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NEUROCHEMICAL CHARACTERIZATION OF THE INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONAL SYSTEM IN THE RAT

By

Ye Tian

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ABSTRACT

NEUROCHEMICAL CHARACTERIZATION OF THE INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONAL SYSTEM IN THE RAT

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Incertohypothalamic dopaminergic (DA) neurons have cell bodies in medial zona incerta (MZI) and axon terminals in the dorsomedial nucleus (DMN). Both regions contain a high density of norepinephrine (NE)-containing nerve terminals which may complicate interpretation of neurochemical changes used in estimating activity of incertohypothalamic DA neurons. Since blockade of aromatic L-amino acid decarboxylase causes 3,4-dihydroxyphenylalanine (DOPA) to accumulate within both DA and NE neurons, this technique cannot be employed to measure selectively the activity of incertohypothalamic DA neurons. Concentrations of a major metabolite of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), in MZI and DMN reflect the activity of incertohypothalamic DA neurons providing NE neurons are not activated. If NE neurons are activated both dopamine and DOPAC accumulate within NE neurons. Therefore, the following approaches were employed to monitor the activity of incertohypothalamic DA neurons: DOPAC and dopamine concentrations were measured in MZI and DMN; alterations in DOPAC concentrations without changes in dopamine concentrations reflect the activity of incertohypothalamic DA neurons. Increases in concentrations of dopamine

and 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG, a major metabolite of NE) reflect an activation of NE neurons. If manipulations increased dopamine and MHPG concentrations, experiments were repeated in animals whose NE neurons projecting to the hypothalamus had been destroyed. In these animals DOPAC concentrations in MZI and DMN reflect changes in the activity of incertohypothalamic DA neurons.

Using these techniques, responses of incertohypothalamic DA neurons to drug treatments were examined. Results are summarized as follows. Although SCH 39166, raclopride or remoxipride administration activate mesotelencephalic DA neurons, only raclopride activates incertohypothalamic DA neurons. These results indicate that dopamine receptor-mediated regulation of incertohypothalamic DA neurons is different from that of mesotelencephalic DA neurons, and suggest that a subgroup of D2 or a novel type of dopamine receptor regulates incertohypothalamic DA neurons. 5-Hydroxytryptamine (5HT) neurons do not tonically regulate incertohypothalamic DA neurons, but do inhibit NE neurons projecting to MZI and DMN. Morphine stimulates incertohypothalamic DA neurons through a μ opioid receptor-mediated mechanism that does not involved 5HT neurons. A neurotensin agonist, Sub-NT_{8-13}, activates both incertohypothalamic DA neurons and NE neurons projecting to MZI and DMN.

To my wife, Yanying, for her love, support and patience; my daughter, Iris, for the joy and happiness she gives to me.

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LIST OF ABBREVIATIONS

5-ADMP	5-amino-2,4-dihydroxy-α-methylphenylethylamine
5HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine
5HTP	5-hydroxytryptophan
5,7-DHT	5,7-dihydroxytryptamine
8-OH-DPAT	(±)-8-hydroxy-(DL-n-propylamino)-tetralin
AAAD	aromatic L-amino acid decarboxylase
COMT	catechol-O-methyltransferase
DA	dopaminergic
DBH	dopamine ß-hydroxylase
DMN	dorsomedial nucleus of the hypothalamus
DOPA	L-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
DOPEG	3,4-dihydroxyphenylglycol
GBL	γ-butyrolactone
HPLC	high performance liquid chromatography
HVA	homovanillic acid
i.c.	intracerebral
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
i.v.	intravenous
MAO	monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenylethyleneglycol
MZI	medial zona incerta
NE	norepinephrine
NSD 1015	m-hydroxybenzylhydrazine
s.c.	subcutaneous
TH	tyrosine hydroxylase

1.INTRODUCTION

Catecholamine-containing cells in the rat brain have been identified by an alphanumeric system suggested by Dahlström and Fuxe (1964, Figure 1.1). Norepinephrine (NE) neurons located caudally in the pons and medulla are identified as A_1-A_7, whereas dopaminergic (DA) neurons located more rostrally from the midbrain to the olfactory bulb are identified as A_8-A_{16}. Axons of NE neurons project diffusely throughout the brain and spinal cord (Moore and Bloom, 1979) whereas the DA neurons have a more precise and selective distribution pattern (Moore and Bloom, 1978).

DA cells located in the mesencephalon in the pars compacta of the substantia nigra (A_8-A_9) and in the ventral tegmental area (A_{10}) give rise to the major ascending DA neuronal systems to the telencephalon; collectively, these neurons are referred to as the mesotelencephalic DA system. The mesotelencephalic DA neurons have been subdivided into: 1) nigrostriatal neurons projecting from the pars compacta of the substantia nigra to the striatum (caudate/putamen), 2) mesolimbic neurons projecting from the ventral tegmental area to a variety of subcortical brain regions, such as nucleus accumbens, and 3) mesocortical neurons projecting to cingulate, prefrontal and pyriform cerebral cortices. Most of what is known about DA neurons has been learned from studies on the mesotelencephalic system.

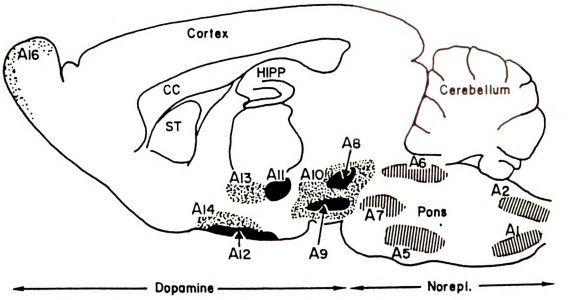

Figure 1.1. Schematic view showing the location of the catecholamine-containing perikarya in a sagittal section of the rat brain (modified from Hökfelt et al., 1984a). A_{1-16}, cell groups according to Dahlström and Fuxe (1964) in which A_{1-7} contain NE whereas A_{8-16} contain DA; CC, corpus callosum; HIPP, hippocampus; ST, striatum.

A. Anatomy of hypothalamic dopaminergic neuronal systems

There are four distinct groups of DA cell bodies located in the hypothalamus - A_{11}, A_{12}, A_{13} and A_{14} (Figure 1.1). In the mediobasal hypothalamus tuberoinfundibular neurons project from A_{12} cell bodies in the arcuate nucleus to the external layer of the median eminence, where they terminate close to the primary capillary loops of the hypophysial portal system. Dopamine released from these neurons is transported in the blood via the hypophysial portal system to the anterior pituitary where it activates D2 receptors on prolactin-secreting cells and thereby inhibits the release of prolactin (Moore, 1987).

Tuberohypophysial DA neurons were originally proposed to project from a sub-population of the A_{12} cells in the rostral arcuate nucleus to the neural and intermediate lobes of the pituitary (Björklund et al, 1973), but more recent anatomical studies suggest that DA neurons terminating in the posterior pituitary originate, not from A_{12} cells in the arcuate nucleus, but from the more rostral and dorsal A_{14} group (Kawano and Daikoku, 1987). The axons of these neurons travel through the median eminence and infundibular stalk and terminate in the posterior pituitary (Björklund et al, 1973). In the intermediate lobe of the pituitary, dopamine released from the tuberohypophysial DA neurons inhibits the secretion of ß-endorphin and α-melanocyte stimulating hormone (Holzbauer and Racké, 1985).

A_{11}, A_{13} and A_{14} DA neurons were collectively referred to as the incertohypothalamic DA system by Björklund et al. (1975; Figure 1.2). The most rostral group of incertohypothalamic DA neurons are the A_{14} cells, which are located in the

periventricular hypothalamic areas on either side of the 3rd ventricle; these cells extend from the level of the anterior commissure caudal to the level of median eminence (Björklund and Nobin, 1973). Rostral A_{14} neurons are reported (Björklund et al., 1975) to project from periventricular region laterally to the medial preoptic area and rostrally to the periventricular and suprachiasmatic preoptic nuclei, whereas, caudal A_{14} neurons project to the posterior pituitary (Kawano and Daikoku, 1987). The A_{11} DA cells are located in the caudal and ventral thalamus, and the caudal and dorsal hypothalamus, principally scattered around the mammillothalamic tract (Björklund and Nobin, 1973). Some A_{11} DA neurons project to the spinal cord (Björklund and Skagerberg, 1980; Lindvall and Björklund, 1983). The DA neurons located in the medial zona incerta (MZI) are the A_{13} group. Axons of the A_{13} DA neurons have been suggested to project only short distances within the diencephalon (Björklund et al., 1975).

B. A_{13} incertohypothalamic dopaminergic neurons

Because the A_{13} DA neurons are located in the MZI, and some of their axon terminals project to the hypothalamus, in this dissertation only this group of DA neurons will be referred to as the incertohypothalamic DA neurons. Incertohypothalamic DA neurons were first identified with the Falck-Hillarp fluorescence technique as densely packed small, round or oval-shaped cells, located in the MZI, dorsal to the dorsomedial nucleus of the hypothalamus (DMN) and ventromedial to the mammillothalamic tract (Björklund and Nobin, 1973). Subsequent to the initial anatomical characterization, it has been shown that incertohypothalamic DA neurons include three morphologically distinct groups of tyrosine hydroxylase (TH) containing cells (Sanghera, 1989). Large

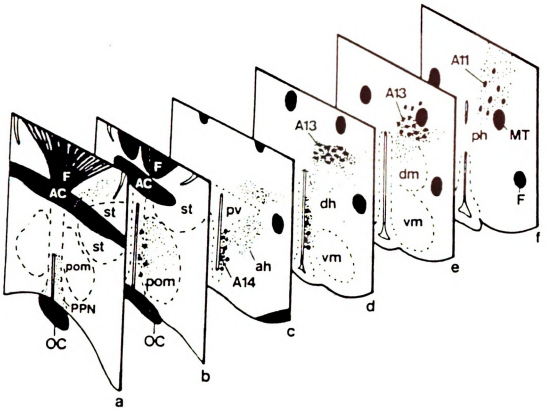

Figure 1.2. Frontal sections through the hypothalamus of rat brain showing the cell bodies and terminals of the incertohypothalamic DA neurons. Abbreviations: AC, anterior commissure; ah, anterior hypothalamic area; A_{11}, A_{13} and A_{14}, dopaminergic cell groups; dh, dorsal hypothalamic area; dm, dorsomedial nucleus; F, fornix; MT, mammillothalamic tract; OC, optic chiasm; ph, posterior hypothalamus; pom, medial preoptic area; PPN, preoptic periventricular nucleus; pv, paraventricular nucleus; st, bed nucleus of stria terminalis; vm, ventromedial nucleus (Björklund et al., 1975).

fusiform cells with a mean diameter 15 μm distribute throughout the rostrocaudal extent of the nucleus. A second group of the TH-positive cells found throughout the MZI are oval-shaped having a mean diameter about 12 μm. A third group of TH-positive cells found only in the most caudal portion of the MZI are small round shaped with a mean diameter about 9 μm. The precise projections of these neurons have not been well defined but Björklund and colleagues (1975) suggested that these neurons project rostrally to the dorsal anterior hypothalamic area and ventrally to the dorsal part of the DMN. Using more precise immunohistochemical techniques the A_{13} DA neurons were found to cluster in the MZI and project locally with lightly stained short dendrites within the MZI as well as other directions (Van den Pol et al., 1984; Chan-Palay et al., 1984). Consistent with early studies (Björklund et al., 1975), immunocytochemical evidence also indicates that DMN receives most TH-positive fiber projections from the MZI (Chan-Palay et al., 1984). It should be noted that the TH-positive fibers in the DMN could be DA nerve terminals, or axons and/or dendrites passing through this brain regions. In the MZI and DMN incertohypothalamic DA cell bodies are located among and their dendrites intermingled with a large number of NE axons projecting from NE neurons originating primarily from A_1 and A_2 groups (Lindvall and Björklund, 1983; Palkovits and Brownstein, 1989).

Figure 1.3, modified from a drawing by Chan-Palay and coworkers (1984), depicts TH immunoreactivity in a frontal section of the rat hypothalamus at approximately the level of 2.4 mm caudal to bregma (Paxinos and Watson, 1986). The right side of this figure shows TH-immunoreactive cell bodies and dendrites, the left side

shows TH-immunoreactive axons and terminals. The cells in the MZI are the A_{13} incertohypothalamic DA neurons. Those in the arcuate nucleus are the A_{12} tuberoinfundibular DA neurons. Axons of A_{13} neurons project ventrally into the DMN (Björklund et al 1975). It should be noted, however, that although the TH-immunoreactive cells in the MZI are incertohypothalamic DA neurons, the dense TH-immunoreactive terminals and axons in the DMN represent projections from both DA and NE neurons. In most regions of the hypothalamus the density of NE terminals exceeds that of DA terminals. The predominance of NE nerve terminals in the hypothalamus presents a major problem when neurochemical techniques are employed to study hypothalamic DA neurons.

