retrochiasmatic deafferentation reduced the concentration of the deaminated metabolite, DOPAC, in the median eminence without altering DA concentrations in this region (Table 2). Serum prolactin concentrations, as well, were unaltered by retrochiasmatic deafferentation. Thus retrochiasmatic deafferentation of the mediobasal hypothalamus decreases TIDA neuronal activity in the female rat as evidenced by the decreased rates of synthesis and turnover, and the metabolism of DA in the terminals of these neurons in the

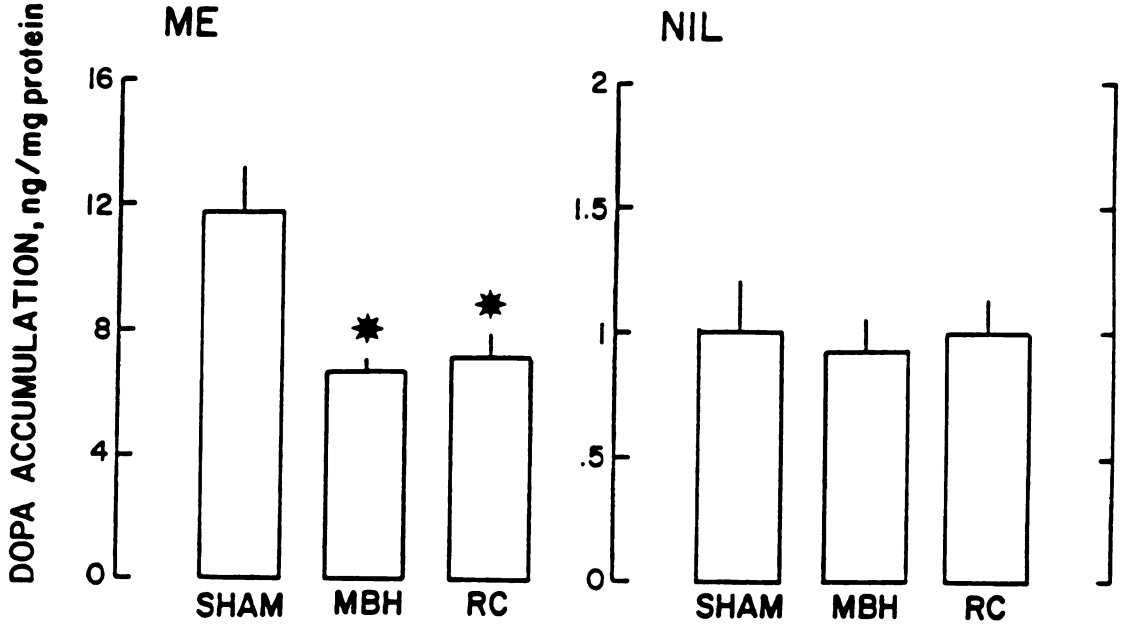

Figure 11. The effect of complete (MBH) and retrochiasmatic (RC) deafferentation of the mediobasal hypothalamus on DOPA accumulation in the median eminence (ME) and neurointermediate lobe of the pituitary (NIL) of female rats. Columns represent the means and the vertical lines 1 SE of 6-8 determinations. *Values significantly different from respective sham control rats, $p < 0.05$.

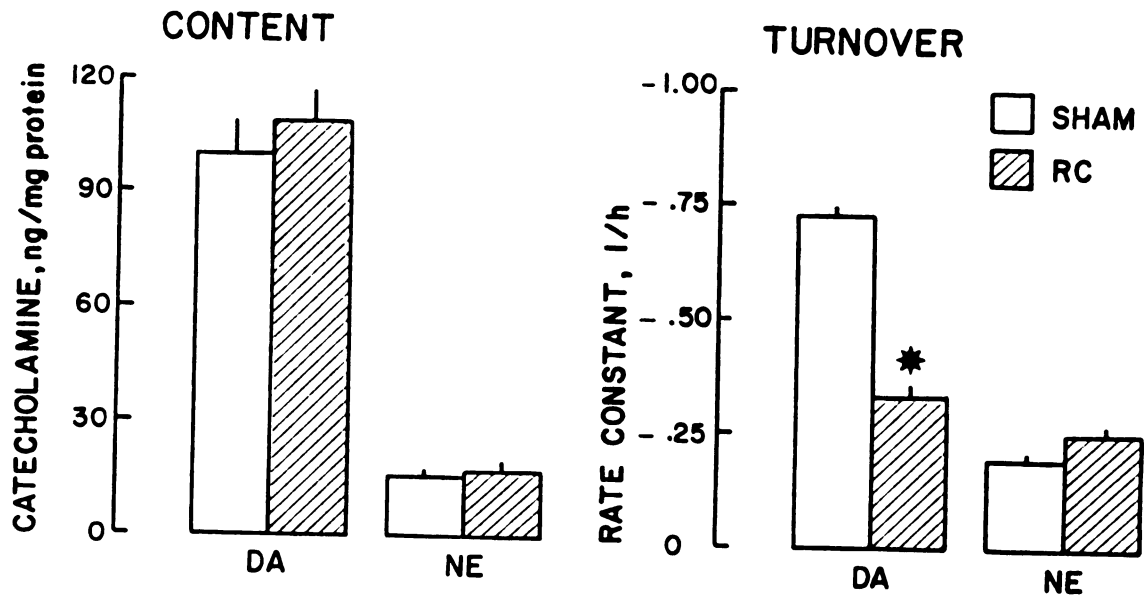

Figure 12. The effect of retrochiasmatic (RC) deafferentation on DA and NE content and turnover in the median eminence of female rats. The columns illustrating DA and NE content represent the means and the vertical lines 1 SE of 8 determinations, while those illustrating DA and NE turnover represent the means and the vertical lines 1 SE of the rate constant calculated from the decline in DA and NE concentrations determined in 8 animals decapitated 0, 30 and 60 minutes after alpha-methyl tyrosine (a total of 24 determinations). *Value significantly different from respective sham control rats, $p < 0.05$.

TABLE 2

The Effect of Retrochiasmatic Deafferentation (RC) on DOPAC and DA Concentrations in the Median Eminence and Serum Prolactin Concentrations of Female Rats

	DOPAC ng/mg protein	DA ng/mg protein	Prolactin ng/ml
Sham	20.7 ± 1.6	97 ± 7.6	6.8 ± 2.8
RC	15.0 ± 1.4*	102 ± 9.8	6.6 ± 1.6

Values represent the means ± 1 SE of 6-8 determinations. *Value significantly different from sham control rats, $p < 0.05$.

median eminence. These results suggest that regions rostral to the retrochiasmatic deafferentation exert a stimulatory influence on TIDA neurons in the female rat.

Studies were undertaken to more completely characterize the effects of retrochiasmatic deafferentation on TIDA neuronal activity and circulating prolactin by examining a time course for the effect of deafferentation. DOPAC and DA concentrations in the median eminence were measured 1, 3, 7, 14 and 21 days after retrochiasmatic deafferentation (Figure 13). DOPAC concentrations were significantly reduced by 3 days and remained decreased for 21 days following deafferentation. A 30% to 35% decrease in DOPAC was observed at all time periods. Consistent with previous results (Figure 12), DA concentrations in the median eminence were unaltered at 7 days and at all other times following retrochiasmatic deafferentation (Figure 13). Circulating prolactin concentrations also remained unchanged at 1 and up to 21 days following retrochiasmatic deafferentation (Figure 14). These results suggest that retrochiasmatic deafferentation acutely interrupts stimulatory afferent input to the TIDA neurons which is maintained for at least 21 days. Since the deafferentation-induced decrease in TIDA neuronal activity does not result in an increase in circulating prolactin concentrations, this procedure may interfere with neurons which contain prolactin-releasing factors or a reserve of DA may be present at the anterior pituitary.

To determine more precisely the region rostral to the mediobasal hypothalamus mediating the stimulatory influence on TIDA neurons in the female rat, anterior hypothalamic knife cuts were made at the retrochiasmatic region and at 0.5 mm increments rostral into the anterior hypothalamus (Figure 15). Retrochiasmatic deafferentation (-1.5 mm to bregma) and placement of this knife cut 0.5 mm rostral into the anterior hypothalamus (-1.0 mm to bregma) decreased

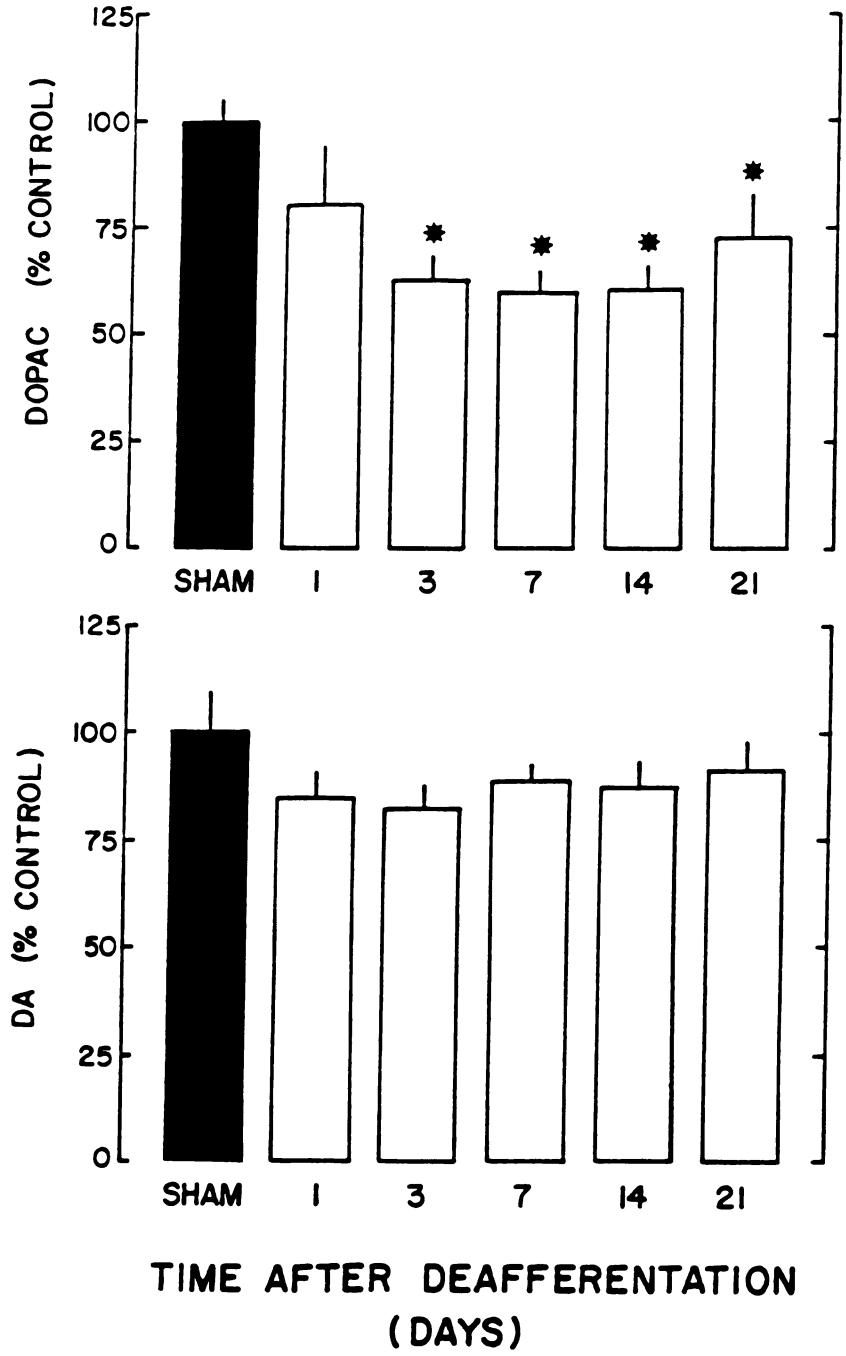

Figure 13. A time course for the effect of retrochiasmatic deafferentation (open columns) on DOPAC and DA concentrations in the median eminence of female rats. Columns represent the means as a percent of the control (DOPAC and DA = 12.73 $\pm$ 0.66 and 97.38 $\pm$ 9.60 ng/mg protein, respectively) and the vertical lines 1 SE of 6-8 determinations. *Value significantly different from sham control rats, $p < 0.05$.

