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John L. Goudreau

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K E Moore

Major professor

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FUNCTIONAL ANATOMY AND REGULATION OF DOPAMINERGIC NEURONS TERMINATING IN THE INTERMEDIATE LOBE OF THE PITUITARY

By

John L. Goudreau

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ABSTRACT

FUNCTIONAL ANATOMY AND REGULATION OF DOPAMINERGIC NEURONS TERMINATING IN THE INTERMEDIATE LOBE OF THE PITUITARY

By

John L. Goudreau

The purpose of the present study was examine the functional anatomy and afferent regulation of dopaminergic (DA) neurons terminating in the intermediate lobe of the pituitary. The *in vivo* activity of DA neurons was estimated by measuring concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) in the intermediate lobe of the pituitary. Plasma concentrations of α-melanocyte-stimulating hormone (αMSH) were also determined to provide a functional correlate to observed changes in DA neuronal activity in the intermediate lobe of the pituitary.

Unilateral injections of *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the periventricular nucleus reveal labelled terminal axonal arbors in the intermediate lobe which are also immunoreactive for tyrosine-hydroxylase. Surgical isolation of the periventricular nucleus from the mediobasal hypothalamus produces a 50% decrease in DOPAC and dopamine in the intermediate lobe and increases αMSH secretion, whereas electrical stimulation of this region increases the activity of intermediate lobe DA neurons and decreases αMSH secretion. Taken together, these results indicate that neurons in the periventricular nucleus terminate in the intermediate lobe of the posterior pituitary and regulate the secretion of αMSH. It is proposed that these DA neurons be referred to as a "periventricular-hypophysial" DA (PHDA) neuronal system which better reflects

the origin and site of termination of these neurons than the heretofore designation of tuberohypophysial DA neurons.

Further studies examined the afferent regulation of PHDA neurons by 5-hydroxytryptaminergic (5HT) and gamma-aminobutyric acid (GABA) inputs under basal and stress conditions. Results are summarized as follows. Activation of the post-synaptic $5HT_2$ and $GABA_B$ receptors inhibits the activity of PHDA neurons and increases the secretion of αMSH. Basal PHDA neuronal activity and αMSH secretion, however, are not tonically inhibited by 5HT neurons or GABAergic neurons acting at $GABA_B$ receptors. Furthermore, afferent inputs from 5HT and GABAergic neurons, acting via $5HT_2$ receptors and $GABA_B$ receptors, respectively, mediate inhibitory effects of stress on PHDA neurons. Finally, $GABA_B$ receptor antagonists block the inhibitory effects of $5HT_2$ receptor stimulation on PHDA neuronal activity, suggesting that activation of $5HT_2$ receptors stimulates inhibitory GABAergic interneurons which, in turn, inhibit PHDA neuronal activity via $GABA_B$ receptors.

In memory of my father,

Clinton L. Goudreau

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ACTH	Adrenocorticotropic hormone
cAMP	Adenosine 3',5'-monophosphate
DAB	3,3'-Diaminobenzidine
DOPA	3,4-Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
5,7-DHT	5,7-Dihydroxytryptamine creatinine sulfate
DOI	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
DA	Dopaminergic
FITC	Fluorescein isothiocyanate
GABA	Gamma-aminobutyric acid
HRP	Horseradish peroxidase
8-OH-DPAT	8-Hydroxy-2-(di-n-(propylamino)-tetralin hydrobromide
5HIAA	5-Hydroxyindoleacetic acid
5HT	5-Hydroxytryptaminergic
icv	Intracerebroventricular
ß-LPH	ß-Lipotropin
αMSH	α-Melanocyte stimulating hormone
MAO	Monoamine oxidase

PHA-L	*Phaseolus vulgarus* leucoagglutinin
PHDA	Periventricular-hypophysial dopaminergic
POMC	Pro-opiomelanocortin
THDA	Tuberohypophysial dopaminergic
TIDA	Tuberoinfundibular dopaminergic
TH	Tyrosine hydroxylase
TBS	Tris-buffered saline
TBS-TX	Tris-buffered saline-0.5% Triton-X100

1. INTRODUCTION

A. ANATOMY OF CENTRAL DOPAMINERGIC NEURONS

Dopaminergic (DA) neuronal systems within the mammalian brain are classified by the location of their cell bodies and axon terminals. Discrete dopamine-containing perikarya are identified according to the alpha numeric nomenclature developed by Dahlström and Fuxe in 1964 (See Figure 1.1). Dopamine-containing cell bodies located within the pars compacta of the substantia nigra (A8-A9) and the ventral tegmentum (A10) comprise the major ascending DA neuronal systems collectively known as the mesotelencephalic DA neurons. The mesotelencephalic neurons have been subdivided, based on major projection sites, into the mesocortical (neurons projecting to several cortical regions), mesolimbic (neurons projecting to various subcortical regions, e.g., the nucleus accumbens, septum, olfactory tubercle), and the nigrostriatal (neurons projecting to the caudate-putamen) DA neuronal systems. Four additional groups of dopamine-containing perikarya are located within the hypothalamus (A11-A14) and constitute the hypothalamic DA neuronal systems.

Hypothalamic DA neurons are divided into three major systems. The largest percentage of DA neurons within the hypothalamus comprise the incertohypothalamic system with perikarya located in the caudal hypothalamus (A11 cell group), medial zona incerta (A13 cell group) and rostral periventricular region (A14 cell group; Björklund et

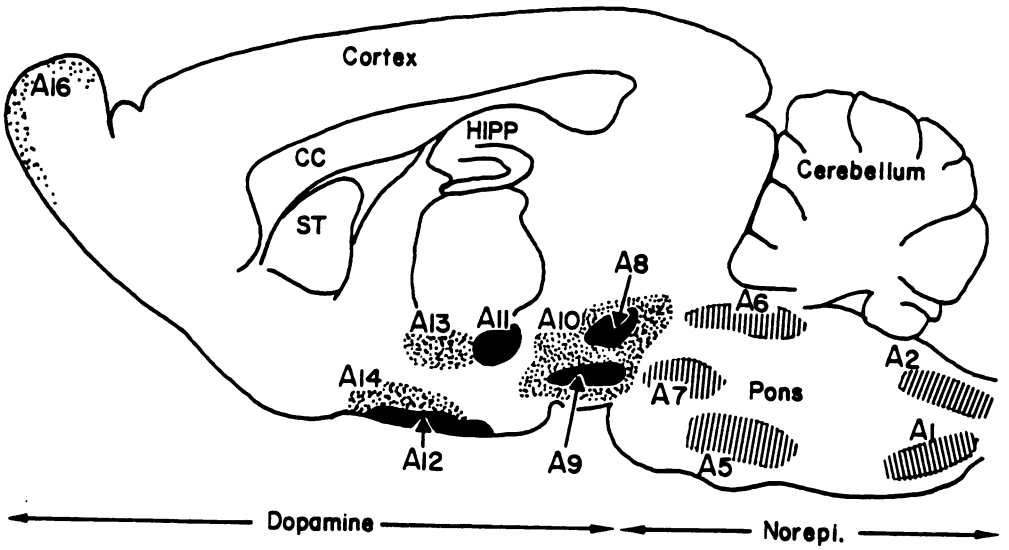


Figure 1.1 Schematic sagittal section of the rat brain depicting the location of catecholamine-containing perikarya (Moore, 1987) Cell groups A_1-A_7 contain norepinephrine and cell groups A_8-A_{16} contain dopamine (Dahlström and Fuxe, 1964). CC, corpus callosum; HIPP, hippocampus; ST, striatum.

al., 1975; Van den Pol et al., 1984). The axonal projections of the incertohypothalamic DA system are not well defined, but are thought to consist of projections within the diencephalon. For example, DA neurons in the rostral periventricular nucleus project laterally toward the medial preoptic regions of the hypothalamus (Björklund et al., 1975), whereas DA neurons in the caudal periventricular nucleus have dendrites and axons oriented in a dorsal-ventral fashion (Van den Pol et al., 1984; Chan-Palay et al., 1984). Some cell of the A11 group project to the spinal cord (Björklund and Skagerburg, 1979). Tuberoinfundibular DA (TIDA) neuronal system has cell bodies located in the arcuate nucleus and the adjacent periventricular region (A12 cell group, Björklund et al., 1973) and have short axons which project to the external layer of the median eminence (Figure 1.2). The remaining hypothalamic DA neuronal system consist of DA neurons innervating the posterior pituitary.

B. INNERVATION OF THE INTERMEDIATE LOBE OF THE PITUITARY

Innervation of the posterior pituitary was first described by Ramòn y Cajal (1894) who identified nerve fibers originating in the brain and terminating in the neural and intermediate lobes of the pituitary. Later studies using fluorescence histochemistry identified the presence of monoamine containing nerve fibers in the pituitary gland (Dahlström and Fuxe, 1966). Biochemical and spectrofluorescence studies revealed that the fluorescence was due predominantly to dopamine-containing fibers with a minor contribution from norepinephrine-containing fibers (Björklund et al., 1967). Ultrastructural studies reveal that catecholamine fibers in the intermediate lobe contain numerous varicose swellings (0.1-0.5 μm diameter) along terminal axon branches. These

spherical swellings were found intimately apposed to glandular cells in the intermediate lobe, suggesting that catecholamine-containing neurons make direct synaptic contact with melanotrophs (Baumgarten et al., 1972). Subsequent studies by Björklund and coworkers (1973) using electrolytic lesions and knife cuts in conjunction with fluorescence histochemistry suggested that the perikarya of DA neurons terminating in the posterior pituitary were located in the tuberal portion of the hypothalamus, specifically in the rostral arcuate nucleus and adjacent periventricular region. Hence, DA neurons terminating in the neural and intermediate lobes were classified as tuberohypophysial DA (THDA) neurons (Figure 1.2).

Recent studies, however, have suggested that the origin of DA innervation of the neural and intermediate lobes of the pituitary may be different than originally proposed by Björklund and coworkers. Injection of the retrograde tracer horseradish peroxidase (HRP) into the neurointermediate lobe of the rat pituitary results in labelling of tyrosine hydroxylase (TH) immunoreactive cells in the periventricular nucleus (Kawano and Daikoku, 1987; Luppi et al., 1986), but not in the arcuate nucleus (Luppi et al., 1986; Kawano and Daikoku, 1987; Kelly and Swanson, 1980; Weigand and Price, 1980). Destruction of DA neuronal cell bodies within the arcuate nucleus following neonatal administration of monosodium glutamate decreases dopamine content in the median eminence, but has no effect on dopamine content (Dawson et al., 1985) or TH immunoreactivity (Davis et al., 1984) in the posterior pituitary. Taken together, these studies suggest that DA innervation of the neural and intermediate lobes of the pituitary may originate from the periventricular region and not the arcuate nucleus.

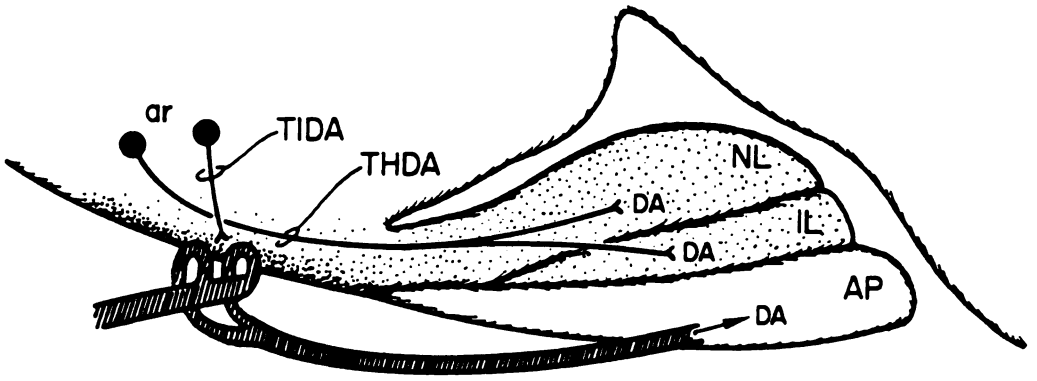


Figure 1.2 Schematic diagram indicating the location of TIDA and THDA neurons on a midsagittal view of the rat hypothalamus and pituitary (Moore, 1987) AL, anterior lobe; IL, intermediate lobe; NL, neural lobe; TIDA, tuberoinfundibular dopaminergic neurons; THDA, tuberohypophysial dopaminergic neurons.

The pituitary intermediate lobe is also innervated by neurons containing a variety of other neurotransmitters including norepinephrine, 5-hydroxytryptamine and gamma-aminobutyric acid (GABA) (for review see Millington and Chronwall, 1988). The origin and function of these neurons has not been completely characterized. For example, the noradrenergic innervation originates from either the central nervous system (Saavedra, 1985), the superior cervical ganglion (Björklund et al., 1973), or both central and peripheral sites (Alper et al., 1980a). The 5-hydroxytryptaminergic (5HT) innervation of the intermediate lobe originates from either cells in the midbrain raphe or dorsomedial nucleus (Mezey et al., 1984) or from cells caudal to the dorsal and medial raphe nuclei (Shannon and Moore, 1987). Central GABAergic neurons originating in the hypothalamus innervate the posterior pituitary (Tappaz et al., 1986) and GABA has been shown to be extensively colocalized with DA in neurons innervating the intermediate lobe (Vuillez et al., 1987; Schimchowitsch et al., 1991).

C. NEUROCHEMISTRY OF DA NEURONS IN THE POSTERIOR PITUITARY

Current concepts of dopamine synthesis and metabolism have been derived primarily from studies in nigrostriatal DA neurons. The synthesis and metabolism of dopamine within neurons terminating in the neural and intermediate lobe are similar to that in nigrostriatal DA neurons (for review see Holzbauer and Racké, 1985; Moore, 1987). As depicted in Figure 1.3, dopamine is synthesized from dietary tyrosine which is transported into the nerve terminal by an active transport mechanism. Once inside the neuron tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by the rate limiting

enzyme TH. DOPA is rapidly decarboxylated by L-aromatic amino acid decarboxylase to form dopamine, such that DOPA does not accumulate in the neuron. The newly synthesized dopamine is stored in synaptic vesicles and released in response to nerve stimulation.

Following arrival of a nerve action potential and release into the synaptic cleft, dopamine may activate post-synaptic DA receptors. Dopamine released from mesotelencephalic DA neurons has been shown to activate inhibitory presynaptic autoreceptors and provide a negative-feedback loop for DA neuronal activity. Activation of DA receptors inhibits the activity of DA neurons terminating in the intermediate lobe, but it is not known if this effect is mediated by presynaptic autoreceptors (Lookingland et al., 1985).

The synaptic actions of dopamine are terminated primarily by an active reuptake mechanism in the neuron terminal, although in neurons terminating in the intermediate lobe of the pituitary this mechanism has lower affinity than those in nigrostriatal DA neurons (Demarest and Moore, 1979b). Recaptured dopamine may be recycled into vesicles for subsequent re-release or metabolized by mitochondrial monoamine oxidase (MAO)-A to the inactive metabolite 3,4-dihydroxyphenylacetic acid (DOPAC; Demarest and Moore, 1981). DOPAC may be further metabolized to homovanillic acid by catechol-O-methyltransferase. A small amount of dopamine that is not recaptured may also be metabolized to 3-methoxytyramine by extraneuronal catechol-o-methyltransferase although this appears to be a minor pathway in the posterior pituitary (Racké and Muscholl, 1986).

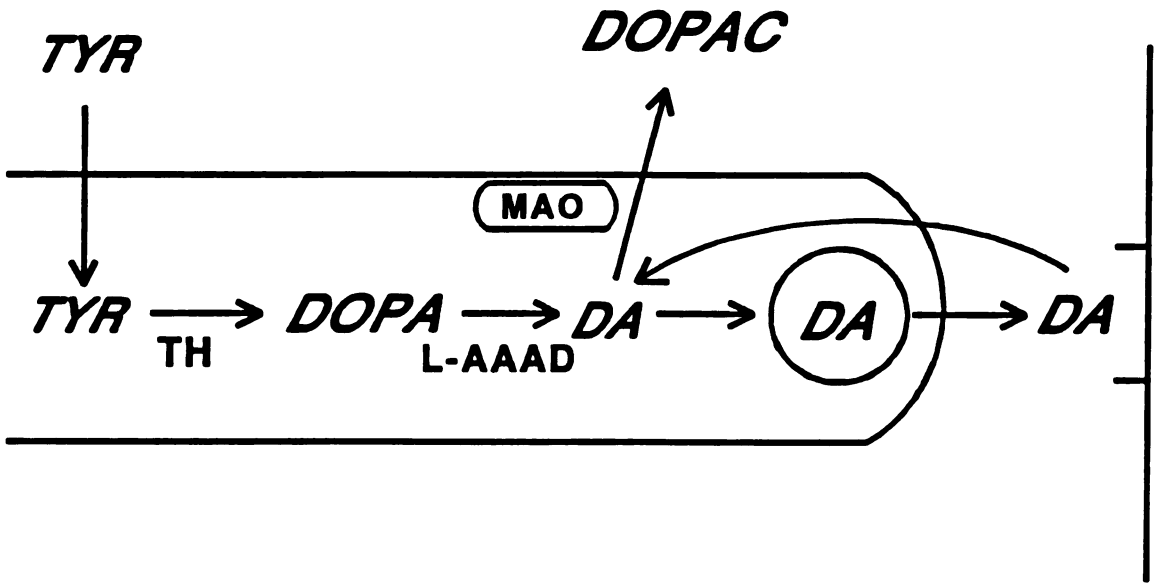


Figure 1.3 Neurochemical synthetic and metabolic pathways in the axon terminal of DA neurons in the pituitary intermediate lobe. MAO, monoamine oxidase; TH, tyrosine hydroxylase; L-AAAD, L-aromatic amino acid decarboxylase; DOPA, 3,4-dihydroxyphenylalanine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; TYR, tyrosine.

Concentrations of dopamine within DA neurons remain fairly constant despite changes in the rate of release of neurotransmitter. This reflects the end-product feedback regulation by cytoplasmic dopamine on the activity of the rate limiting enzyme TH. For example, acute increases in cytoplasmic dopamine concentrations decrease TH activity and, conversely, acute decreases in cytoplasmic dopamine concentrations increase TH activity (Demarest and Moore, 1979a; Demarest et al., 1979). Thus, the release of dopamine from terminals in the intermediate lobe is closely coupled to dopamine synthesis which maintains a steady-state level of neurotransmitter. Accordingly, *in vivo* neurochemical estimation of TH activity, e.g., the accumulation of DOPA following decarboxylase inhibition (Carlsson et al., 1972), has been used as an index of the DA neuronal activity in the intermediate lobe of the pituitary (Gunnet et al., 1987; Demarest et al., 1979).

The formation of dopamine metabolites such as DOPAC is also closely coupled to intermediate lobe DA neuronal activity. Approximately one-third of DOPAC concentrations in the intermediate lobe result from released dopamine that is recaptured by DA nerve terminals and oxidized by mitochondrial MAO (Lindley et al., 1990a). The small contribution of released dopamine to the total pool of dopamine metabolized to DOPAC may be due to a lack of high affinity uptake sites for dopamine in the posterior pituitary (Demarest and Moore, 1979b). In addition, metabolism of dopamine to DOPAC occurs in the absence of DA neuronal activity and contributes to the basal concentrations of DOPAC (Chrapusta et al., 1992). A majority of DOPAC formed in the intermediate lobe reflects the fraction of newly synthesized dopamine that is not

packaged into synaptic vesicles and is thus exposed to MAO (Racké et al., 1987; Lindley et al., 1990b). Since this pool of dopamine is dependent upon *de novo* synthesis, procedures which increase or decrease the activity of DA neurons terminating in the intermediate lobe produce corresponding increases or decreases in the concentrations of DOPAC in the intermediate lobe *in vitro* (Racké and Muscholl, 1986) and *in vivo* (Lindley et al., 1990a). Taken together, these empirical studies indicate that the concentrations of DOPAC in the intermediate lobe reflect neuronal activity of DA neurons terminating in this region and that changes in concentrations of DOPAC in the intermediate lobe provide a relative index of changes in DA neuronal activity.

D. NEUROENDOCRINE FUNCTION OF HYPOTHALAMIC DA NEURONS TERMINATING IN THE MEDIAN EMINENCE AND POSTERIOR PITUITARY

TIDA neurons project to the external layer of the median eminence and terminate close to the primary capillary loops of the hypothalamic-hypophysial portal system. Dopamine is released from TIDA neurons into the portal blood and is transported to the anterior pituitary where it activates D_2 receptors located on lactotrophs, resulting in the inhibition of prolactin release (for review see Gudelsky, 1981 and Ben Jonathan, 1985). The inhibition of prolactin secretion by dopamine released from TIDA neurons provides the primary hypothalamic control of lactotroph function.

DA neurons innervating the intermediate lobe participate in the regulation of melanotroph function. DA fibers in the intermediate lobe make numerous synaptic contacts with melanotrophs (Baumgarten et al., 1972; Luppi et al., 1986) which

synthesize pro-opiomelanocortin (POMC)-derived peptides, e.g., α-melanocyte stimulating hormone (αMSH) and ß-endorphin (Eberle, 1988). *In vitro* experiments have demonstrated that dopamine inhibits the release of POMC-derived peptides (Farah et al., 1982; Randle et al., 1983a). *In vivo* experiments indicate that activation of DA neurons innervating the intermediate lobe inhibits αMSH secretion, whereas inhibition of the activity of these neurons increases αMSH secretion (Lindley et al., 1988). It has also been suggested that dopamine released from neurons terminating in the intermediate lobe may reach the anterior pituitary via the short portal vessels and inhibit the secretion of prolactin (Ben-Jonathan, 1991). Most of the dopamine reaching the anterior pituitary, however, originates from the terminals of the TIDA neurons and is transported by blood in the long hypothalamic-hypophysial portal vessels.

The function of DA neurons terminating in the neural lobe is less well defined. The neural lobe of the pituitary contains diffuse varicose DA fibers which terminate near neurosecretory axons but do not appear to form true synaptic structures (Baumgarten et al., 1972; Luppi et al., 1986). A modulatory role for neural lobe dopamine on oxytocin and vasopressin release has been suggested, but the nature of this role is not clear (for review see Holzbauer and Racké, 1985). Indeed, dopamine agonists have been shown to increase (Bridges, et al., 1976), decrease (Lightman, et al., 1982) or have no effect (Pitzel and König, 1984) on neural lobe secretion of vasopressin and oxytocin. These discrepancies may be due to heterogeneity of DA receptors within the neural lobe (Holzbauer and Racké, et al., 1985). At the present time, however, the source and role of dopamine in the neural lobe remains unresolved.