Little is known about the physiological function of the incertohypothalamic DA neurons. These neurons have been suggested to play a role in the regulation of pituitary-gonadal systems. Naumenko and Serova (1976) reported that injection of dopamine or apomorphine into the MZI increases plasma testosterone levels in male rats. Destruction of MZI decreases the level of gonadotropin releasing hormone in the median eminence (Wilkes et al., 1979). Injection of dopamine into the MZI on the morning of proestrus overcomes the phenobarbital blockade of ovulation, whereas in another study, injection of the dopamine receptor antagonist haloperidol into the same region in the morning of the proestrus blocks ovulation the next day (MacKenzie et al., 1984). Dopamine injected into the MZI increases plasma luteinizing hormone and prolactin concentrations in ovariectomized and estrogen primed rats (MacKenzie et al., 1984) and electrical lesion of the MZI abolishes proestrus surges of these hormones (Sanghera et al., 1991) and

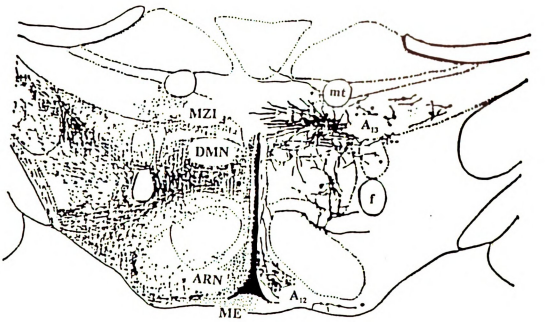

Figure 1.3. Frontal section through the rat hypothalamus showing the TH-immunoreactive cell bodies and dendrites (right) and axons and terminals (left; modified from Chan-Palay et al., 1984). Abbreviations: A_{12} and A_{13}, dopaminergic neurons; ARN, arcuate nucleus; DMN, dorsomedial nucleus of the hypothalamus; f, fornix; mt, mammillothalamic tract; MZI, medial zona incerta.

thereby induce constant diestrous in female rats (MacKenzie et al., 1984). Accordingly, DA neurons in the MZI seem to be important in regulation of luteinizing hormone secretion, and therefore, ovulation in female rats. Injection of apomorphine locally into the medial preoptic area, one possible terminal region of incertohypothalamic DA neurons, has a facilitatory effect on male sexual behavior (Bitran et al., 1988). Moreover, bilateral injections of ibotenic acid (an excitatory neurotoxin that destroys cell bodies at the site of injection) into the MZI reduce the lordosis behavior in ovariectomized estrogen- and progesterone-treated female rats (Dornan et al., 1991). Therefore, incertohypothalamic DA neurons also play a role in reproductive behaviors in rats.

Very little is known about the regulation of the incertohypothalamic DA neurons. Like nigrostriatal DA neurons, incertohypothalamic DA neurons are responsive to dopamine receptor agonists and antagonists (Lookingland and Moore, 1984b). Haloperidol, a dopamine receptor antagonist, increases and apomorphine, a dopamine receptor agonist, decreases the turnover rate of dopamine in the MZI and DMN. Treatment with apomorphine reverses the γ-butyrolactone (GBL)-induced increase in dopamine concentrations in the MZI and DMN, suggesting incertohypothalamic DA neurons are regulated by dopamine autoreceptors (Roth, 1979; Lookingland and Moore, 1984b). This idea is supported by electrophysiological observations; dopamine and apomorphine inhibit the firing rate of neurons located in the MZI, presumably incertohypothalamic DA neurons, and haloperidol prevents the inhibitory effect of dopamine on these neurons (Sanghera, 1989; Eaton and Moss, 1989). The

incertohypothalamic DA neurons have also been reported to be stimulated by estrogen through a prolactin-mediated mechanism (Lookingland and Moore, 1984a).

C. Biochemistry of dopaminergic and norepinephrine neurons

The following is a description of the biochemical events that occur at the terminals of DA neurons; most of this information has been obtained from studies on nigrostriatal DA neurons for the past 20 years (see Figure 1.4, top). Tyrosine is transported into the nerve terminal by an active transport process (Guroff et al., 1961), where it is converted to 3,4-dihydroxyphenylalanine (DOPA) by TH. DOPA is then rapidly decarboxylated to dopamine; this reaction is catalyzed by aromatic L-amino acid decarboxylase (AAAD). The newly synthesized dopamine is packaged in synaptic vesicles and either stored or released in response to the arrival of nerve action potentials. TH is the rate limiting enzyme for dopamine synthesis so that as soon as DOPA is formed it is quickly decarboxylated to dopamine (Nagatsu, 1973). Under normal conditions, therefore, the DOPA concentration in DA neurons is very low. Once dopamine is released it is free to activate either 1) receptors located on membranes of postsynaptic neurons, or 2) presynaptic-autoreceptors. Activation of these autoreceptors reduces the synthesis and possibly the release of dopamine (Roth, 1979; Wolf and Roth, 1990).

Dopamine is removed from the synaptic cleft principally by a high affinity uptake system located on the DA nerve terminal. The recaptured dopamine can be repackaged into synaptic vesicles and re-utilized by the neuron, or metabolized by mitochondrial monoamine oxidase (MAO) to 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC, in

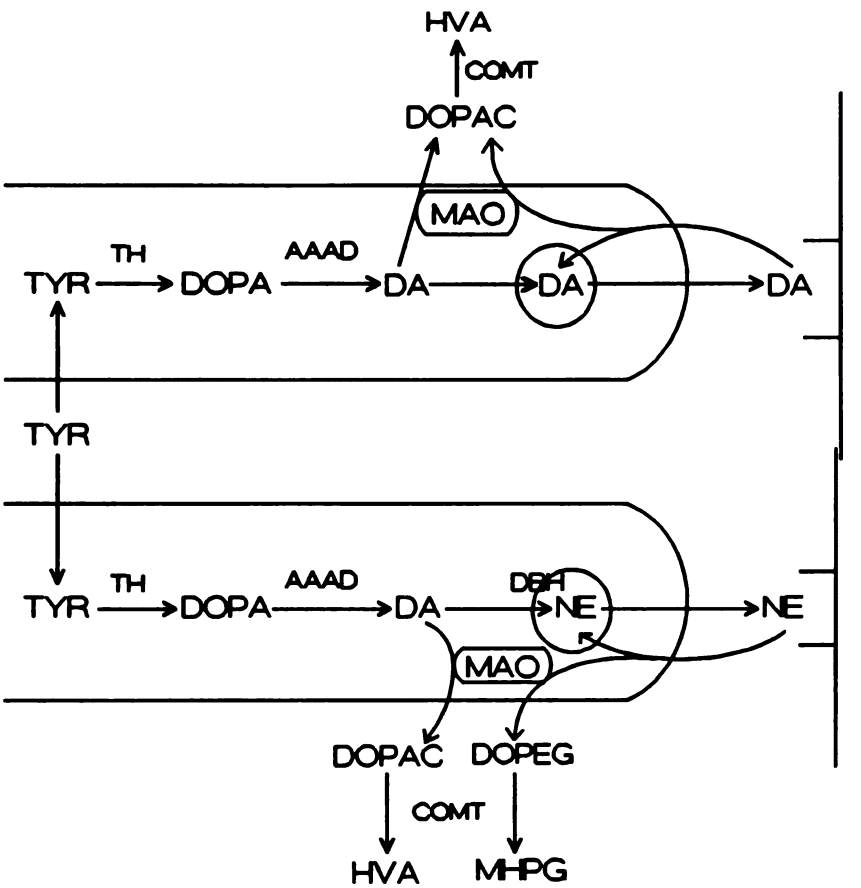

Figure 1.4. Schematic diagrams of the neurochemical events that occur in terminals of DA (top) and NE (bottom) neurons. Abbreviations: AAAD, aromatic L-amino acid decarboxylase; COMT, catechol-O-methyltransferase; DBH, dopamine ß-hydroxylase; DA, dopamine; DOPA, dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPEG, 3,4-dihydroxyphenylglycol; HVA, homovanillic acid; MAO, monoamine oxidase; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; TH, tyrosine hydroxylase; TYR, tyrosine.

turn, is lost from the neuron, and can be further metabolized to homovanillic acid (HVA) by a reaction catalyzed by catechol-O-methyltransferase (COMT; Westerink, 1985). A small portion of dopamine can also be oxidatively deaminated by extraneuronal MAO to DOPAC or directly methylated by COMT to form 3-methoxytyramine (Westerink and Korf, 1976; Westerink, 1979; Waldmeier et al., 1981). 3-Methoxytyramine can also be further oxidized to HVA.

In terminals of NE neurons, as in DA neurons, tyrosine is converted to dopamine by TH and AAAD. Once dopamine is formed, it is transported into synaptic vesicles and within these vesicles dopamine is further hydroxylated at the ß carbon to form NE, this reaction is catalyzed by dopamine ß-hydroxylase (DBH). The newly synthesized NE can either be stored in the vesicle or released upon the arrival of an action potential. Like dopamine, released NE can activate both post- and presynaptic receptors. Activation of presynaptic adrenergic autoreceptors inhibits the activity of NE neurons (for review see Salzman and Roth, 1979; Scatton, 1990).

The actions of NE in the synaptic cleft are terminated, like dopamine, mainly by a high affinity uptake system on the nerve terminal (Kopin, 1985). The recaptured NE can either be restored in synaptic vesicles for future use or be metabolized. Non-vesicular NE can be deaminated by MAO intraneuronally to form 3,4-dihydroxyphenylglycol (DOPEG) which is then lost from neurons. DOPEG is taken up by glial cells and further metabolized to 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) by COMT. Released NE can also be methylated directly by COMT to form normetanephrine, which is further oxidized by MAO to MHPG. MHPG can either

diffuse out of the brain as a free acid or be conjugated (Nagatsu, 1973). In the rat, MHPG is mainly present in the brain as a sulfonate conjugate and represents the major metabolite of NE (Schanberg et al., 1968a,b). In NE nerve terminals, newly synthesized dopamine can be converted by mitochondrial MAO to DOPAC. Under basal conditions, newly synthesized dopamine is quickly transported into synaptic vesicles and converted to NE and very little dopamine is metabolized by MAO to DOPAC. On the other hand, when NE neurons are activated, TH is activated and the reaction catalyzed by this enzyme is no longer the rate limiting step. The transport of dopamine into synaptic vesicle and/or DBH may become rate limiting (for review see Salzman and Roth, 1979). Therefore, when NE neurons are activated dopamine accumulates within NE neurons and substantial amount of dopamine metabolites can be formed within NE neurons (Andén and Grabowska-Andén, 1983; Boulat et al., 1990). Similarities in the synthesis and metabolism of dopamine and NE make it difficult to study incertohypothalamic DA neurons biochemically since they are located in regions which are densely innervated by NE neurons.

D. Neurochemical techniques to estimate dopaminergic neuronal activity

In order to study incertohypothalamic DA neurons one must have a way of quantifying their activity. The activity of mesotelencephalic DA neurons has been estimated in anesthetized animals by recording extracellular unit activity from electrodes placed in the tightly packed A_8, A_9, and A_{10} cell bodies (Bunney, 1979). There have been very few efforts to record electrical activity from identified DA neurons in the hypothalamus, although there are two recent reports on electrical activity of putative DA

neurons located in the MZI. Sanghera (1989) recorded extracellular electrical activity from MZI cells in anesthetized rats, and Eaton and Moss (1989) recorded extracellular unit activity from the same region in hypothalamic slices. Unfortunately, these investigators were unable to determine if they were recording from DA neurons. An *in vivo* microdialysis technique has been developed to measure the release of dopamine directly from terminal regions of DA neurons (Di Chiara, 1990; Imperato and Di Chiara, 1985), but to date, this technique has only been used effectively in relatively large brain regions that contain predominant DA nerve terminals, such as striatum and nucleus accumbens. Up to the present time, no one has attempted to use the microdialysis technique to detect release of dopamine from incertohypothalamic DA neurons. Therefore, to estimate activity of hypothalamic DA neurons *in situ* in unanesthetized animals one is limited to the use of postmortem biochemical methods.

Biochemical methods for estimating DA neuronal activity are based upon the observations that the rates of synthesis, turnover and metabolism of dopamine in the terminals of DA neurons are proportional to the impulse traffic in these neurons (Murrin and Roth, 1976; Roth et al, 1976). These biochemical techniques have been used to estimate the activity of nigrostriatal DA neurons:

1) To estimate dopamine synthesis the rate of DOPA accumulation after the administration of m-hydroxybenzylhydrazine (NSD 1015), an inhibitor of AAAD, has been employed. In the absence of an AAAD inhibitor the concentration of DOPA in the striatum is essentially zero, but after injecting NSD 1015 the concentration of DOPA increases linearly with time at a rate that is proportional to nigrostriatal DA nerve activity

(Murrin and Roth, 1976).

2) To estimate dopamine metabolism the concentration of dopamine metabolites, e.g., DOPAC, has been measured in the striatum. Increased activity of nigrostriatal DA neurons, and thus increased release of dopamine from the terminals, is accompanied by an increased concentration of DOPAC in the striatum (Roth et al, 1976).

3) To estimate dopamine turnover α-methyltyrosine, which blocks dopamine synthesis at the TH step, has been administered. Following α-methyltyrosine dopamine is released but cannot be replaced by synthesis. Thus, the concentration of dopamine in nerve terminals declines at a rate that is proportional to nerve activity (Brodie et al, 1966).

To summarize, the rates of synthesis and turnover of dopamine and the concentration of DOPAC in brain regions containing DA nerve terminals reflect impulse traffic (or activity) of these neurons (for review see Moore, 1987). These techniques have been employed successfully to estimate activity of nigrostriatal (Roth et al., 1976; Murrin and Roth, 1976), tuberoinfundibular (Demarest and Moore, 1980; Lookingland et al., 1987) and tuberohypophysial DA neurons (Alper et al., 1980b; Lindley et al., 1990). It should be noted, however, that these DA neurons terminate in regions (striatum, median eminence, posterior pituitary) where the density of NE nerve terminals is comparatively low. In the striatum there is essentially no NE (Westerink and De Vries, 1985; Palkovits and Brownstein, 1989). In the median eminence and posterior pituitary the dopamine content is approximately 3 times that of NE, and rates of synthesis and turnover of dopamine in this brain region are much higher than that of NE (Demarest

et al., 1979; Lookingland and Moore, 1984b). Accordingly, neurochemical studies of nigrostriatal DA neurons terminating in striatum, tuberoinfundibular DA neurons terminating in median eminence and tuberohypophysial DA neurons terminating in posterior pituitary are not complicated by interference from NE neurons. On the other hand, in the hypothalamus the density of NE terminals is higher than that of the DA terminals (Palkovits and Brownstein, 1989) and this could present problems when neurochemical techniques are employed to estimate DA neuronal activity.