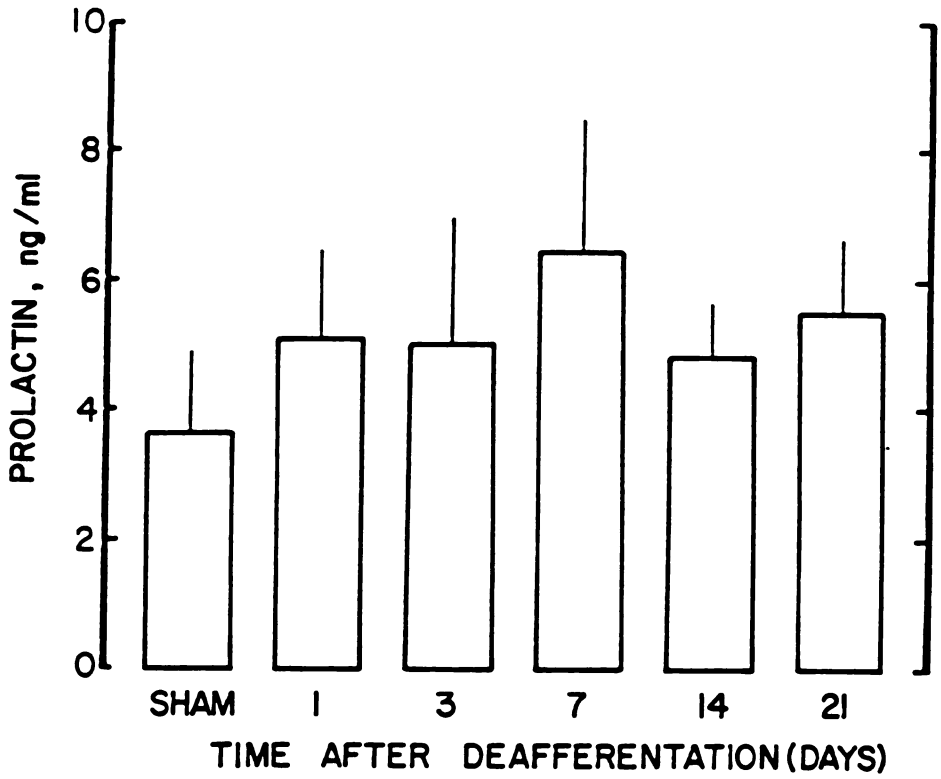

Figure 14. A time course for the effect of retrochiasmatic deafferentation on serum prolactin concentrations in female rats. Columns represent the means and the vertical lines 1 SE of 6-8 determinations. Values are from the same animals used for determinations shown in Figure 13.

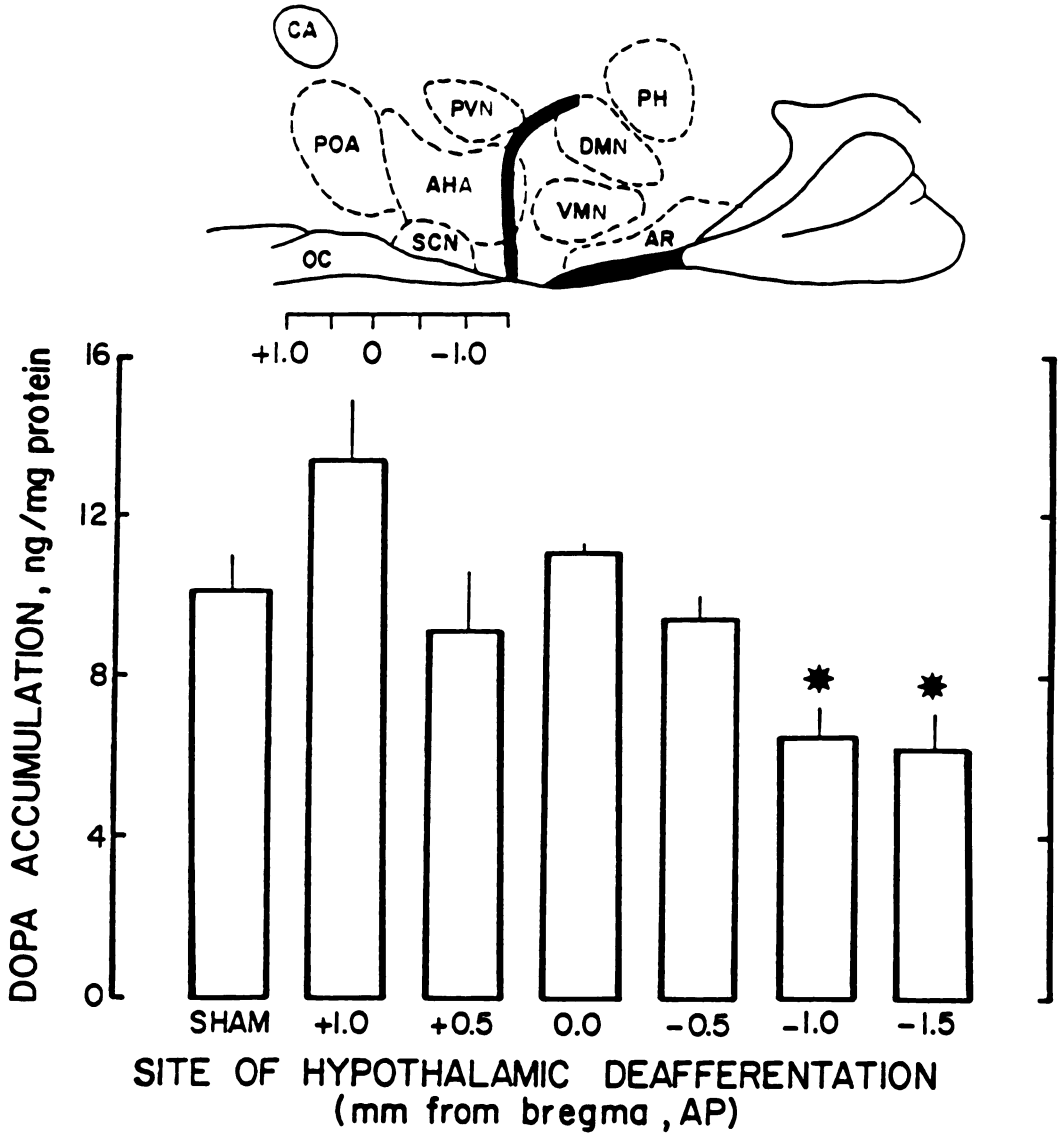

Figure 15. The effect of knife cuts made at the retrochiasmatic region (-1.5 mm to bregma) and at 0.5 mm increments rostral to this region on DOPA accumulation in the median eminence of female rats. Columns represent the means and the vertical lines 1 SE of 6-8 determinations. *Values significantly different from sham control rats, $p < 0.05$.

the basal rate of DOPA accumulation in the median eminence. Deafferentations made in more rostral regions (i.e., -0.5, 0.0, +0.5 and +1.0 mm from bregma), however, did not reduce basal TIDA neuronal activity. These results suggest that afferent input originating in or coursing through the caudal portion of the anterior hypothalamus mediates a stimulatory influence on the basal activity of TIDA neurons in the female rat.

A number of neurons from different anterior hypothalamic nuclei have axons which project laterally and caudally before converging at the lateral retrochiasmatic area and entering into the median eminence (Palkovits, 1982). Since the afferent input which stimulates TIDA neurons in the female rat could originate in these more rostral areas and project to the TIDA neurons via this lateral route, the effect of deafferentation of the lateral retrochiasmatic area on basal TIDA neuronal activity was examined in female rats (Figure 16). Retrochiasmatic deafferentation decreased the basal rate of DOPA accumulation in the median eminence while deafferentation of the lateral retrochiasmatic area had no effect. In agreement with results from the anterior hypothalamic deafferentation studies, these data provide evidence that the stimulatory afferent input to the TIDA neurons originates in the caudal portion of the anterior hypothalamus.

2. Effect of electrolytic lesions of anterior hypothalamic nuclei on basal tuberoinfundibular dopaminergic neuronal activity

To attempt to identify the origin of stimulatory afferent input to TIDA neurons in the female rat, the effect of electrolytic lesions of nuclei located in the caudal region of the anterior hypothalamus was examined. In these experiments, the effect of electrolytic lesions of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA) and paraventricular nucleus (PVN) on the rate of DOPA accumulation in the median eminence was examined (Figure 17). A bilateral electrolytic lesion of the SCN decreased the basal rate of DOPA

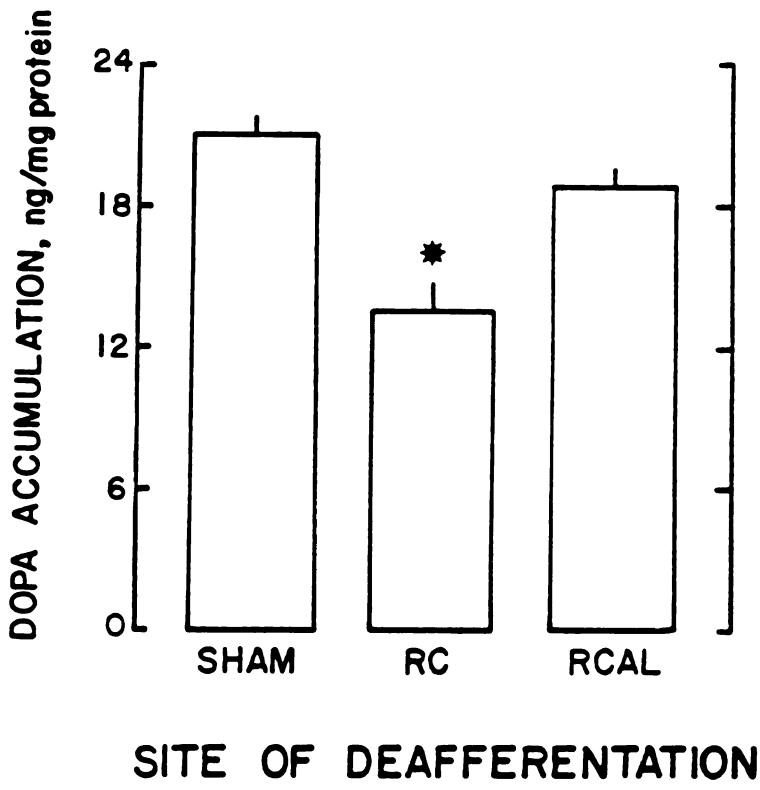

Figure 16. The effect of retrochiasmatic (RC) deafferentation and deafferentation of the lateral retrochiasmatic area (RCAL) on DOPA accumulation in the median eminence of female rats. Columns represent the means and the vertical lines 1 SE of 7-9 determinations. *Value significantly different from sham control rats, $p < 0.05$.