E. REGULATION OF MELANOTROPH FUNCTION

Melanotrophs in the intermediate lobe and corticotrophs in the anterior lobe synthesize the pre-prohormone POMC (Figure 1.4). POMC is initially cleaved by POMC converting enzyme (Loh et al., 1985) into adrenocorticotropic hormone (ACTH), ß-lipotropin (ß-LPH), and an N-terminal fragment. In the intermediate lobe, but not in the anterior lobe, ACTH is further cleaved into corticotropin-like intermediate lobe peptide and αMSH, ß-LPH is cleaved to gamma-LPH and ß-endorphin$_{1\text{-}31}$, and the N-terminal fragment is metabolized to gamma-MSH. ß-endorphin$_{1\text{-}31}$ is further metabolized to ß-endorphin$_{1\text{-}27}$ and ß-endorphin$_{1\text{-}26}$ (for review see Akil et al., 1984). In addition to these cleavage steps, the intermediate lobe melanotrophs contain peptide acetyltransferase which acetylates both ß-endorphin peptides and αMSH which increases the melanocyte-stimulating properties and behavioral activity of αMSH (e.g., grooming, stetching/yawning, increased acquisition in active and passive avoidance paradigms; review Eberle, 1988) while eliminating the opiate analgesic properties of ß-endorphin peptides (Akil et al., 1981; O'Donohue et al., 1979). Intermediate lobe melanotrophs secrete all of the end products of POMC processing (review, Eberle, 1988).

Melanotroph function is under direct inhibitory control from the hypothalamus. Lesions of the hypothalamus and pituitary stalk increase plasma concentrations of αMSH in rats (for review, see Eberle, 1988). Dopamine or DA agonists inhibit, while DA antagonists increase the *in vivo* secretion of POMC-derived peptides, including αMSH (Lindley et al., 1988). The effects of DA on melanotroph function *in vitro* include: 1) inhibition of spontaneous electrical activity (Douglas and Tarasckevich, 1978; Davis and

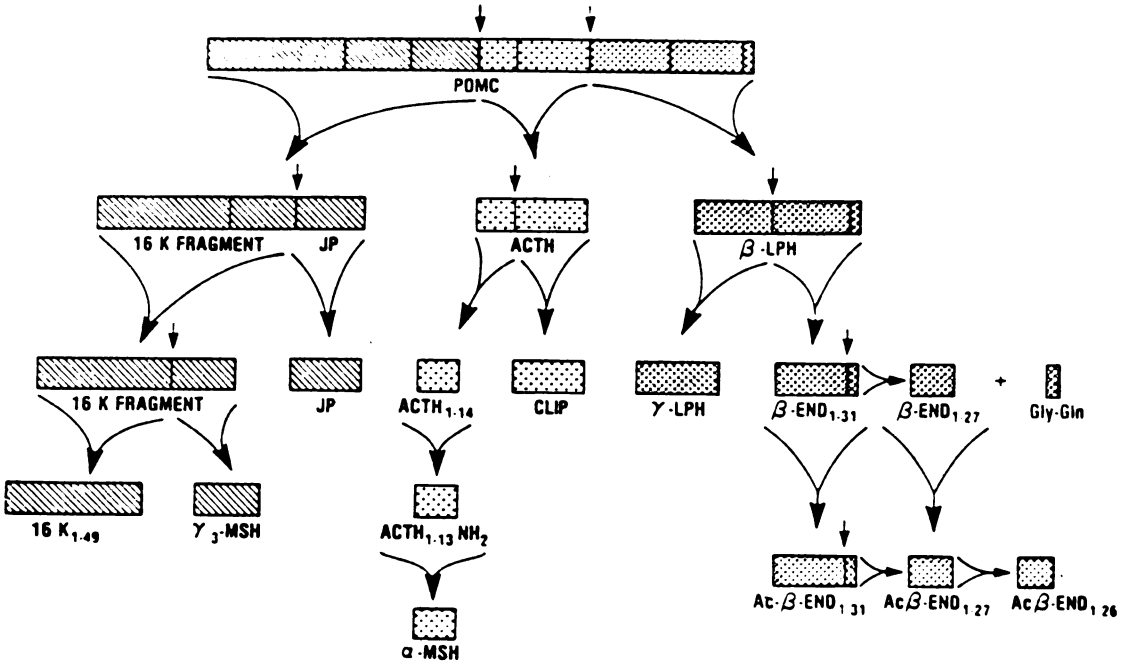


Figure 1.4 Post-translational processing of POMC in the intermediate lobe of the pituitary (Millington and Chronwall, 1988). Arrows mark sites of endoproteolytic cleavage. JP, joining peptide; ACTH, adrenocorticotropic hormone; ß-LPH, ß-lipotropin; CLIP, corticotropin-like intermediate lobe peptide; α-MSH, α-melanocyte stimulating hormone; ß-END, ß-endorphin; Ac, acetylated.

Hadley, 1976); 2) reduction in the number of melanotrophs with high secretory activity (Chronwall et al., 1988); 3) inhibition of POMC-processing enzymes (Millington et al., 1986a; Mains et al., 1985); and 4) reduction of POMC mRNA concentrations (Beaulieu et al., 1984). DA_2 receptors have been identified on melanotrophs in the intermediate lobe (Munemura et al., 1980; Tiligada and Wilson, 1989) and are negatively coupled to adenylate cyclase. Stimulation of the DA_2 receptor decreases the intracellular concentration of adenosine cyclic 3', 5'-monophosphate (cAMP), and thus decreases the synthesis and secretion of POMC-derived peptides from melanotrophs (Coté et al., 1982). More recent *in vivo* studies, however, suggest that there may be a heterogeneity of DA receptors in the intermediate lobe. The reportedly selective D_2 antagonists remoxipride and raclopride produce differential effects on αMSH secretion; i.e., administration of raclopride but not remoxipride increases αMSH secretion (Eaton et al., 1992). These data suggest that there may be a subtype of the "classical" D_2 receptor on melanotrophs in the intermediate lobe.

Various other neurotransmitters have direct effects on melanotroph secretion. The adrenal medulla releases epinephrine which activates $ß_2$ receptors on melanotrophs and stimulates αMSH secretion by increasing the intracellular concentrations of cAMP (Bowers et al., 1974; Coté et al., 1982; Kvetnansky et al., 1987). Corticotropin-releasing hormone stimulates the secretion of αMSH *in vitro* (Meunier et al., 1982) and *in vivo* (Proulx-Ferland et al., 1982), although at concentrations greater than are required to stimulate anterior lobe corticotroph secretion (Vale et al., 1983). High doses of 5-hydroxytryptamine also have a modest stimulatory effects on melanotroph secretion *in*

vitro (Randle et al., 1983b). GABA exerts both stimulatory and inhibitory effects on melanotroph secretion *in vitro* (Tomiko et al., 1983; Demeniex et al., 1984).

The intermediate lobe is an attractive system for studying the neuroendocrine regulation of hypothalamic DA neurons. It is a homogenous endocrine tissue secreting characteristic peptides which are under direct inhibitory control of hypothalamic DA neurons. Despite the virtual absence of the intermediate lobe in the adult human, the physiologic function of αMSH continues to be a focus of ongoing research. In addition to the known role of αMSH in controlling skin pigmentation, αMSH has been shown to have neurotrophic effects in regenerating peripheral nerves (Strand et al., 1976; Bijlsma et al., 1984). Further studies suggest that αMSH may be an important regulator of immune function; i.e., αMSH antagonizes many of the peripheral and central effects of interleukin-1ß (for review see Eberle, 1988). Although the physiological role of αMSH in humans remains uncertain, αMSH secretion from melanotrophs provides a useful endpoint to asses the activity of DA neurons terminating in the intermediate lobe.

F. REGULATION OF DA NEURONS TERMINATING IN THE INTERMEDIATE LOBE OF THE PITUITARY

1. Differential regulation of hypothalamic DA neurons

The regulation of intermediate lobe DA and TIDA neurons are distinct. For example, the activity of TIDA neurons is regulated by circulating levels of prolactin such that endocrinological or pharmacological manipulations that increase (i.e. prolactin, estrogen, or haloperidol administration) or decrease (i.e. hypophysectomy, bromocriptine administration, or prolactin antiserum) circulating prolactin concentrations produce

corresponding changes in the activity of TIDA neurons. In contrast, the basal activity of intermediate lobe DA neurons is not regulated by prolactin, but is responsive to the acute administration of dopamine agonists and antagonists (for review see Moore, 1987). Furthermore, icv administration of αMSH activates TIDA neurons, but not DA neurons terminating in the intermediate lobe (Lindley et al., 1990c).

DA neurons terminating in the neural and intermediate lobe are also regulated by divergent mechanisms. For example, DA agonists and antagonists decrease or increase, respectively, the activity of DA neurons terminating in the intermediate, but not neural lobe (Lookingland et al., 1985). On the other hand, mu-opioid agonists inhibit the activity of DA neurons terminating in the neural, but not intermediate lobe (Lookingland and Moore, 1985). Indeed, it has been proposed that the DA neurons terminating in the intermediate and neural lobe are functionally distinct (Holzbauer and Racké, 1985).

2. Pharmacological manipulations

A number of pharmacological manipulations affect the activity of DA neurons terminating in the intermediate lobe. Administration of kappa (Manzanares et al., 1991a) but not mu opioid agonists (Lookingland and Moore, 1985) inhibits the activity of intermediate lobe DA neurons and increases αMSH secretion. Furthermore, endogenous kappa opioids, e.g., dynorphins, tonically inhibit the activity of intermediate lobe DA neurons (Manzanares et al., 1991a; Manzanares et al., 1992a). Intermediate lobe DA neurons also receive tonic inhibitory input from endogenous glutamatergic neurons via the non-n-methyl-D-aspartate receptor subtype (Wagner et al., 1993). Histamine antagonists and synthesis inhibitors block stress-induced increases in αMSH secretion

(Knigge et al., 1991; Knigge et al., 1989), but do not alter the basal activity of intermediate lobe DA neurons or αMSH secretion (Fleckenstein et al., 1993). Several peptide neurotransmitters also appear to alter the basal activity of intermediate lobe DA neurons. The tridecapeptides neurotensin and bombesin activate intermediate lobe DA neurons and decrease αMSH secretion (Pan et al., 1992; Manzanares et al., 1991b). The neurochemical anatomy, physiologic relevance and mechanisms involved in the above pharmacological manipulations are the focus of ongoing research.

3. Physiological manipulations

a. Stress

Stress produces differential effects on the activities of central DA neuronal systems. Neurons comprising mesocortical and mesolimbic but not nigrostriatal DA neuronal systems are activated by stressful manipulations (Thierry et al., 1976; Fadda et al., 1978; Reinhard et al., 1982; Deutch et al., 1985). In contrast, stress decreases the activity of TIDA neurons of female but not male rats (Demarest et al., 1985; Lookingland et al., 1990). Stress also decreases the activity of DA neurons terminating in the intermediate, but not the neural lobe of the pituitary of both male and female rats (Lookingland et al., 1991).

Stressful manipulations consistently increase the secretion of αMSH (Tilders et al., 1985), and this is accompanied by a decrease in the activity of DA neurons terminating in the intermediate lobe (Lookingland et al., 1991). Full expression of stress-induced secretion of αMSH is dependent on a decrease in DA neuronal activity in the intermediate lobe and stimulation of the $ß_2$ receptor by epinephrine released from the

adrenal medulla (Lindley et al., 1990b). For example, adrenal medullectomy attenuates, but does not abolish the stress induced increase in αMSH secretion (Kvetnansky et al., 1987). Hypothalamic deafferentation blocks the stress-induced inhibition of intermediate lobe DA neuronal activity and attenuates the stress-induced increase in αMSH secretion. The latter findings further suggest that afferent neuronal inputs mediate the effects of stress on these neurons (Lindley et al., 1990b). The exact nature, however, of the afferent neuronal regulation of intermediate lobe DA neuronal activity under basal or stress conditions has not been determined. Restraint stress, therefore, may prove to be a useful model for elucidating physiologically relevant afferent inhibitory inputs to DA neurons terminating in the intermediate lobe.

b. Gonadal Steroids

Initial studies suggested that there is no sexual difference in the activity of DA neurons terminating in the intermediate lobe (Gunnet et al., 1986). More recent studies, however, indicate that the activity of DA neurons is slightly higher in the intermediate lobe of female rats compared to male rats. In contrast to the sexual difference found TIDA neuronal activity, the sexual difference in the activity of intermediate lobe DA neurons is not dependent upon the presence of circulating gonadal steroids (Manzanares et al., 1992b).

While it is known that dopamine released from TIDA neurons tonically inhibits the release of prolactin, DA neurons terminating in the posterior pituitary have also been implicated in this process (Peters et al., 1981). For example, it has been reported that the *in vitro* activity of DA neurons terminating in the posterior pituitary is diminished

during surges of prolactin that occur during proestrus, pregnancy and lactation (for review see Ben-Jonathan, 1991). These observed changes in posterior pituitary DA activity in the female rat have been associated with changes in circulating estrogen (Barden et al., 1982; Saavedra et al., 1984; Garris and Ben-Jonathan, 1991) Furthermore, it appears that the effects of posterior pituitary dopamine on prolactin secretion are mediated by a prolactin-releasing factor derived from the posterior pituitary (Hyde et al., 1987). The role of posterior pituitary DA neurons in the control of prolactin secretion is minor compared to the influence of TIDA neurons and remains to be fully characterized.

c. Diurnal Variation

The secretion of αMSH was proposed to occur in a continuous, non-pulsatile manner (Wilson and Harry, 1981). However, both monophasic (Wilson and Morgan, 1979; Millington et al., 1986b) and biphasic (Usategui et al., 1976; Monnet et al., 1981) diurnal variations in the secretion of αMSH have been reported. In addition, diurnal variations in the concentration of dopamine in the posterior pituitary have also been documented (Barden et al., 1982; Koulu et al., 1989). The pattern of these diurnal variations, however, is not consistent among studies and may reflect differences in strain and experimental protocol. Furthermore, Lindley has reported that there is no diurnal variation in the secretion of αMSH or the activity of PHDA neurons (1989).

d. Dehydration

Physiologic stimuli which increase neurohypophysial peptide secretion, e.g., dehydration and salt loading, also increase dopamine concentrations and synthesis within

the posterior pituitary (Alper et al., 1980b). In addition, dehydration has been reported to increase the secretion of αMSH in some species (Leenders et al., 1990). More recent studies, however, indicate that dehydration does not alter the activity of DA neurons terminating in the neural or intermediate lobes, but does increase the concentration of dopamine in the neural lobe (Manazanres et al., 1990). An alternative source of increased neural lobe dopamine synthesis has been proposed. Young and coworkers (1987) have suggested that increases in neural lobe dopamine content following hyperosmotic stimuli may result from induction of TH-mRNA expression in the supraoptic and paraventricular nuclei which project to the neural lobe. The functional significance of *de novo* synthesis of dopamine within vasopressin neurosecretory cells terminating in the neural lobe following water deprivation remains to be determined.

G. STATEMENT OF PURPOSE

Although much attention has been focused on the DA regulation of melanotroph secretion in the intermediate lobe, few studies have examined the afferent neuronal regulation of DA neurons terminating in the intermediate lobe. The purpose of the present studies is to test the hypothesis that DA neurons terminating in the intermediate lobe receive a convergence of afferent inhibitory inputs which mediate the inhibition of these neurons during stress. As an initial step the functional anatomy of DA neurons innervating the intermediate lobe will be determined using anatomical and neurochemical techniques. Following this, the afferent neuronal regulation of DA neurons terminating in the intermediate lobe will be examined under basal conditions and during stressful manipulations. Characterizing the afferent neuronal regulation of DA neurons

terminating in the intermediate lobe will provide further insight into the differential regulation of DA neuronal systems in general.

2. MATERIALS AND METHODS

A. Animals

All experiments were conducted using male or female Long-Evans rats (initial body weight 200-225 g) obtained from either Charles River Laboratories (Wilmington, MA) or Harlan Laboratories (Indianapolis, IN) and were allowed to accommodate to the animal quarters for at least two days before being used in experiments. Animals were housed four per cage, maintained in a temperature- (22 ± 1 °C) and light-controlled (illumination between 06.00-18.00 hr) environment, and provided with food (Purina Rat Chow) and tap water *ad libitum*. Vaginal cytology was monitored daily in female animals to determine the stage of the estrous cycle, and only those animals displaying a vaginal smear consistent with the first day of diestrus after two consecutive cycles were used.

B. Drugs

5,7-Dihydroxytryptamine creatinine sulfate (5,7-DHT; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.3% saline containing 0.1% ascorbic acid, kept cold on ice with minimal exposure to light prior to administration. Desipramine hydrochloride (Sigma Chemical Co.), (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI),8-hydroxy-2-(di-n-(propylamino)-tetralinhydrobromide(8-OH-DPAT)isoguvacine hydrochloride, 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridaziniumbromide (SR-95,531; Research Biochemical Inc., Natick, MA), ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge IA), xylazine hydrochloride (Mobay Co.,Animal Health Division, Shawnee, KA), 1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1H-imidazol-1-

yl)methyl]-4H-carbazol-4-one hydrochloride dihydrate (ondansetron; kindly supplied by Drs. Michael Tyers and B.M. Bain of Glaxo Research Ltd.), and P-(3-aminopropyl)-P-diethoxymethyl-phosphinic acid (CGP-35,348; kindly supplied by Dr. Anna Suter, CIBA-GIEGY Ltd., Basel, Switzerland) were dissolved in water. α-Phenyl-1-(2-phenylethyl)-4-piperidine methanol (MDL-11,939; kindly supplied by Drs. Michael G. Palfreyman and Ekkhard H.W. Bohme of Merrel Dow Pharmaceuticals) was dissolved in 0.01 N HCl and ritanserin (Research Biochemicals Inc.) was dissolved in 0.1% tartaric acid. Baclofen and 2-hydroxysaclofen (Research Biochemicals, Inc.) were dissolved in 0.03 N and 0.01 N NaOH, respectively. Drugs were administered as indicated in the legends of the appropriate figures. MDL-11,939 and ritanserin were calculated as the free base, while baclofen and 2-hydroxysaclofen were calculated as the acid. All other doses of drugs were calculated as the appropriate salt.

The anesthetic Equithesin was prepared by stirring 42.51 g chloral hydrate, 9.72 g sodium pentobarbital (Sigma Chemical Co.) and 21.26 g magnesium sulfate in 443 ml of warm propylene glycol. After these compounds were completely dissolved, 120 ml of 95% ethanol was added and the total volume was brought to 1000 ml with double distilled water.

C. Surgical Manipulations

1. Intracerbroventricular cannula

Intracerebroventricular (icv) injections were administered to freely-moving rats via cannula guides which were implanted 3-5 days prior to the experiment. Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and positioned in a stereotaxic apparatus

(David Kopf Instruments, Tujunga, CA) with the incisor bar set at 2.4 mm below the horizontal plane. A 23-gauge stainless-steel guide cannula was implanted 1.4 mm lateral to the bregma and 3.2 mm below the dura mater (Paxinos and Watson, 1986) and anchored to the skull with stainless-steel screws and dental cement. A stainless-steel stylet was used to occlude the guide cannula prior to icv injections. At the time of the experiment, drug or vehicle was injected to freely-moving rats over a period of 1 min in a volume of 5 μl. This volume was delivered with a 10 μl Hamilton microsyringe connected by a 50 cm length of PE-10 tubing to a 30-gauge stainless-steel injector which protruded 1 mm beyond the tip of the guide cannula and into the ventricle. Cannula placement was verified post-mortem in appropriate frozen frontal brain sections with the aid of a dissecting microscope and only those animals with the tip of the cannula tract in the right lateral cerebral ventricle were used in the study.

2. Neurotoxin lesions

5,7-DHT was administered to selectively destroy central 5HT neurons (Baumgarten, 1973). Rats were pretreated with the norepinephrine uptake inhibitor desipramine hydrochloride (25 mg/kg; i.p.) 45 min prior to neurotoxin administration, anesthetized with ketamine hydrochloride (44 mg/kg; i.p.) and xylazine hydrochloride (10 mg/kg; ip), and placed in a stereotaxic frame with the incisor bar set 2.4 mm below the horizontal plane. The needle of a 5 μl Hamilton syringe was inserted into the lateral ventricle with the tip located at coordinates A 0.0 mm, L 1.4 mm , and V -3.2 mm (König and Klippel, 1963) and 5,7-DHT (200 μg/rat) or its vehicle (0.3% saline containing 1% ascorbic acid; 5 μl/rat) were injected over a period of 1 min. The needle

remained in the ventricle for an additional 10 min to prevent fluid reflux. The wound was then sealed with bone wax and surgical clips, and the animals were allowed to recover for seven days. The correct placement of the needle tip was determined in frontal frozen brain sections with the aid of a dissecting microscope, and only those animals with needle tracts terminating in the lateral ventricle were included in the study.

3. Halasz knife lesions

Rats were anesthetized with Equithesin (3 ml/kg; i.p.), placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar 8 mm below the intraaural line. A modified Halasz knife (height, 0.5 mm; radius, 1.5 mm; Halasz and Pup, 1965) was lowered in the midsagittal plane to the base of the skull 1.8 mm posterior to bregma and rotated $\pm$ 90° twice with a stereotaxic micromanipulator (David Kopf Instruments). Sham surgery consisted of lowering the knife 6.0 mm below the surface of the brain 1.8 mm posterior to bregma without rotating the blade. Following the operation the skull was sealed with bone wax and the skin with surgical clips, and the rats were allowed to recover for 7 days before decapitation. Surgical lesions were verified postmortem in frozen brain sections; animals in which knife cuts were not visible lateral and superior to the arcuate nucleus or which damaged the arcuate nucleus were not used (15 % of the animals).

D. Electrical Stimulation

Rats were anesthetized with gamma-butyrolactone (1000 mg/kg; i.p.; Sigma Chemical Co.) positioned in a stereotaxic frame with the incisor bar placed 3.3 mm below the intraaural line. Rats were implanted with bipolar stainless steel electrodes

placed bilaterally in the periventricular nucleus (A - 1.3 mm; L $\pm$ 0.5 mm, V - 8.6 mm relative to bregma, midline and dura, respectively; König and Klippel, 1963). Electrical stimulation consisted of biphasic pulses of 200 μamp intensity, 1 ms duration, applied at 10 Hz frequency for 15 minutes (Model S9 and SD9, Grass Square Wave Stimulators, Quincy, MA), and was continuously monitored with an oscilloscope (Gunnet et al., 1987). Sham stimulation consisted of electrode placement at the same stereotaxic coordinates without application of current. Electrode placement was verified postmortem in frozen brain sections with the aid of a dissecting microscope.

E. IMMUNOHISTOCHEMISTRY AND ANTEROGRADE TRACT TRACING

1. *Phaseolus vulgaris leucoagglutinin* (PHA-L) injections

Rats were anesthetized with Equithesin (3 ml/kg; i.p.) and placed in a stereotaxic frame with the incisor bar set 3.3 mm below the intraaural line. Neuronal and axonal labelling was performed by a modification of the method of Gerfen and Sawchenko (1984). A glass microelectrode having a tip diameter of 15-20 μm was filled with a solution of 2.5 mg% PHA-L (Vector Labs, Burlingame, CA lot # L-1110) dissolved in a solution of 0.05 M phosphate buffered saline (pH 7.4). Injection coordinates were 6.8-6.9 mm anterior and 0.8-0.9 mm above the intra-aural line and 0.2 mm lateral to the midline (Paxinos and Watson, 1986). The injection site was chosen based upon previous anatomical studies (Luppi et al., 1986; Kawano and Daikoku, 1987). PHA-L was iontophoretically injected using a pulsatile square wave positive current of 5 μA intensity (0.5 seconds on; 0.5 seconds off; Midgard, Transkinetics Model CS3) for 20 minutes. The microelectrode was left in place for an additional 10 minutes to minimize spread of

the lectin upon removal from the brain.