E. Statement of the goal of the thesis

The objective of this dissertation was to neurochemically characterize the regulation of incertohypothalamic DA neurons by comparing it with that of other DA neuronal systems in the rat brain. Since incertohypothalamic DA neurons are located in brain regions which contain dense NE axons and terminals, it was realized that appropriate techniques would have to be developed to distinguish drug effects on DA neurons from those on NE neurons. The appropriateness of using classical neurochemical measurements of the metabolism and synthesis of dopamine to estimate the activity of incertohypothalamic DA neurons was investigated. Based on the results of studies on the synthesis and metabolism of dopamine and NE, experimental protocols were developed. Using these experimental protocols responses of incertohypothalamic DA neurons to a variety of pharmacological treatments were examined.

2. MATERIALS AND METHODS

Animals

Male and female Long-Evans rats weighing between 200-225 g (Harlan Breeding Laboratories, Minneapolis, MN), maintained in a temperature- (22 ± 1°C) and light- (lights on between 0600 and 2000 h) controlled environment, were provided food and tap water *ad libitum*. Daily vaginal lavage was employed to determine the days of the estrous cycle in female rats. Male rats were used in most of the studies and in a few studies regular cycling female rats were used at the first day of diestrus.

Drugs

Pargyline HCl (Sigma Chemical Co., St. Louis, MO), nomifensine maleate (kindly supplied by Dr. S. Fielding; Hoechst-Roussel Pharmaceuticals, Somerville, NJ), apomorphine (Eli Lilly Co, Indianapolis, Ind) and NSD 1015 HCl (Sigma Chemical Co., St. Louis, MO), were dissolved in 0.9 % saline. Apomorphine HCl (Eli Lilly Co, Indianapolis, IN), idazoxan HCl (Reckitt & Colman Pharm Div, Hull, U.K.), morphine sulfate (Mallinckrodt, St. Louis, MO), naltrexone HCl (Endo Laboratories, Garden City, NY), (±)-8-hydroxy-2(DL-n-propylamino)-tetralin HBr (8-OH-DPAT; Sigma Chemical Co., St. Louis, MO), desipramine HCl (Sigma Chemical Co., St. Louis, MO); remoxipride HCl and raclopride tartrate (generously provided by Dr. S.O. Ögren, Astra Laboratory, Södertälje, Sweden) were dissolved in distilled water. Neurotensin acetate (Sigma Chemical Co., St. Louis, MO), Sub-NT_{8-13} trifluoroacetate (generously provided by Dr. M. Duff Davis, Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI),

5-amino-2,4-dihydroxy-α-methylphenylethylamine dihydrobromide (5-ADMP (U72632E);synthesized by John R. Palmer, The Upjohn Co., Kalamazoo, MI) and 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT; Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% saline containing 0.1% ascorbic acid. Haloperidol (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.3% tartaric acid. SCH 39166 HCl (generously provided by Dr. A. Barnett, Schering-Plough Corporation, Bloomfield, New Jersey) was dissolved in 50% ethanol. Drugs were administered as indicated in the legends of the appropriate figures and tables. Doses of drugs refer to their respective salts except those indicated as free base.

Neurochemical lesions of the ventral norepinephrine bundle

Animals receiving intracerebral (i.c.) injections of 5-ADMP or its vehicle were anesthetized with Equithesin (3 ml/kg; intraperitoneal (i.p.)) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set -3 mm below the ear bar. The needle of a 5 μl Hamilton syringe was inserted into the ventral NE bundle at coordinates A: 0.0 mm from interaural line, L: $\pm$ 1.3 mm from midline, V: -7.0 mm from dura (Paxinos and Waston, 1986), and bilateral injections of either 5-ADMP (8 μg free base/side) or its vehicle (0.3 μl/side) were made over a 1 min period. The syringe needle remained in the brain for an additional 10 min after the injection to reduce reflux of neurotoxin into the needle tract.

Neurochemical lesion of the 5-hydroxytryptamine neurons

Animals receiving intracerebroventricular (i.c.v.) injections of 5,7-DHT or its vehicle were anesthetized with Equithesin (1.5 ml/kg; i.p.) and positioned in a stereotaxic

frame (David Kopf Instruments, Tujunga, CA) with the incisor bar set -2.4 mm below the ear bar. The needle of a 5 μl Hamilton syringe was inserted into the right lateral ventricle at coordinates A: 0.0 mm from bregma, L: 1.4 mm from midline, V: -3.2 mm from dura (Paxinos and Waston, 1986). Either 5,7-DHT (200 μg in 5 μl) or its vehicle was injected 45 min after an injection of desipramine (25 mg/kg; i.p.) with a 5 μl Hamilton syringe over a 5 min period. The syringe needle remained in the brain for an additional 10 min after the injection to reduce reflux of neurotoxin into the needle tract.

Routes of drug administration

Drugs were administered subcutaneously (s.c.) or i.p. in volumes of 1 ml/kg or 2 ml/kg body weight as indicated in the legends to the figures or tables. In experiments requiring intravenous (i.v.) drug injections, animals were implanted with a polyethylene catheter inserted into the right atrium via the right jugular vein under diethyl ether anesthesia 2 days prior to injections. The i.v. injection volume was 2 ml/kg.

Tissue dissection

Following appropriate treatments, animals were decapitated, and their brains quickly removed from the skull and frozen on aluminum foil 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 MZI and DMN as well as other brain regions were dissected from these sections using a modification (Lookingland and Moore, 1984b) of the technique of Palkovits (1973). Tissue samples were placed in 30 or 60 μl of 0.1 M phosphate/citrate buffer (pH 2.5) containing 15% methanol and stored at -20°C until assayed.

Acid hydrolysis procedure

MHPG-sulfate conjugate is electrochemically inactive and, therefore, cannot be detected by electrochemical methodology (Warnhoff, 1984). Accordingly, deconjugation procedures such as acid hydrolysis (Artigas et al., 1986; Lookingland et al., 1991) which remove the sulfate moiety and liberate free MHPG have to be employed before samples can be analyzed by high performance liquid chromatography (HPLC) coupled with electrochemical detection. The optimal conversion of MHPG-sulfate to free MHPG using acid hydrolysis occurs when samples are heated at 94°C for 5 min in the presence of 0.16 M perchloric acid (Lookingland et al., 1991), and these incubation conditions were used to hydrolyze brain tissue extracts in the present study.

Assays

The contents of dopamine, DOPAC, DOPA, NE, MHPG, 5-hydroxytryptamine (5HT), 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophan (5HTP) in extracts of brain regions were determined by HPLC coupled to electrochemical detection as described previously (Chapin et al., 1986; Lindley et al., 1990; Lookingland et al., 1991). On the day of the assay, tissue samples were thawed, sonicated for 3 s (Sonicator Cell Disrupter, Heat Systems-Ultrasonic, Plainview, NY), and centrifuged for 30 s in a Beckman 152 Microfuge. For samples to be analyzed for MHPG and NE, supernatants (30 μl) were acidified with an equal volume of 0.32 M perchloric acid (0.16 M final acid concentration), heated for 5 min at 94°C, and immediately frozen on dry ice (Lookingland et al., 1991). Fifty 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, West Lafayette, IN) which was protected by a precolumn cartridge filter (5 μm spheres; 30 x 4.6 mm). The HPLC column was coupled to a single coulometric electrode conditioning cell in series with dual electrode analytical cells (ESA, Bedford, MA). The conditioning cell electrode was set at +0.40 V, while the analytical electrodes were set at +0.12 V and -0.40 V, respectively, relative to internal silver reference electrodes. The HPLC mobile phase consisted of 0.1 M phosphate/citrate buffer (pH 2.7) containing 0.1 mM EDTA, 0.035% sodium octylsulfate and 10% to 25% methanol. The amounts of amines, their precursors and metabolites in samples were determined by comparing peak heights (recorded from the -0.40 V analytical electrode by a Hewlett Packard Integrator, Model 3393A) with those obtained from standards run the same day. The lower limit of sensitivity for these compounds was approximately 0.5 pg/sample. Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein (Lowry et al., 1951).

Statistical Analyses

Statistical analyses were conducted using Student's t-test for comparisons between two groups and one-way analysis of variance followed by Student-Neuman-Keuls test for comparisons among three or more groups (Steel and Torrie, 1960). Differences were considered significant if the probability of error was less than 5%.

3. DOPA ACCUMULATION NON-SELECTIVELY MEASURES THE OVERALL DOPAMINE SYNTHESIS IN BOTH DOPAMINERGIC AND NOREPINEPHRINE NEURONS

Introduction

As mentioned in the Chapter 1, the synthesis of dopamine in DA neurons is coupled with the activity (impulse traffic) of these neurons (Murrin and Roth, 1976). As a result, when animals are treated with an AAAD inhibitor at a dose which blocks the conversion from DOPA to dopamine, DOPA accumulates within DA nerve terminals at a rate that is proportional to the activity of these neurons. Therefore, the rate of DOPA accumulation after NSD 1015 in brain regions containing a predominance of DA nerve terminals reflects the rate of dopamine synthesis or the activity of TH within DA neurons. Since Carlsson and his colleagues (1972) introduced this technique, DOPA accumulation has been used successfully to estimate the activity of mesotelencephalic (Murrin and Roth, 1976), tuberoinfundibular (Demarest and Moore, 1980) and tuberohypophysial DA neurons (Demarest et al., 1979; Alper et al., 1980b). Since NE neurons share a common synthetic pathway with DA neurons, inhibition of AAAD also causes DOPA to accumulate within NE neurons. In the striatum, median eminence and posterior pituitary utilization of DOPA accumulation to estimate the activity of DA neurons is not complicated by the interference from NE neurons because of the low density of NE terminals (Palkovits and Brownstein, 1989; Moore and Bloom, 1979) and/or a low rate of NE turnover and synthesis in these regions (Demarest and Moore, 1980). In the MZI and DMN the density of NE fibers is high. It was predicted, therefore, that there would be difficulties interpreting results of DOPA accumulation

when this procedure was used to study incertohypothalamic DA neurons. Accordingly, the contributions from DA and NE neurons to the DOPA accumulation in the MZI and DMN were studied so as to evaluate the potential use of DOPA accumulation to estimate the activity of incertohypothalamic DA neurons.

Results

As depicted in the Table 3.1, the concentrations of NE in the MZI and DMN were much higher than that of dopamine, reflecting a high density of NE terminals in these regions. Incertohypothalamic DA neurons are regulated by autoreceptors (Lookingland and Moore, 1984b). Stimulation of these autoreceptor decreases, whereas blockade of these receptors increases the activity of incertohypothalamic DA neurons. Alterations in DOPA accumulation in the MZI and DMN were examined when the

Table 3.1. Dopamine and NE concentrations (ng/mg protein) in the MZI and DMN of male rats.

	MZI	DMN
Dopamine	3.7 ± .1	5.1 ± .3
NE	14.2 ± .5	59.9 ± 5.1

Values represent means ± 1 S.E.M. of 7-8 determinations.

activity of these DA neurons were manipulated pharmacologically. Two hr after injection of the dopamine receptor antagonist haloperidol DOPA accumulation increased

in the DMN, while 30 min after injection of the dopamine receptor agonist apomorphine DOPA accumulation decreased in the MZI and DMN (Figure 3.1). These data indicate that the rate of DOPA accumulation in the MZI and DMN following administration of a dopamine receptor antagonist or agonist alters in a way similar to that seen in the striatum (Wolf and Roth, 1990), and indicate that changes in the activity of incertohypothalamic DA neurons can be reflected by this measurement.

NE neurons are regulated by autoreceptors which belong to the α_2-adrenergic subtype (Cedarbaum and Aghajanian, 1977; Scatton, 1990). Activation of α_2-adrenergic receptors decreases the activity of NE neurons. Idazoxan is an α_2-adrenergic receptor antagonist, which when injected into animals increases the activity of NE neurons by blocking these inhibitory autoreceptors (Chapleo et al., 1981; Dettmar et al., 1983). Clonidine is an α_2-adrenergic receptor agonist. Treatment with this compound decreases the activity of NE neurons by activation of α_2-adrenergic autoreceptors (Unnerstall et al., 1984). To study the contribution of NE neurons to the DOPA accumulation in the MZI and DMN, the activity of central NE neurons was modulated by these α_2-adrenergic agonist and antagonist. Consistent with their α_2-adrenergic receptor antagonist and agonist properties, when rats were injected with idazoxan DOPA accumulation increased, whereas with clonidine DOPA accumulation decreased in the MZI and DMN. These results indicate that alterations in the activity of NE neurons projecting to these brain regions produce corresponding changes in DOPA accumulation (Figure 3.2).