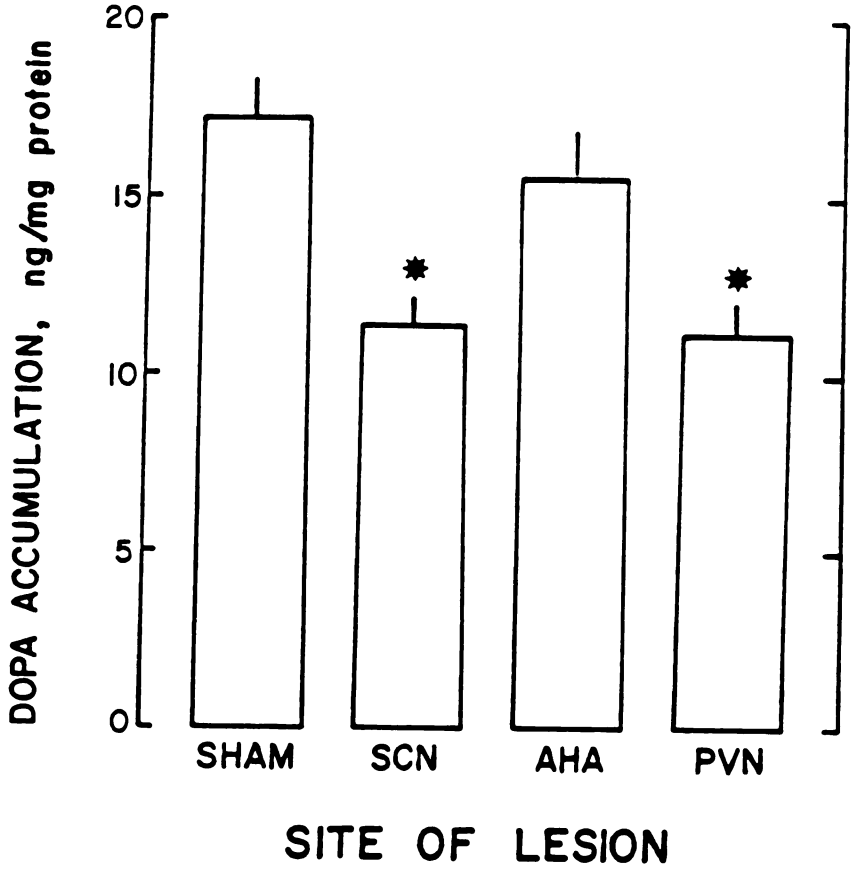

Figure 17. The effect of electrolytic lesions of the suprachiasmatic nucleus (SCN), anterior hypothalamic area (AHA) and paraventricular nucleus (PVN) on DOPA accumulation in the median eminence of female rats. Columns represent the means and the vertical lines $\pm$ 1 SE of 6-9 determinations. *Values significantly different from sham control rats, $p < 0.05$.

accumulation in the median eminence. Electrolytic ablation of the PVN produced a similar decrease in DOPA accumulation in the median eminence while lesioning the AHA had no effect . These results suggest that stimulatory afferent input to the TIDA neurons in the female rat originates in the SCN and/or PVN.

B. Afferent Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity in the Female Rat: Role of Prolactin

1. Effect of retrochiasmatic deafferentation on the delayed activation of tuberoinfundibular dopaminergic neurons by prolactin

TIDA neuronal activity is believed to be primarily regulated by the stimulatory action of prolactin (Moore and Demarest, 1982). This action of prolactin is divided into two components: a delayed "induction" component and a rapid "tonic" component (Demarest et al., 1984d, 1986). Since the deafferentation-induced decrease in TIDA neuronal activity may be the consequence of interrupting afferent input mediating either the delayed or rapid activation of TIDA neurons by prolactin, the effect of retrochiasmatic deafferentation on the ability of prolactin to stimulate TIDA neuronal activity was examined.

The effect of retrochiasmatic deafferentation on the delayed activation of TIDA neurons by prolactin was examined in female rats receiving an icv injection of rat prolactin (Figure 18). Retrochiasmatic deafferentation produced a characteristic decrease in the rate of DOPA accumulation in the median eminence. The administration of exogenous prolactin directly into the lateral ventricle increased median eminence DOPA accumulation in both sham-operated and retrochiasmatic-deafferentated rats. Although the overall magnitude of responsiveness of the TIDA neurons in the retrochiasmatic-deafferentated rats was reduced, the percent increase in median eminence DOPA accumulation was approximately the same for both groups. Similar results were observed when endogenous prolactin concentrations were elevated by haloperidol administration

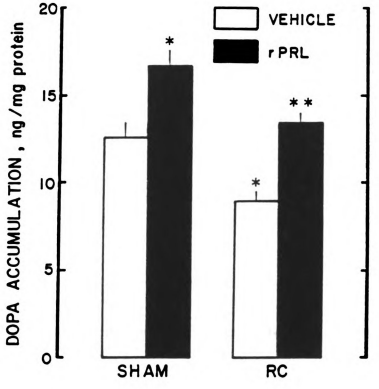

Figure 18. The effect of retrochiasmatic (RC) deafferentation on the prolactin-induced increase in DOPA accumulation in the median eminence of female rats. Rat prolactin (rPRL, 10 µg/3 µl) or its 0.9% saline vehicle was administered via an intracerebroventricular cannula 12 hours prior to sacrifice. Columns represent the means and the vertical lines 1 SE of 8-10 determinations. *Values significantly different from sham vehicle-treated rats; **value significantly different from RC vehicle-treated rats, $p < 0.05$.

(Figure 19). Haloperidol is a DA antagonist which blocks the tonic inhibitory action of DA on lactotrophs in the anterior pituitary gland and thereby increases secretion of prolactin. The increase in serum prolactin levels then stimulates TIDA neuronal activity (Demarest and Moore, 1980; Demarest et al., 1984d). Haloperidol produced a significant increase in the rate of DOPA accumulation in the median eminence of both sham-operated and retrochiasmatic-deafferentated female rats. Thus, although the overall magnitude of responsiveness of the TIDA neurons to the stimulatory actions of prolactin was decreased in the retrochiasmatic-deafferentated rats, the percent increase in TIDA neuronal activity to prolactin was not altered by the knife cut. Thus, retrochiasmatic deafferentation significantly decreases the basal rate of TIDA neuronal activity in female rats yet is ineffective in blocking the delayed prolactin-induced activation of these neurons.

2. Effect of retrochiasmatic deafferentation on the tonic activation of tuberoinfundibular dopaminergic neurons by prolactin

The effect of retrochiasmatic deafferentation on the rapid or "tonic" activation of TIDA neurons by prolactin was also determined. Following the administration of bromocriptine, a DA agonist, the concentration of prolactin in the serum is reduced, thereby removing the stimulatory influence of endogenous prolactin on TIDA neurons (Demarest et al., 1984d). As a consequence of lowered prolactin levels, the activity of TIDA neurons is decreased (Demarest et al., 1984d; 1985c). Bromocriptine administration significantly decreased the rate of DOPA accumulation in the median eminence of sham-operated female rats (Figure 20). Retrochiasmatic deafferentation also decreased the rate of DOPA accumulation in the median eminence and this was further reduced following bromocriptine administration. Prolactin reversed the bromocriptine-induced decrease in DOPA accumulation in the median eminence of both sham-operated rats and retrochiasmatic-deafferentated rats. Although the overall magnitude of

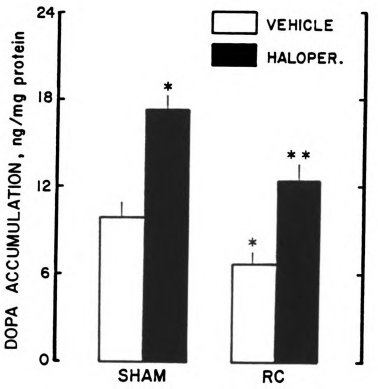

Figure 19. The effect of retrochiasmatic (RC) deafferentation on the haloperidol-induced increase in DOPA accumulation in the median eminence of female rats. Haloperidol (2.5 mg/kg, s.c.) or its 0.3% tartaric acid vehicle was administered 16 hours prior to sacrifice. Columns represent the means and the vertical lines 1 SE of 8 determinations. *Values significantly different from sham vehicle-treated rats; **value significantly different from RC vehicle-treated rats, $p < 0.05$.

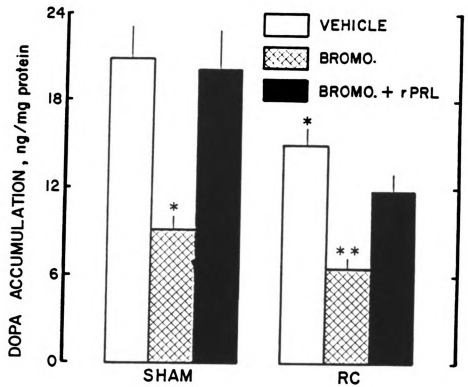

Figure 20. The effect of retrochiasmatic (RC) deafferentation on the bromocriptine-induced decrease and the prolactin-induced increase in DOPA accumulation in the median eminence of female rats. Bromocriptine (3 mg/kg, s.c.) or its 0.1% ascorbic acid vehicle was administered 24 hours prior to sacrifice. Rat prolactin (rPRL, 10 µg/3 µl) or its 0.9% saline vehicle was administered via an intracerebroventricular cannula 4 hours prior to sacrifice. Columns represent the means and the vertical lines 1 SE of 7-10 determinations. *Values significantly different from sham vehicle-treated rats; **value significantly different from RC vehicle-treated rats, $p < 0.05$.

the response of TIDA neurons to prolactin was decreased in retrochiasmatic-deafferentated rats prolactin produced similar percentage increases in the rate of DOPA accumulation in the median eminence of both groups. These results suggest that the rapid or "tonic" activation of TIDA neurons by prolactin is not mediated by rostral afferent input to the mediobasal hypothalamus.