2. Tissue preparation

Following surgery the skull was sealed with bone wax, and the skin clamped with surgical clips. The rats were allowed to survive for 14 days at which time they were again anesthetized with Equithesin and perfused transcardially with 80 ml each of the following series of solutions at 4 °C: 1) 0.05 M tris buffered saline (TBS, pH 7.4); 2) 4 mg% paraformaldehyde in 0.1 M acetate buffer (pH 6.5); 3) 4 mg% paraformaldehyde and 0.2 mg% glutaraldehyde in 0.1 M borate buffer (pH 9.5) as described by Bérod (1981). The brains and pituitaries were rapidly removed and post-fixed in the second fixative solution for 1 day. In addition, the pituitaries were placed in a cryoprotectant solution of 20 mg% sucrose in TBS for two days and imbedded in Tissue-Tek (Miles Inc., Elkhart, IN). Frontal sections (50-75 μm thick) through the hypothalamus (beginning at approximately A9220 König and Klippel, 1963) and pituitary were prepared with aid of a Vibratome (series 1000, Technical Products International, St. Louis, MO) and cryostat (- 20 °C, International Equipment Corporation, Model 2488, Needham, MA), respectively, and collected in TBS.

3. PHA-L immunohistochemistry

After three 5 minute washes in 0.5% Triton-X-100 in TBS (TBS-TX), sections to be immunoreacted for PHA-L were preabsorbed with 3% normal rabbit serum for 30 minutes at room temperature. Following three 5 minute washes in TBS-TX, sections were incubated in a solution containing goat anti-PHA-L IgG (Vector Labs, Lot #B0822) at a dilution of 1:2000 in TBS-TX for 48 hours at 4 °C. Prior to incubation in

secondary antiserum, sections were washed for 5 minutes three times in TBS-TX. Sections were then incubated in biotin-conjugated rabbit anti-goat serum diluted to 1:200 in TBS-TX for 2 hours at room temperature, washed three times for 5 minutes with TBS-TX, and incubated in avidin-conjugated HRP (Vectastain Kit # PK-4005, Vector Labs) diluted to 1:1000 in TBS-TX for 90 minutes. After four 5 minute washes with TBS-TX the sections were incubated in a solution containing 100 mg% 3,3'-diaminobenzidine (DAB), 200 mg% ß-d-glucose, 40 mg% NH_4Cl, 38 mg% imidazole, 3 mg% glucose oxidase, 0.2 mg% thimerosal in 0.15 M TBS for 20 to 40 minutes until the background began to darken. The DAB reaction was terminated by four 5 minute washes with TBS-TX.

4. TH immunohistochemistry

TH immunoreactivity was identified using a mouse monoclonal anti-rat TH primary antibody (Incstar, Stillwater, MN) as described previously (Hökfelt et al., 1975). After three 5 minute washes in TBS-TX, sections were preabsorbed with 3 % normal horse serum for 30 minutes at room temperature. Following three 5 minute washes in TBS-TX, sections were incubated in a solution containing mouse anti-TH IgG at a dilution of 1:500 in TBS-TX for 24 hours at 4 °C. Prior to incubation in secondary antiserum, sections were washed three times for 5 minutes in TBS-TX. Sections were then incubated in bioinylated horse anti-mouse serum diluted to 1:400 in TBS-TX for 2 hours at room temperature, washed three times for 5 minutes with TBS-TX and incubated for 90 minutes in either 1:500 avidin-conjugated rhodamine or 1:1000 avidin-conjugated HRP (Vector Labs). Following incubation in avidin-conjugated rhodamine and four 5

minutes washes with TBS-TX, sections were stored in TBS-TX with minimal illumination until mounting and visualization. In addition, the latter sections incubated in avidin-conjugated HRP were washed four times (5 minutes each) with TBS-TX and then incubated in a solution containing 100 mg% DAB, 200 mg% ß-d-glucose, 40 mg% NH_4Cl, 38 mg% imidazole, 3 mg% glucose oxidase, 0.2 mg% thimerosal in 0.15 M TBS for 20 to 40 minutes until the background began to darken. The DAB reaction was terminated by four five minute washes with TBS-TX.

5. Combined PHA-L/TH immunohistochemistry

After three five minute washes in TBS-TX, sections to be immunoreacted for PHA-L and TH were preabsorbed with 3% normal rabbit serum and 3% normal horse serum for 30 minutes at room temperature. Following three 5 minute washes in TBS-TX, sections were incubated in a solution containing 1:2000 goat anti-PHA-L IgG and 1:500 mouse anti-TH IgG (Incstar, Stillwater, MN) in TBS-TX for 48 hours at 4 °C. Prior to incubation in secondary antiserum, sections were washed three times for 5 minutes in TBS-TX, and were then incubated in 1:200 fluorescein isothiocyanate (FITC)-conjugated rabbit anti-goat serum and 1:400 biotin-conjugated horse anti-mouse serum in TBS-TX for 2 hours at room temperature. After three 5 minute washes with TBS-TX, sections were incubated in 1:500 avidin-conjugated rhodamine diluted in TBS-TX for 90 minutes at room temperature. Sections were then washed a final three times for 5 minutes with TBS-TX and stored at 4 °C with minimal illumination until mounting and visualization.

6. Mounting and visualization

After the appropriate treatments, sections were floated onto a gelatin-coated glass slide and allowed to dry overnight. Sections reacted for DAB were counterstained with thionin. All slide-mounted sections were then dehydrated in a graded series of ethanol baths (70, 95, 100%), lipids were removed by rinsing in chloroform for 10 minutes, and glass coverslips were mounted with DPX (Fluka, St. Louis, MO). Axons and injection sites were visualized with either a standard light microscope (Leitz Laborlux 12, Leipzig, Germany) or a fluoromicroscope (Nikon Labophot with Episcopic/Fluorescence Attachment D, Tokyo, Japan) and photographed using Kodak tri-X pan or Ektachrome 400 film, respectively. Where appropriate, axons and injection sites were traced using a drawing tube attached to the light microscope. Detailed drawings were made using either a 40X objective lens or a 100X oil-immersion objective lens at a magnification of 400X and 1250X, respectively and were photographed using Kodak Ortholith high contrast film.

F. STRESS PROCEDURE

On the day of the experiment, non-stressed rats were removed from their home cages and immediately decapitated. Rats that were to be stressed were removed from their home cages and placed in a glass desiccator containing cotton batting saturated with diethylether for 1-3 minutes. These animals lost their righting reflex within 30 s and remained anesthetized for 2-4 min after being removed from the desiccator. While anesthetized, the rats were immobilized in the supine position on the laboratory bench top with adhesive tape for 30 min and then decapitated.

G. TISSUE PREPARATION

Following appropriate treatments, animals were decapitated and trunk blood was collected in tubes containing sodium heparin and 150 μg bacitracin. Brains and pituitaries were quickly removed from the skull and frozen on aluminum foil and glass slides, respectively, placed directly over dry ice. Frontal brain sections (600 μm) beginning at approximately A9220 μm (Kõnig and Klippel, 1963) were prepared in a cryostat (-9 °C) and the median eminence, arcuate and periventricular nuclei were dissected from these sections according to a modification (Lookingland and Moore, 1984) of the method of Palkovits (1973). The intermediate and neural lobes were dissected from the frozen pituitary glands as described previously (Lookingland, et al.,1985). Tissue samples were placed in 60 μl of 0.1 M phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at -20 °C until assayed.

Trunk blood samples were centrifuged (model DPR-6000, International Equipment Co., Needhan, MA) on the day of the experiment at 2000 rpm for 20 min. Plasma was removed, placed in a glass vial containing 100 μl of saturated sodium citrate and stored at - 20 °C until assayed.

H. NEUROCHEMICAL ANALYSES

On the day of the assay, tissue samples were thawed, sonicated for 3 s (Sonicator Cell Disrupter, Heat Systems-Ultrasonics, Plainview, NY) and centrifuged for 1 min in a Beckman Microfuge. Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein (Lowry et al., 1951).

DOPAC, dopamine, 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxy-

tryptamine concentrations in the supernatants were determined by high-performance liquid chromatography with electrochemical detection as described previously (Chapin et al., 1986; Lindley et al., 1990a). Briefly, 50 μl of the supernatant was injected onto a C_{18} reverse phase analytical column (5 μm spheres; 250 x 4.6 mm; Biophase ODS, Bioanalytical Systems, Inc., West Lafayete, IN) which was protected by a precolumn cartridge filter (5 μm spheres; 30 x 4.6 mm). High-performance liquid chromatography mobile phase consisted of 0.05 M sodium phosphate, 0.03 M citric acid buffer adjusted to pH 2.7, with 0.1 mM disodium ethylenediamine-tetraacetic acid, 0.35% sodium octyl sulfate and 25% methanol. Depending on the condition of the column, components of the mobile phase were slightly adjusted to maintain separation of compounds of interest and minimize total retention times (Chapin et al., 1986)

Amine content in the median eminence, arcuate nucleus and periventricular nucleus was detected using an electrochemical detector (LC4A, Bioanylytical Systems, Inc.) equipped with a TL-5 glassy carbon electrode set at a potential of +0.75 V relative to a Ag/AgCl reference electrode. Because neural and intermediate lobes of the pituitary contain lesser amounts of DOPAC and dopamine, a more sensitive electrochemical detection system was used which consisted of a single coulometric electrode in series with dual electrode analytical cells (models #5021 and 5011, respectively, ESA, Bedford, MA). The current signal from the second electrode of the analytical cell was monitored by a Hewlett-Packard 3390A Integrator (Hewlett-Packard, Avondale, PA). Use of coulometric electrodes increases the sensitivity of the measurement of compounds of interest, and with the analytical electrodes set up in an oxidation-reduction mode, only

compounds oxidized at the first electrode and reduced at the second electrode are detected, resulting in an increased selectivity and reduced noise signal (Lindley et al., 1990a).

The amounts of DOPAC, dopamine, 5HIAA and 5-hydroxytryptamine in each sample were determined by comparing peak heights measured by the integrator with those of the standards run on the same day. The lower limit of sensitivity of this assay for DOPAC and dopamine was approximately 2-5 pg per sample; the limit of sensitivity for 5HIAA and 5-hydroxytryptamine was approximately 10-20 pg/sample.

I. RADIOIMMUNOASSAYS FOR PROLACTIN AND αMSH

Prolactin concentrations were measured in plasma by a double antibody radioimmunoassay using procedures and reagents supplied by Drs. A. Parlow and S. Raiti of the National Hormone and Pituitary Program (NIDDK). NIDDK rat prolactin RP-3 was used as the standard. Using a 100 μl aliquot of plasma, the lower limit of sensitivity for prolactin was 0.1 ng/tube, and the intraassay and interassay variations were approximately 12.4 and 14.5%, respectively.

Concentrations of αMSH in plasma were determined using a double antibody radioimmunoassay modified (Lindley et al., 1990a) from a procedure originally described by Penny and Thody (1978). Antisera to αMSH were kindly supplied by Dr. G. Mueller, Uniformed Services University for the Health Sciences, Bethesda, MD. These antisera cross-react on a equimolar basis with des-acetyl-αMSH and diacetyl-αMSH, but do not detect up to 30 ng/tube of any of the following: deaminated αMSH, βMSH, ACTH, ACTH 1-10, ACTH 1-13 or ACTH 1-24, β-endorphin peptides, or β-LPH. Using an

aliquot of 200 μl, the lower limit of sensitivity of this assay was approximately 9.5 pg/tube, and the intra-assay and interassay variabilities were approximately 12.6% and 17.9%, respectively.

J. STATISTICS

Statistical analyses between two groups were conducted using Students's t-test. Statistical analyses among groups were conducted using one-way or two-way analysis of variance and followed by the Least Significant Difference Test or Student-Newman-Keuls Test (Steel and Torrie, 1979). Differences were considered significant if the probability of error was less than 5%.

3. FUNCTIONAL ANATOMY OF DA NEURONS TERMINATING IN THE INTERMEDIATE LOBE OF THE PITUITARY

A. ANATOMICAL EVIDENCE THAT PERIVENTRICULAR DA NEURONS TERMINATE IN THE INTERMEDIATE LOBE OF THE PITUITARY

Introduction

DA neurons terminating in the median eminence, neural lobe and intermediate lobe were originally described as originating from the arcuate nucleus (Björklund et al., 1973). While the common origin of these distinct DA neuronal systems has been widely accepted, several lines of evidence (see chapter 1, section A) suggest that DA neurons terminating in the intermediate lobe may originate from the periventricular nucleus (A_{14} cell group; Björklund and Nobin, 1973) rather than the arcuate nucleus.

The purpose of the studies detailed in this section was to provide direct anatomical evidence that DA neurons situated in the periventricular hypothalamus have axons which terminate in the intermediate lobe of the rat posterior pituitary. To this aim, discrete unilateral injections of the anterograde tracer PHA-L were made into the periventricular nucleus. PHA-L immunoreactive terminal axonal arbors were identified in the pituitary intermediate lobe. In addition, terminal axonal arbors and varicosities containing both PHA-L and TH were identified. For comparison, PHA-L and TH immunoreactivity were also assessed in the periventricular, paraventricular, arcuate nuclei, and the pituitary neural lobe.

Results

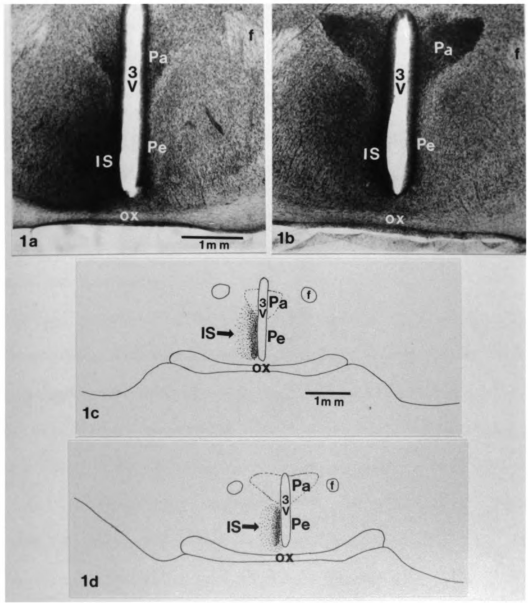
PHA-L Injection Sites

PHA-L injection sites from two animals were confined to and located within the caudal portion of the hypothalamic periventricular nucleus (Figure 3.1 a-d) which is proposed to contain the DA neurons projecting to the intermediate lobe of the pituitary (Luppi et al., 1986; Kawano and Daikoku, 1987). The injection sites measured approximately 300 μm rostrocaudally. Distinct PHA-L-immunoreactive cells were evident at the injection site at a distance approximately 25-75 μm lateral to the third ventricle. The injection sites from these animals did not infiltrate the adjacent arcuate or paraventricular nuclei. An additional injection site was located within the paraventricular nucleus, with little spread into the adjacent periventricular nucleus (Figure 3.4a). This latter injection site served as a useful control due to the well known peptidergic innervation of the pituitary neural lobe which originates in the paraventricular nucleus (Pittman et al., 1981; Silverman, 1983).

PHA-L Terminal Arbors

After traversing the infundibular stalk, numerous PHA-L immunoreactive parent axons gave rise to terminal axonal arbors in the pituitary intermediate lobe (Figure 3.2 a-e) from animals with injection sites within the periventricular nucleus. These highly branched terminal arbors ramified amongst the intermediate lobe melanotrophs and along the ends of these branches (1-3 μm in diameter) were multiple varicosities measuring 1-2 μm in diameter. Terminal arborizations were found in large numbers throughout the intermediate lobe, with a slightly higher density ipsilateral to the injection site.

Figure 3.1. Photomicrographs and corresponding drawings of a PHA-L injection site situated in the hypothalamic periventricular nucleus (Pe) taken from two 50-70 μm thick sections through the hypothalamus at approximately 6.8 (1a, 1c) and 6.9 mm (1b, 1d) anterior to the intra-aural line. These drawings represent the center of the injection site which extends approximately 300 μm rostrocaudally. Pa, paraventricular nucleus; f, fornix; ox, optic chiasm; IS, injection site; 3V, third ventricle. Scale: 1 mm.


f
Pa
3
V
IS
Pe
ox
1a
1mm
1b
1c
1d

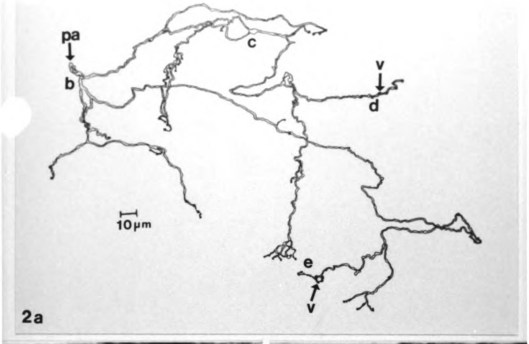
Very few PHA-L immunoreactive axons were found within the pituitary neural lobe. In contrast, numerous PHA-L-immunoreactive terminal axonal arbors with large varicosities (3-5 μm in diameter) were found in the neural lobe, but not the intermediate lobe from animals in which PHA-L was injected into the paraventricular nucleus (Figure 3.4 a-b).

PHA-L/TH Immunoreactivity

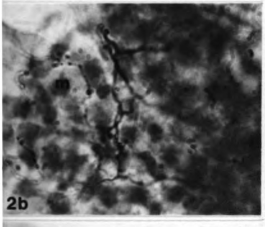
Sections of the intermediate lobe were examined for both TH and PHA-L immunoreactivity to determine if catecholamines are contained within the aforementioned axonal branches and varicosities. As shown in figure 3.3 (a-d), PHA-L immunoreactive branches and varicosities within the intermediate lobe were also immunoreactive for TH. All identified PHA-L positive axonal branches and varicosities in the intermediate lobe were also immunoreactive for TH.

There was no evidence for inappropriate cross-reactions between primary and secondary antibodies. TH-immunoreactive axonal branches were observed in the pituitary intermediate lobe which were not immunoreactive for PHA-L (Figure 3.3 c-d, Figure 3.4 e-f) and could represent either axons of periventricular DA neurons which were not labelled with PHA-L, axons which contain norepinephrine or DA neurons outside of the periventricular nucleus which project to the intermediate lobe. In addition, several PHA-L immunoreactive axonal branches and varicosities were evident in the neural lobe following injections of the lectin into the paraventricular nucleus. These axonal branches and varicosities, however, were not immunoreactive for TH (Figure 3.4 c-d). Taken together, these observations indicate that the primary and secondary antibodies for PHA-L do not bind nonspecifically to TH-immunoreactive axonal branches

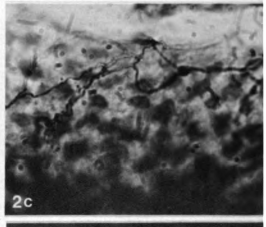
Figure 3.2. A drawing (2a) and representative photomicrographs (2b-2e) of a PHA-L terminal arbor in the pituitary intermediate lobe. The parent axon (pa, 2b) gives rise to highly branched axons (2c) approximately 1-3 μm in diameter and these branches (2d, 2e) contain multiple varicosities (v) 1-2 mm in diameter located near melanotrophs (m). Scale: 10 μm.



2a



2b



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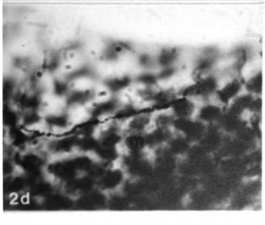
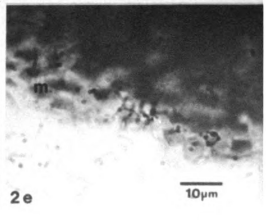


2d



2e

Figure 3.3. Photomicrographs of terminal axonal branches (3a, 3b) and varicosities (3c, 3d) in the pituitary intermediate lobe which demonstrate colocalization of both PHA-L and TH. Arrows labelled b and d (3a and 3c) indicate PHA-L-immunoreactive fibers and varicosities which correspond to TH-immunoreactive fibers and varicosities shown by arrows labelled a and c (3b and 3d). Scale: 10 μm. PHA-L, ***Phaseolus vulgaris*** leucoagglutinin; TH, tyrosine hydroxylase.

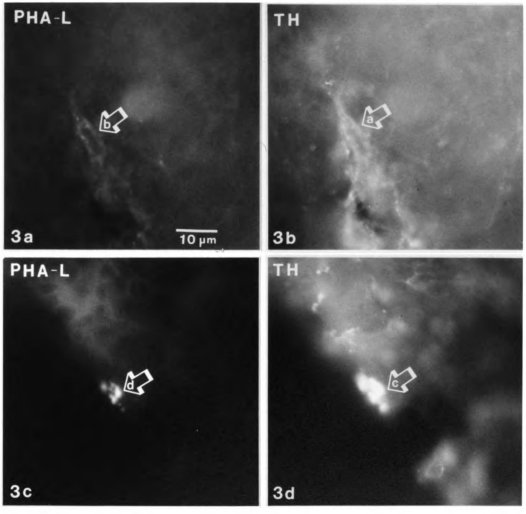
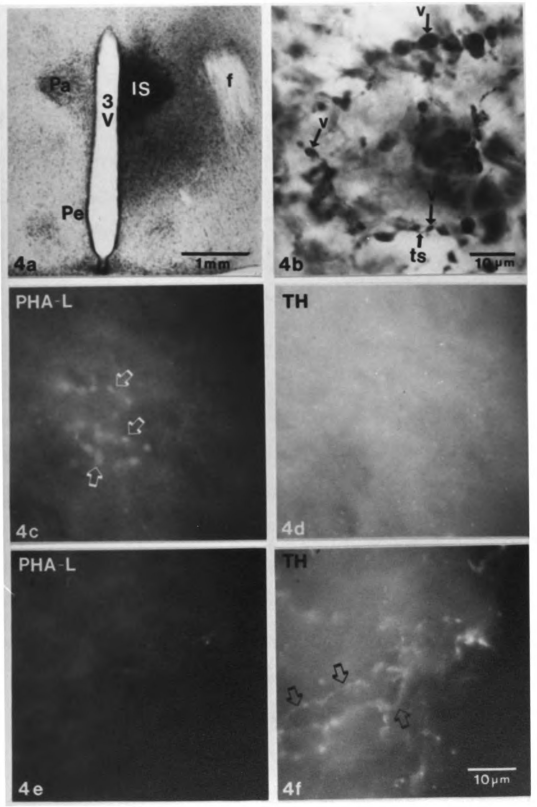

PHA-L
TH
3a
10 µm
3b
PHA-L
TH
3c
3d

Figure 3.4. Photomicrographs of a PHA-L injection site located in the paraventricular nucleus and the corresponding PHA-L- and TH-immunoreactive terminal axonal arbors in the neural and intermediate lobes of the pituitary. 4a, injection site location on a 50-70 μm thick frontal section through the hypothalamus at approximately 6.8 mm anterior to the intra-aural line. PHA-L immunoreactivity extends approximately 400 μm rostrocaudally. 4b, PHA-L immunoreactive terminal arbors in the neural lobe of the pituitary containing large varicosities (v) along terminal strands (ts). 4c, PHA-L immunoreactive terminal axonal arbors and varicosities in the neural lobe that are not immunoreactive for TH (4d). 4f, TH-immunoreactive terminal arbors and varicosities in the intermediate lobe that are not PHA-L-immunoreactive.