A selective neurotoxin 5-ADMP (Jarry et al., 1986) was used to destroy NE neurons projecting to the hypothalamus. Seven days after bilateral injections of 5-ADMP

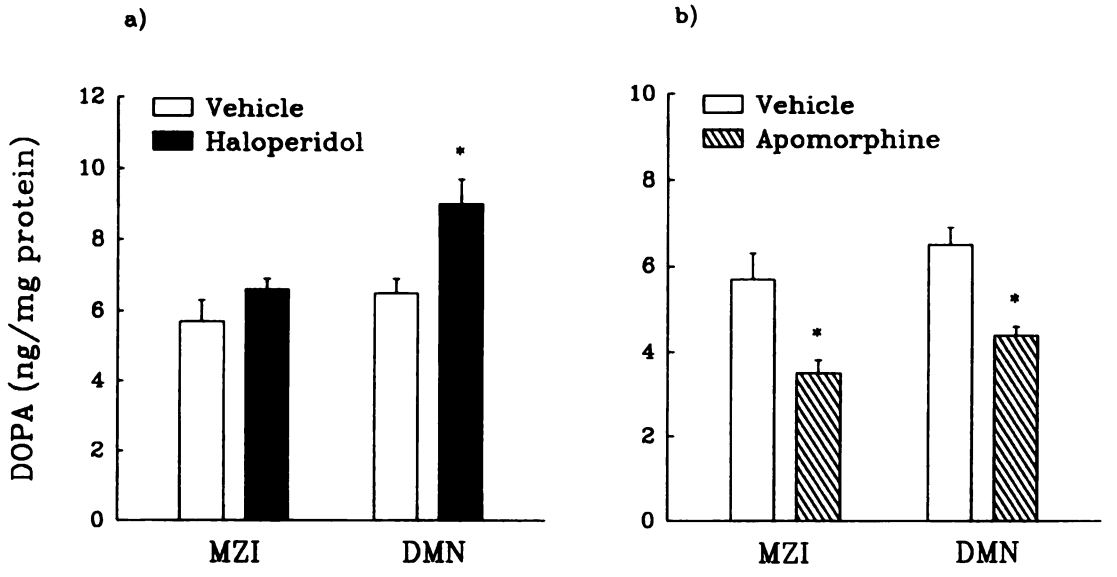

Figure 3.1. Effects of haloperidol and apomorphine on DOPA accumulation in the MZI and DMN. Male rats were injected with a) haloperidol (0.1 mg/kg; s.c.) or its 0.3% tartaric acid vehicle (1 ml/kg; s.c.) and killed 120 min later, or b) with apomorphine (2 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 30 min later. Thirty min prior to decapitation animals received an injection of NSD 1015 (100 mg/kg; i.p.). Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for drug-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

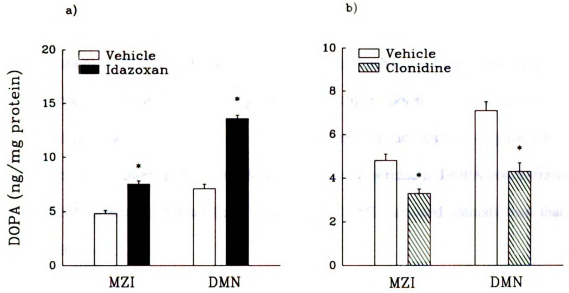

Figure 3.2. Effects of idazoxan and clonidine on DOPA accumulations in the MZI and DMN. Male rats were injected with a) idazoxan (20 mg/kg; s.c.) or b) clonidine (0.3 mg/kg; s.c.) or their water vehicle (1 ml/kg; s.c.) and killed 60 min later. Thirty min prior to decapitation animals received an injection of NSD 1015 (100 mg/kg; i.p.). Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for drug-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

into the ventral NE bundle, the NE content in the MZI and DMN was reduced to less than 20% of that in intact animals, indicating a marked loss of NE neurons projecting to these regions (Figure 3.3). Although the NE content was depleted over 80% after the injection of 5-ADMP, dopamine concentrations remain unaltered in these brain regions. The injection of 5-ADMP into the ventral NE bundle reduced DOPA accumulation by 40% in the MZI and 60% in the DMN.

After the destruction of NE neurons by 5-ADMP, although the absolute concentrations of DOPA in the MZI and DMN were much lower than that in intact rats, DOPA concentrations in these regions were still responsive to haloperidol and apomorphine in a manner that is similar to that seen in intact animals (Figure 3.4). It should be noted, however, that the haloperidol-induced increase in DOPA concentrations in the DMN seemed to be smaller in ventral NE bundle lesioned animals than that in intact animals.

Discussion

Since blockade of AAAD causes DOPA to accumulate within both DA and NE neurons, the measurement of the rate of DOPA accumulation in the MZI and DMN reflects the activity of TH in whole tissue; that is the overall synthetic capacity of dopamine of both neuronal systems. It has been shown that the activity of DA neurons is higher than that of NE neurons in the hypothalamus (Lookingland and Moore, 1984b). In this chapter, the possibility of using DOPA accumulation to study incertohypothalamic DA neurons was explored by examining contributions from both DA and NE neuronal

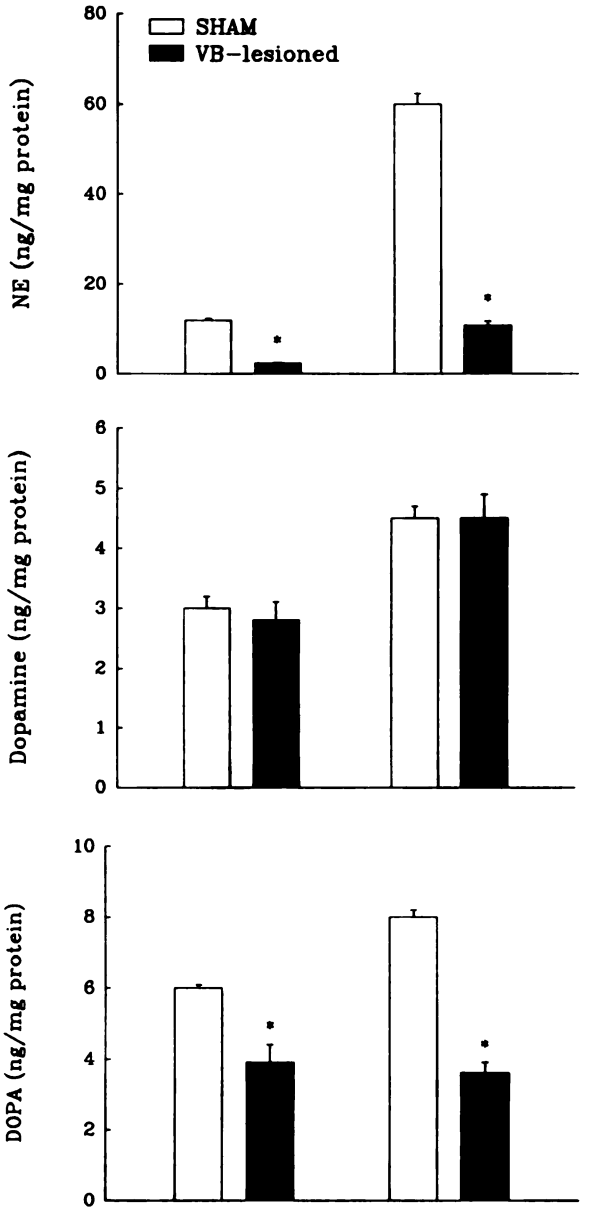

Figure 3.3. Effects of bilateral injections of 5-ADMP into the ventral NE bundle on NE and dopamine concentrations, and on DOPA accumulation in the MZI and DMN. Male rats were injected bilaterally with 5-ADMP (8 μg free base/side; i.c.; VB-lesioned) or its saline containing 0.1% ascorbic acid vehicle (0.3 μl/side; i.c.; SHAM). Seven days later rats were injected with NSD 1015 (100 mg/kg, i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 5-ADMP-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

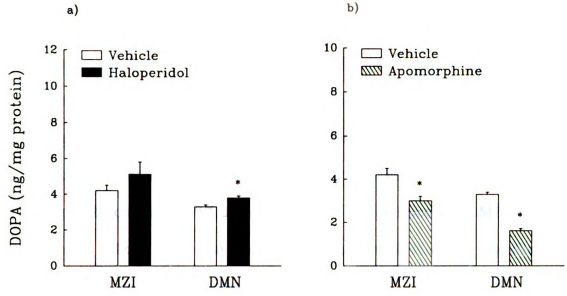

Figure 3.4. Effects of haloperidol and apomorphine on DOPA accumulation in the MZI and DMN of ventral NE bundle-lesioned rats. Seven days after an bilaterally injection of 5-ADMP (8 μg free base/site;i.c.), rats were injected with a) haloperidol (0.1 mg/kg; s.c.) or its 0.3% tartaric acid vehicle (1 ml/kg; s.c.) and killed 120 min later, or b) with apomorphine (2 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 30 min later. Thirty min prior to decapitation animals received an injection of NSD 1015 (100 mg/kg, i.p.). Columns represent the means and vertical lines 1 S.E.M. of 5-8 determinations. *, values for drug-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

system to DOPA accumulation. Injection of 5-ADMP, a selective neurotoxin (Jarry et al., 1987), into the ventral NE bundle greatly reduces the NE content in the MZI and DMN, confirming that NE neurons innervating these regions project mainly through ventral NE bundle (Moore and Bloom, 1979). A large reduction in DOPA accumulation, i.e. about half of that in intact animals, was observed in the MZI and DMN after the lesion of NE neurons, indicating that at least half of DOPA synthesis in these brain regions occurs in NE neurons. These data are consistent with the fact that the NE content in the MZI and DMN is 4-12 times that of dopamine (Table 3.1; Lookingland and Moore, 1984b). Accordingly, it is not surprising that a large amount of DOPA accumulates within NE nerve terminals after blockade of AAAD. It is also noted that more than 80% reduction in NE content after the ventral NE bundle-lesion is accompanied by only about 50% decrease in DOPA accumulation in the MZI and DMN. These results indicate either that the relatively small numbers of DA neurons have a higher level of activity (as indicated by Lookingland and Moore, 1984b) or the NE neurons remaining after the lesion increase their activity in a compensatory manner.

Incertohypothalamic DA neurons are regulated by dopamine receptor-mediated mechanisms (Lookingland and Moore, 1984b). In the present study, pharmacological manipulations which alter the activity of incertohypothalamic DA neurons produce characteristic changes in DOPA accumulation in the MZI and DMN. An increase in the activity of incertohypothalamic DA neurons after haloperidol increases the rate of DOPA accumulation in the DMN whereas a decrease in the activity of these neurons after apomorphine reduces the rate of DOPA accumulation in the MZI and DMN. Moreover,

after NE neurons projecting to the hypothalamus have been destroyed, DOPA accumulation in the MZI and DMN is still responsive to haloperidol and apomorphine treatments. It is unknown why the haloperidol-induced increase in DOPA accumulation in ventral NE neurons lesioned animals seems to be smaller. But it is unlikely that haloperidol has any stimulatory effect on NE neurons (see next chapter).

The activity of NE neurons is modulated by α_2-adrenergic autoreceptors (Scatton, 1990). Alterations in the activity of NE neurons also change DOPA accumulation in the MZI and DMN. The α_2-adrenergic receptor antagonist idazoxan increases, while the α_2-adrenergic receptor agonist clonidine decreases DOPA accumulation in these regions. It is noted, however, that the activation of incertohypothalamic DA neurons by haloperidol induces a smaller elevation in DOPA accumulation than that induced by activating NE neurons with idazoxan, reflecting the fact that there are more NE than DA neuronal fibers in the MZI and DMN. Since the number of NE fibers greatly exceeds that of DA fibers and the activity of NE neurons is low, when NE neurons are activated a relatively large signal is picked up by the measurement of DOPA accumulation. It could be that changes in the rate of DOPA accumulation after inhibition of AAAD in the MZI and DMN are more sensitive to alterations in the activity of NE neurons than of DA neurons. Nevertheless, alterations in the activity of both DA and NE neurons cause changes in the rate of DOPA accumulation. Thus, when an increased or a decreased rate of DOPA accumulation is observed in the MZI and DMN, it is difficult to determine if the change reflects alterations in NE or DA neuronal activity.

In summary, DOPA accumulates in both DA and NE neurons after blockade of

the AAAD. Alterations in activities of either DA or NE neurons causes corresponding changes in the DOPA accumulation in the MZI and DMN. Since an alteration in DOPA accumulation in these brain regions reflects an alteration in the overall activity of TH in both DA and NE neurons this technique cannot be employed to estimate selectively the activity of DA and NE neurons. In order to use this measurement to estimate the activity of incertohypothalamic DA neurons, it must be used in combination with other techniques so as to eliminate confounding data resulting from changes in NE neuronal activity.

4. CONTRIBUTION OF DOPAMINERGIC AND NOREPINEPHRINE NEURONS TO DOPAC CONCENTRATIONS IN THE MZI AND DMN

Introduction

In terminals of the major ascending mesotelencephalic DA neurons dopamine is metabolized by mitochondrial MAO to DOPAC (for review see Kopin, 1985). The rate of metabolism of dopamine is coupled to the release of dopamine from the mesotelencephalic DA neurons (Roth et al., 1976; Moore and Wuerthele, 1979). Accordingly, the DOPAC concentration in the striatum provides a good index of the activity of nigrostriatal DA neurons (Roth et al., 1976).

Since dopamine is a precursor of NE and can be metabolized to DOPAC in NE neurons (Andén and Grabowska-Andén, 1983; Curet et al., 1985; Scatton et al., 1984; Westerink and De Vries, 1985), DOPAC concentrations in the MZI and DMN may originate in NE neurons. This could confound interpretation of results from experiments employing DOPAC to estimate the activity of incertohypothalamic DA neurons. The purpose of studies described in this chapter was to examine the appropriateness of using DOPAC concentrations in the MZI and DMN as an estimate of the activity of incertohypothalamic DA neurons. If DOPAC can be used to estimate the activity of incertohypothalamic DA neurons, this technique offers the following advantages over other neurochemical techniques:

a) it can be used to measure rapid changes of neuronal activity,

b) it is more economical than employing the α-methyltyrosine procedure to calculate turnover rates,

c) it does not require pharmacological manipulations that disrupt catecholamine synthesis (i.e., with α-methyltyrosine or NSD 1015) and secretion of hormones (i.e., prolactin, α-melanocyte stimulating hormone).

Results

The concentrations of DOPAC, dopamine, MHPG and NE (and the ratios of DOPAC to dopamine and MHPG to NE) in acid hydrolyzed extracts of the MZI and DMN tissues microdissected from the brain of the male rat are listed in Table 4.1. In agreement with the report by Lookingland and Moore (1984b), the concentration of NE in the DMN was about six times that in the MZI, and the concentration of NE was much higher than that of dopamine in both regions. The concentrations of dopamine were three to four times greater than that of DOPAC and the concentrations of dopamine and DOPAC were about the same in the MZI and DMN. MHPG was detected in the MZI and DMN after deconjugation by acid hydrolyzation and its concentration was similar in both regions.