3. Identification of prolactin binding sites in rat brain

The target site for the prolactin-induced activation of TIDA neurons appears to be contained in the mediobasal hypothalamus since both complete (Gudelsky et al., 1978) and retrochiasmatic (Barton et al., 1988) deafferentations of this region do not block the stimulatory action of prolactin on these neurons. Thus, prolactin may exert its effects on TIDA neurons via an action on specific receptors localized within the mediobasal hypothalamus. The present study was designed to identify the presence or absence of specific binding sites for prolactin in a discrete hypothalamic region(s) of rat brain following the in vitro labeling of these sites with ^{125}I-ovine prolactin. Autoradiographs of tissue sections incubated in 0.4 nM ^{125}I-ovine prolactin exhibited an intense reaction over the choroid plexus of the lateral ventricle (Figure 21-A). Incubation of an adjacent brain section with labeled prolactin in the presence of excess unlabeled hormone inhibited the binding reaction in the choroid plexus (Figure 21-B). More caudal tissue sections also showed a reaction for prolactin binding in the choroid plexus of the third ventricle and in the median eminence (Figure 22-A). Incubation of an adjacent brain section with excess unlabeled prolactin inhibited the binding reaction in the choroid plexus and significantly reduced the binding in the median eminence (Figure 22-B). A similar pattern of total and nonspecific binding was observed when tissue sections were incubated in 0.04 nM ^{125}I-ovine prolactin (data not shown).

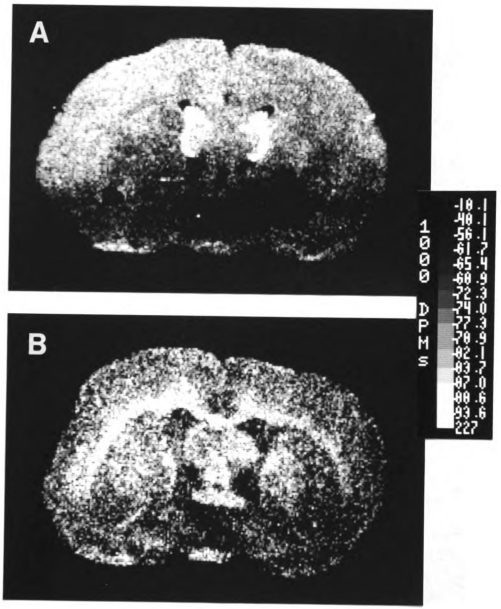

Figure 21. Autoradiographs of consecutive frontal sections of rat brain taken 0.3 mm posterior to bregma with respect to the atlas of Paxinos and Watson (1982). (A) represents total binding of ^{125}I-ovine prolactin, while (B) represents nonspecific binding of ^{125}I-ovine prolactin. The binding scale refers to Figures 21 and 22.

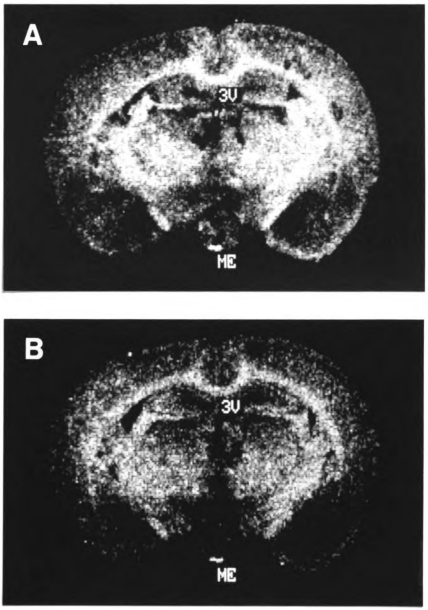

Figure 22. Autoradiographs of consecutive frontal sections of rat brain taken 2.8 mm posterior to bregma with respect to the atlas of Paxinos and Watson (1982). (A) represents total binding of ^{125}I-ovine prolactin, while (B) represents nonspecific binding of ^{125}I-ovine prolactin.

Specific binding of ovine prolactin was quantified in selected brain regions (Table 3) and significant binding was observed only in the choroid plexus of the lateral ventricle and in the median eminence. Although specific binding was present in the choroid plexus of the third ventricle it was not significant when compared to the other brain regions which demonstrated no specific binding for prolactin. These results suggest that prolactin may influence the activity of the TIDA neurons directly via a receptor-mediated interaction at the terminal region of these neurons in the median eminence.

C. Role of Afferent Neuronal Input in the Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity in the Male Rat

1. Effect of complete and retrochiasmatic deafferentation of the mediobasal hypothalamus on basal tuberoinfundibular dopaminergic neuronal activity

The basal rate of DA synthesis and turnover in the median eminence is several times greater in the female than in the male rat (Demarest et al., 1981a; Gunnet et al., 1986). Since the higher level of basal activity in the female may be propagated by stimulatory afferent input from the rostral hypothalamus it was important to determine whether a similar input regulates TIDA neuronal activity in the male. As demonstrated previously (Figure 10) complete and retrochiasmatic deafferentations of the mediobasal hypothalamus decreased the basal rate of DOPA accumulation in the median eminence of female rats (Figure 23). The basal rate of DOPA accumulation in the median eminence of sham-operated male rats was much less than that of sham-operated female rats and neither the complete nor the retrochiasmatic deafferentation reduced the rate of DOPA accumulation in the median eminence of male rats. Similarly, retrochiasmatic deafferentation reduced the basal rate of DA turnover in the median eminence of female rats but had no effect in male rats (Figure 24). Steady state concentrations of DA in the median eminence were not altered by retrochiasmatic deafferentation in either

TABLE 3

Specific Binding of ^{125}I Ovine Prolactin in Various Regions of the Female Rat Brain

Region	Average Sample Area (mm^2)	n	Specific Binding (fmol/mg protein)
Choroid plexus			
Lateral ventricle	0.10	6	11.8 ± 2.1*
Third ventricle	0.04	8	nd
Striatum	6.34	12	nd
Thalamus	5.17	18	nd
Hippocampus	3.05	10	nd
Amygdala	4.43	10	nd
Hypothalamus			
Median eminence	0.05	9	2.0 ± 0.2
Arcuate nucleus	0.28	8	nd
Ventromedial nucleus	0.30	6	nd
Dorsomedial nucleus	0.70	6	nd
Anterior hypothalamic area	0.94	7	nd
Paraventricular nucleus	0.70	5	nd

Values for specific binding represent the means of (n) determinations. Specific binding in the choroid plexus (lateral ventricle) and median eminence represent 43,018 dpm/mg protein and 7,312 dpm/mg protein, respectively, and represent 66% and 69% of the total binding. *, depicts significant specific binding as compared to binding in all brain regions sampled. nd, statistically significant specific binding was not detectable.

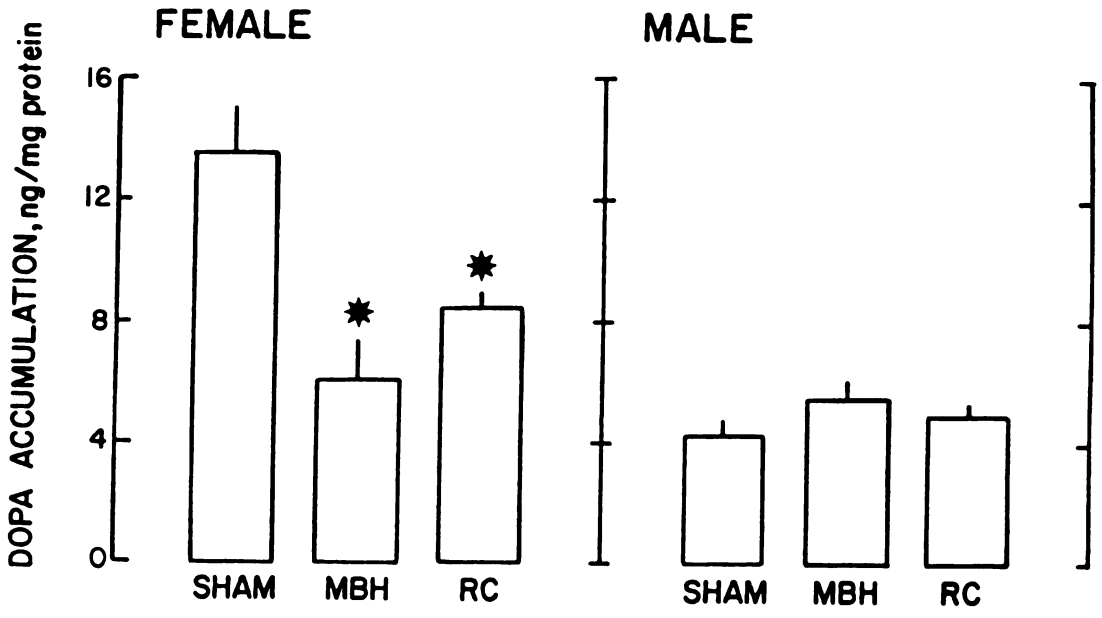

Figure 23. The effect of complete (MBH) and retrochiasmatic (RC) deafferentation of the mediobasal hypothalamus on DOPA accumulation in the median eminence of female and male rats. Columns represent the means and the vertical lines 1 SE of 6-8 determinations. *Values significantly different from respective sham control rats, $p < 0.05$.