Pa
3
V
IS
f
Pe
1mm
4a
v
v
ts
10μm
4b
PHA-L
4c
TH
4d
PHA-L
4e
TH
10μm
4f

and varicosities and that the primary and secondary antibodies for TH do not bind nonspecifically to PHA-L immunoreactive axonal branches and varicosities.

Discussion

Results presented in this section indicate that neurons originating in the hypothalamic periventricular nucleus have axons which arborize in the intermediate lobe but not the neural lobe of the rat pituitary. This conclusion is based upon the observations that discrete injections of PHA-L into the periventricular nucleus result in labelled axonal arbors in the pituitary intermediate lobe and not the neural lobe. Since axons of passage may also transport PHA-L, DA neurons originating outside, but passing through the periventricular nucleus en route to the intermediate lobe cannot be discounted. The observed terminal axonal arbors, however, most likely represent the terminal branches of periventricular DA neurons innervating the intermediate lobe. This hypothesis is consistent with anatomical studies which identified retrogradely labelled perikarya in the periventricular nucleus following injections of HRP into the pituitary posterior lobe (Kelly and Swanson, 1980; Weigand and Price; 1980).

The pituitary intermediate lobe is innervated by neurons containing a variety of neurotransmitters including dopamine, norepinephrine, 5-hydroxytryptamine, GABA, and corticotropin-releasing hormone (for review see Millington and Chronwall, 1988). The morphology of terminal arborizations identified in the present study resembles catecholamine-containing axons and varicosities originally described in the intermediate lobe (Björklund, et al., 1968, Baumgarten et al., 1972). Accordingly, the results of the

present study indicate that neurons projecting from the periventricular nucleus to the intermediate lobe are catecholaminergic since the intermediate lobe fibers anterogradely labelled with PHA-L are also immunoreactive for TH, a marker for catecholamine-synthesizing cells (Hökfelt et al., 1975). It is most likely that these TH-immunoreactive terminal arbors are DA since noradrenergic nerve terminal arbors comprise only a small percentage of the catecholaminergic fibers found in the intermediate lobe (Björklund et al., 1967; Björklund et al., 1970; Baumgarten et al, 1972) and the hypothalamic periventricular nucleus does not contain noradrenergic perikarya (Swanson and Hartman, 1975; Hökfelt et al., 1976). This conclusion is consistent with recent anatomical studies using retrograde tracing (Luppi et al., 1986; Kawano and Daikoku, 1987) which indicate that terminal arbors of DA neurons in the posterior pituitary originate from the periventricular A_{14} cell group rather than the arcuate A_{12} cell group as originally proposed by Björklund and coworkers (1973). The present studies, however, do not rule out the possibility that some DA neurons in the arcuate nucleus project to the intermediate lobe.

The existence of two anatomically distinct DA neuronal systems (i.e. TIDA and THDA neurons) originating from the A_{12} cell group in the arcuate nucleus has long been puzzling. As discussed in Chapter 1.F., there are major differences in the role of these DA neurons in controlling pituitary hormone secretion and in the mechanisms that regulate their activity (for review see Moore, 1987). The differences in regulation and function of these two hypothalamic DA neuronal systems could be reflected in the distinct anatomic segregation of their perikarya.

In conclusion, the results of the present study provide direct anatomical evidence that catecholaminergic neurons in the periventricular nucleus innervate the intermediate lobe of the posterior pituitary. It is not known, however, if these catecholaminergic neurons release dopamine or if they play a role in regulating the secretion of αMSH.

B. FUNCTIONAL AND NEUROCHEMICAL EVIDENCE THAT PERIVENTRICULAR DA NEURONS TERMINATE IN THE INTERMEDIATE LOBE OF THE PITUITARY

Introduction

Results summarized in the previous section (Chapter 3.A.) indicate that catecholaminergic neurons in the periventricular nucleus project to the pituitary intermediate lobe. Although the neurotransmitter contained within these neurons has not been definitively characterized, it is likely that these neurons contain dopamine and correspond to the neurons identified by Björklund and coworkers as the "THDA" neurons (1973). Previous studies have demonstrated that DA neurons terminating in the intermediate lobe tonically inhibit αMSH secretion (Lindley et al., 1988). DA neurons situated in the periventricular nucleus and projecting to the intermediate lobe could, therefore, regulate αMSH secretion.

The purpose of studies in this section was to provide neurochemical and endocrinological evidence that DA neurons in the periventricular nucleus terminate in the intermediate lobe of the posterior pituitary and regulate αMSH secretion. To this aim, concentrations of dopamine and DOPAC in the intermediate lobe and of αMSH in plasma were determined following procedures that disrupt (i.e. surgical isolation of the periventricular nucleus from the posterior pituitary) or activate (i.e. electrical stimulation of the periventricular nucleus) DA neurons. For comparison, dopamine and DOPAC concentrations in the median eminence and prolactin concentrations in plasma were also

determined.

Results

A photomicrograph depicting the location of a Halasz knife cut in the mediobasal hypothalamus and TH-immunoreactive cells of the A_{12} group in the arcuate nucleus is presented in Figure 3.5. The lesion was placed rostral and dorsolateral to the arcuate nucleus so as to disrupt axonal projections from periventricular A_{14} DA neurons to the mediobasal hypothalamus, while sparing those of A_{12} DA neurons in the arcuate nucleus. Seven days following the knife cut, there was a 50% reduction in dopamine concentrations in the intermediate lobe (Figure 3.6), and this was accompanied by an increase in plasma αMSH concentrations (Table 3.1). In contrast, mediobasal hypothalamic lesions had no effect on dopamine concentrations in the median eminence (Figure 3.6) or prolactin concentrations in the plasma (Table 3.1). These results reveal that at least one-half of all the DA neurons terminating in the intermediate lobe originate from regions rostral and dorsal to the arcuate nucleus. Furthermore, these results indicate that DA neurons terminating in the median eminence originate entirely from A_{12} DA neurons in the arcuate nucleus.

Depletion of dopamine concentrations in the intermediate lobe following the knife cut was accompanied by a comparable depletion of DOPAC concentrations such that the ratio of DOPAC/dopamine (or the amount of dopamine metabolized relative to that stored within neurons) was not altered (Figure 3.6). Since changes in DOPAC/dopamine ratio are indicative of alterations in the activity of DA neurons that terminate in the

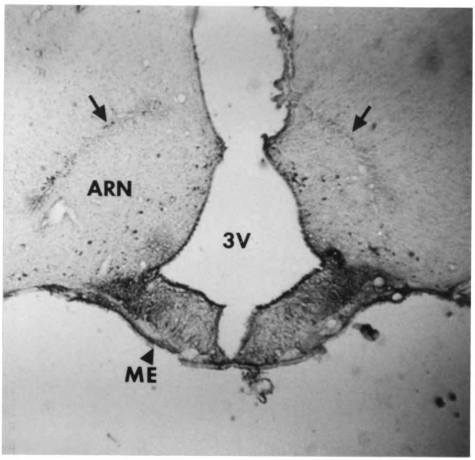


Figure 3.5. Photomicrograph depicting the caudal location of a Halasz knife cut lesion (arrows) in the mediobasal hypothalamus and TH-immunoreactive cells in the arcuate nucleus. The knife extends 1.5 mm rostrally in the midline position. Non-reactive cells were stained with thionin. Magnification, 40X. Abbreviations: ARN, arcuate nucleus; ME, median eminence; 3V, third ventricle.

Table 3.1. Effects of mediobasal hypothalamic lesions on αMSH and prolactin concentrations in the plasma of male rats.

	Sham	Lesion
αMSH (pg/ml)	103.3 ± 11.8	311.5 ± 35.7 *
prolactin (ng/ml)	2.6 ± 0.8	3.2 ± 0.8

Rats received a knife cut or sham surgery and were killed by decapitation 7 days later. Values represent means ± 1 S.E. of 6-8 determinations of αMSH and prolactin in the plasma from sham or lesioned rats. *, values in lesioned rats that are significantly different from sham-lesioned controls ($p < 0.05$)n

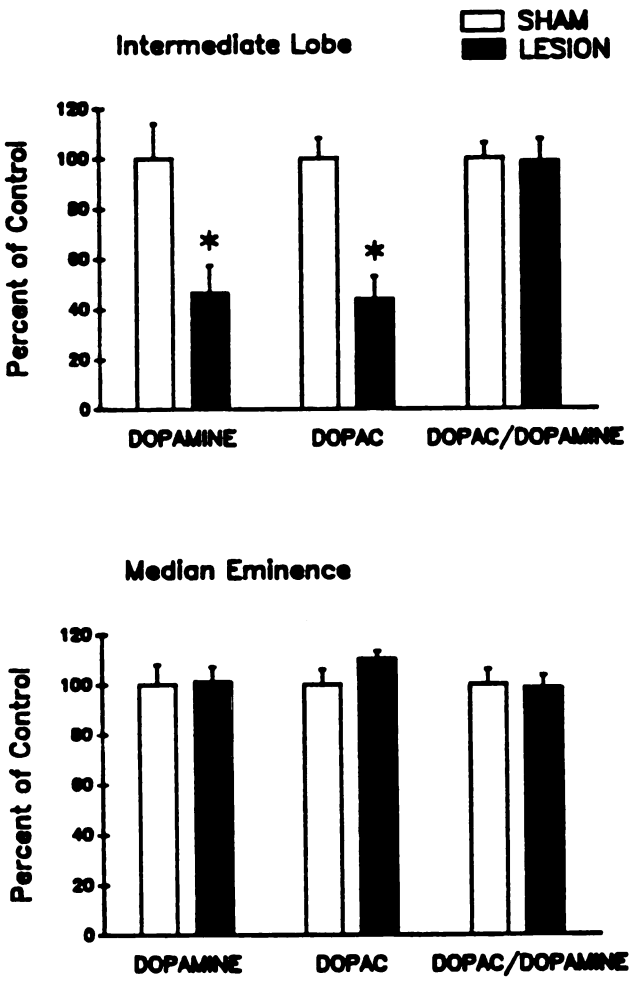


Figure 3.6. Effects of mediobasal hypothalamic lesions on the concentrations of DOPAC, dopamine, and the ratio of DOPAC/dopamine in the intermediate lobe (top) and median eminence (bottom) of male rats. Rats received a knife cut (closed bars) or sham surgery (open bars) 7 days prior to decapitation. Columns represent means and vertical lines 1 S.E. of 7-9 determinations of DOPAC and dopamine concentrations and the ratio of DOPAC/dopamine expressed as a percentage change from sham-treated control values. Control values for intermediate lobe dopamine, DOPAC, and DOPAC/dopamine are 15.3 $\pm$ 2.1 ng/mg protein, 1.00 $\pm$ 0.08 ng/mg protein, and 0.065 $\pm$ 0.004, respectively. Control values for median eminence dopamine, DOPAC, and DOPAC/dopamine are 102.3 $\pm$ 8.2 ng/mg protein, 15.3 $\pm$ 1.0 ng/mg protein, and 0.154 $\pm$ 0.010, respectively. *, Values that are significantly different from sham-treated controls ($p < 0.05$).

intermediate lobe (Lindley et al., 1990a), these results demonstrate that following disruption of DA innervation, there is no compensatory change in the activity of remaining unlesioned DA neurons that terminate in the intermediate lobe.

To determine whether periventricular DA neurons provide functional input to the intermediate lobe, the ratio of DOPAC/dopamine in the intermediate lobe and concentrations of αMSH in the plasma were determined following activation of these neurons by electrical stimulation. As shown in Figure 3.7, bilateral stimulation of the periventricular nucleus increased the ratio of DOPAC/dopamine in the intermediate lobe and decreased plasma αMSH concentrations, but had no effect on the ratio of DOPAC/dopamine in the median eminence or concentrations of prolactin in plasma. These results provide correlative neurochemical and endocrinological evidence that DA neurons originating in or projecting through the periventricular nucleus of the rat hypothalamus terminate in the intermediate lobe of the posterior pituitary. Furthermore, these results indicate that these DA neurons regulate the secretion of αMSH from intermediate lobe melanotrophs, but have no effect on the secretion of prolactin from the anterior pituitary.

Discussion

The results of experiments described in this section indicate that DA neurons originating in or projecting through the periventricular nucleus provide functional innervation to the intermediate lobe of the rat pituitary. This conclusion is based upon observations that surgical isolation of the periventricular nucleus from the mediobasal

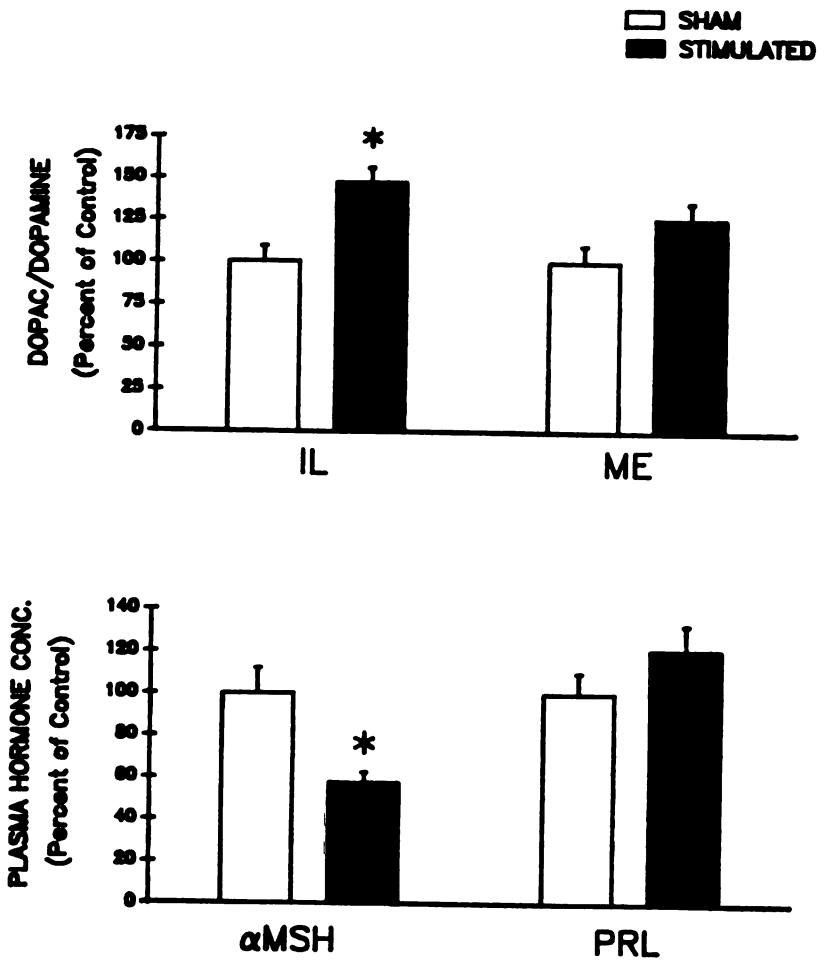


Figure 3.7. Effects of electrical stimulation of the periventricular nucleus on the ratio of DOPAC/dopamine in the intermediate lobe and median eminence, and the concentration of αMSH and prolactin in the plasma of male rats. Rats were anesthetized with gamma-butyrolactone (1000 mg/kg; i.p.; 45 min prior to decapitation), placed in a stereotaxic frame, and implanted with coaxial electrodes into the periventricular nucleus. The periventricular nucleus was stimulated bilaterally and rats were killed by decapitation 15 min later. Sham-stimulated rats were killed 15 min after electrode placement. Columns represent means and vertical lines 1 S.E. of 7-9 determinations of the ratio of DOPAC/dopamine, PRL, and αMSH concentrations in electrically-stimulated rats (solid columns) expressed as a percentage of sham-stimulated control values (open columns). Control values for DOPAC/dopamine were 0.066 ± 0.006 for the intermediate lobe and 0.060 ± 0.006 for the median eminence. Control values for αMSH and PRL were 608 ± 36 pg/ml and 24.5 ± 3.6 ng/ml plasma, respectively. *, Values that are significantly different from sham-stimulated controls ($p < 0.05$).

hypothalamus produces a 50% decrease in DOPAC and dopamine in the intermediate lobe, and electrical stimulation of this region increases the activity of DA neurons that terminate in the intermediate lobe (i.e. as estimated from the DOPAC/dopamine ratio) and decreases the secretion of αMSH. These conclusions are consistent with anatomical evidence (above; Kawano and Daikoku, 1987) indicating that terminal axons of DA neurons in the intermediate lobe originate from the periventricular A_{14} cell group rather than the arcuate A_{12} cell group as originally proposed by Björklund and coworkers (Björklund et al., 1973).

The results in this section also reveal that as much as 50% of the DA innervation to the intermediate lobe may originate from within the mediobasal hypothalamus. Although it is possible that A_{12} DA neurons are a source of the unlesioned DA neurons following the knife cut, this conclusion is not consistent with previous studies which exclude the arcuate nucleus as a source of DA neurons innervating the posterior pituitary (Kawano and Daikoku, 1987; Kelly and Swanson, 1980; Weigand and Price, 1980; Dawson et al., 1985; Davis et al., 1984; see chapter 1.B). Considering this, in the present study it is likely that knife cut lesions did not disrupt all of the periventricular DA neurons innervating the intermediate lobe (i.e., A_{14} periventricular DA neurons located caudal or ventral to the knife cut).

Intermediate lobe DA neurons do not appear to be important in regulating PRL secretion. Plasma prolactin concentrations are unaltered by procedures which disrupt or activate DA neurons in the intermediate lobe. These data do not agree with reports that dopamine from the posterior pituitary regulates the secretion of prolactin (Peters et al.,

1981, review Ben-Jonathan, 1991). This discrepancy may reflect differences in protocol since the latter findings were based upon posterior pituitary lobectomized animals and *in vitro* culture systems.

In conclusion, neurochemical and endocrinological evidence indicates that A_{14} DA neurons in the periventricular nucleus terminate in the intermediate lobe of the posterior pituitary and regulate the secretion of αMSH. On the basis of these results and supporting anatomical studies (Section 3.A.; Kawano and Daikoku, 1987), it is proposed that these DA neurons be referred to as a "periventricular-hypophysial" DA (PHDA) neuronal system (see Figure 3.8) which better reflects the sites of origin and termination of these neurons than the heretofore designation of THDA neurons.

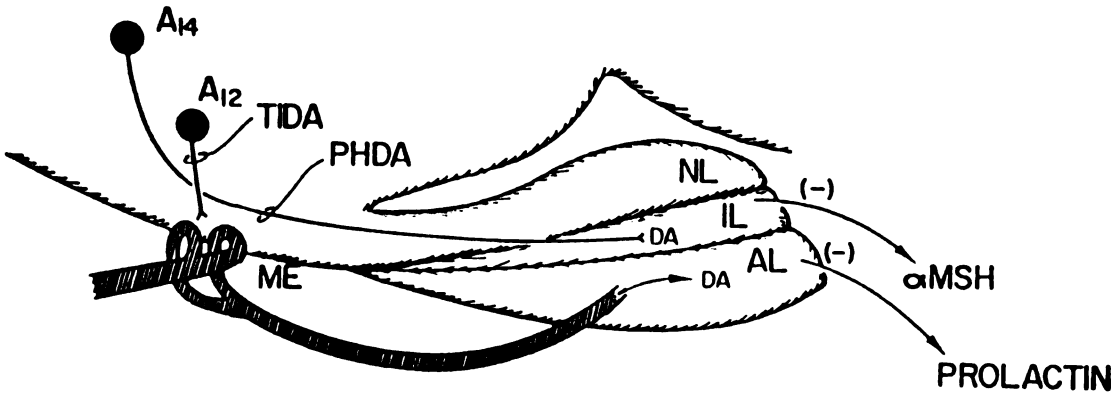


Figure 3.8. Parasagittal section through the mediobasal hypothalamus and pituitary of the rat illustrating schematically the location of axonal projections of A_{12} and A_{14} DA neurons, and their role in the regulation of prolactin and αMSH secretion. Abbreviations: AL, anterior lobe; IL, intermediate lobe; ME, median eminence; NL, neural lobe; PHDA, periventricular-hypophysial dopaminergic neurons; TIDA, tuberoinfundibular dopaminergic neurons.

4. AFFERENT REGULATION OF PHDA NEURONS: 5HT

A. 5-HT_2 RECEPTOR-MEDIATED REGULATION OF BASAL PHDA NEURONAL ACTIVITY AND THE SECRETION OF αMSH

Introduction

5HT neurons originating in the midbrain raphe nuclei make direct synaptic contact with catecholamine neurons in the mediobasal hypothalamus (Bosler et al., 1984) and participate in the neuroendocrine regulation of pituitary hormone secretion (for review see Van de Kar, 1991). 5HT releasing agents and post-synaptic receptor agonists stimulate prolactin secretion (Clemens et al., 1978; Van de Kar et al., 1989a; Pan and Tai, 1992; Jorgensen, et al. 1993), and these stimulatory effects could be mediated by inhibiting TIDA neurons or by stimulating neurons containing prolactin-releasing factors such as vasoactive intestinal polypeptide. For example, 5HT agonists decrease the activity of hypothalamic DA neurons (Lynch et al., 1984). In addition, 5HT neurons, by inhibiting TIDA neurons, are necessary for the stress-induced release of prolactin in female rats (Demarest et al., 1985). On the other hand, 5HT neurons stimulate the secretion of prolactin by increasing the release of a prolactin releasing factor in the absence of a change in TIDA neuronal activity (Clemens et al., 1978; Pilotte and Porter, 1981; Ohta et al., 1985). The 5HT stimulation of prolactin secretion appears to be mediated by several 5HT-receptor subtypes including the $5HT_{1b}$, $5HT_{1c}$, $5HT_2$ and possibly $5HT_3$ receptors (Quattrone et al., 1981; Van de Kar et al., 1989b; Jorgensen et

al., 1992; Jorgensen et al., 1993).

In contrast, little is known about the 5HT regulation of either PHDA neuronal activity or αMSH secretion. Administration of 5HT precursors or agonists stimulate the secretion of other POMC peptides such as ß-endorphin and ß-LPH (Sapun, et al., 1981; Sapun-Malcolm, et al., 1983). Recently, Carr and coworkers (1992) have shown that nonselective 5HT agonists (e.g., MK-212) increase the secretion of αMSH *in vivo*. 5-Hydroxytryptamine, however, has only a mild stimulatory effect on αMSH secretion from melanotrophs *in vitro* (Randle, et al., 1983b). This suggests that the stimulatory effects of 5HT agents on αMSH secretion have a central component.

The purpose of studies in this section was to examine 5HT receptor-mediated regulation of basal PHDA neuronal activity. To this end, the effects of the $5HT_{2/1c}$ agonist DOI (Glennon et al., 1992) were examined on DOPAC concentrations in the intermediate lobe and αMSH secretion. For comparison, the effects of DOI on DOPAC concentrations in the median eminence and PRL secretion were also examined.