The concentration of DOPAC in the brain is dependent upon the balance between its rate of synthesis and its rate of removal. Following blockade of its synthesis with the MAO inhibitor pargyline DOPAC concentrations decreased rapidly in the MZI and DMN (Fig. 4.1a) with rate constants 3.3 min^{-1} in the MZI and 1.2 min^{-1} in the DMN (calculated from first-order decline kinetics).

DOPAC in brain tissues originates from the metabolism of either the dopamine that has been released and recaptured by nerve terminals; or newly synthesized non-vesicular dopamine that is metabolized intraneuronally. The contribution of recaptured

Table 4.1. DOPAC, dopamine, MHPG and NE concentrations (ng/mg protein) in acid hydrolyzed extracts of the MZI and DMN of male rats.

	MZI	DMN
DOPAC	.75 ± .04	.88 ± .05
Dopamine	3.3 ± .3	4.3 ± .3
DOPAC/Dopamine	.234 ± .009	.210 ± .010
MHPG	1.69 ± .03	1.74 ± .07
NE	11.0 ± .5	65.1 ± 3.1
MHPG/NE	.149 ± .005	.039 ± .002

Values represent means ± 1 S.E.M. of 7-8 determinations.

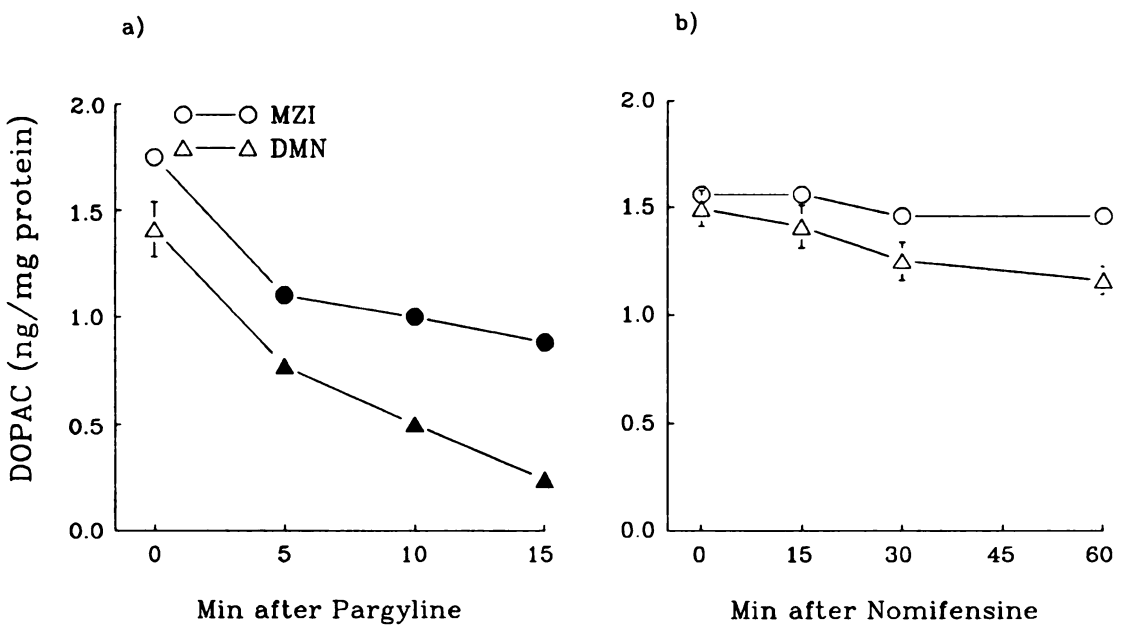

Figure 4.1. Time course effects of pargyline and nomifensine on DOPAC concentrations in the MZI and DMN. a) Male rats were injected with pargyline (50 mg free base/kg; i.v.) and killed 5, 10 or 15 min later. Zero time control animals were injected with 0.9% saline (2 ml/kg; i.v.) and killed 15 min later. b) Male rats were injected with nomifensine (25 mg/kg; i.p.) and killed 15, 30 or 60 min later. Zero time control animals for nomifensine were injected with 0.9% saline (2 ml/kg; i.p.) and killed 30 min later. Symbols represent the means and vertical lines 1 S.E.M. of 6-10 determinations; where no vertical lines are depicted, 1 S.E.M. is less than the radius of the symbol. Solid symbols represent the DOPAC concentrations in drug-treated animals that are significantly different ($P<0.05$) from vehicle-treated controls.

dopamine to DOPAC concentration was estimated by measuring DOPAC concentrations after administration of a dopamine uptake inhibitor nomifensine. DOPAC concentrations in the MZI and DMN were not altered for up to 60 min after injection of nomifensine (Figure 4.1b). These results indicate that the recapture of dopamine released from incertohypothalamic DA neurons does not contribute significantly to DOPAC concentrations in the MZI and DMN.

Alteration of the activity of incertohypothalamic DA neurons was achieved by administration of haloperidol and apomorphine. Two hr after an injection of haloperidol (0.1 mg/kg; s.c.), DOPAC concentrations increased in the MZI and DMN, whereas concentrations of dopamine, MHPG and NE were not altered (Figure 4.2). This result is consistent with reports that haloperidol is a D2 receptor antagonist which increases the activity of incertohypothalamic DA neurons without altering the activity of NE neurons projecting to the MZI and DMN. Thirty min after an injection of apomorphine (2 mg/kg; s.c.) DOPAC concentrations decreased in the MZI and DMN, but dopamine concentrations remained unaltered (Figure 4.3). These results indicate that an increase or decrease in the activity of incertohypothalamic DA neurons is reflected in the changes in DOPAC concentrations in the MZI and DMN.

Idazoxan, which activates NE neurons by blocking α_2-adrenergic autoreceptors (Jacobo, 1987), increased MHPG and decreased NE concentrations in the MZI and DMN (Figure 4.4). This is consistent with the report that MHPG is an index of the activity of NE neurons in other hypothalamic nuclei (Lookingland et al., 1991). The decrease in the NE concentrations following idazoxan administration suggests that when NE

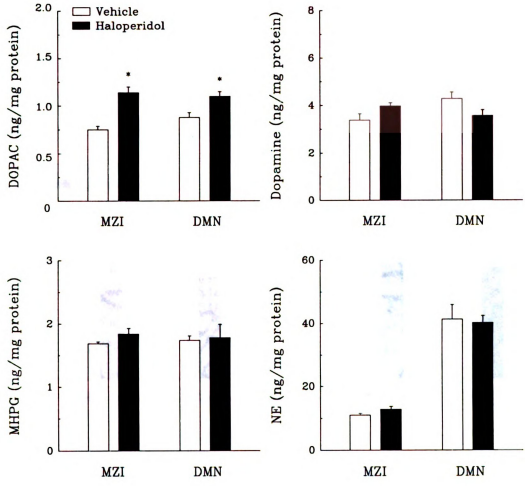

Figure 4.2. Effects of haloperidol on DOPAC, dopamine, MHPG and NE concentrations in the MZI and DMN. Male rats were injected with haloperidol (0.1 mg/kg; s.c.) or its 0.3% tartaric acid vehicle (1 ml/kg; s.c.) and killed 120 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for haloperidol-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

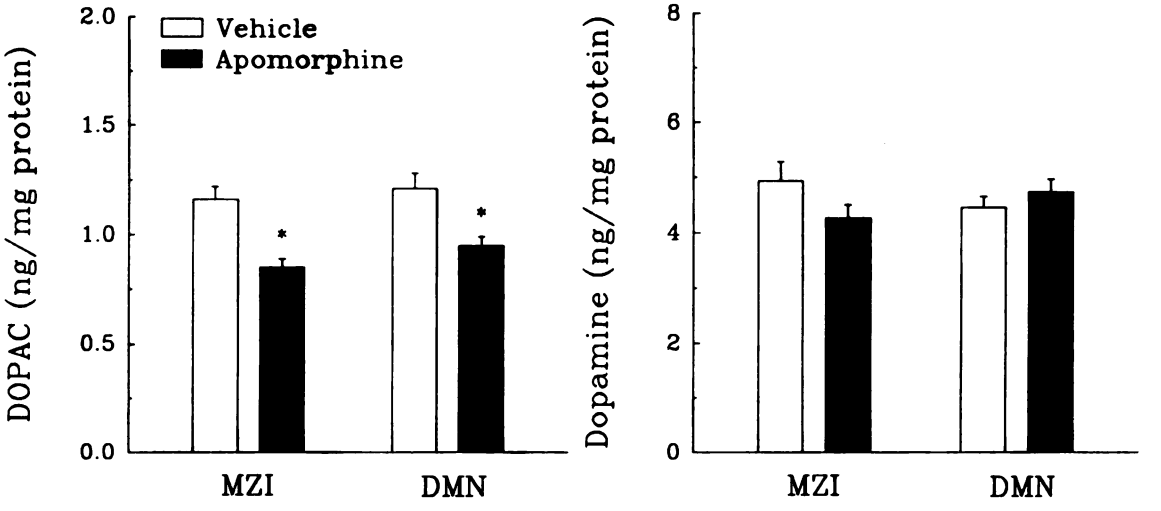

Figure 4.3. Effects of apomorphine on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with apomorphine (2 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 30 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for apomorphine-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

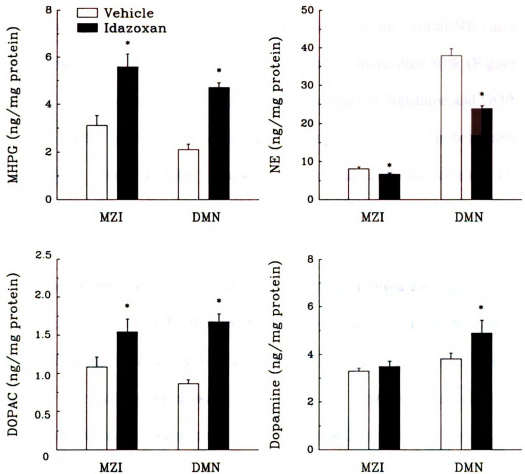

Figure 4.4. Effects of idazoxan on MHPG, NE, DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with idazoxan (20 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for idazoxan-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

neurons are activated synthesis of the amine cannot keep pace with release and, therefore, results in a net decrease in NE in neurons. Idazoxan also increased the DOPAC concentrations in the MZI and DMN, and dopamine concentrations in the DMN.

Seven days after bilateral injections of 5-ADMP into the ventral NE bundle NE concentrations in both MZI and DMN were depleted by more than 80% (Figure 4.5). Depletion of NE, however, did not alter the concentrations of dopamine and DOPAC in the MZI and DMN. In animals in which NE neurons projecting to the hypothalamus had been destroyed with 5-ADMP, haloperidol was still able to increase DOPAC concentrations in the MZI and DMN (Figure 4.6) in a manner similar to that seen in intact animals, whereas in 5-ADMP-treated animals idazoxan failed to increase DOPAC and dopamine concentrations in these brain regions (compare Figure 4.7 and Figure 4.4). These data suggest that after destruction of NE neurons projecting to the MZI and DMN incertohypothalamic DA neurons are still responsive to dopamine receptor blockade, and as a result their activity increases. In contrast, the idazoxan-induced increase in dopamine and DOPAC concentrations in the MZI and DMN of non-lesioned animals (Figure 4.3) occurs in NE neurons. Accordingly, destruction of a majority of NE neurons projecting to the MZI and DMN abolished the idazoxan-induced increases in dopamine and DOPAC concentrations in these brain regions.

Discussion

In dopamine-rich regions containing comparatively little or no NE terminals DOPAC originates predominantly from the metabolism of dopamine in DA neurons. Accordingly, alterations in concentrations of DOPAC reflect the activity of DA neurons

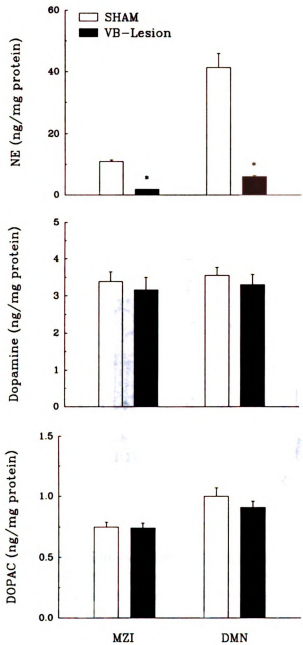

Figure 4.5. Effects of bilateral injections of 5-ADMP into the ventral NE bundle on NE, dopamine and DOPAC concentrations in the MZI and DMN. Male rats were injected bilaterally with 5-ADMP (8 μg free base/side; i.c.; VB-lesion) or its saline containing 0.1% ascorbic acid vehicle (0.3 μl/side; i.c.; SHAM) and killed 7 days later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 5-ADMP-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

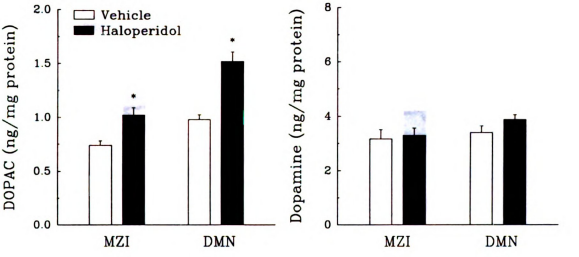

Figure 4.6. Effects of haloperidol on DOPAC and dopamine concentrations in the MZI and DMN of 5-ADMP-treated rats. Seven days after bilateral injections of 5-ADMP into ventral NE bundles, male rats were injected with haloperidol (0.1 mg/kg; s.c.) or its 0.3% tartaric acid vehicle (1 ml/kg; s.c.) and killed 120 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations.