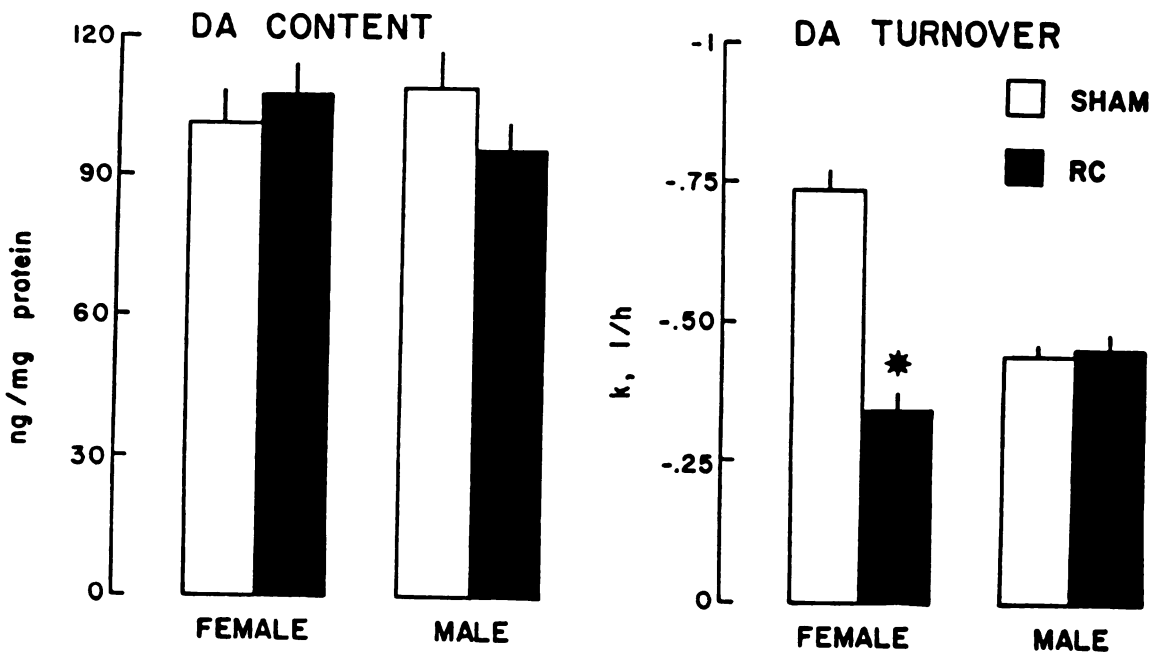

Figure 24. The effect of retrochiasmatic (RC) deafferentation on DA content and turnover in the median eminence of female and male rats. Columns illustrating DA content represent the means and the vertical lines 1 SE of 8 determinations, while those illustrating DA turnover represent the means and the vertical lines 1 SE of the rate constant calculated from the decline in DA concentrations determined in 8 animals decapitated 0, 30 and 60 minutes after αMT (a total of 24 determinations). *Value significantly different from respective sham control rats, $p < 0.05$.

female or male rats. In addition, steady state concentrations and the turnover of NE in the median eminence was not altered by retrochiasmatic deafferentation in the male rat (Table 4). These results suggest that regions rostral to the retrochiasmatic deafferentation exert a stimulatory influence on TIDA neurons in the female but not the male rat.

2. Effect of retrochiasmatic deafferentation on the prolactin-induced activation of tuberoinfundibular dopaminergic neurons.

Consistent with previous experiments, retrochiasmatic deafferentation decreased the rate of DOPA accumulation in the median eminence of female but not male rats (Figure 25). An icv injection of rat prolactin increased the rate of DOPA accumulation in the median eminence of both sham-operated and retrochiasmatic-deafferentated female and male rats. These results indicate that the ability of prolactin to stimulate TIDA neuronal activity is not blocked by retrochiasmatic deafferentation in either male or female rats. Thus, the deafferentation-induced decrease in basal TIDA neuronal activity in female rats appears to be the consequence of removing afferent input to these neurons which is independent of the action of prolactin.

3. Effect of retrochiasmatic deafferentation on tuberoinfundibular dopaminergic neuronal activity in castrated female and male rats

Gender differences in TIDA neuronal activity result, in part, from differences in the hormonal environment of the adult rat. In particular, estrogen has a stimulatory influence (Fuxe et al., 1969a; Eikenburg et al., 1977; Demarest and Moore, 1980) while testosterone has an inhibitory influence (Brawer et al., 1986; Nakahara et al., 1976) on TIDA neuronal activity. Since differences in the actions of the gonadal steroids on TIDA neurons may be responsible for the ability to observe a retrochiasmatic deafferentation-induced decrease in TIDA neuronal activity in female but not male rats, the effect of deafferentation on gonadally-intact and castrated female and male rats was examined.

TABLE 4

The Effect of Retrochiasmatic Deafferentation (RC) on NE Content and Turnover in the Median Eminence of Male Rats

	Steady State Concentration ng/mg protein	Rate Constant. k h^{-1}
Sham	15.6 ± 1.0	0.11 ± 0.01
RC	12.5 ± 0.6	0.17 ± 0.01

Steady state concentrations represent the means ± 1 SE of 8 determinations. The rate constants, an index of NE turnover, represent the means ± 1 SE calculated from the decline in NE concentrations determined in 8 animals decapitated 0, 30 and 60 minutes after αMT (a total of 24 determinations).

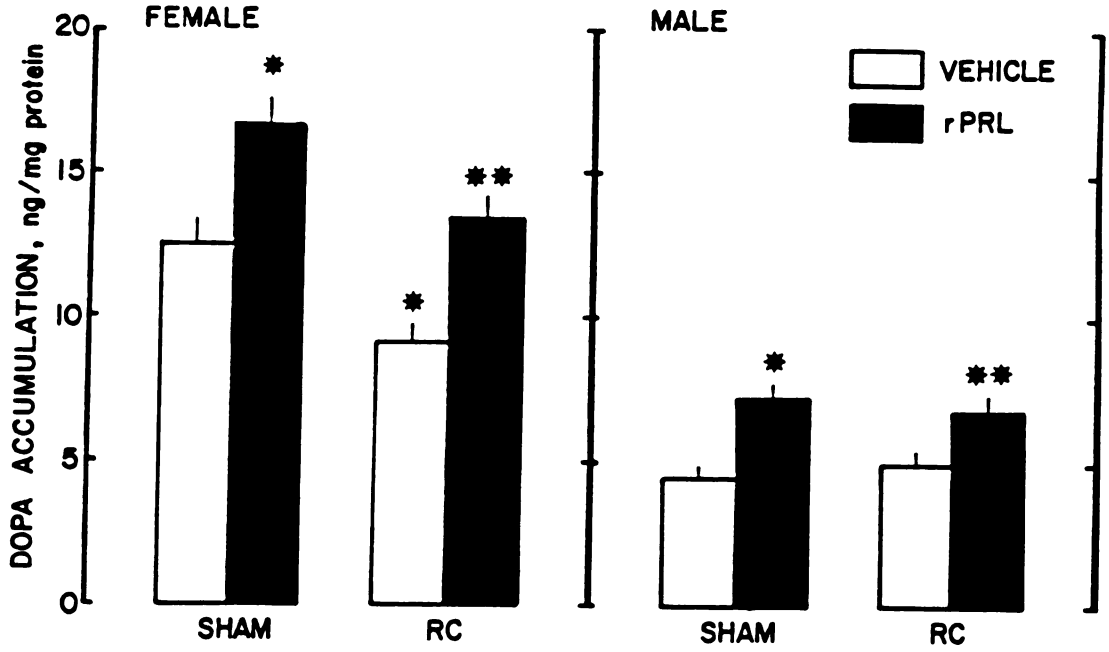

Figure 25. The effect of retrochiasmatic (RC) deafferentation on the prolactin-induced increase in DOPA accumulation in the median eminence of female and male rats. Rat prolactin (rPRL, 10 µg/3 µl) or its 0.9% saline vehicle was administered via an intracerebroventricular cannula 12 hours prior to sacrifice. Columns represent the means and the vertical lines 1 SE of 7-10 determinations. *Values significantly different from respective sham vehicle-treated rats; **values significantly different from respective RC vehicle-treated rats, $p < 0.05$.

Both ovariectomy and retrochiasmatic deafferentation decreased the rate of DOPA accumulation in the median eminence of female rats (Figure 26). Retrochiasmatic deafferentation, however, did not produce a further decrease in the rate of DOPA accumulation in the median eminence of ovariectomized rats suggesting that retrochiasmatic deafferentation and ovariectomy may decrease TIDA neuronal activity via a common neuronal pathway. This common pathway may involve a direct stimulatory action of estrogen at the level of the anterior hypothalamus.

The effect of retrochiasmatic deafferentation on the rate of DOPA accumulation in the median eminence of intact and orchidectomized male rats is shown in Figure 27. Orchidectomy increased the rate of DOPA accumulation in the median eminence of male rats; retrochiasmatic deafferentation, however, did not alter the rate of DOPA accumulation in either intact or orchidectomized male rats. Thus, the inhibitory influence of testosterone in the adult male does not appear to be responsible for the failure to observe a deafferentation-induced decrease in TIDA neuronal activity, suggesting that the afferent input which stimulates TIDA neurons in the female rat may not be present and/or operational in the male rat.

D. Inhibitory Afferent Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity in the Female Rat

1. Effect of complete deafferentation of the mediobasal hypothalamus on the stress-induced changes in tuberoinfundibular dopaminergic neuronal activity and pituitary prolactin secretion

TIDA neuronal activity is regulated, in part, by inhibitory afferents which impinge on these neurons to decrease their activity during stress. This decrease may facilitate the stress-induced increase in prolactin secretion. The origin of inhibitory afferents to the TIDA neurons was investigated by examining the effect of complete deafferentation of the mediobasal hypothalamus on the

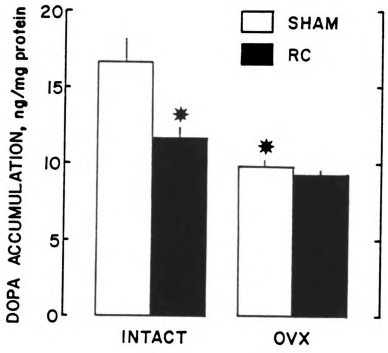

Figure 26. The effect of retrochiasmatic (RC) deafferentation on DOPA accumulation in the median eminence of intact and ovariectomized (OVX) female rats. Columns represent the means and the vertical lines 1 SE of 7-10 determinations. *Values significantly different from intact sham control rats, $p < 0.05$.

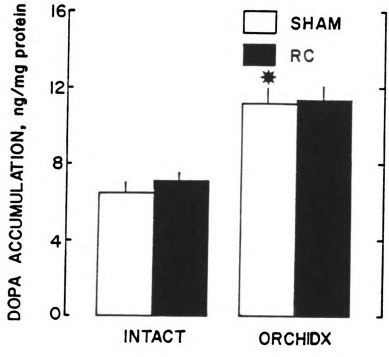

Figure 27. The effect of retrochiasmatic deafferentation on DOPA accumulation in the median eminence of intact and orchidectomized (ORCHIDX) male rats. Columns represent the means and the vertical lines 1 SE of 6-9 determinations. *Values significantly different from intact sham control rats, $p < 0.05$.

stress-induced decrease in TIDA neuronal activity and increase in plasma prolactin. As demonstrated previously (Demarest et al., 1985a), acute restraint stress decreased DOPA accumulation in the median eminence of sham-operated rats but failed to alter DOPA accumulation in animals which had received complete deafferentation (Figure 28). Furthermore, restraint stress decreased DOPAC concentrations in the median eminence of sham-operated rats, but failed to produce a decrease in DOPAC concentrations in the median eminence of rats receiving complete deafferentation (Figure 29). These results suggest that complete deafferentation of the mediobasal hypothalamus blocks the stress-induced decrease in TIDA neuronal activity.