Results

The effects of the $5HT_{2/1c}$ agonist DOI (Glennon et al., 1992) on concentrations of αMSH and prolactin in plasma and of DOPAC in the intermediate lobe of the pituitary and the median eminence are summarized in Figure 4.1. Acute administration (30 min; s.c.) of DOI produced a dose-dependent increase in plasma concentrations of both αMSH and prolactin; this was accompanied by a reduction in DOPAC concentrations in the intermediate lobe of the pituitary but not the median eminence. The same general pattern

of effects was observed when the time course of the effects of DOI (300 μg/kg) was determined. Results presented in Figure 4.2 reveal that the DOI-induced increase in αMSH concentrations in plasma and decrease in DOPAC concentrations in the intermediate lobe of the pituitary occurred promptly (within 30 min) and lasted for at least 1 hr. The results presented in Figure 4.3, obtained from the same animals depicted in Figure 4.2, indicate that DOI caused a rapid (within 15 min) increase in circulating levels of prolactin that are sustained for 60 but returned to values similar to control by 120 minutes. These results indicate that activation of either $5HT_2$ or $5HT_{1c}$ receptors decreases the activity of PHDA, but not TIDA neurons and increases αMSH and PRL secretion.

The results of experiments designed to determine the postsynaptic 5HT receptor subtypes (i.e., $5HT_2$ versus $5HT_{1c}$ mediating the effects of DOI are summarized in Figures 4.4 and 4.5. Pretreatment with the $5HT_{2/1c}$ antagonist ritanserin (Leysen, et al., 1985; Pierce, et al., 1992) blocked the effects of DOI on plasma concentrations of αMSH and prolactin as well as DOPAC concentrations in the intermediate lobe of the pituitary (Figure 4.4). On the other hand, pretreatment with the selective $5HT_2$ antagonist, MDL-11,939 (Dudley, et al., 1988; Pierce et al., 1992) blocked the effects of DOI on plasma αMSH concentrations and intermediate lobe DOPAC concentrations but failed to alter the DOI-induced increase in plasma concentrations of prolactin (Figure 4.5). Administration of either ritanserin or MDL-11,939 did not alter basal plasma concentrations of αMSH and prolactin or DOPAC concentrations in the intermediate lobe of the pituitary and the median eminence.

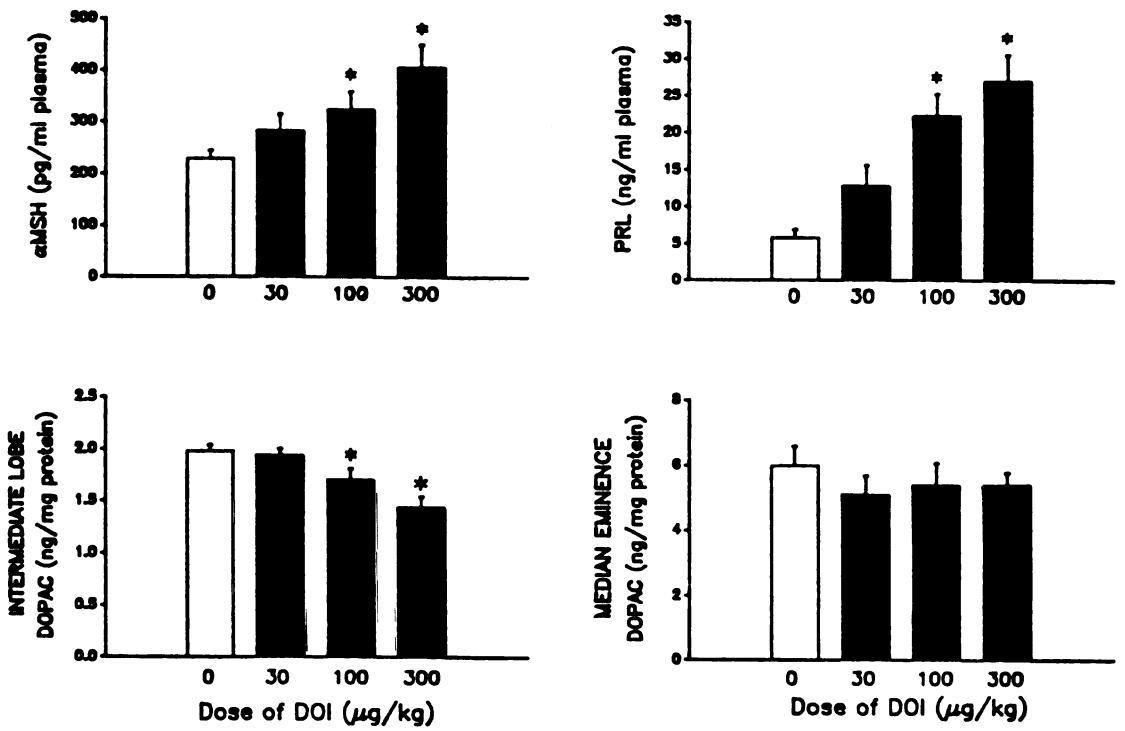


Figure 4.1. Dose-response effects of DOI on plasma αMSH and prolactin concentrations (TOP) and on intermediate lobe and median eminence DOPAC concentrations (BOTTOM). Rats were injected with DOI (30, 100, 300 μg/kg; s.c.) or its water vehicle (1 ml/kg) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH and prolactin concentrations and of DOPAC concentrations in the intermediate lobe and median eminence of 7-9 rats. *, Values from rats treated with DOI (solid columns) which are significantly different ($P < 0.05$) from water-treated controls (open columns).

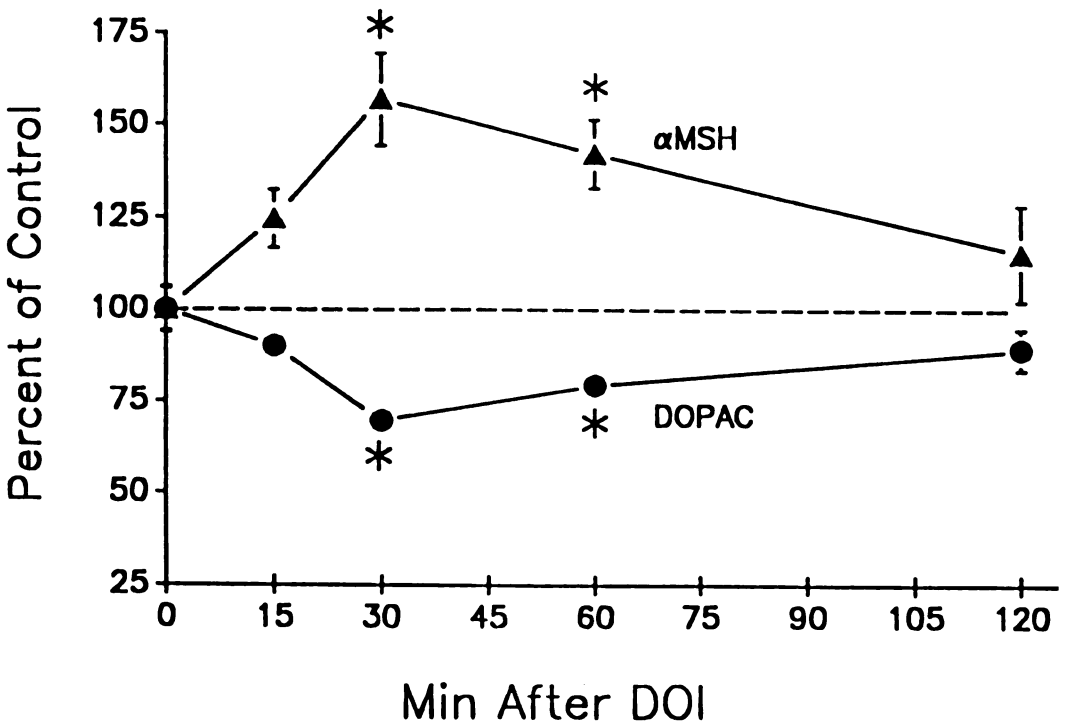


Figure 4.2. Time course effects of DOI on plasma αMSH and intermediate lobe DOPAC concentrations. Rats were injected with DOI (300 μg/kg; s.c.) 15, 30, 60, or 120 min prior to decapitation. Zero-time control rats were injected with water (1 ml/kg; s.c.) 30 min prior to decapitation. Symbols represent means and vertical lines ± 1 S.E. of intermediate lobe DOPAC (●) and plasma αMSH (▲) concentrations of 7-9 rats expressed as a percentage of zero-time control values. Where no vertical lines are depicted 1 S.E. is less than the radius of the symbol. Control values for intermediate lobe DOPAC and plasma αMSH concentrations were 1.78 ± 0.11 ng/mg protein and 239 ± 14 pg/ml plasma, respectively. *, Values from rats treated with DOI which are significantly different ($P < 0.05$) from zero time control.

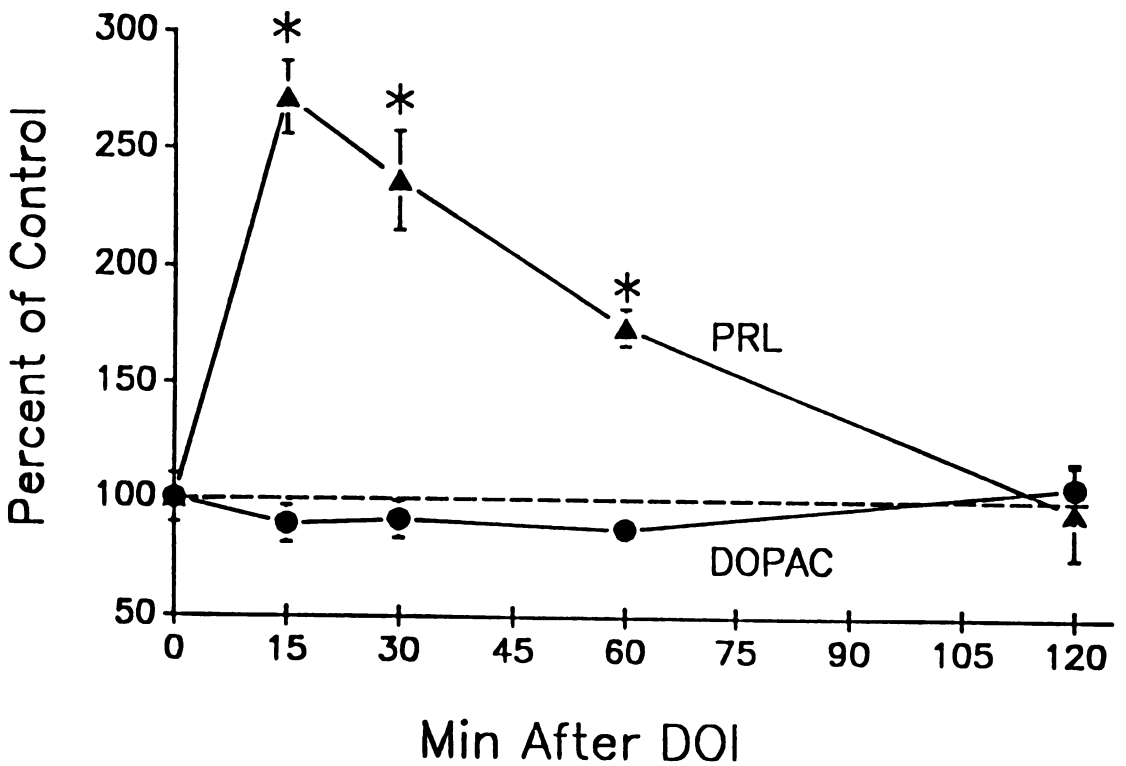


Figure 4.3. Time course effects of DOI on plasma prolactin and median eminence DOPAC concentrations. Rats were injected with DOI (300 μg/kg; s.c.) 15, 30, 60, or 120 min prior to decapitation. Zero time control rats were injected with water (1 ml/kg; s.c.) 30 min prior to decapitation. Symbols represent means and vertical lines $\pm$ 1 S.E. of median eminence DOPAC (●) and plasma prolactin (▲) concentrations of 7-9 rats expressed as a percentage of zero-time control values. Where no vertical lines are depicted 1 S.E. is less than the radius of the symbol. Control values for median eminence DOPAC and plasma prolactin concentrations were 10.0 $\pm$ 0.07 ng/mg protein and 11.4 $\pm$ 1.2 ng/ml plasma, respectively. *, Values from rats treated with DOI which are significantly different ($P < 0.05$) from zero time control.

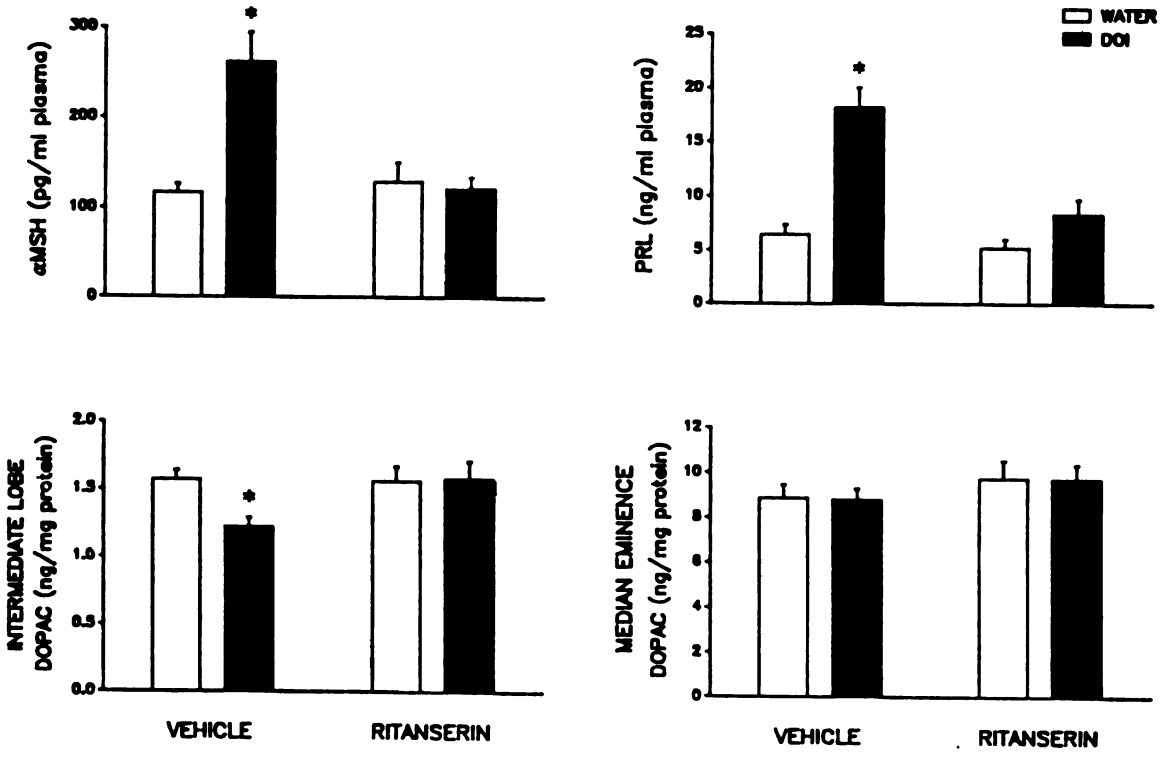


Figure 4.4. Effects of DOI on plasma αMSH and prolactin concentrations (TOP) and on DOPAC concentrations in intermediate lobe and median eminence (BOTTOM) of ritanserin-pretreated rats. Animals were injected with ritanserin (1.0 mg/kg; i.p.) or its 0.1% tartaric acid vehicle (1 ml/kg; i.p.) 90 min prior to decapitation, and with DOI (300 μg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH and prolactin concentrations and of DOPAC concentrations in the intermediate lobe and median eminence of 7-9 rats. *, Values from DOI-treated animals that are significantly different ($P < 0.05$) from water-treated controls.

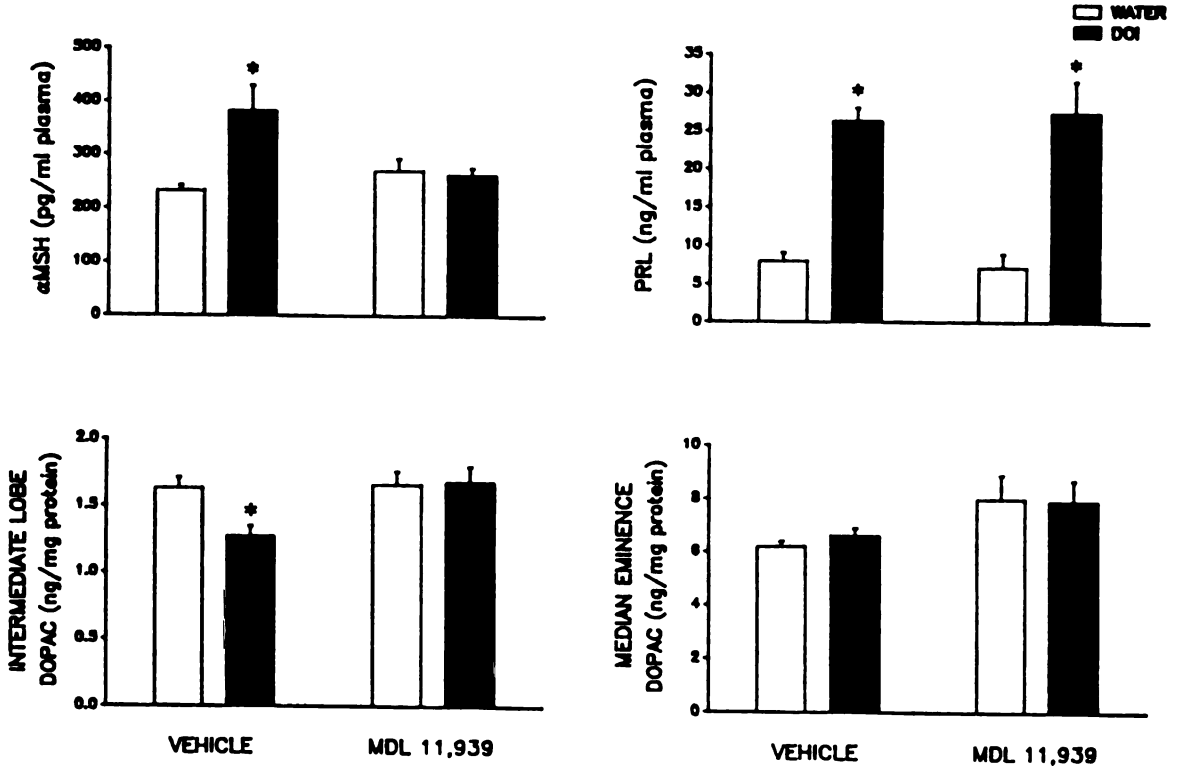


Figure 4.5. Effects of DOI on plasma αMSH and prolactin concentrations (TOP) and on DOPAC concentrations in the intermediate lobe and median eminence (BOTTOM) of MDL-11,939-pretreated rats. Animals were injected with MDL-11,939 (1.0 mg/kg; i.p.) or its 0.01 N HCl vehicle (1 ml/kg; i.p.) 60 min prior to decapitation, and with DOI (300 μg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH and prolactin or intermediate lobe and median eminence DOPAC concentrations of 7-9 rats. *, Values from DOI-treated animals that are significantly different ($P < 0.05$) from water-treated controls.

Discussion

The results of studies presented in this section reveal that $5HT_{2/1c}$ agonist DOI increases secretion of αMSH from melanotrophs in the intermediate lobe. This conclusion is based on the observation that DOI administration produces a dose- and time-dependent increase in plasma αMSH concentrations. These results are consistent with the report by Carr, et. al. (1991) that non-selective 5HT agonists stimulate αMSH secretion *in vivo*. In addition to increasing αMSH secretion, DOI produces a corresponding dose- and time-dependent decrease in intermediate lobe DOPAC concentrations. This suggests that the DOI-induced secretion of αMSH is related to the decrease in the activity of PHDA neurons which tonically inhibit secretion of POMC-derived hormones from melanotrophs. DOI may stimulate melanotroph secretion directly, but since 5HT agonists exert only modest stimulatory effects on melanotroph secretion *in vitro* (Randle, 1983b) an inhibitory action on the PHDA neurons that tonically regulate melanotroph secretion appears to be reasonable.

Results from studies in this section also indicate that the effects of DOI on αMSH secretion and PHDA neuronal activity are mediated by activation of post synaptic $5HT_2$ receptors. Pretreatment with both the $5HT_{2/1c}$ antagonist ritanserin and the $5HT_2$ antagonist MDL-11939 blocks both the DOI-induced increase in αMSH secretion and the associated decrease in PHDA neuronal activity when given at a doses and times reported to block central $5HT_2$ receptors (Dudley, et al., 1988; Leysen, 1985). The effects of $5HT_2$ receptor activation on PHDA neuronal activity is likely to be indirect since activation of the $5HT_2$ receptor depolarizes neuronal membranes and would be expected

to excite, not inhibit a post synaptic cell (McCall and Aghajanian, 1980). An intervening inhibitory neuron, therefore, could be mediating the inhibitory effects of $5HT_2$ receptor activation on PHDA neurons.

In comparison, data in this section also reveal that DOI stimulates prolactin secretion from lactotrophs in the anterior pituitary. Consistent with reports by other investigators (Pan and Tai, 1992), DOI produces a dose- and time-dependent increase in plasma prolactin concentrations. The DOI-induced increase in plasma prolactin concentrations, however, is not associated with a decrease in median eminence DOPAC concentrations. This suggests that DOI stimulates prolactin secretion by a mechanism which is independent of an inhibition of TIDA neuronal activity. This finding supports the proposal that 5-hydroxytryptamine increases prolactin secretion by stimulating the release of a prolactin-releasing factor from the hypothalamus (Clemens et al., 1979; Ohta et al., 1985).

Furthermore, it appears that the stimulatory effects of DOI on prolactin secretion may be mediated by the post-synaptic $5HT_{1c}$ receptor. Pretreatment with the $5HT_{2/1c}$ antagonist ritanserin blocks the DOI-induced increase in plasma prolactin concentrations, while the more selective $5HT_2$ antagonist was without effect. This indicates that the DOI-induced increase in prolactin secretion is not mediated by the $5HT_2$ receptor. Given that DOI has equal affinity for the $5HT_{1c}$ and $5HT_2$ receptors (Glennon, et al., 1992), it may be inferred that the DOI-induced increase in prolactin secretion is mediated by $5HT_{1c}$ receptor activation. Unfortunately, the lack of a selective $5HT_{1c}$ antagonist (Hoyer, 1988) precludes a definitive confirmation of this proposal.

Other studies report that the stimulatory effects of DOI and other 5HT agonists on prolactin secretion are mediated, in part, by $5HT_2$ receptor activation (Van de Kar et al., 1989b; Pan and Tai, 1992; Jorgensen et al., 1992; Jorgensen et al., 1993). The antagonists employed in these studies, however, have appreciable affinity (Ki) for both the $5HT_2$ and $5HT_{1c}$ receptors and do not differentiate $5HT_2$ versus $5HT_{1c}$ mediated drug effects. MDL-11,939, in contrast, is relatively selective for the $5HT_2$ receptor (Dudley et al., 1988). This compound has a Ki of 12.2 nM in binding assays in rat brain cortical membranes and compares favorably with other potent $5HT_2$ antagonists. In addition, MDL 11,939 offers an advantage over traditional $5HT_2$ antagonists such as ketanserin and ritanserin since it has relatively low affinity for the $5HT_{1C}$ receptor (the ratio of the Ki for $5HT_{1C}/5HT_2$ is 160 for MDL 11,939, 20 for ketanserin, and 0.5 for ritanserin, Pierce et al., 1992). Although the results of studies in this section clearly indicate that $5HT_2$ receptor activation is not involved in the stimulatory effects of DOI on prolactin secretion, $5HT_2$ receptors may play a minor role in the response to other 5HT agonists or releasing agents.