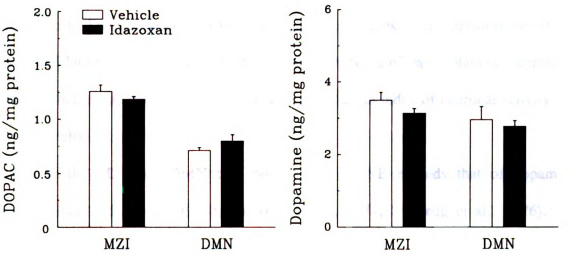

Figure 4.7. Effects of idazoxan on DOPAC and dopamine concentrations in the MZI and DMN of 5-ADMP-treated rats. Seven days after bilateral injections of 5-ADMP into ventral NE bundles, male rats were injected with idazoxan (20 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations.

that terminate in these regions (Roth, 1979; Lookingland and Moore, 1987; Moore and Wuerthele, 1979; Lindley et al., 1990). On the other hand, in NE-rich regions containing comparatively little or no DA neurons (e.g. cortex, brainstem, hippocampus and cerebellum), DOPAC has been determined to originate from the metabolism of dopamine in NE neurons, and the concentration of this dopamine metabolite increases when NE neurons are activated (Andén and Grabowska-Andén, 1983; Curet, et al., 1985; Scatton, et al., 1984; Westerink and De Vries, 1985). Thus, the metabolism of dopamine to DOPAC occurs in both NE and DA neurons, and determination of the source of DOPAC in brain regions containing both types of catecholamine neurons is essential before the results of studies using DOPAC as an index of neuronal activity can be interpreted.

In the MZI and DMN the concentration of NE exceeds that of dopamine (Lookingland and Moore, 1984b; Palkovits et al., 1974; Versteeg et al., 1976). In agreement, the results of the present study reveal that the concentration of NE in the MZI and DMN is 4-12 times higher than that of dopamine. The activity of incertohypothalamic DA neurons is higher that of NE neurons projecting to the MZI and DMN, which is evidenced by a higher rate of turnover of dopamine than that of NE in these brain regions (Lookingland and Moore, 1984b). Although incertohypothalamic DA neurons are tonically more active than NE neurons in the MZI and DMN, the potential exists for the more abundant NE neurons to contribute to the overall concentrations of DOPAC measured in these brain regions.

The results presented in this chapter indicate that when NE neurons are not

activated, DOPAC concentrations measured in the MZI and DMN originate predominantly from the metabolism of dopamine in DA neurons. Indeed, destruction of up to 85% of the NE neurons projecting to the MZI and DMN following 5-ADMP injection into the ventral NE bundle failed to alter DOPAC concentrations in these regions. These results are consistent with those of Westerink and De Vries (1985) who reported that electrolytic lesions of the locus ceruleus have no effect on DOPAC concentrations in the frontal cortex, a NE-rich region that also contains sparse DA innervation. Therefore, under basal conditions, once dopamine is formed in NE neurons it is quickly transported into synaptic vesicles and converted to NE by DBH.

The rapid decline in the concentrations of DOPAC in the MZI and DMN following inhibition of MAO reveals that 'steady state' concentrations of DOPAC represent a dynamic balance between the rapid synthesis and removal of DOPAC in these regions. The high rate of removal of DOPAC from these regions, either by further metabolism to HVA and/or rapid diffusion as suggested for other brain regions (Westerink, 1979; Westerink & Korf, 1976), makes it possible for the DOPAC concentration in the MZI and DMN to be used as a good index of the activity of incertohypothalamic DA neurons.

DOPAC concentrations in the MZI and DMN were not altered following the blockade of dopamine uptake with nomifensine suggesting that released dopamine from incertohypothalamic DA neurons does not contribute to DOPAC concentrations in these regions. Instead it would appear that most of the DOPAC is formed intraneuronally from newly synthesized non-vesicular dopamine; a similar suggestion has been made for the

striatum (Zetterström et al, 1988).

The activity of incertohypothalamic DA neurons in the MZI and DMN is regulated by dopamine receptor-mediated mechanisms. Activation of dopamine receptors with agonists decreases impulse flow (Sanghera, 1989) and turnover rate of dopamine (Lookingland and Moore, 1984b), i.e. the activity of these neurons. Conversely, blockade of dopamine receptors with antagonists increases the activity (dopamine turnover (Lookingland and Moore, 1984b)) and abolishes dopamine receptor-mediated inhibition of impulse flow (Sanghera, 1989). Consistent with these results, the present study shows that treatment with apomorphine decreases, and with haloperidol increases DOPAC concentrations in the MZI and DMN. Accordingly, alterations in the activity of incertohypothalamic DA neurons are accompanied by corresponding changes in the rate of metabolism of dopamine, and suggest that the concentration of DOPAC in the MZI and DMN is an index of the activity of incertohypothalamic DA neurons. It should be noted that treatment with haloperidol or apomorphine does not alter dopamine concentrations in the MZI and DMN. The release and synthesis of dopamine in mesotelencephalic neurons are coupled. Released dopamine is replaced by newly synthesized and/or recaptured dopamine; as a result the concentration of dopamine in nerve terminals remains relatively constant (Murrin and Roth, 1976; and for review see Moore and Wuerthele, 1979).

On the other hand, activation of NE neurons (as evidenced by an increase in MHPG concentrations) following administration of the α_2-adrenergic receptor antagonist idazoxan also increases DOPAC concentrations in the MZI and DMN. When NE

neurons are activated, however, an increased dopamine concentration is also observed (e.g., in the DMN). Evidently, when NE neurons are activated the rate of dopamine synthesis exceeds the rate of dopamine uptake and/or conversion to NE by DBH in synaptic vesicles and significant amounts of precursor dopamine accumulate in the cytoplasm where some of it is metabolized to DOPAC by mitochondrial MAO. Consequently, the amount of NE synthesized is inadequate to replenish the amount released and this results in a decrease in NE concentrations. Thus, increases in DOPAC concentrations in brain may reflect the deamination of dopamine in activated NE neurons, but these increases are usually associated with corresponding increases in dopamine concentrations and decreases in NE concentrations. Accordingly, when increased DOPAC concentrations are observed with increased dopamine concentrations, it implies a possible activation of NE neurons. It should be noted that immobilization stress which activates central NE neurons (Paré and Glavin, 1986) or direct electrical stimulation of NE axons produce time-dependent increases in MHPG concentrations and a corresponding decrease in NE concentrations in the hypothalamus (Tanaka et al., 1982; Lookingland et al., 1991).

Haloperidol increases DOPAC concentrations in the MZI and DMN by activating incertohypothalamic DA neurons. Therefore, the haloperidol-induced increase in DOPAC concentrations in the MZI and DMN should not be affected by the destruction of NE neurons projecting to these brain regions. Results presented in this chapter are consistent with this hypothesis. The results presented here also indicate that haloperidol has no direct effect on NE neurons. Furthermore, results presented in this chapter

indicate that the presence of NE terminals in the MZI and DMN is not necessary for the dopamine receptor-mediated activation of incertohypothalamic DA neurons. In contrast, as discussed in the previous chapter when NE neurons projecting to the MZI and DMN are destroyed, the effect of haloperidol on DOPA accumulation in these regions seems to be attenuated. The discrepancy of using DOPA and DOPAC to measure response of incertohypothalamic DA neurons to haloperidol treatment is likely because DOPA is not as good an index as DOPAC for the activity of incertohypothalamic DA neurons. Although the activity of NE neurons are low, after blockade of AAAD the relative abundance of NE fibers in the MZI and DMN makes significant contribution to DOPA accumulation in these regions. On the other hand, when NE neurons are not activated dopamine is quickly transported into synaptic vesicles and converted to NE in NE neurons. As a result, little dopamine is exposed to MAO and metabolized to DOPAC in NE neurons. DOPAC in the MZI and DMN represents the metabolism of dopamine in DA neurons and its concentration in these brain regions reflects the activity of incertohypothalamic DA neurons providing NE neurons are not activated. In this respect, the concentration of DOPAC is a better index of the activity of incertohypothalamic DA neurons than DOPA accumulation.

The ability of neurotoxin-induced lesions of the ventral NE bundle to block the effect of idazoxan on DOPAC and dopamine concentrations in the MZI and DMN indicate that the idazoxan-induced increases in DOPAC and dopamine concentrations in intact animals are occurring in NE neurons in these brain regions.

In summary, results described in this chapter indicate that when NE neurons are

not activated, DOPAC in the MZI and DMN originates in incertohypothalamic DA neurons and, therefore, reflects the activity of incertohypothalamic DA neurons. When NE neurons are activated, significant amounts of dopamine are metabolized to DOPAC in NE terminals, and under these conditions DOPAC may reflect the activity of NE neurons. Since the activity of NE neurons can be monitored by measuring MHPG concentrations in NE nerve terminals (Lookingland et al., 1991), DOPAC concentrations in the MZI and DMN in combination with MHPG technique can be used to study incertohypothalamic DA neurons.

Experimental Protocol

Based on the results from the Chapters 3 and 4, the following experimental protocol was developed for subsequent studies:

1) Possible drug effects on incertohypothalamic DA neurons were examined by measuring DOPAC concentrations in the MZI and DMN. When NE neurons are not activated, increases in DOPAC concentrations in the MZI and DMN reflect increases in the activity of incertohypothalamic DA neurons. When an increased DOPAC concentration is detected with an increased dopamine concentration in the MZI or DMN, which implies an activation of NE neurons, further experiments need to be undertaken to determine if NE neurons are activated.

2) To determine if NE neurons projecting to the MZI and DMN are activated the MHPG concentration is measured. If the drug to be examined has no effect on MHPG concentrations in the MZI and DMN, alterations in the DOPAC concentrations in these regions indicate drug effects on incertohypothalamic DA neurons. An increased MHPG concentration indicates that the drug to be examined activates NE neurons, and the following step is needed to examine a possible effect on incertohypothalamic DA neurons.

3) To examine a drug effect on incertohypothalamic DA neurons when the drug stimulates NE neurons projecting to the MZI and DMN, a neurochemical lesion of NE axons is employed. 5-ADMP, a selective neurotoxin for NE neurons, is injected bilaterally into the ventral NE bundle to destroy NE neurons projecting

to the hypothalamus. Alterations in the DOPAC concentration in the MZI and DMN in animals whose NE neurons projecting to the hypothalamus have been destroyed reflects drug effects on incertohypothalamic DA neurons.

Using this experimental protocol, drug effects on incertohypothalamic DA neurons and NE neurons projecting to the MZI and DMN can be estimated separately.

5. DOPAMINE RECEPTOR-MEDIATED REGULATION OF INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS

Introduction

DA neurons are regulated by dopamine receptor-mediated mechanisms. Both pre- and postsynaptic dopamine receptors have been suggested to be involved (Wolf and Roth, 1990; Carlsson, 1975). Three possible mechanisms have been proposed to explain dopamine receptor-mediated events. Firstly, presynaptic autoreceptors located on terminals of DA neurons control the synthesis (Kehr et al., 1972, Wolf and Roth, 1990) and release of their transmitter (Imperato and Di Chiara, 1985, 1988); Secondly, autoreceptors located on membranes of cell bodies or dendrites control the nerve impulse flow (Aghajanian and Bunney, 1977; Bunney et al., 1973; Mereu et al., 1983; Morelli et al., 1987); Thirdly, receptors located on postsynaptic target cells may regulate DA neurons through neuronal feedback loops (Carlsson and Lindquist, 1963; Carlsson, 1975; Bunny et al., 1973).

It was originally believed that there were two distinct dopamine receptor subtypes, D1 and D2, which are classified according to the second messenger system to which they are coupled. D1 receptors are positively coupled to the adenylate cyclase system (Stoof and Kebabian, 1984), and activation of these receptors increases adenosine 3',5'-cyclic monophosphate concentrations in the striatum (Clark and White, 1987). In contrast, D2 receptors are either negatively coupled (Onali et al., 1985) or not coupled to adenylate cyclase (Memo et al., 1986; Clark and White, 1987). Both D1 and D2 receptors are widely distributed in the central nervous system. Using receptor autoradiographic

methods, D1 and D2 receptors are shown to have overlapping but distinct anatomical distributions. High density of both receptor subtypes are present in the basal ganglia (Bouthenet et al., 1987; Wamsley et al., 1989; Charuchinda et al., 1987). D1 receptors are generally considered to be located postsynaptically on the membrane of target cells, whereas D2 receptors are located both postsynaptically and presynaptically (Seeman, 1980). Presynaptic receptors functioning as "autoreceptors" to modulate the synthesis and release of dopamine are considered to be of D2 subtype (Stoof and Kebabian, 1984). Support for this hypothesis has come from findings that D2 receptors are located on DA cells (Reisine et al., 1979), and application of selective D2 agonists decreases cell firing (White and Wang, 1983) and dopamine synthesis and release (Wolf and Roth, 1990; Brown et al., 1985; Stoof et al., 1982).

Recently novel dopamine receptors, e.g. D3, D4 and D5, have been discovered through molecular biology technology. The D3 receptor shares 75% and the D4 receptor 52% of their putative transmembrane sequences with the D2 receptor (Sokoloff et al., 1990; Van Tol et al., 1991). Since this transmembrane region is believed to encode the ligand binding site, it is not surprising that D3, and D4 receptors have similar pharmacological properties as D2 receptors (for review see Civelli et al., 1991). Accordingly, D3 and D4 receptors can be considered "D2-like" dopamine receptors.

In the MZI, both D1 and D2 binding sites are detected, although only D2 receptor mRNA has been found (Weiner, et al., 1991). Like mesotelencephalic DA neurons incertohypothalamic DA neurons are regulated by dopamine receptor-mediated mechanisms (Lookingland and Moore, 1984b). Dopamine receptor agonists decrease

whereas antagonists increase the activity of these neurons (Chapter 4, Figure 4.2 and 4.3). Local application of dopamine or its agonist inhibits firing of neurons in the MZI, presumably incertohypothalamic DA neurons by activating cell body/dendritic autoreceptors on these neurons (Eaton and Moss, 1989; Sanghera, 1989). Nevertheless, very little is known about the precise and specific dopamine receptor-mediated regulations of incertohypothalamic DA neurons. In this chapter, comparisons were made between responses of incertohypothalamic and mesotelencephalic DA neurons to selective D1 and D2 receptor antagonists.