In addition to the decrease in TIDA neuronal activity, restraint stress increased circulating prolactin concentrations (Table 5). Complete deafferentation of the mediobasal hypothalamus had no effect on basal prolactin concentrations in sham-operated rats, but this procedure attenuated the rise in prolactin due to restraint stress. These results suggest that complete deafferentation of the mediobasal hypothalamus may blunt the stress-induced increase in prolactin by blocking the stress-induced decrease in TIDA neuronal activity. In addition, complete deafferentation may interrupt neurons which contain prolactin-releasing factors whose increased activity may stimulate prolactin release during stress.

2. Effect of retrochiasmatic deafferentation on the stress-induced changes in tuberoinfundibular dopaminergic neuronal activity and pituitary prolactin secretion

The prolactin response to various stressful stimuli is attenuated by interruption of neural connections originating rostral to the mediobasal hypothalamus (Kawakami and Higuchi, 1981; Mioduszewski and Critchlow, 1981; Kaler et al., 1984). Thus, inhibitory afferent input originating rostral to the mediobasal hypothalamus may project to and decrease TIDA neuronal activity and increase prolactin secretion in response to stress. As such, the ability of retrochiasmatic

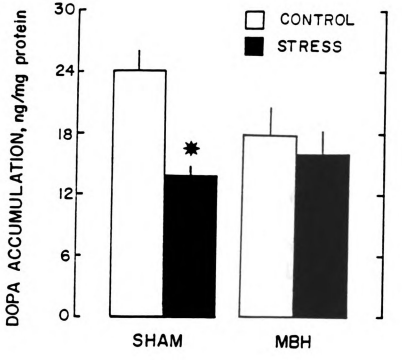

Figure 28. The effect of complete (MBH) deafferentation of the mediobasal hypothalamus on the stress-induced decrease in DOPA accumulation in the median eminence of female rats. Columns represent the means and the vertical lines 1 SE of 5-8 determinations. *Value significantly different from sham control rats, $p < 0.05$.

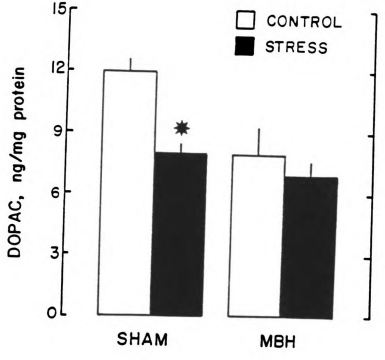

Figure 29. The effect of complete (MBH) deafferentation of the mediobasal hypothalamus on the stress-induced decrease in DOPAC concentrations in the median eminence of female rats. Columns represent the means and the vertical lines 1 SE of 4-8 determinations. *Value significantly different from sham control rats, $p < 0.05$.

TABLE 5

The Effect of Complete Deafferentation of the Mediobasal Hypothalamus (MBH) on the Stress-Induced Increase in Plasma Prolactin Concentrations

	ng/ml	
	Control	Stress
Sham	2.8 ± 0.5	107 ± 10.5*
MBH	5.9 ± 1.2	28 ± 7.1**

Values represent the means ± 1 SE of 5-8 determinations. *Value significantly different from sham control rats, **value significantly different from RC control rats, $p < 0.05$.

deafferentation to block the stress-induced decrease in TIDA neuronal activity and increase in prolactin was examined. Restraint stress and retrochiasmatic deafferentation decreased the rate of DOPA accumulation in the median eminence of female rats (Figure 30). Restraint stress did not decrease DOPA accumulation in the median eminence of retrochiasmatic-deafferentated rats. As demonstrated previously (Figure 13), retrochiasmatic deafferentation did not alter basal prolactin concentrations, however, deafferentation attenuated the stress-induced increase in prolactin (Table 6). These results suggest that the neuronal mechanism(s) which decreases TIDA neuronal activity and increases prolactin secretion during stress originates rostral to the mediobasal hypothalamus.

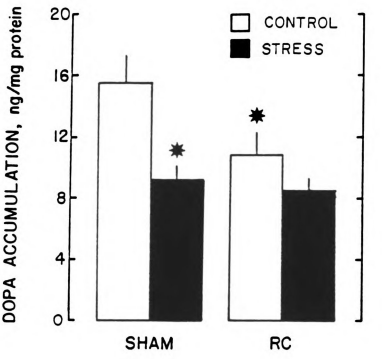

Figure 30. The effect of retrochiasmatic (RC) deafferentation on the stress-induced decrease in DOPA accumulation in the median eminence of female rats. Columns represent the means and the vertical lines 1 SE of 8-10 determinations. *Values significantly different from sham control rats, $p < 0.05$.

TABLE 6

The Effect of Retrochiasmatic Deafferentation (RC) on the Stress-Induced Increase in Plasma Prolactin Concentrations

	ng/ml	
	Control	Stress
Sham	0.9 ± 0.1	73.1 ± 23.1*
RC	6.3 ± 2.0	21.6 ± 6.3

Values represent the means ± 1 SE of 7-10 determinations.
*Value significantly different from sham control rats, $p < 0.05$.

DISCUSSION

A. Afferent Neuronal Regulation of Basal Tuberoinfundibular Dopaminergic Neuronal Activity in the Female Rat: Anterior Hypothalamic Origin

Results of the present studies demonstrate that afferent neurons originating rostral to the mediobasal hypothalamus provide stimulatory input to TIDA neurons in the gonadally-intact female rat. Retrochiasmatic deafferentation, a procedure which interrupts afferent connections from the anterior hypothalamus to the mediobasal hypothalamus, reduced basal neuronal activity of the TIDA neurons in the female rat. This was demonstrated by a decrease in DA synthesis, turnover and metabolism in the terminal region of the TIDA neurons in the median eminence. The deafferentation-induced decrease is specific for the TIDA neurons since the tuberohypophysial DA neurons, which are related anatomically to the TIDA neurons (Moore and Demarest, 1982), are not influenced by the knife cuts.

A state of constant estrus is observed in animals receiving retrochiasmatic deafferentation (Halasz and Gorski, 1967; Brownstein et al., 1976a). A similar state is observed in rats chronically treated with estrogen (Demarest et al., 1984b). Both retrochiasmatic deafferentation and long-term (18 days) estrogen treatment decrease basal TIDA neuronal activity and reduce the effectiveness of the prolactin feedback mechanism in the female rat (Demarest et al., 1984b; Barton et al., 1988). Estrogen-induced changes in TIDA neuronal activity are biphasic; short-term (6 days) treatment with estradiol increases TIDA neuronal activity while long term (18 days) treatment decreases the activity of these neurons (Demarest et al., 1984b). The deafferentation-induced decrease in TIDA

neuronal activity is observed 3 days after deafferentation at a time when estrogen would be expected to have a stimulatory influence on TIDA neurons. Thus, it is unlikely that the elevated estrogen levels during the state of continuous estrus induced by retrochiasmatic deafferentation is responsible for the deafferentation-induced decrease in TIDA neuronal activity.

The decrease in TIDA neuronal activity and the interruption of cyclicity following retrochiasmatic deafferentation are probably not the result of direct insult to the TIDA neurons or of perturbations in the hypophysial vascular supply. Neither the complete nor the retrochiasmatic deafferentation altered TIDA neuronal activity in male rats. Furthermore, the decrease in TIDA neuronal activity following complete (Weiner et al., 1972; Gudelsky et al., 1978) and retrochiasmatic (Barton et al., 1988) deafferentation in the female rat is not associated with an alteration in DA content in the median eminence suggesting that there is no loss of TIDA neurons. In addition, the deafferentation-induced decrease in TIDA neuronal activity is observed at 3 and up to 21 days after deafferentation, at a time when blood flow to the hypothalamus/anterior pituitary is maintained at normal levels (Nikitovitch-Winer and Goldman, 1986).

The DA which is released from TIDA neurons inhibits the secretion of prolactin from the anterior pituitary (MacLeod, 1976; Ben-Jonathan et al., 1977). Despite the decrease in TIDA neuronal activity at 3 and up to 21 days after retrochiasmatic deafferentation, serum prolactin concentrations remained unaltered. Although Caligaris and Taleisnik (1976) reported a significant elevation in circulating prolactin concentrations, several investigators observed no change in circulating levels of this hormone following retrochiasmatic deafferentation (Siegel et al., 1979; Siegel et al., 1980; Mioduszewski and Critchlow, 1981). Not only does the deafferentation-induced decrease in TIDA neuronal activity fail to elevate circulating prolactin concentrations, but destruction of half of these

neurons also produces no change in serum prolactin concentrations (Demarest et al., 1980; Fenske and Wuttke, 1976). Pretreatment with 6-hydroxydopamine produces a 50% decline in DA content in the median eminence yet no alteration in circulating prolactin. Thus, a reserve of DA at the anterior pituitary may compensate for the decrease in TIDA neuronal activity after retrochiasmatic deafferentation as well as for the partial destruction of TIDA neurons following 6-hydroxydopamine treatment. On the other hand, retrochiasmatic deafferentation may not only interfere with the regulation of basal TIDA neuronal function, but may also interrupt neurons containing prolactin-releasing factors. A number of such neurons have cell bodies in the anterior hypothalamus with neuronal projections to the median eminence (for review, see Shin et al., 1987). Thus, the deafferentation-induced decrease in TIDA neuronal activity may be counterbalanced by a decrease in the activity of neurons which contain prolactin-releasing factors to result in no net change in circulating prolactin concentrations. In addition, DA released from the posterior pituitary has been reported to inhibit prolactin release (Peters et al., 1981; Ben-Jonathan and Peters, 1982). An enhanced activity of the tuberohypophysial DA neurons, however, does not appear to compensate for the decreased TIDA neuronal activity since neither complete nor retrochiasmatic deafferentation altered DA synthesis in the terminal region of the tuberohypophysial DA neurons.

Several studies were done to determine the origin of stimulatory afferent input to the TIDA neurons in the female rat. DA synthesis in the median eminence was decreased following retrochiasmatic deafferentation and in animals receiving deafferentations 0.5 mm rostral to the retrochiasmatic region. Placement of the deafferentation more rostral into the anterior hypothalamus and bilateral deafferentation of the lateral retrochiasmatic area did not alter the basal activity of TIDA neurons. Thus, afferent input originating in or coursing

through the caudal region of the anterior hypothalamus mediates a stimulatory influence on TIDA neurons in the intact female rat. The caudal portion of the anterior hypothalamus contains 3 distinct areas, the SCN, AHA and PVN. Bilateral electrolytic lesions of the SCN and PVN decreased the basal rate of DA synthesis in the median eminence, while bilateral lesions of the AHA had no effect. The afferent neuronal input which stimulates TIDA neurons in the female rat may, therefore, originate in the SCN and PVN or in either the SCN or PVN and course through the other on route to the arcuate nucleus/median eminence. This latter route is most likely the one taken by stimulatory afferent neurons which project to the TIDA neurons. Electrolytic lesions of the SCN and PVN each produced a 30% to 40% decrease in TIDA neuronal activity. If unique afferent projections to the TIDA neurons originated in both the SCN and PVN, deafferentation of this input would be expected to produce a 60% decrease in TIDA neuronal activity. Retrochiasmatic deafferentation generally produced a 30 to 40% decrease in TIDA neuronal activity suggesting that stimulatory afferent input to the TIDA neurons in the female rat originates in either the SCN or PVN. Anatomical evidence supports reciprocal connections between the SCN and PVN (Berk and Finkelstein, 1981; Silverman et al., 1981; Watts et al., 1987; Watts and Swanson, 1987), as well as from the SCN and PVN to the arcuate nucleus/median eminence complex (Swanson and Cowman, 1975; Swanson and Sawchenko 1980; Weigand and Price, 1980; Stephan et al., 1981).