In summary, the results from studies described in this section reveal that DOI, acting via $5HT_2$ receptors, inhibits PHDA neuronal activity and increases the secretion of αMSH. In contrast, DOI does not alter TIDA neuronal activity, but does stimulate prolactin secretion presumably by activation of $5HT_{1c}$ receptors.

B. $5HT_2$ RECEPTORS MEDIATE THE EFFECTS OF STRESS ON THE ACTIVITY OF PHDA NEURONS AND THE SECRETION OF α-MSH

Introduction

Supine restraint stress decreases the activity of PHDA neurons and increases αMSH secretion (Lookingland, et al., 1991), but little is known about the mechanisms underlying this effect. There is evidence to suggest that 5HT neurons may be involved in the inhibitory effects of stress on hypothalamic DA neuronal activity; for example, 5HT neuronal activity increases within the mediobasal hypothalamus following stressful manipulations (Houdouin et al., 1991; Johnston and Negro-Villar, 1986), and non-selective 5HT antagonists block the stress-induced decrease in TIDA neuronal activity and the resulting increase in plasma concentrations of prolactin (Demarest et al., 1985). In addition, administration of 5HT agonists inhibits the activity of PHDA neurons and increases the secretion of αMSH (Section 4.A.; Carr et al., 1991). The role of 5HT neurons in the stress-induced inhibition of PHDA neurons, however, is unknown.

The purpose of studies in this section was to determine the role of 5HT neurons and receptor subtypes in mediating the effects of stress on the activity of PHDA neurons and αMSH secretion. To this end, the acute effects of supine restraint stress were examined following experimental procedures that disrupt 5HT neurons or selectively block postsynaptic 5HT receptors. PHDA neuronal activity was estimated by measuring concentrations of the dopamine metabolite DOPAC in the intermediate lobe of the posterior pituitary whereas the activity of 5HT neurons projecting to the arcuate and

periventricular nuclei of the rat hypothalamus was estimated by measuring the concentrations of 5HIAA (Shannon et al., 1986).

Results

To determine if central 5HT neurons play any role in the stress-induced inhibition of PHDA neuronal activity and the secretion of αMSH, animals were pretreated with 5,7-DHT so as to destroy central 5HT neurons (Baumgarten et al., 1973). Seven days after icv injection of this neurotoxin the concentrations of 5-hydroxytryptamine, but not of norepinephrine or dopamine were reduced in the arcuate and periventricular nuclei of the hypothalamus (Table 4.1). These neurochemical data reveal the selective destruction of 5HT neurons terminating in these representative hypothalamic regions.

Vehicle and 5,7-DHT-pretreated rats were subjected to brief exposure to diethylether followed by 30 min of supine restraint. In the vehicle-treated group, this stressful manipulation decreased DOPAC concentrations in the intermediate lobe and increased αMSH concentrations in the plasma (Figure 4.6). In the 5,7-DHT-pretreated group, basal concentrations of DOPAC in the intermediate lobe and αMSH in the plasma of non-stressed (SHAM) rats were unaltered. On the other hand, prior administration of 5,7-DHT blocked the effects of stress on both intermediate lobe DOPAC concentrations and plasma αMSH concentrations.

The effects of stress on PHDA neuronal activity and αMSH secretion were also examined following inhibition of 5HT neuronal activity with the administration of the $5HT_{1A}$ agonist 8-OH-DPAT (Hjorth, et al., 1982; Hjorth and Magnussun, 1988).

TABLE 4.1. Effects of 5-7-DHT administration on 5-hydroxytryptamine, norepinephrine and dopamine concentrations in the arcuate (ARN) and periventricular (PeVN) nuclei of the rat hypothalamus

	ARN		PeVN	
	CONTROL	5,7 DHT	CONTROL	5,7-DHT
5-Hydroxytryptamine	2.2 ± 0.1	ND	4.0 ± 0.3	ND
Norepinephrine	11.4 ± 0.4	12.0 ± 0.4	26.2 ± 1.4	35.0 ± 3.3
Dopamine	4.5 ± 0.5	4.0 ± 0.8	4.3 ± 0.2	4.2 ± 0.2

Rats were pretreated with desipramine (25 mg/kg; ip) 45 min prior an injection of either 5,7-DHT (200 μg/rat; icv) or its saline vehicle (5 μl/rat; icv) and were decapitated 7 days later. Values represent means ± 1 S.E. (n= 6-9) of 5-hydroxytryptamine, norepinephrine and dopamine concentrations (ng/mg protein). ND, values below the level of sensitivity of the neurochemical assay (< 20 pg/sample, or < 0.4 ng/mg for the ARN and < 0.6 ng/mg for the PeVN).

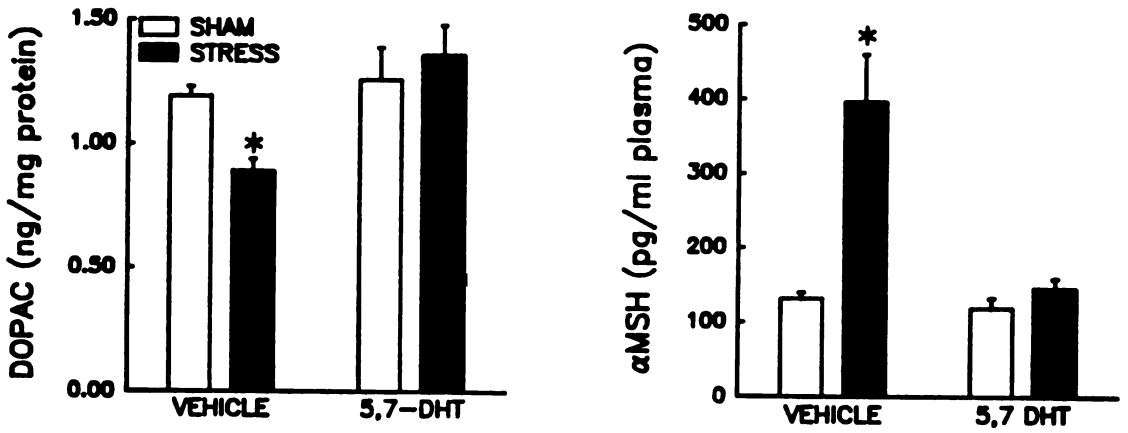


Figure 4.6. Effect of 5,7-DHT administration on stress-induced changes in DOPAC concentrations in the intermediate lobe and plasma αMSH concentrations. Female rats were pretreated with desipramine (25 mg/kg; ip) 45 min prior to injection of either 5,7-DHT (200 μg/rat; icv) or its 0.9% saline vehicle (5 μl/rat; icv) and were decapitated 7 days later. On the day of the experiment animals were removed from their home cages and immediately decapitated (SHAM) or were briefly anesthetized with diethylether, restrained in the supine position for 30 min and then decapitated (STRESS). Columns represent means and vertical lines + 1 S.E. (n= 6-9) of DOPAC and αMSH concentrations. *, values from stressed animals that are significantly different ($p < 0.05$) from sham controls.

Administration of 8-OH-DPAT at a dose and time which suppresses the firing of raphe 5HT neurons (Hjorth et al., 1982) decreased concentrations of the 5-hydroxytryptamine metabolite 5HIAA in the arcuate and periventricular nuclei (Figure 4.7). The concentrations of 5HIAA in animals treated with 8-OH-DPAT may reflect basal metabolism of cytoplasmic 5-hydroxytryptamine which occurs in the absence of 5HT neuronal activity (Lookingland et al., 1986). 8-OH-DPAT administration did not alter basal concentrations of DOPAC in the intermediate lobe or αMSH in plasma of non-stressed (SHAM) rats (Figure 4.8). Consistent with the results in 5,7-DHT lesioned rats, 8-OH-DPAT pretreatment blocked the stress-induced decrease in intermediate lobe DOPAC concentrations and the concomitant increase in plasma αMSH concentrations.

As shown in Figure 4.9, the $5HT_2$ antagonist MDL-11,939 blocked the stress-induced decrease in DOPAC concentrations in the intermediate lobe and the stress-induced increase in plasma αMSH concentrations, while the $5HT_3$ antagonist ondansetron was without effect (Figure 4.10). In the non-stressed (SHAM) group, neither of these antagonists *per se* altered basal concentrations of 5HIAA in hypothalamic nuclei (Table 4.2), DOPAC in the intermediate lobe, or αMSH in the plasma (Figures 4.9 and 4.10). These results indicate that the effects of stress on PHDA neurons and αMSH secretion are mediated by $5HT_2$ receptors.

Discussion

The results of studies in this section reveal that 5HT neuronal function is necessary for the inhibitory effects of stress on PHDA neurons. This conclusion is

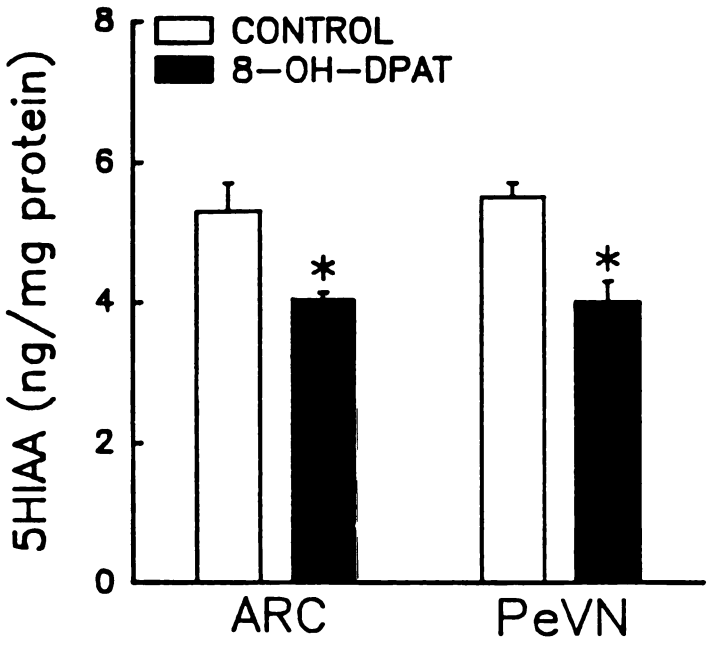


Figure 4.7. Effects of 8-OH-DPAT administration on 5HIAA concentrations in the arcuate (ARN) and periventricular (PeVN) nuclei of the rat hypothalamus. Female rats were injected with either 8-OH-DPAT (0.3 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. Columns represent means and vertical lines + 1 S.E. (n= 6-9) of 5HIAA concentrations. *, values from 8-OH-DPAT-treated animals that are significantly different ($p < 0.05$) from vehicle-treated control.

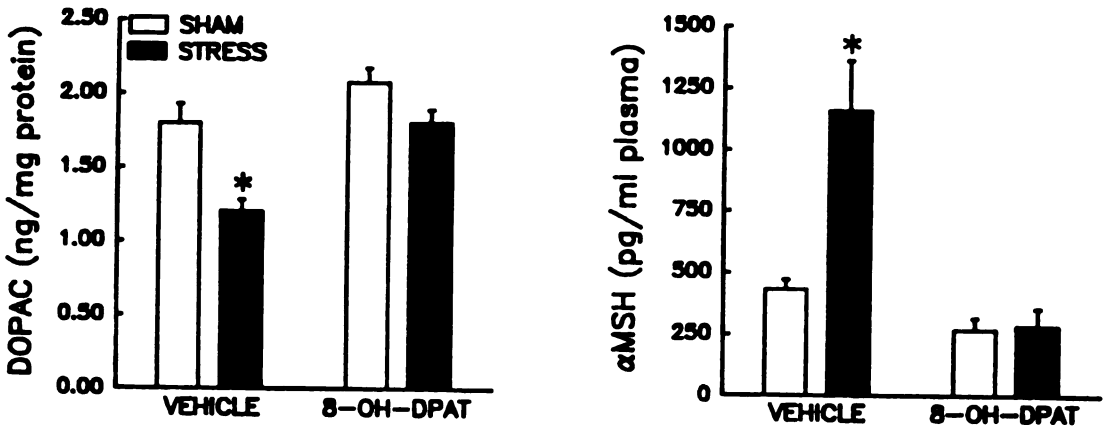


Figure 4.8. Effect of 8-OH-DPAT pretreatment on stress-induced changes in DOPAC concentrations in the intermediate lobe and plasma αMSH concentrations. Female rats were injected with either 8-OH-DPAT (0.3 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. Animals were removed from their home cages and immediately decapitated (SHAM) or were briefly anesthetized with diethylether, restrained in the supine position for 30 min and then decapitated (STRESS). Columns represent means and vertical lines + 1 S.E. (n= 6-9) of DOPAC and αMSH concentrations. *, values from stressed animals that are significantly different ($p < 0.05$) from sham controls.

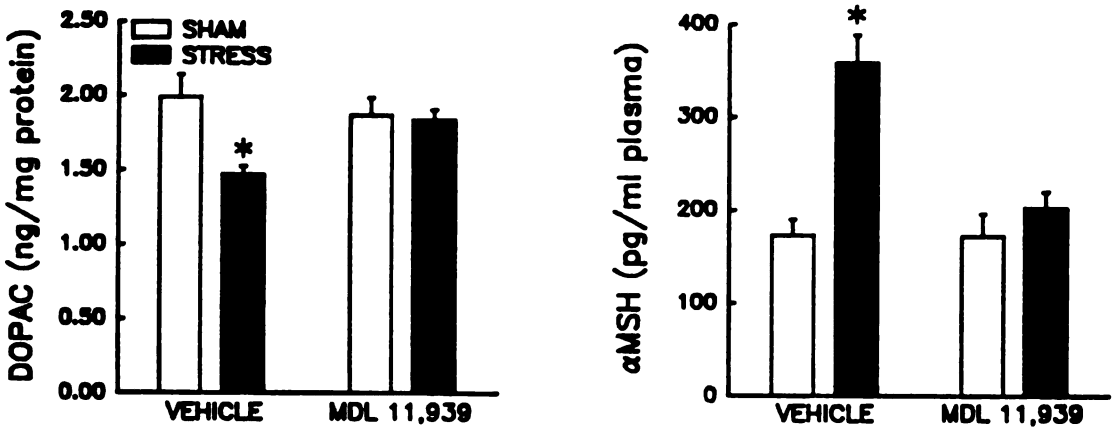


Figure 4.9. Effect of MDL-11,939 pretreatment on stress-induced changes in DOPAC concentrations in the intermediate lobe and plasma αMSH concentrations. Female rats were injected with either MDL-11,939 (1.0 mg/kg; i.p.) or its 0.01 N HCl vehicle (1 ml/kg; i.p.) 60 min prior to decapitation. Animals were removed from their home cages and decapitated immediately (SHAM) or were briefly anesthetized with diethylether, restrained in the supine position for 30 min and then decapitated (STRESS). Columns represent means and vertical lines + 1 S.E. (n= 6-9) of DOPAC and αMSH concentrations. *, values from stressed animals that are significantly different ($p < 0.05$) from sham controls.

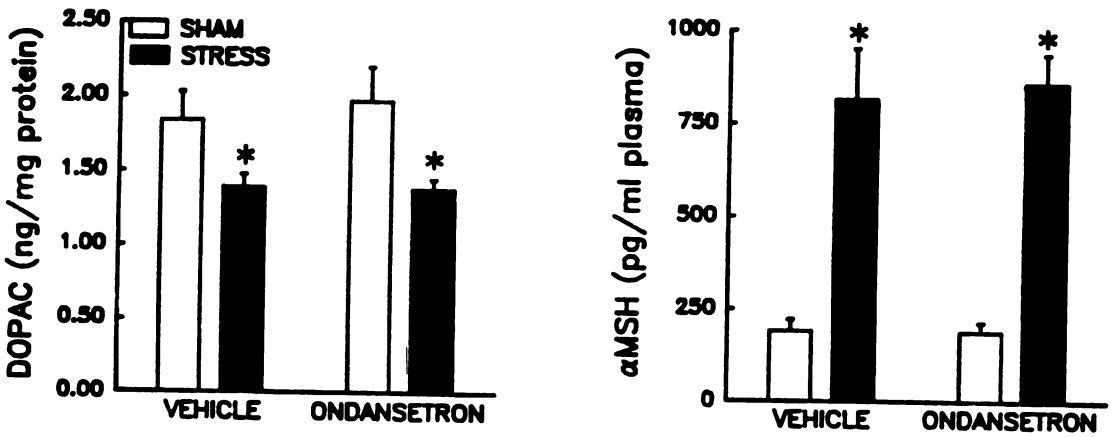


Figure 4.10. Effect of ondansetron pretreatment on stress-induced changes in DOPAC concentrations in the intermediate lobe and plasma αMSH concentrations. Female rats were injected with either ondansetron (0.1 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. Animals were removed from their home cages and decapitated immediately (SHAM) or were briefly anesthetized with diethylether, restrained in the supine position for 30 min and then decapitated (STRESS). Columns represent means and vertical lines represent + 1 S.E. (n= 6-9) of DOPAC and αMSH concentrations. *, values from stressed animals that are significantly different ($p < 0.05$) from sham controls.

TABLE 4.2. Effects of ondansetron and MDL-11,939 on the concentrations of 5HIAA in the arcuate (ARN) and periventricular (PeVN) nuclei of the rat hypothalamus

Region	Control	Ondansetron	Control	MDL-11,939
ARC	2.7 ± 0.2	2.9 ± 0.2	2.7 ± 0.3	2.7 ± 0.2
PeVN	5.3 ± 0.5	6.0 ± 0.5	4.9 ± 0.3	5.6 ± 0.7

Rats were injected with either ondansetron (0.1 mg/kg; s.c.), MDL-11,939 (1.0 mg/kg; i.p.) or their respective vehicles (1 ml/kg water; s.c.; or 0.01 N HCL; i.p.) and decapitated 60 min later. Values represent means ± 1 S.E. (n= 6-9) of 5HIAA concentrations (ng/mg protein).

based upon the observations that either 5,7-DHT-induced lesions of 5HT neurons or inhibition of 5HT neuronal activity following administration of the $5HT_{1A}$ receptor agonist 8-OH-DPAT blocks the stress-induced decrease in DOPAC concentrations in the intermediate lobe of the posterior pituitary. This suggests that the chain of neuronal events which translates stressful stimuli into functional alterations in hypothalamic DA neuronal activity involves inhibitory 5HT neurons. This hypothesis is consistent with results of studies on other hypothalamic DA neuronal systems which demonstrate that disruption of 5HT neuronal function prevents the stress-induced decrease in TIDA neuronal activity (Demarest, et al., 1985).

The $5HT_2$ receptor appears to be the receptor subtype involved in mediating the inhibitory effects of 5-hydroxytryptamine on PHDA neurons during stress. MDL-11939, a selective $5HT_2$ antagonist (Dudley et al., 1988; Pierce et al., 1992), prevents the stress-induced decrease in PHDA neuronal activity when given at a dose and time known to block central $5HT_2$ receptors (Dudley, et al., 1988). In contrast, ondansetron, a selective $5HT_3$ antagonist (Brittain, et al., 1987; Butler et al., 1988), does not alter the inhibitory effects of stress on PHDA neuronal activity when given at a dose and time known to block central $5HT_3$ receptors (Tyers et al., 1989; Jones et al., 1988; Costall, et al., 1987). These results indicate that activation of post-synaptic $5HT_2$ receptors is a key step in mediating the stress-induced inhibition of PHDA neuronal activity. This conclusion is supported by the previous section which demonstrated that activation of the post-synaptic $5HT_2$ receptor inhibits PHDA neuronal activity and increases αMSH secretion (Section 4.A.).

5HT neurons are also involved in the stress-induced increase in αMSH secretion since disruption of 5HT neuronal function with either 5,7-DHT or 8-OH-DPAT prevents the stress-induced increase in plasma αMSH concentrations. In addition, blockade of $5HT_2$ but not the $5HT_3$ receptors, prevents the stress-induced increase in αMSH secretion. Previous studies indicate that a decrease in PHDA neuronal activity and an increase in circulating epinepherine are both necessary for the full expression of stress-induced increases in αMSH secretion (Lindley, et al., 1990b). Therefore, it is likely that the effects of 5HT on αMSH secretion during stress are mediated, in part, by changes in PHDA neuronal activity. Given that the above manipulations to 5HT neurons completely block the stress-induced increase in αMSH, it is possible that 5HT neurons are also important for the stress-induced activation of the peripheral sympathetic nervous system. Finally, since 5HT neurons innervate the intermediate lobe (Palkovits, et al., 1986) and 5-hydroxytryptamine has been shown to slightly increase αMSH secretion from melanotrophs *in vitro* (Randle, et al., 1983b), a direct stimulatory effect of 5-hydroxytryptamine on melanotroph secretion during stress cannot be ruled out.

Consistent with data from the previous section (4.A.), the results of the present section indicate that 5HT neurons do not tonically inhibit PHDA neuronal activity. Disruption of 5HT neuronal function with either 8-OH-DPAT or 5,7-DHT pretreatment does not alter basal PHDA neuronal activity. Furthermore, blockade of post-synaptic $5HT_2$ or $5HT_3$ receptors with selective antagonists has no effect on basal PHDA neuronal activity. Thus, 5HT neurons do not regulate the basal activity of PHDA neurons in non-stressed rats. Apparently, quiescent 5HT neurons (Aghajanian, 1982) become activated

during stress (Houdouin et al., 1991; Johnston and Negro-Villar, 1986) and these activated 5HT neurons, in turn, inhibit PHDA neurons.

In summary, the results from studies in this section reveal that 5HT neurons, acting via $5HT_2$ receptors, mediate the inhibitory effects of stress on PHDA neurons and the corresponding increase in secretion of αMSH. Basal PHDA neuronal activity and melanotroph secretion of αMSH are not tonically inhibited by 5HT neurons.

5. AFFERENT REGULATION OF PHDA NEURONS: GABA

A. GABA RECEPTOR-MEDIATED REGULATION OF PHDA NEURONAL ACTIVITY AND αMSH SECRETION

Introduction

The hypothalamus contains high concentrations of GABAergic neurons, located in short interneurons primarily in the arcuate, periventricular and supraoptic nuclei (Tappaz, et al., 1977; Vincent et al., 1982). GABA has been identified as a dominant hypothalamic neurotransmitter, present at approximately half of the synapses found within the hypothalamus (Decavel and Van den Pol, 1990). In addition, central GABAergic neurons innervate the posterior pituitary (Tappaz et al., 1986) and GABA has been shown to be extensively colocalized with dopamine in neurons innervating the intermediate lobe (Vuillez et al., 1987; Schimchowitsch et al., 1991). Receptors for GABA have been identified in the hypothalamus (Beaumont et al., 1978; Bowery et al., 1987) and posterior pituitary (Anderson and Mitchell, 1986a) and GABAergic synapses are found on periventricular hypothalamic neurons (Tappaz et al., 1982), suggesting GABAergic neurons may be important in the regulation of both PHDA neuronal activity and αMSH secretion.