Raclopride (Ögren et al., 1986; Köhler, et al., 1985) and remoxipride (Ögren et al., 1984; Köhler et al., 1990) have much higher affinity for D2 receptors over D1 receptors; they antagonize apomorphine-induced behavior and reverse the blockade by D2 receptor agonist B-HT 920 on GBL-induced increase of dopamine synthesis (Ögren et al., 1984, 1986; Magnusson et al., 1988; Wadworth and Heel, 1990). As a result, raclopride and remoxipride are classified as "selective" D2 receptor antagonists. SCH 39166, a benzonaphthazepine, is a selective D1 receptor antagonist (Chipkin et al., 1988). It is a relatively long-lasting drug and has less affinity for $5HT_2$ receptors when compared with the first selective D1 antagonist SCH 23390 (Clark and White, 1987). In this chapter these selective D1 and D2 receptor antagonists were employed to study dopamine receptor-mediated regulation of incertohypothalamic DA neurons.

Results

Two hr after injections of various doses of raclopride, DOPAC concentrations increased in the striatum and nucleus accumbens in a dose-dependent manner (Figure

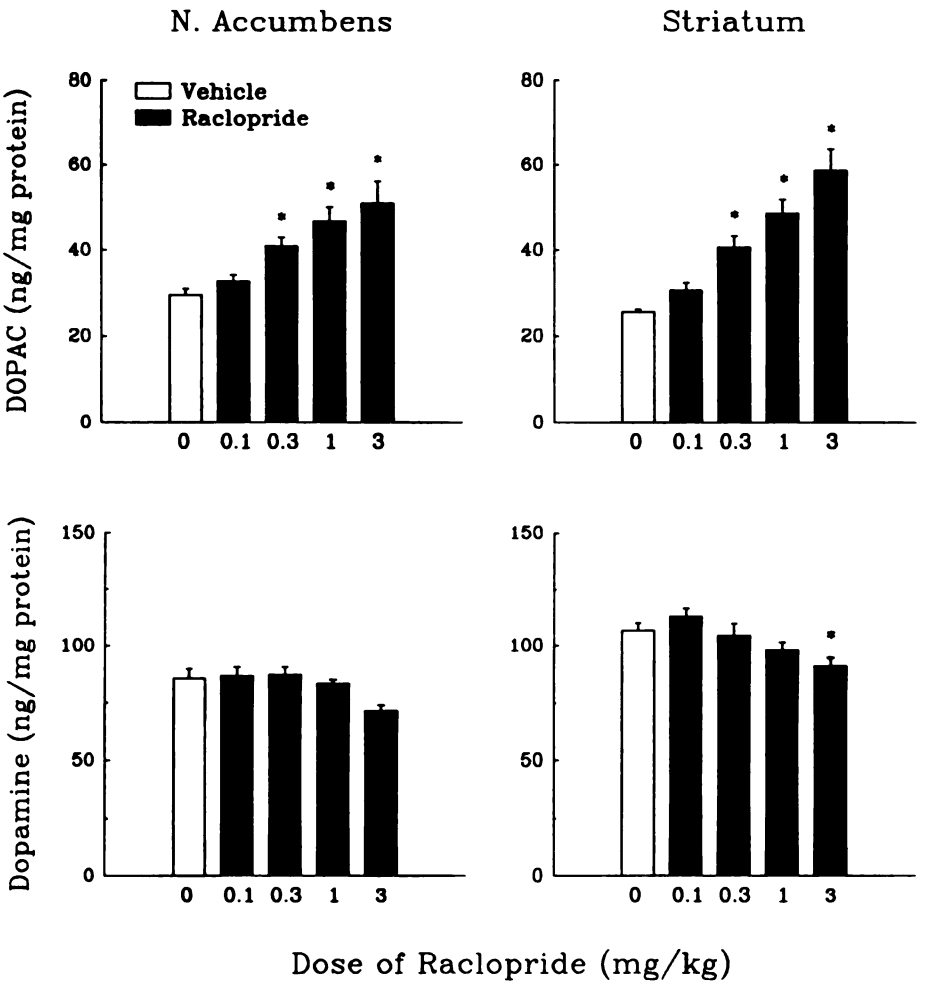

Figure 5.1. Dose-response effects of raclopride on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected i.p. with raclopride .1, .3, 1 and 3 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for raclopride-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

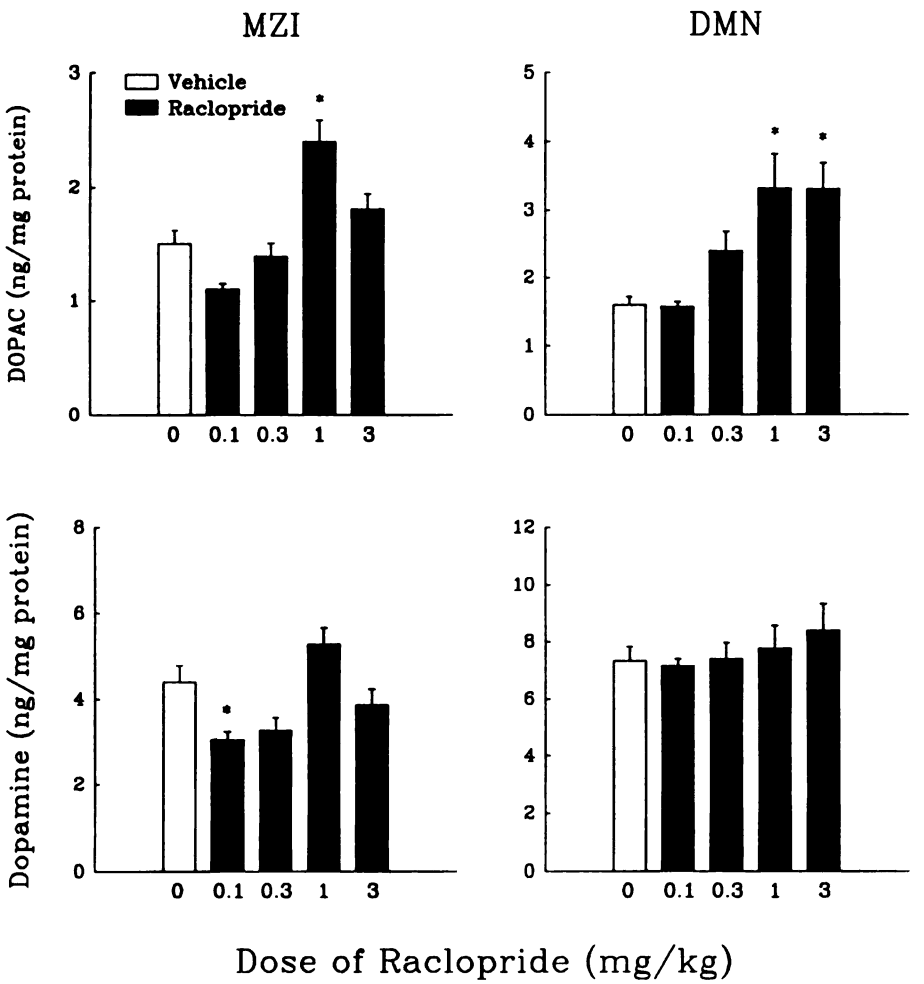

Figure 5.2. Dose-response effects of raclopride on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected i.p. with raclopride .1, .3, 1 and 3 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for raclopride-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

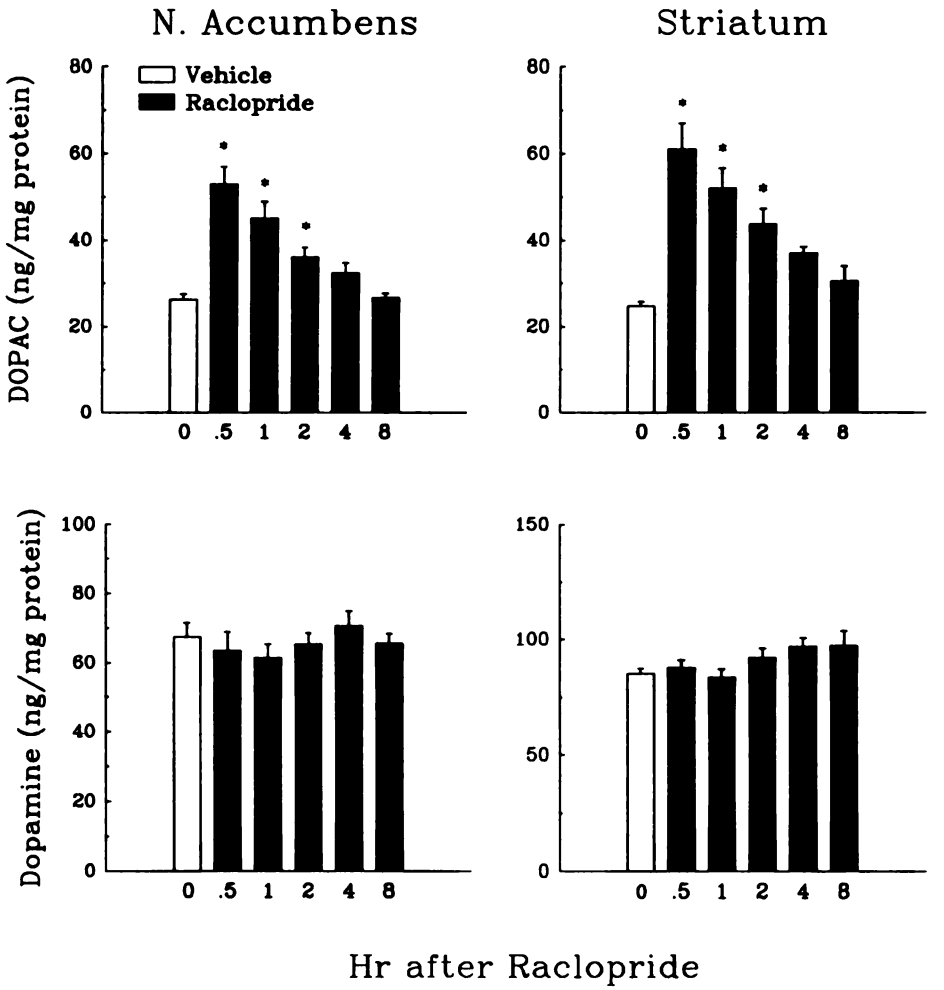

Figure 5.3. The time-course effects of raclopride on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected with raclopride (1 mg/kg; i.p.) and killed 0.5, 1, 2, 4 or 8 hr later. Rats received water injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for raclopride-treated rats that are significantly different ($p < 0.05$) from zero-time controls.

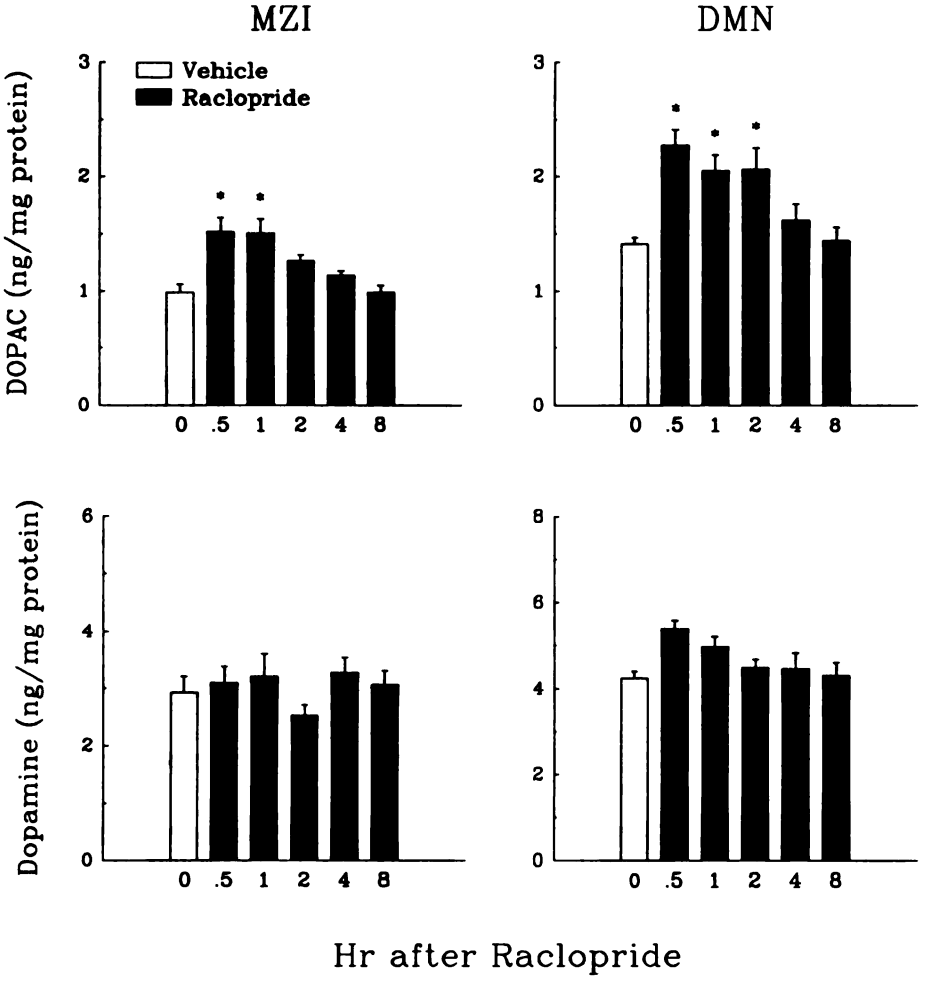

Figure 5.4. The time-course effects of raclopride on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with raclopride (1 mg/kg; i.p.) and killed 0.5, 1, 2, 4 or 8 hr later. Rats received water injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for raclopride-treated rats that are significantly different ($p < 0.05$) from zero-time controls.