Several neuropeptides are possible candidates for mediating the stimulatory afferent neuronal regulation of TIDA neurons in the female rat. Atrial natriuretic factor in the median eminence is contained in neurons which originate in the PVN (Palkovits et al., 1987). It has been suggested that the prolactin-inhibitory effect of atrial natriuretic factor is mediated by a dopaminergic mechanism (Samson et al., 1988). Similarly, vasopressin, which is of known PVN origin, and neurotensin,

which is widely distributed throughout the CNS (Lazarus et al., 1977) presumably stimulate DA release from TIDA neurons and thereby inhibit prolactin secretion (Tojo et al., 1986; DePaolo et al., 1986). The role of these neuropeptides and the more classical neurotransmitters in mediating the stimulatory afferent neuronal regulation of TIDA neurons remains to be elucidated.

B. Stimulatory Afferent Input Does Not Mediate the Action of Prolactin on Tuberoinfundibular Dopaminergic Neurons

1. Delayed and tonic activation of tuberoinfundibular dopaminergic neurons in the absence of afferent input from the anterior hypothalamus

TIDA neuronal activity is believed to be primarily regulated by the positive feedback action of prolactin (Moore and Demarest, 1982). Increases in circulating concentrations of prolactin stimulate the synthesis and turnover of DA in the median eminence (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976; Selmanoff, 1981) while decreases in prolactin are associated with a decline in these indices of TIDA neuronal activity (Demarest and Moore, 1981a; Demarest et al., 1985c). The stimulatory action of prolactin on TIDA neurons is divided into two interrelated components: a rapid "tonic" component and a delayed "induction" component (Demarest et al., 1984d; 1986). The rapid component mediates relatively short-term changes in TIDA neuronal activity in response to acute increases or decreases in circulating prolactin concentrations. This rapid component can be demonstrated as early as 4 hours when one examines the effect of prolactin on TIDA neuronal activity in a bromocriptine-treated rat. The delayed component is activated by long-term changes in prolactin and has been demonstrated by a variety of in vivo techniques where prolactin increased DA synthesis and turnover in the terminal region of the TIDA neurons after a delay of 12 to 16 hours (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976; Johnston et al., 1980).

Results of the present studies indicate that the retrochiasmatic deafferentation-induced decrease in TIDA neuronal activity is not the consequence of interrupting afferent input mediating either the rapid or delayed components of prolactin's actions. Despite the decrease in basal TIDA neuronal activity following retrochiasmatic deafferentation, manipulations which increase prolactin levels increase activity of the TIDA neurons, while those which decrease prolactin levels are associated with a further decrease in TIDA neuronal activity in the deafferentated female rat. For example, 12 to 16 hours after a single injection of either prolactin or haloperidol respectively, DA synthesis in the median eminence was increased by approximately the same relative amount in both sham-operated and retrochiasmatic-deafferentated rats. In addition, removing the tonic stimulatory influence of endogenous prolactin on TIDA neurons by pretreating the animals with bromocriptine, decreased median eminence DA synthesis in sham-operated and retrochiasmatic-deafferentated rats. This decrease was reversed by prolactin administration, which produced similar percentage increases in the rate of DA synthesis in the median eminence of both groups. These results demonstrate that both the delayed and rapid activation of TIDA neurons by prolactin is still operating when afferent input from areas rostral to the mediobasal hypothalamus is removed.

Although prolactin increases TIDA neuronal activity proportionally in retrochiasmatic-deafferentated and sham-operated rats, TIDA neurons in animals receiving retrochiasmatic deafferentation cannot be stimulated to the same absolute magnitude. This reduced magnitude of response in deafferentated rats resembles that observed in an aged rat (Demarest et al., 1987). Aging produces a similar decrease in basal TIDA neuronal function as does retrochiasmatic deafferentation and, although these neurons in the aged rat are still responsive to changes in prolactin, they cannot be stimulated to the same absolute magnitude.

The aging-induced decrease in basal TIDA neuronal function is associated with a decrease in DA content (Demarest et al., 1980; 1982; Estes and Simpkins, 1980) presumably the result of a decrease in the number of viable terminals of TIDA neurons (Hoffman and Sladek, 1980; Selemon and Sladek, 1981). Retrochiasmatic deafferentation is not associated with a decrease in DA content suggesting that the TIDA terminals are intact. Rather, the deafferentation-induced decrease in TIDA neuronal function is apparently the result of a decrease in stimulatory input to the TIDA neurons.

2. Site of action of prolactin

Deafferentation studies (Gudelsky et al., 1978; Barton et al., 1988) provide evidence to suggest that the target site for the prolactin-induced activation of TIDA neurons is contained in the mediobasal hypothalamus. This is consistent with the identification of specific binding sites for prolactin in the median eminence of the female rat brain. Autoradiographic reactions were observed in both the median eminence and choroid plexus after labeling with ^{125}I-ovine prolactin and the intensity of the reactions was significantly reduced after coincubation with excess unlabeled ovine prolactin.

The demonstration of prolactin binding sites in the choroid plexus is in agreement with previous reports (Walsh et al., 1978; 1984; 1987; Dube et al., 1980). Prolactin receptors on choroid plexus appear to be present on epithelial cells (Walsh et al., 1978; 1984) where they function to transport prolactin from the circulation to the cerebrospinal fluid (CSF; Walsh et al., 1984; 1987). It has been suggested that a prerequisite step for all prolactin-brain interactions lies in the receptor-mediated uptake and transport of prolactin from the vasculature to CSF via the choroid plexus. For example, prolactin in the CSF can increase the activity of the TIDA neurons in the mediobasal hypothalamus (Annunziato and Moore, 1978). In turn, the dopaminergic neurons of the TIDA system inhibit

prolactin secretion from the anterior pituitary (MacLeod, 1976; Gudelsky and Porter, 1980; 1981). The autoregulation of prolactin secretion via TIDA neuronal activation may be mediated by prolactin in the CSF (Nicholson et al., 1980). Increases in prolactin in the plasma produce a delayed elevation of CSF prolactin (Belchetz et al., 1982; Kalin et al., 1982). Thus, the increase in CSF prolactin may be responsible, in part, for the delayed activation of TIDA neurons by prolactin (Hökfelt and Fuxe 1972; Gudelsky et al., 1976). On the other hand, the median eminence is outside the blood-brain barrier providing free access of this region to prolactin in the circulation. In fact, prolactin is in higher concentrations in the hypophysial portal vasculature than in the systemic circulation and this is due, in part, to retrograde flow of prolactin from the pituitary to the hypothalamus (Oliver et al., 1977; Mezey and Palkovits, 1982). Nevertheless, the exact mechanism by which prolactin activates the TIDA neurons has yet to be elucidated.

The present results suggest that prolactin influences the activity of the TIDA neurons directly via a receptor-mediated interaction at the terminal region of the TIDA neurons in the median eminence. VanHouten and coworkers (1980) identified receptors in the rat median eminence which they termed "lactogen receptors" since they were identified using ^{125}I-human growth hormone as the ligand, and antagonized by unlabeled human growth hormone and ovine prolactin. Binding of human growth hormone was found in both the external and internal layers of the median eminence and unlabeled hormone inhibited binding in the external layer but not in the internal layer. The findings in the present studies are consistent with this observation in that incubation with unlabeled prolactin did not prevent but only reduced ^{125}I-prolactin binding in the median eminence. The binding signal for prolactin which persists in the presence of excess unlabeled hormone may represent nonspecific binding of prolactin to the

internal zone of the median eminence at the arcuate nucleus border. The specific binding for prolactin may, therefore, represent binding to receptor sites in the external zone of the median eminence, a region known to contain projections of the TIDA system (Björklund et al., 1973b). Together with the observation that prolactin can augment dopamine release from median eminence synaptosomes (Gregerson and Selmanoff, 1988), these results suggest the possibility of a direct interaction of prolactin on the terminals of the TIDA neurons.

Previous attempts to identify prolactin binding sites in rat hypothalami using ^{125}I-prolactin as the radiolabeled ligand have been unsuccessful. Traditional in vitro competitive binding assays (DiCarlo et al., 1983), in vivo autoradiographic analysis of whole hypothalami (Dube et al., 1980) and in vivo autoradiographic analysis of the median eminence (Walsh et al., 1978; 1987) failed to demonstrate significant specific binding for prolactin. The difficulty in demonstrating specific prolactin binding sites in earlier in vitro procedures is most likely due to a low concentration of receptors in the rat hypothalamus, as well as to their discrete localization in the median eminence. Autoradiographic reactions were observed in rat median eminence both 5 minutes (Walsh et al., 1978) and 1 hour (Walsh et al., 1987) after the in vivo administration of ^{125}I-ovine prolactin. Unfortunately, the intensity of nonspecific binding was so high that it may have obscured identification of specific prolactin binding sites in this region. In addition, the total binding of prolactin in the median eminence was low suggesting that prolactin was not reaching its site of action. The rapid metabolism of prolactin (Koch et al., 1971) may have contributed to this finding or perhaps a longer time after the in vivo administration of prolactin is required for the transport of prolactin into the CSF for subsequent interaction of this hormone at binding sites in the median eminence. The potential requirement for prolactin transport into CSF is not a problem in the present in vitro analysis and

the concentration of labeled prolactin in the incubation solution, as well as the incubation parameters, were optimized in the present study to reduce the amount of nonspecific binding.

C. Sexual Difference in the Afferent Neuronal Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity: Influence of the Gonadal Steroids

Afferent neuronal input originating rostral to the mediobasal hypothalamus stimulates TIDA neurons in gonadally-intact female but not male rats. That is, retrochiasmatic deafferentation, a procedure which interrupts neuronal connections from regions rostral to the mediobasal hypothalamus, reduced DA synthesis and turnover in the terminal region of the TIDA neurons in the median eminence of intact female but not male rats.