Although GABA does not alter the release of dopamine from the posterior pituitary *in vitro* (Sharman et al., 1982; Anderson and Mitchell, 1985), GABA could regulate the activity of PHDA neurons via actions on centrally located receptors. While

little is known about $GABA_A$ receptor-mediated regulation of PHDA neurons, the inhibitory actions of the $GABA_B$ receptor agonist baclofen (Hill and Bowery, 1981) on extrahypothalamic DA neurons have been well documented (Dray and Straughan, 1976, Waldmeier and Maitre, 1978; Moore and Demarest; 1980). In addition, activation of $GABA_B$ receptors hyperpolarizes mediobasal hypothalamic neurons *in vitro* (Loose et al., 1991) and inhibits neuronal impulse flow in hypothalamic DA neurons, including those terminating in the posterior pituitary (Demarest and Moore, 1979a; Moore and Demarest, 1980; Lindley et al., 1988).

GABA exerts both stimulatory and inhibitory effects on melanotroph secretion *in vitro* (Tomiko et al., 1983). Although most of the *in vitro* effects of GABA on melanotroph secretion and electrical activity appear to be mediated by $GABA_A$ receptors (Tomiko et al., 1983; Taraskevich and Douglas, 1982; MacVicar and Pittman, 1986), activation of $GABA_B$ receptors has also been reported to inhibit melanotroph secretion *in vitro* (Demeneix et al., 1984; Shibuya et al., 1991). These latter studies are surprising since the posterior pituitary contains $GABA_A$, but not $GABA_B$ binding sites (Anderson and Mitchell, 1986a). Furthermore, since *in vitro* studies are performed in a milieu devoid of neuronal and hormonal inputs normally found in the intact animal, extrapolation of these data to *in vivo* physiology can be problematic. Clearly, the effects of GABA receptor activation on αMSH secretion need to be characterized *in vivo*.

The purpose of the studies in this chapter was to examine $GABA_A$- and $GABA_B$-receptor mediated regulation of PHDA neuronal activity and the secretion of αMSH *in vivo*. To this end, the effects of the $GABA_A$ agonist isoguvacine, and antagonist SR-

95,531 or the $GABA_B$ agonist baclofen, and antagonists 2-hydroxysaclofen or CGP-35,348 on PHDA neuronal activity and αMSH secretion were characterized.

Results

As shown in Table 5.1, administration of the $GABA_A$ antagonist SR-95,531 (Wermuth and Bizière, 1986) did not alter concentrations of αMSH in plasma or of DOPAC in the intermediate lobe at doses of 1.0 and 3.0 mg/kg. Higher doses of SR-95,531 (10 mg/kg) produced lethal seizures. Central administration of the $GABA_A$ agonist isoguvacine (Krogsgaard-Larsen et al., 1977) also did not alter concentrations of αMSH in plasma or of DOPAC in the intermediate lobe (Table 5.2).

The effects of the $GABA_B$ agonist baclofen (Hill and Bowery, 1981) on concentrations of αMSH in plasma and of DOPAC in the intermediate lobe of the pituitary are summarized in Figure 5.1 Acute administration of baclofen produced a dose-dependent increase in plasma αMSH concentrations and this was accompanied by a reduction in DOPAC concentrations in the intermediate lobe of the pituitary. The same general pattern of effects was observed when the time course of the effects of baclofen were determined. Results presented in Figure 5.2 reveal that the baclofen-induced increase in plasma αMSH concentrations and decrease in DOPAC concentrations in the intermediate lobe of the pituitary occurred promptly (within 30 min for both indices) and lasted for at least 2 hr.

Administration of the $GABA_B$ antagonist 2-hydroxysaclofen (Kerr et al., 1988) did not alter basal concentrations of plasma αMSH or intermediate lobe DOPAC

Table 5.1. Lack of effects of SR-95,531 on plasma αMSH and intermediate lobe DOPAC concentrations of male rats.

	αMSH (pg/ml)	DOPAC (ng/mg protein)	N
CONTROL	92 ± 8	1.15 ± 0.05	7
1 mg/kg	90 ± 12	0.99 ± 0.07	8
3 mg/kg	90 ± 8	1.26 ± 0.05	9

Rats were injected with either SR-95,531 (1 or 3 mg/kg) or water (1ml/kg; s.c.) 30 min prior to decapitation. Values represent means ±'1 S.E. of plasma αMSH or intermediate lobe DOPAC concentrations from 7-9 rats.

Table 5.2. Lack of effects of isoguvacine on plasma αMSH and intermediate lobe DOPAC concentrations of male rats.

	αMSH (pg/ml)	DOPAC (ng/mg protein)	N
CONTROL	167 ± 19	1.59 ± 0.10	8
3 μg/rat	155 ± 21	1.46 ± 0.05	8
10 μg/rat	190 ± 19	1.39 ± 0.07	8
30 μg/rat	154 ± 20	1.37 ± 0.12	8

Rats were injected with either isoguvacine (10, 30, 100 μg/rat; icv) or its water vehicle (5μl/rat; icv) 30 min prior to decapitation. Values represent means ± 1 S.E. of plasma αMSH or intermediate lobe DOPAC concentrations from 8 rats.

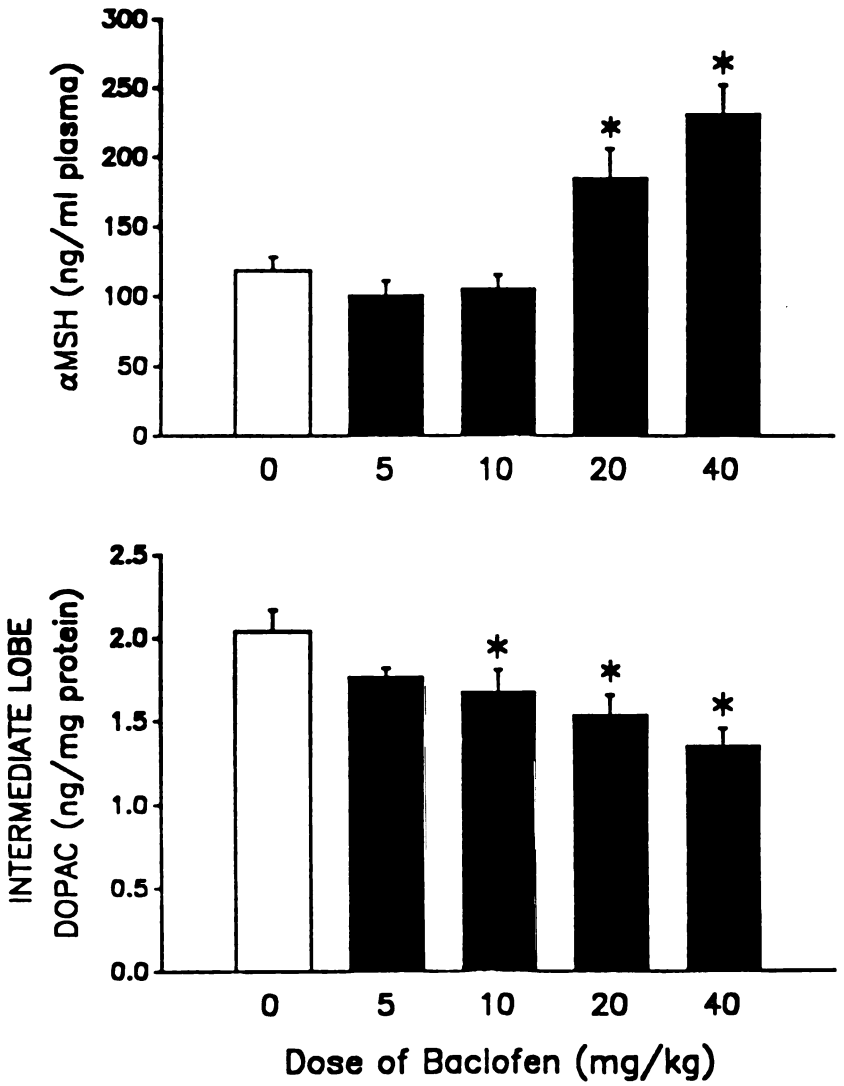


Figure 5.1. Dose-response effects of baclofen on plasma αMSH (TOP) and intermediate lobe DOPAC (BOTTOM) concentrations. Rats were injected with baclofen (5, 10, 20, and 40 mg/kg; i.p.) or its 0.03 N NaOH vehicle (2ml/kg, i.p.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH and of DOPAC concentrations in the intermediate lobe of 7-9 rats. *, Values from rats treated with baclofen (solid columns) which are significantly different ($P < 0.05$) from vehicle-treated controls (open columns).

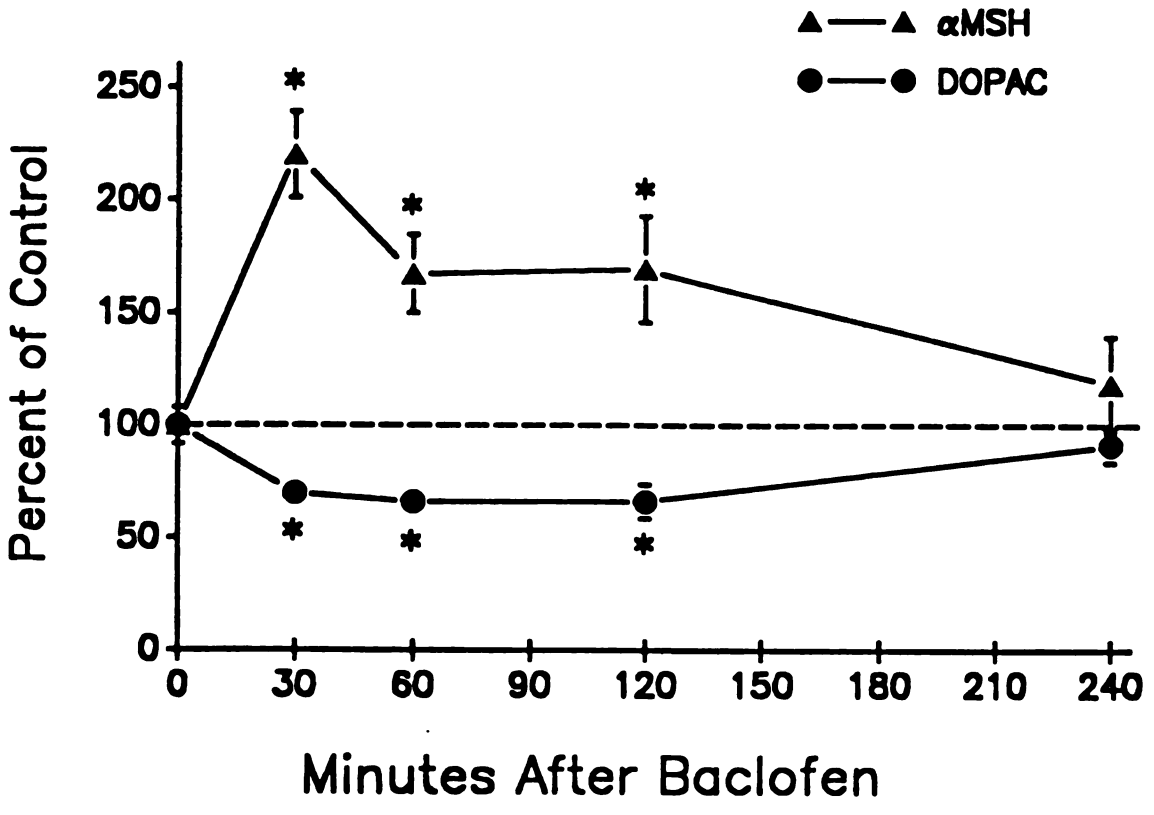


Figure 5.2. Time course of effects of baclofen on plasma αMSH and intermediate lobe DOPAC concentrations. Rats were injected with baclofen (40 mg/kg; i.p.) 30, 60, 120, or 240 min prior to decapitation. Zero-time control rats were injected with 0.03 N NaOH vehicle (2 ml/kg; i.p.) 60 min prior to decapitation. Symbols represent means and vertical lines ± 1 S.E. of intermediate lobe DOPAC (●) and plasma αMSH (▲) concentrations of 7-9 rats expressed as a percentage of zero-time control values. Where no vertical lines are depicted 1 S.E. is less than the radius of the symbol. Control values for intermediate lobe DOPAC and plasma αMSH concentrations were 1.83 ± 0.05 ng/mg protein and 98 ± 8 pg/ml plasma, respectively. *, Values from rats treated with baclofen which are significantly different ($P < 0.05$) from zero time controls.

concentrations (Table 5.3). The 30 min time point was chosen based upon the reported short duration of action of 2-hydroxysaclofen on neuroendocrine systems (Akema and Kimura, 1991). Treatment with the 2-hydroxysaclofen, however, reversed the effects of baclofen on plasma concentrations of αMSH and on DOPAC concentrations in the intermediate lobe of the pituitary (Figure 5.3). This latter result was confirmed using a novel, centrally-active $GABA_B$ antagonist CGP-35,348 (Olpe et al., 1990); treatment with CGP-35,348 reversed the effects of baclofen on plasma αMSH concentrations and on DOPAC concentrations in the intermediate lobe of the pituitary (Figure 5.4).

Discussion

The results from studies in this chapter reveal that activation of $GABA_B$, but not $GABA_A$ receptors inhibits the activity of PHDA neurons. This conclusion is based upon observations that baclofen, but not isoguvacine produces a dose- and time-dependent decrease in pituitary intermediate lobe DOPAC concentrations. Furthermore, the effects of baclofen on PHDA neuronal activity are reversed by the selective $GABA_B$ receptor antagonists 2-hydroxysaclofen and CGP-35,348. These results are consistent with the known inhibitory effects of $GABA_B$ receptor activation on other DA neuronal systems (Waldemier and Maitre; 1978; Moore and Demarest; 1980; Demarest and Moore 1979a).

Results presented in this section indicate that central administration of $GABA_A$ receptor agonists do not alter αMSH secretion *in vivo*. Although *in vitro* studies demonstrate that $GABA_A$ receptor activation produces a brief stimulation then prolonged inhibition of αMSH release (Tomiko et al., 1983), this was not observed in the present

Table 5.3. Lack of effects of 2-hydroxysaclofen on plasma αMSH and intermediate lobe DOPAC concentrations of male rats.

	αMSH (pg/ml)	DOPAC (ng/mg protein)	N
CONTROL	127 ± 7	1.31 ± 0.05	8
10 μg/rat	139 ± 18	1.34 ± 0.05	7
30 μg/rat	132 ± 17	1.45 ± 0.11	7
100 μg/rat	121 ± 12	1.41 ± 0.08	8

Rats were injected with either 2-hydroxysaclofen (10, 30, 100 μg/rat; icv) or its 0.01 N NaOH vehicle (5μl/rat; icv) 30 min prior to decapitation. Values represent means ± 1 S.E. of plasma αMSH or intermediate lobe DOPAC concentrations from 7-8 rats.

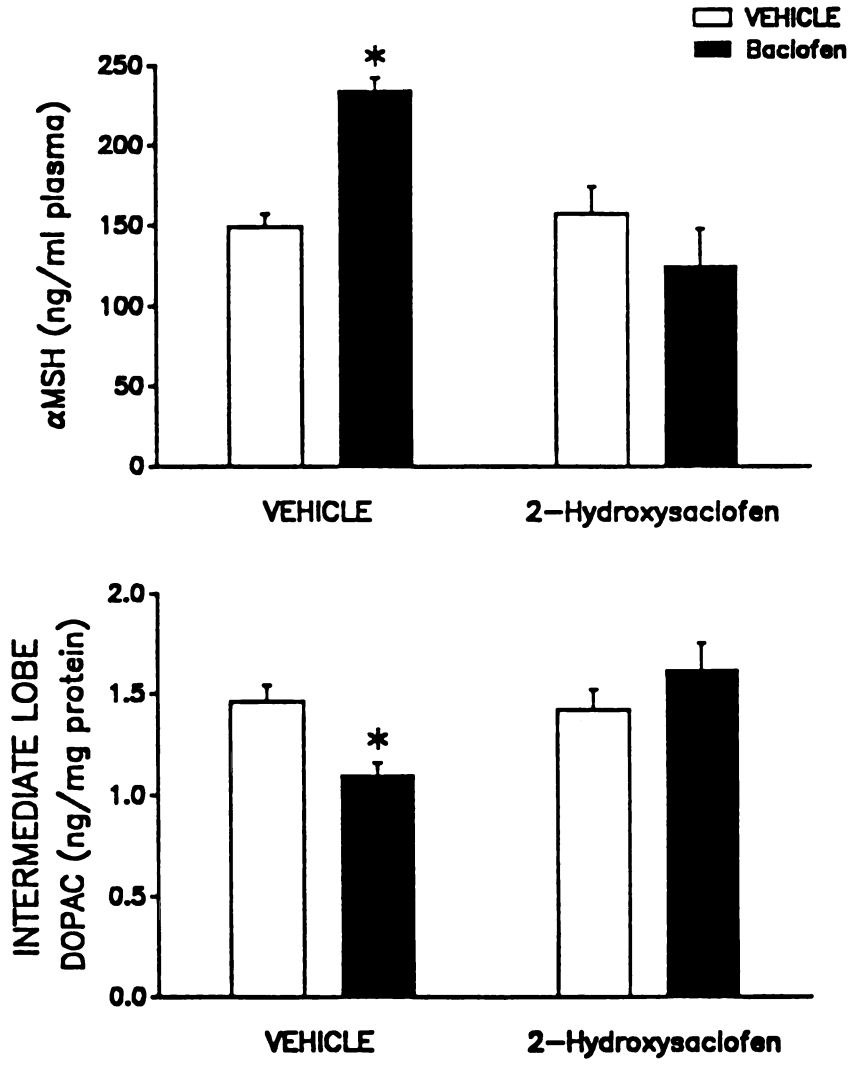


Figure 5.3. Effects of baclofen on plasma αMSH (TOP) and intermediate lobe DOPAC concentrations (BOTTOM) of 2-hydroxysaclofen-treated rats. Animals were injected with baclofen (20 mg/kg; i.p.) or its 0.03 N NaOH vehicle (2 ml/kg; i.p.) 60 min prior to decapitation, and with 2-hydroxysaclofen (100 μg/rat; icv) or its 0.01 N NaOH vehicle (5 μl/rat; icv) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH concentrations and of DOPAC concentrations in the intermediate lobe of 7-9 rats. *, Values from baclofen-treated animals (solid columns) that are significantly different ($P < 0.05$) from vehicle-treated controls (open columns).

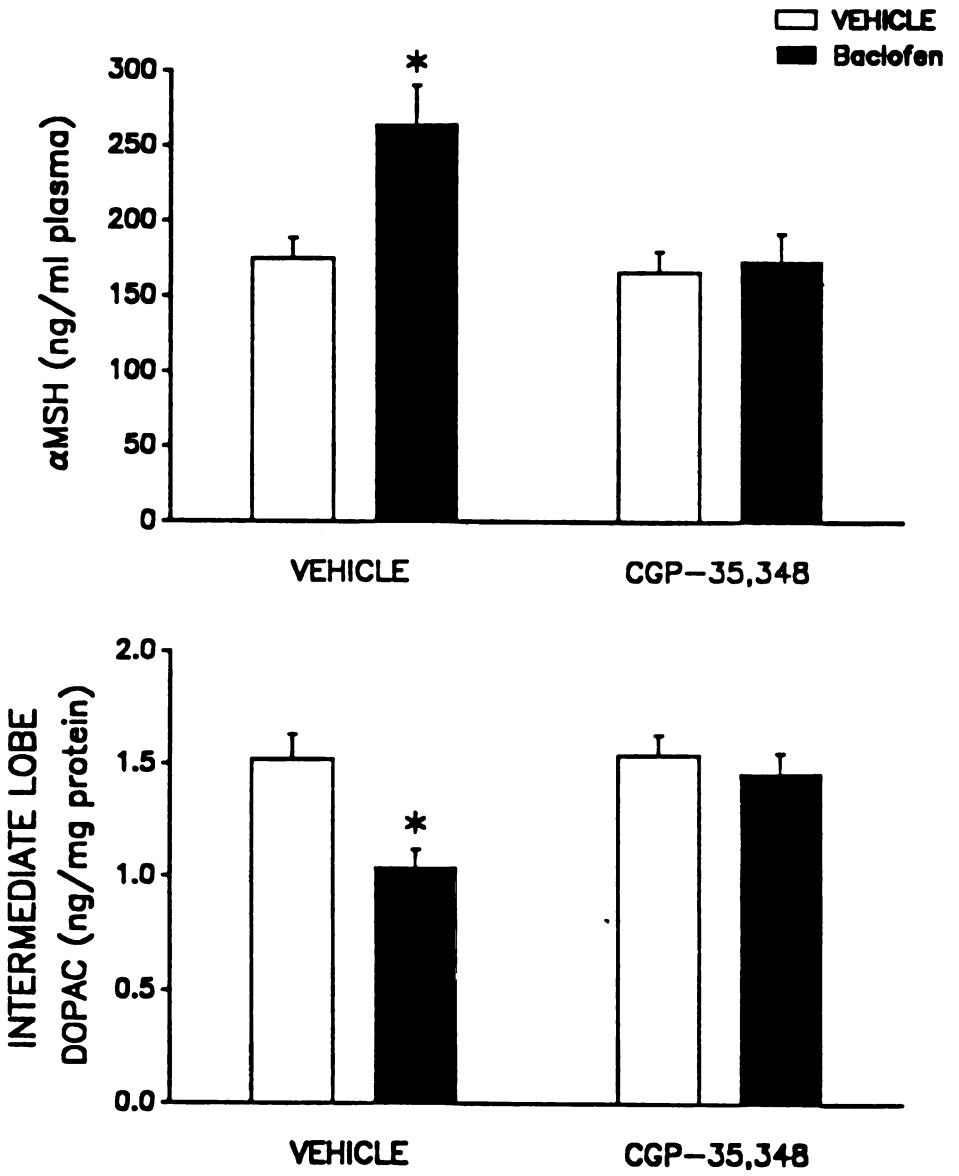


Figure 5.4. Effects of baclofen on plasma αMSH (TOP) and intermediate lobe DOPAC concentrations (BOTTOM) of CGP-35,348-treated rats. Animals were injected with baclofen (20 mg/kg; i.p.) or its 0.03 N NaOH vehicle (2 ml/kg; i.p.), and with CGP-35,348 (100 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH concentrations and of DOPAC concentrations in the intermediate lobe of 7-9 rats. *, Values from baclofen-treated animals (solid columns) that are significantly different ($P < 0.05$) from vehicle-treated controls (open columns).

study; i.e., isoguvacine does not alter plasma concentrations of αMSH when given at doses known to be effective in the central nervous system (Zarrindast and Oveissi, 1988). The lack of effect of isoguvacine on αMSH secretion could, however, reflect insufficient concentrations of this agonist reaching the pituitary following icv administration.