TIDA neurons appear to be primarily regulated by prolactin and, in the female rat, these neurons are more sensitive to the stimulatory actions of this hormone (Demarest and Moore, 1981a). Thus, an increased sensitivity to and a continuous activation by prolactin has been suggested to be the basis for the higher level of basal activity of TIDA neurons in the female rat. Results of the present studies demonstrate that a prolactin-independent afferent neuronal input may also play a role in propagating the sexual difference in TIDA neuronal activity since retrochiasmatic deafferentation decreases the basal activity of TIDA neurons only in the intact female rat.

Results of the present studies suggest that the hormonal environment may play a role in the retrochiasmatic deafferentation-induced decrease in TIDA neuronal activity in intact female rats. For example, ovariectomy decreased the rate of DA synthesis in the median eminence of female rats and retrochiasmatic deafferentation did not produce a further decrease. The decrease in TIDA neuronal activity following ovariectomy may be due to a removal of the

stimulatory action of prolactin on the TIDA system or to removal of the stimulatory action of estrogen at some region(s) in the hypothalamus. The DA agonist, bromocriptine, decreases TIDA neuronal activity by reducing the stimulatory action of prolactin on TIDA neurons (Demarest and Moore, 1981a), and this decrease is further reduced following retrochiasmatic deafferentation. Thus, bromocriptine and retrochiasmatic deafferentation together exert an additive effect in decreasing TIDA neuronal activity. Bromocriptine reduces circulating levels of prolactin thereby reducing the stimulatory action of this hormone on TIDA neurons while retrochiasmatic deafferentation removes stimulatory afferent input to the TIDA neurons which is not mediated by prolactin. Since ovariectomy and retrochiasmatic deafferentation do not exert additive effects in decreasing TIDA neuronal activity they may decrease the activity of these neurons via a similar mechanism. This mechanism may involve a direct stimulatory action of estrogen at the level of the anterior hypothalamus. In fact, a moderate to high density of estrogen receptors have been identified in the PVN and SCN/preoptic nucleus of the female rat (Rainbow et al., 1982).

The inability to observe a retrochiasmatic deafferentation-induced decrease in TIDA neuronal activity does not appear to be influenced by the hormonal environment of the adult male rat. The TIDA neurons in the male rat have a lower basal rate of activity. Following castration, DA synthesis in the median eminence of male rats was increased to a level similar to that observed in an intact female. In the castrated male, however, retrochiasmatic deafferentation did not decrease DA synthesis in the median eminence. Thus, the inhibitory influence of testosterone in the adult male rat is not responsible for the failure to observe a deafferentation-induced decrease in TIDA neuronal activity. Retrochiasmatic deafferentation removes stimulatory input to the TIDA neurons in the intact female rat which is either not present or not functional in the male rat.

The absence of stimulatory afferent input to TIDA neurons in the male rat may be due to sexual differentiation resulting from neonatal androgen exposure. For example, male rats castrated 12 hours after birth display a level of TIDA neuronal activity similar to that of an adult intact female (Demarest et al., 1981). Conversely, females receiving testosterone on day 5 after birth display a male profile of TIDA neuronal activity in adulthood. Thus, neonatal androgen exposure may result in the sexual differentiation of stimulatory afferent neuronal input to the TIDA neurons which may then result in a higher (female) or a lower (male) rate of neuronal activity.

D. Inhibitory Afferent Neuronal Regulation of Tuberoinfundibular Dopaminergic Neuronal Activity: Anterior Hypothalamic Origin

Inhibitory afferent neuronal input originating rostral to the mediobasal hypothalamus appears to mediate the stress-induced decrease in TIDA neuronal activity and increase in plasma prolactin concentrations in the female rat. Restraint stress decreased DA synthesis and metabolism in the median eminence of sham-operated female rats yet failed to decrease DA synthesis and/or metabolism in the median eminence of rats which had received either complete or retrochiasmatic deafferentation of the mediobasal hypothalamus. In addition, both deafferentation procedures markedly attenuated the stress-induced elevation in plasma prolactin.

Although the origin of inhibitory afferent input to the TIDA neurons has yet to be identified, several studies have been done to examine the role of neural connections to the mediobasal hypothalamus in mediating the prolactin response to stress. The increase in circulating prolactin in response to ether stress (Krulich et al., 1975), heat stress (Siegel et al., 1979) and 10 minutes of leg restraint (Mioduszewski and Critchlow, 1981) was blocked by complete deafferentation and

attenuated by retrochiasmatic deafferentation. In the present study, the prolactin response to stress was attenuated by both complete and retrochiasmatic deafferentation. The discrepancy in the findings of these early studies and those of the present study may result from the different stressors used to elicit the prolactin response. Demarest et al. (1985a) have observed a graded increase in the stress-induced rise in prolactin with increasing intensities of stress. It is possible, therefore, that the graded prolactin response to stress is the consequence of a gradual recruitment of different hypothalamic and/or pituitary mechanisms which stimulate prolactin-releasing factors and/or inhibit prolactin-inhibiting factors. The recruitment of additional mechanisms may explain why the increase in prolactin in response to a 30 minute period of intense restraint is only attenuated by complete deafferentation of the mediobasal hypothalamus.

The hypothalamic PVN plays a pivotal role in the regulation of prolactin secretion in response to a variety of stimuli. PVN lesions attenuate the stress-induced increase in prolactin secretion (Meyerhoff et al., 1987; Minamitani et al., 1987). In addition, PVN lesions attenuate prolactin release in lactating rats (Kiss et al., 1986) and in rats which have received an injection of the serotonin precursor, 5-hydroxytryptophan (Minamitani et al., 1987). Serotonin stimulates prolactin release (Caligaris and Taleisnik, 1974; Chen and Meites, 1975) presumably by stimulating the release of a prolactin-releasing factor (Clemens et al., 1978). Although cell bodies for many prolactin-releasing factors are located in the PVN (Mezey and Kiss, 1985; Brownstein et al., 1982; Shin et al., 1987), only vasoactive intestinal polypeptide (VIP) is reported to mediate prolactin secretion induced by serotonin (Shimatsu et al., 1984). Anti-serum generated against VIP attenuates both the serotonin-induced (Shimatsu et al., 1984) and the stress-induced (Kaji et al., 1985) increases in prolactin secretion. These findings are in agreement with those of Demarest et al. (1985a) who found that the action of

restraint stress to increase prolactin secretion as well as to decrease TIDA neuronal activity is mediated by a serotonergic mechanism. It is interesting to speculate that stress stimulates a serotonergic projection to the PVN to either stimulate an inhibitory afferent or inhibit a stimulatory afferent (ie. the one which maintains basal TIDA neuronal activity in the female rat) to then produce a decline in TIDA neuronal activity. Simultaneously, serotonin may stimulate VIP-containing neurons to enhance the release of VIP from identified terminals in the median eminence (Ceccatelli et al., 1988) which then potentiates the elevation in circulating prolactin levels associated with stress. VIP may, in fact, influence TIDA neuronal activity directly via a presynaptic mechanism similar to that which has been reported for galanin (Nordström et al., 1987).

The stress-induced increase in plasma prolactin was not entirely blocked by the loss of afferent input to the mediobasal hypothalamus. These results suggest that stressful stimuli can act within the mediobasal hypothalamus or at the level of the pituitary to mediate, in part, the stress-induced rise in prolactin. The posterior pituitary contains both DA and a prolactin-releasing factor (Murai and Ben-Jonathan, 1987; Hyde et al., 1987) which may play a pivotal role in the regulation of prolactin secretion during certain physiologic conditions. Extracts of posterior pituitary stimulate prolactin secretion in vitro (Hyde et al., 1987) and removal of the posterior pituitary interferes with the suckling-induced increase in prolactin secretion (Murai and Ben-Jonathan, 1987). Taken together, these results suggest that a decrease in the DA tone of the posterior pituitary or stimulation of a prolactin-releasing factor from this region may mediate, in part, the increase in prolactin secretion in response to stress. In addition, recent reports have demonstrated that VIP is synthesized by the pituitary gland (Aranout et al., 1986) and it appears to act there to stimulate prolactin secretion (Hagen et al., 1986; Nagy et al., 1988). Thus, restraint stress may increase the activity of a prolactin-

releasing factor (VIP) at the level of the anterior pituitary to elevate circulating prolactin concentrations.

SUMMARY AND CONCLUSIONS

Interruption of afferent input originating rostral to the mediobasal hypothalamus decreases the basal rate of activity on TIDA neurons in gonadally-intact female rats. This is evidenced by a decrease in the synthesis, turnover and metabolism of DA in the median eminence. Deafferentations made 1 mm rostral to and bilateral cuts of the retrochiasmatic area failed to alter basal TIDA neuronal activity suggesting that afferent neuronal input to these neurons originates in the caudal region of the anterior hypothalamus. Electrolytic lesions of the SCN and PVN produced a similar percentage decrease in TIDA neuronal activity as did retrochiasmatic deafferentation. Thus, afferent neuronal input originating in the SCN or PVN mediates a stimulatory influence on TIDA neurons in the intact female rat.

Although prolactin appears to be the primary physiological factor which regulates TIDA neuronal activity, the stimulatory action of this hormone on TIDA neurons is still functional when afferent input from areas rostral to the mediobasal hypothalamus is removed. These findings together with the identification of specific binding sites for prolactin in the median eminence provides evidence for a direct action of this hormone on the terminals of the TIDA neurons.

The deafferentation-induced decrease in TIDA neuronal activity in female rats may operate via the same neuronal mechanism that functions to decrease TIDA neuronal activity following ovariectomy. Retrochiasmatic deafferentation and ovariectomy decreased TIDA neuronal activity yet both procedures together produce no further decrease suggesting that these manipulations act via a similar

neuronal pathway. This common pathway may involve a direct action of estrogen at the level of the anterior hypothalamus.

The neuronal mechanism which stimulates TIDA neuronal activity in intact female rats is either not present or not operational in intact and orchidectomized male rats. Even after removal of the inhibitory influence of testosterone (when TIDA neuronal activity in the male approximates that observed in an intact female) retrochiasmatic deafferentation had no effect. The absence or quiescence of stimulatory afferent input to the TIDA neurons in the male could be the consequence of neonatal androgen exposure.

Inhibitory afferent neurons which decrease TIDA neuronal activity during stress, as well as additional afferent inputs to the median eminence which may regulate prolactin secretion during stress, originate rostral to the mediobasal hypothalamus. Complete and retrochiasmatic deafferentation of the mediobasal hypothalamus blocked the stress-induced decrease in TIDA neuronal activity and attenuated the rise in prolactin. The stress-induced increase in prolactin which persists after complete and retrochiasmatic deafferentation may result from alterations in prolactin-releasing and/or inhibiting factors at the level of the mediobasal hypothalamus and/or anterior pituitary.

In summary, the TIDA neurons are not simply part of a hormonal/neuronal feedback system controlling prolactin release independent of afferent neuronal influences. The TIDA neurons are regulated by both stimulatory and inhibitory afferent neurons which appear to originate in the anterior hypothalamus and which influence the activity of these neurons under basal conditions and during certain physiological states.

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