In contrast, the studies in this section demonstrate that activation of $GABA_B$ receptors increases αMSH secretion; baclofen produces a dose- and time-dependent increase in plasma αMSH concentrations and this effect is reversed by 2-hydroxysaclofen and CGP-35,348. Other studies have suggested that activation of $GABA_B$ receptors may also inhibit αMSH secretion (Demeneix et al., 1984; Shibuya et al., 1991), although the intermediate lobe contains $GABA_A$ but not $GABA_B$ receptors (Anderson and Mitchell, 1986a). The *in vivo* effects of baclofen on αMSH secretion observed in this section are centrally mediated since these effects are reversed by the central administration of 2-hydroxysaclofen which does not readily cross the blood-brain barrier (Kerr et al., 1988). It is likely, then, that *in vivo* baclofen administration increases αMSH secretion by decreasing PHDA neuronal activity and removing the tonic inhibition of melanotroph secretion. Evidently, the removal of the tonic inhibition provided by PHDA neurons outweighs any direct $GABA_B$-mediated inhibition of melanotroph secretion.

Alternatively, baclofen may activate $GABA_B$ autoreceptors and decrease the release of endogenous GABA (Waldmeier et al., 1988) in the intermediate lobe and remove further GABA-receptor mediated inhibition of melanotroph secretion. This alternative is not consistent with reports that autoreceptor regulation of endogenous GABA release in the posterior pituitary is mediated by the $GABA_A$ receptor (Anderson

and Mitchell, 1986b). Furthermore, if $GABA_B$ autoreceptors regulate the release of endogenous GABA *in vivo*, then application of $GABA_B$ antagonists alone should increase endogenous GABA release and suppress melanotroph secretion. The data presented in this chapter, however, indicate that central and peripheral administration of 2-hydroxysaclofen or CGP-35,348, respectively, does not alter basal plasma αMSH concentrations when given at doses known to block $GABA_B$ receptors. Therefore, the observed effects of baclofen are not due to activation of autoreceptors on GABAergic neurons terminating in the intermediate lobe of the pituitary.

The activity of PHDA neurons and the secretion of αMSH are not tonically inhibited by endogenous GABA acting at either $GABA_A$ or $GABA_B$ receptors. Administration of the either the $GABA_A$ antagonist SR-95,531 or the $GABA_B$ antagonists 2-hydroxysalcofen and CGP-35,348 does not alter the basal concentrations of DOPAC in the intermediate lobe or αMSH in the plasma when given at doses and times known to block central $GABA_A$ and $GABA_B$ receptors, respectively (Wermuth and Bizière, 1986; Akema and Kimura, 1991). It is possible that hypothalamic GABAergic neurons are quiescent under basal conditions, leaving $GABA_A$ and $GABA_B$ receptors unoccupied and unresponsive to antagonist administration. Although endogenous GABAergic neurons do not appear to tonically regulate the basal activity of PHDA neurons or αMSH secretion, hypothalamic GABAergic neurons acting at $GABA_B$ receptors may play a role in mediating inhibitory inputs to PHDA neurons during stress.

In summary, the results of studies in this chapter reveal that GABAergic neurons, acting via $GABA_B$ and not $GABA_A$ receptors, inhibit the activity of PHDA neurons and

increase the secretion of αMSH. In addition, basal PHDA neuronal activity and αMSH secretion are not tonically inhibited by GABA neurons acting at either $GABA_A$ or $GABA_B$ receptors.

B. ACTIVATION OF $GABA_B$-RECEPTORS MEDIATES THE INHIBITORY EFFECTS OF STRESS AND DOI ON PHDA NEURONAL ACTIVITY.

Introduction

Supine restraint stress inhibits the activity of PHDA neurons and increases αMSH secretion and these effects are mediated by activation of $5HT_2$ receptors (Chapter 4.B.). Furthermore, activation of postsynaptic $5HT_2$ receptors inhibits the activity of PHDA neurons and increases αMSH secretion (Chapter 4.A.). $5HT_2$ receptor activation is unlikely to have a direct inhibitory effect on PHDA neurons since stimulation of $5HT_2$ receptors depolarizes and excites postsynaptic membranes (McCall and Aghajanian, 1980). It is possible that the effects of $5HT_2$ receptor stimulation, and consequently stress, are mediated by activation of an inhibitory interneuron.

The hypothalamus contains large numbers of GABA interneurons which could provide a point of convergence for the inhibitory actions of both stress and $5HT_2$ receptors stimulation. As shown in the previous section (5.A), activation of $GABA_B$ receptors inhibits PHDA neuronal activity and increases αMSH secretion. The purpose, therefore, of studies in this section is to determine if the inhibitory effects of stress or $5HT_2$ agonist administration on PHDA neuronal activity are mediated by endogenous GABAergic neurons acting at $GABA_B$ receptors. To this end, the inhibitory effects of both stress and $5HT_{2/1c}$ agonist administration on PHDA neuronal activity were examined in 2-hydroxysaclofen-treated animals.

Results

Results of experiments designed to examine the possible physiological role of GABAergic regulation of PHDA neuronal activity and αMSH secretion are shown in Figures 5.5 and 5.6. Animals were subjected to both physiological (i.e., supine restraint stress) and pharmacological (i.e., administration of the $5HT_{2/1c}$ agonist DOI) manipulations that are known to inhibit PHDA neuronal activity and increase the secretion of αMSH (Lookingland et al., 1991; Section 4.A.). Indeed, both supine restraint stress (Figure 5.5) and administration of DOI (Figure 5.6) increased plasma concentrations of αMSH and decreased concentrations of DOPAC in the intermediate lobe of the pituitary. Treatment with 2-hydroxysaclofen blocked the effects of both supine restraint stress and administration of DOI on plasma concentrations of αMSH and DOPAC concentrations in the intermediate lobe of the pituitary.

Discussion

Results from studies in this section reveal that the effects of supine restraint stress on PHDA neuronal activity and αMSH secretion are blocked by central administration of a $GABA_B$ receptor antagonist; stress decreases intermediate lobe DOPAC concentrations and increases plasma αMSH concentrations in vehicle-, but not 2-hydroxysaclofen-treated animals. This finding suggests that the chain of neuronal events which translates stressful stimuli into functional alterations in hypothalamic DA neuronal activity involves activation of inhibitory GABAergic neurons which, in turn, inhibit PHDA neuronal activity via $GABA_B$ receptor activation. This hypothesis is consistent

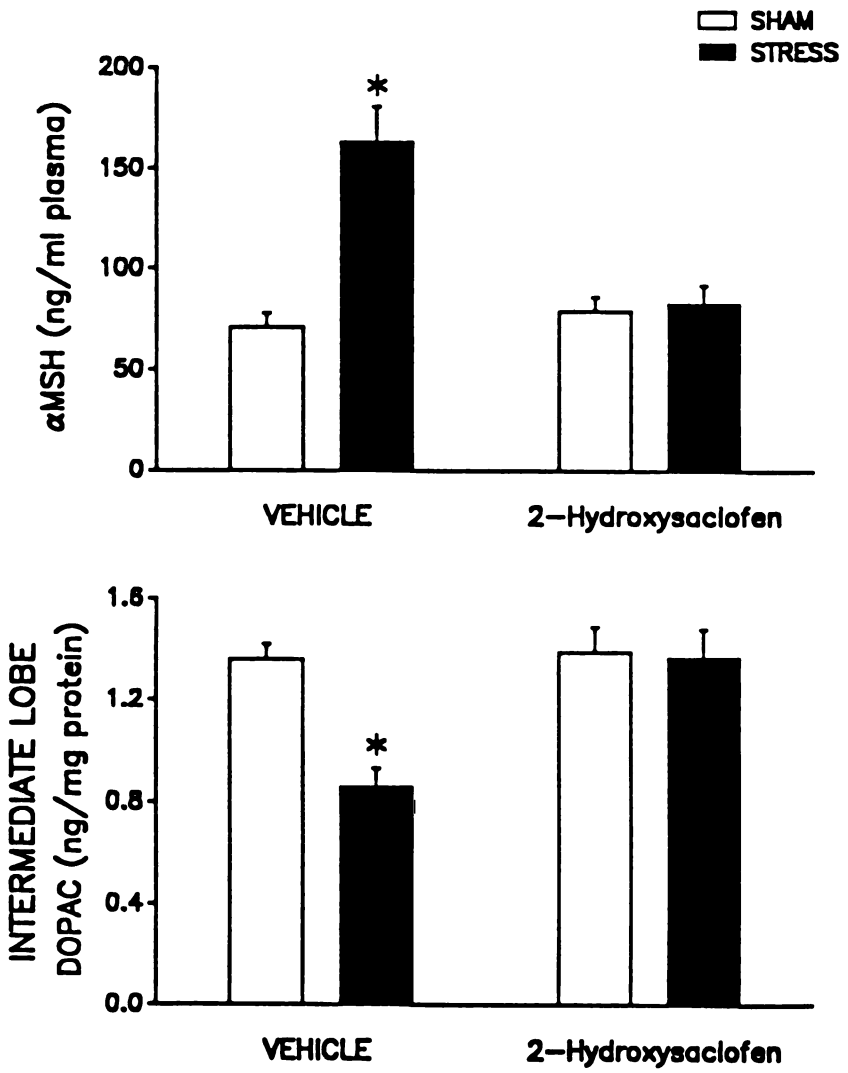


Figure 5.5. Effect of 2-hydroxysaclofen treatment on stress-induced changes in DOPAC concentrations in the intermediate lobe and plasma αMSH concentrations. Rats were injected with either 2-hydroxysaclofen (100 μg/rat; icv) or its 0.01 N NaOH vehicle (5 μl/rat; icv) 30 min prior to decapitation. Animals were removed from their home cages and decapitated immediately (SHAM) or were briefly anesthetized with diethylether, restrained in the supine position for 30 min and then decapitated (STRESS). Columns represent means and vertical lines 1 S.E. of plasma αMSH concentrations and of DOPAC concentrations in the intermediate lobe of 7-9 rats. *, Values from stressed animals (solid columns) that are significantly different ($p < 0.05$) from sham controls (open columns).

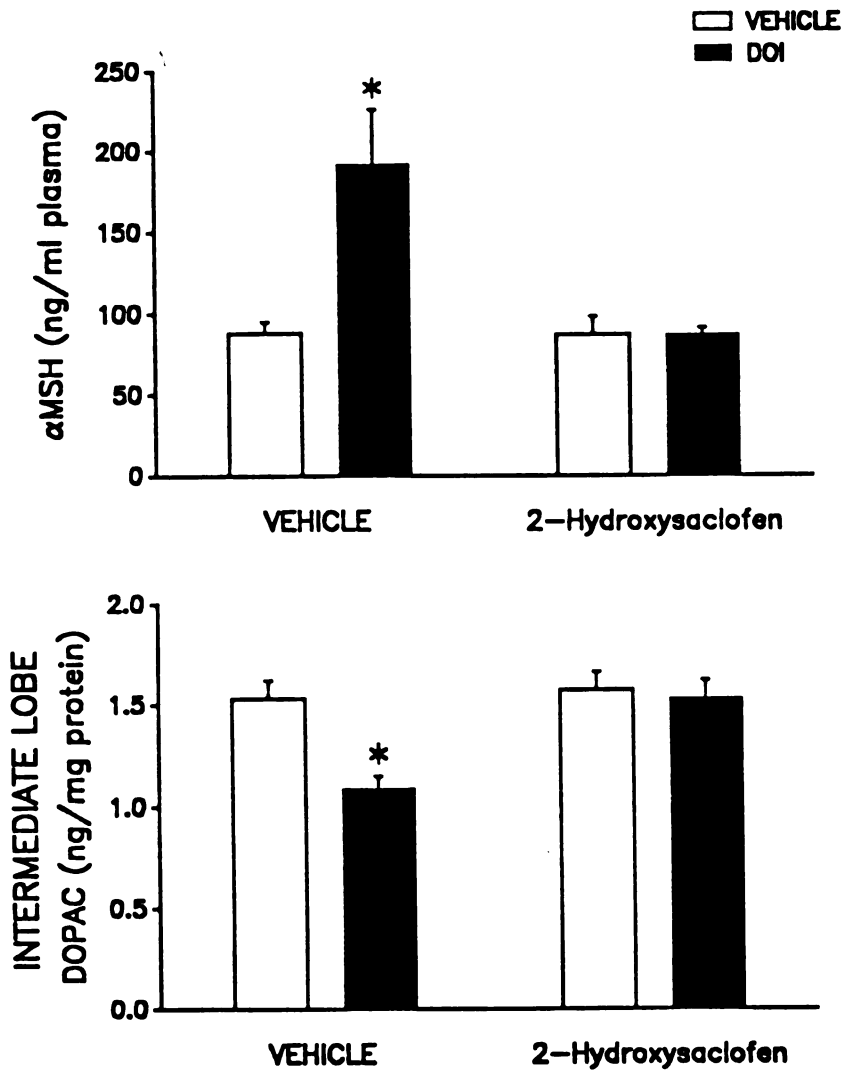


Figure 5.6. Effects of DOI on plasma αMSH (TOP) and intermediate lobe DOPAC concentrations (BOTTOM) of 2-hydroxysaclofen-treated rats. Animals were injected with 2-hydroxysaclofen (100 μg/rat; icv) or its 0.01 N NaOH vehicle (5 μl/rat; icv), and with DOI (300 μg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E. of plasma αMSH and of DOPAC concentrations in the intermediate lobe of 7-9 rats. *, Values from DOI-treated (solid columns) animals that are significantly different ($P < 0.05$) from water-treated controls (open columns).

with studies that demonstrate increases in hypothalamic GABAergic neuronal activity following stressful manipulations (Manev and Pericic, 1983; Yoneda et al., 1983).

PHDA neurons receive inhibitory input from both 5HT and GABAergic neurons acting at $5HT_2$ and $GABA_B$ receptors, respectively. The data in the present study indicate that these inhibitory neuronal inputs are arranged in series; i.e., the inhibitory effects of $5HT_2$ agonists on PHDA neurons are mediated by $GABA_B$ receptor stimulation. This conclusion is based upon the observation that DOI-induced decreases in intermediate lobe DOPAC concentrations and increases αMSH secretion are blocked by central administration of the $GABA_B$ receptor antagonist 2-hydroxysalcofen. Taken together, these data suggest that activation of the $5HT_2$ receptor stimulates inhibitory GABAergic interneurons, which, in turn inhibit PHDA neuronal activity via $GABA_B$ receptors. A similar synaptic scenario has been recently described for the inhibitory effects of $5HT_2$ receptor activation on cortical pyramidal neurons (Gellman and Aghajanian, 1993).

The release of endogenous GABA from neurons and activation $GABA_B$ receptors, may provide a point of convergence for physiologically relevant afferent inhibitory inputs to PHDA neurons. Stressful manipulations activate a chain of neural events which inhibit the activity of PHDA neurons and increase αMSH release. 5HT and GABAergic neurons are key inhibitory inputs to PHDA neuronal activity during stress. Taken together, the data presented in Chapters 4 and 5 suggest that stress releases 5-hydroxytryptamine which stimulates of $5HT_2$ receptors that are inhibitory to PHDA neurons, and that these inhibitory effects, in turn, are mediated by GABAergic neurons

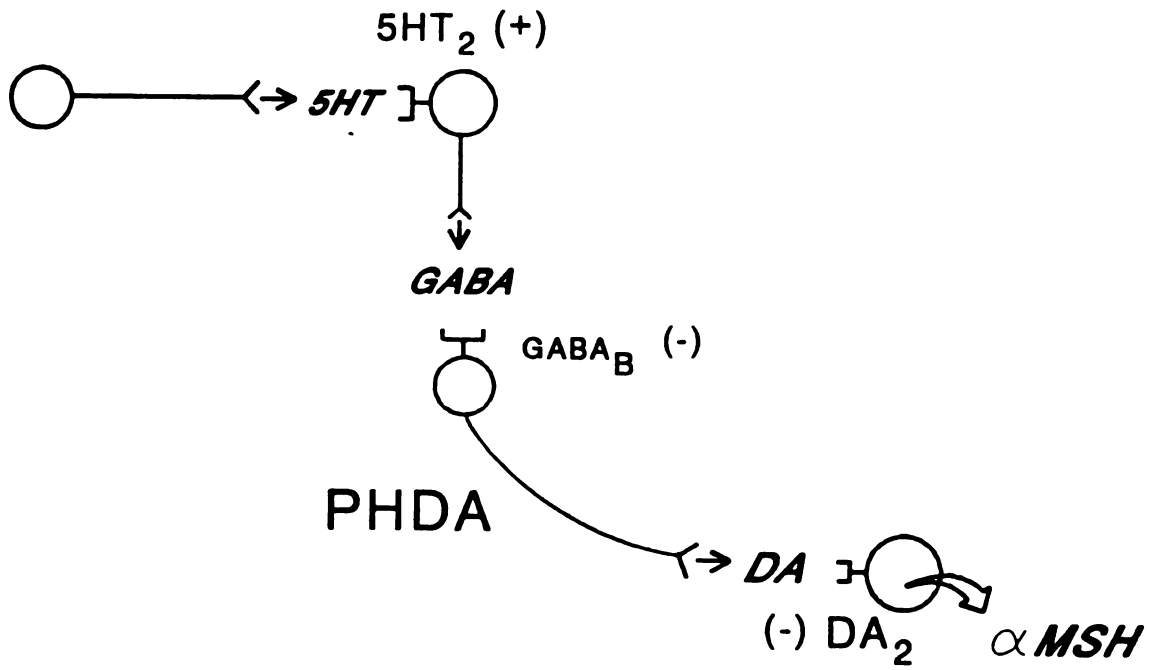


Figure 5.7. Schematic of the afferent inputs to PHDA neurons identified in the present studies. PHDA, periventricular-hypophysial dopaminergic; 5HT, 5-hydroxytryptamine; GABA, gamma-aminobutyric acid; DA, dopamine; αMSH, α-melanocyte-stimulating hormone; (+), stimulatory; (-), inhibitory.

which activate inhibitory $GABA_B$ receptors (Figure 5.7).

In summary, GABAergic neurons, via the $GABA_B$ receptor, mediate the inhibitory effects of both stress and $5HT_2$ receptor activation on PHDA neuronal activity and thus may provide a point of convergence for afferent inhibitory inputs to the PHDA neuronal system.

6. SUMMARY AND CONCLUDING DISCUSSION

A. SUMMARY

The functional anatomy of DA neurons terminating in the intermediate lobe was examined and the afferent neuronal regulation of these neurons was evaluated under basal and stress conditions. The significant findings and conclusions are summarized below.

a. Studies using PHA-L reveal that DA neurons situated in the hypothalamic periventricular nucleus project to the intermediate lobe of the pituitary where they form terminal axonal arborizations. Hypothalamic knife cut lesions which interrupt the axons of these neurons result in a decrease in dopamine concentrations in the intermediate lobe and an increase in αMSH secretion. Conversely, electrical stimulation of the cell bodies in the periventricular nucleus increases the activity of DA neurons terminating in the pituitary and decreases αMSH secretion. Taken together, these results indicate that DA neurons originating in the periventricular nucleus terminate in the intermediate lobe of the posterior pituitary and regulate the secretion of αMSH. On the basis of these results, it is proposed that these DA neurons be referred to as a "periventricular-hypophysial" (PHDA) neuronal system which better reflects the origin and site of termination of these neurons than the heretofore designation of THDA neurons.

b. Activation of the post-synaptic $5HT_2$ receptor inhibits the activity of PHDA neurons and increases αMSH secretion. Basal PHDA neuronal activity and αMSH secretion, however, are not tonically inhibited by 5HT neurons. Furthermore, afferent input from 5HT neurons, acting via $5HT_2$ receptors, mediates the inhibitory effects of

stress on PHDA neurons and the corresponding increase in αMSH secretion.

c. Activation of $GABA_B$ but not $GABA_A$ receptors inhibits the activity of PHDA neurons and increases αMSH secretion. Basal PHDA neuronal activity and αMSH secretion, however, are not tonically inhibited by GABA neurons acting at either $GABA_A$ or $GABA_B$ receptors. Furthermore, afferent input from GABAergic neurons, acting via $GABA_B$ receptors also mediates the effects of stress on PHDA neurons and the corresponding increase in the secretion of αMSH.

d. Activation of $GABA_B$ receptors mediates the inhibitory effects of $5HT_2$ receptor stimulation on PHDA neuronal activity. This suggests that activation of $5HT_2$ receptors stimulates inhibitory GABAergic interneurons, which, in turn inhibit PHDA neuronal activity via $GABA_B$ receptors.

B. CONCLUDING DISCUSSION

Considerable attention has focused on the regulation and function of ascending DA neurons due to their relevance in neuropsychiatric disorders. It is difficult, however, to study both DA neuronal activity and functional consequences of any changes in DA neuronal activity in these systems since the potential changes involve complex motor and behavioral responses that are difficult to monitor, quantify and interpret. On the other hand, DA neurons which terminate in the intermediate lobe are unique in that they directly innervate endocrine cells; i.e., the melanotrophs. Studying intermediate lobe DA neurons, therefore, allows monitoring of not only DA neuronal activity, but also a functional consequence (αMSH secretion) to observed changes in DA neuronal activity that is relatively easy to measure, quantify and interpret.

Although the regulation of melanotroph secretion by PHDA neurons has been well characterized, few studies have examined the afferent neuronal regulation of these neurons. The study of afferent neuronal regulation has been hampered, in part, by the obscure location of perikarya of DA neurons projecting to the intermediate lobe. Indeed, previous studies indicated that perikarya projecting to the intermediate lobe are situated amongst other DA neurons which subserve different endocrinological functions and display differential regulation. In the light of the experiments presented in chapter 3, it is evident that the DA neurons which project to the intermediate lobe have a distinct location within the mediobasal hypothalamus. Furthermore, the differences in regulation and function of hypothalamic DA neuronal systems could be reflected by the distinct anatomical segregation of their perikarya.

Although activation of both $5HT_2$ and $GABA_B$ receptors inhibits the activity of PHDA neurons, these neurons are not tonically regulated by either 5HT or GABAergic neurons. Indeed a modulatory, rather than tonic role has been proposed for both $5HT_2$ and $GABA_B$ receptors (Leysen, 1990; Bowery, 1989). This conclusion suggests that afferent inhibitory inputs to PHDA neurons are quiescent under normal physiological situations, but can become activated during certain situations (e.g., stress) thereby decreasing the activity of PHDA neurons and removing the tonic inhibition of αMSH secretion.

Stressful manipulations activate a chain of neural events that are eventually translated into a hormonal response; i.e., increase in the release of αMSH. The present studies have identified that 5HT and GABAergic neurons are key links in neural mechanisms which inhibit PHDA neuronal activity and thereby, at least in part, increase melanotroph secretion observed during stress. Taken together, the data presented in Chapters 4 and 5 suggest that stress activates 5HT neurons which result in the stimulation of $5HT_2$ receptors that are inhibitory to PHDA neurons, and that these inhibitory effects, in turn, are mediated by GABAergic neurons which activate inhibitory $GABA_B$ receptors (Figure 5.7). Moreover, these results suggest that PHDA neurons receive a convergence of inhibitory afferent inputs which are important for removing the tonic inhibition of melanotroph secretion during stress. Further investigation may reveal other important inhibitory afferent inputs to PHDA neurons.

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