5.1), indicating activation of mesolimbic and nigrostriatal DA neurons. Despite the large increase in DOPAC concentrations, dopamine concentrations remained unaltered or slightly decreased in these regions. Similar results were obtained in the MZI and DMN; that is, two hr after injections of raclopride DOPAC concentrations increased in the MZI and DMN while dopamine concentrations remained relatively constant (Figure 5.2).

The stimulatory effects of raclopride on mesolimbic and nigrostriatal DA neurons had a rapid onset. Thirty min after injection of raclopride DOPAC concentrations increased in the nucleus accumbens and striatum and remained elevated for at least two hr (Figure 5.3). A similar time-course effect of raclopride on DOPAC concentrations was seen in the MZI and DMN (Figure 5.4).

Remoxipride, injected two hr before decapitation, also increased DOPAC concentrations in the nucleus accumbens and striatum in a dose-related fashion, and was without effect on dopamine concentrations (Figure 5.5). In contrast to raclopride, remoxipride, at doses producing dramatic increases in DOPAC concentrations in the nucleus accumbens and striatum, was totally ineffective in increasing DOPAC concentrations in the MZI and DMN (Figure 5.6). When time-course effects were examined, remoxipride produced a rapid and sustained increase in DOPAC concentrations in the nucleus accumbens and striatum (Figure 5.7). Consistent with the results of the dose-response study, remoxipride failed to alter DOPAC concentrations in the MZI and DMN at any time after the administration of a high dose (10 mg/kg) of this drug (Figure 5.8).

When various doses of SCH 39166 were administered there was a moderate

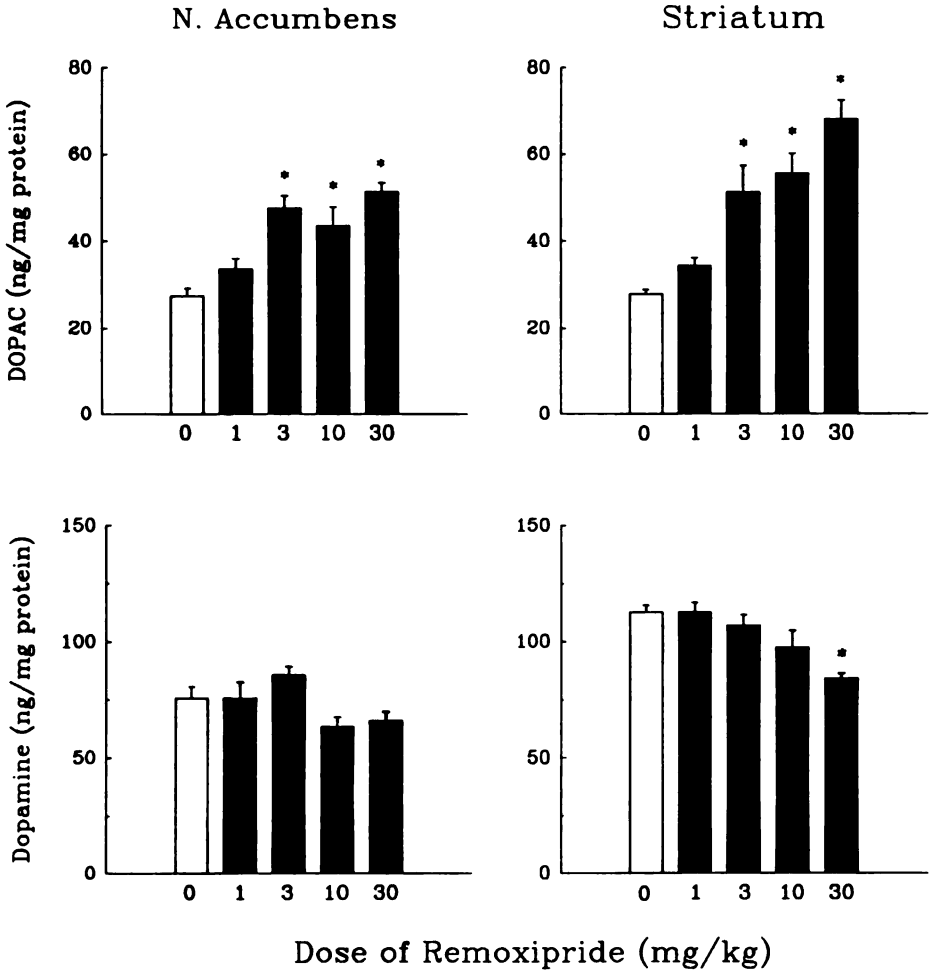

Figure 5.5. Dose-response effects of remoxipride on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected i.p. with remoxipride 1, 3, 10 and 30 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for remoxipride-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

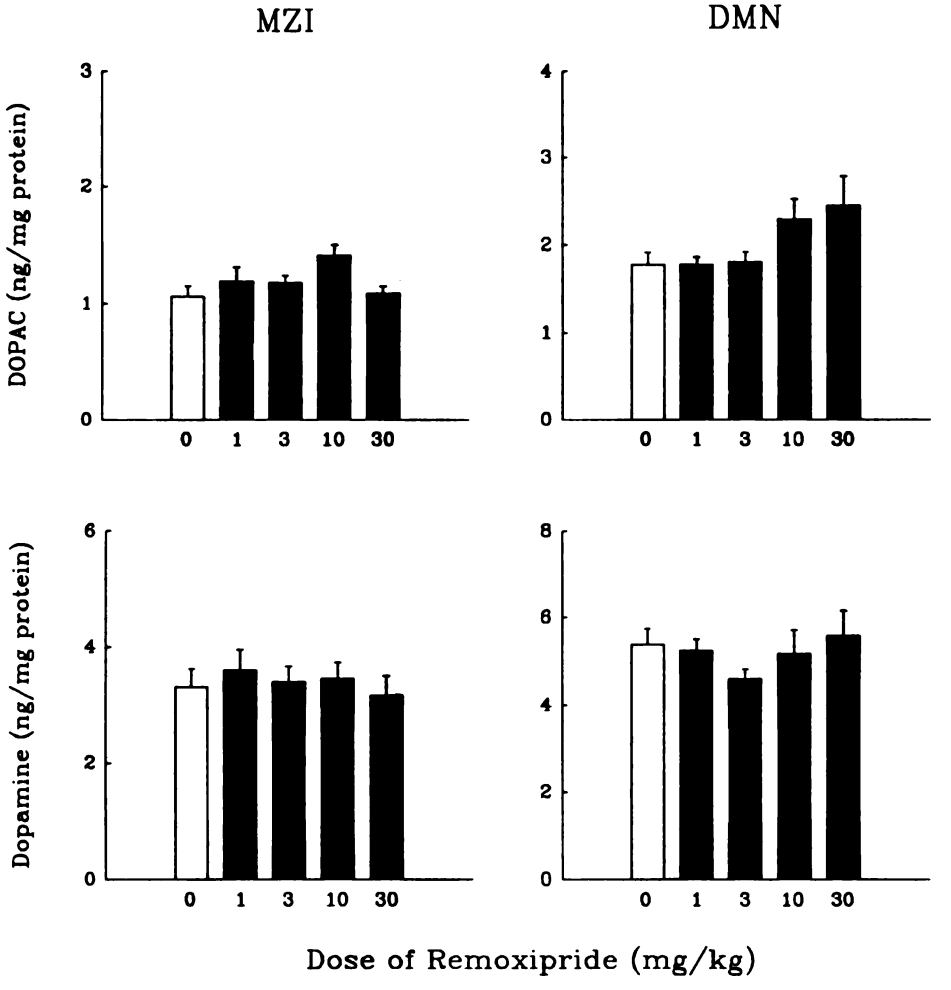

Figure 5.6. Dose-response effects of remoxipride on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected i.p. with remoxipride 1, 3, 10 and 30 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations.

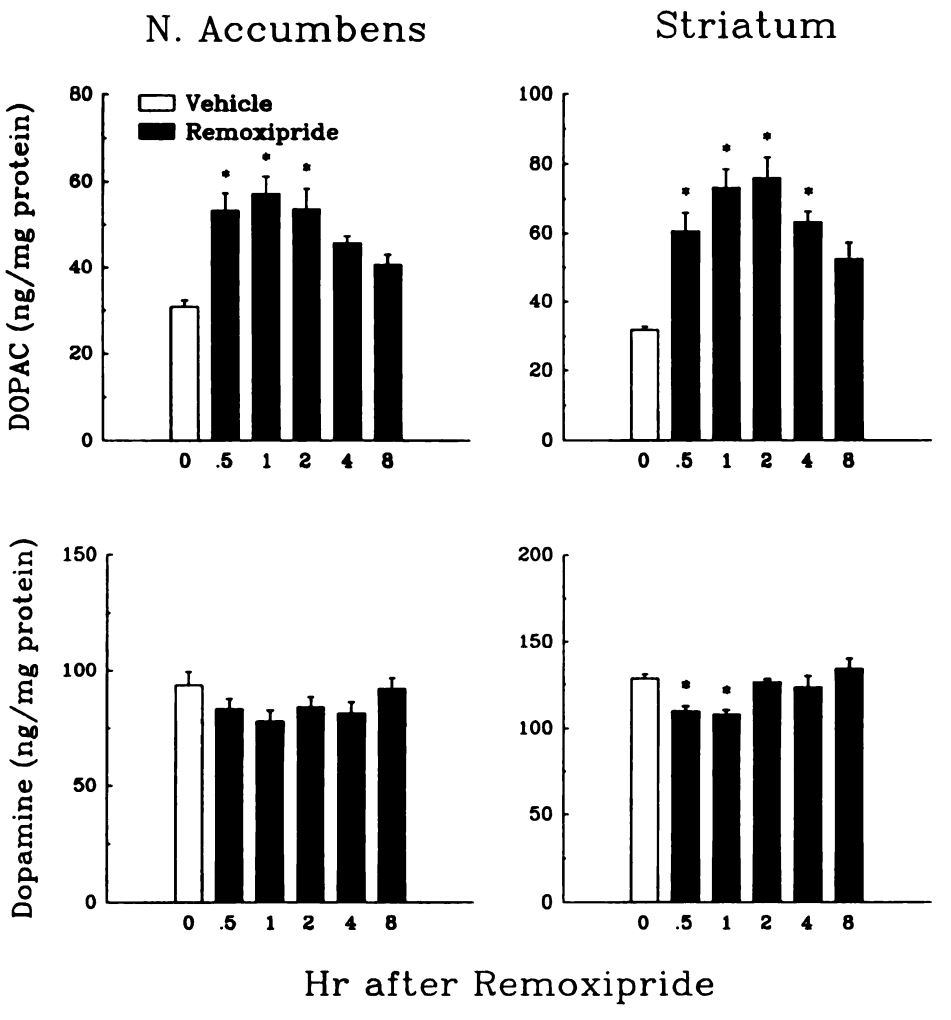

Figure 5.7. The time-course effects of remoxipride on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected with remoxipride (10 mg/kg; i.p.) and killed 0.5, 1, 2, 4 or 8 hr later. Rats received water injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for remoxipride-treated rats that are significantly different ($p < 0.05$) from zero-time controls.

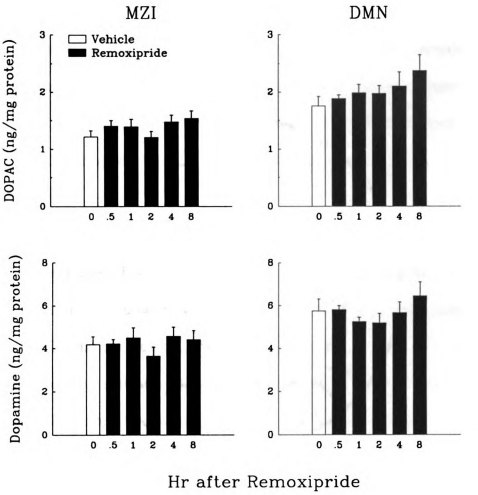

Figure 5.8. The time-course effects of remoxipride on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with remoxipride (10 mg/kg; i.p.) and killed 0.5, 1, 2, 4 or 8 hr later. Rats received water injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations.

increase in DOPAC concentration in the nucleus accumbens and striatum by 1 hr (Figure 5.9). Under the same experimental conditions the DOPAC concentrations in the MZI and DMN were not altered by SCH 39166 (Figure 5.10). The differential effects of SCH 39166 on mesotelencephalic and incertohypothalamic DA neurons were also evident from the results of the time course studies. SCH 39166 increased DOPAC concentrations in the nucleus accumbens and striatum for at least two hr after an injection (Figure 5.11), whereas this drug was without effect on DOPAC concentrations in the MZI and DMN for up to 16 hr after an injection (Figure 5.12).

Discussion

Although incertohypothalamic DA neurons have been reported to be regulated by dopamine receptor-mediated mechanisms in a manner similar to those of mesotelencephalic DA neurons (Lookingland and Moore, 1984b), results presented in this chapter with D1 and D2 receptor antagonists reveal subtle differences in the responses of these two neuronal systems to dopamine receptor blockade. Raclopride (Ögren et al., 1986) and remoxipride (Ögren et al., 1984) are "selective" D2 receptor antagonists based on binding and neurochemical studies. Nevertheless, raclopride and remoxipride have differential effects on incertohypothalamic DA neurons; raclopride stimulates these neurons whereas remoxipride has no effect. The selective D1 receptor antagonist SCH 39166 increases the activity of mesotelencephalic DA neurons as evidenced by the increased DOPAC concentrations in the nucleus accumbens and striatum, but was without effect in the MZI and DMN.

Raclopride and remoxipride have much higher affinity for D2 receptor than for

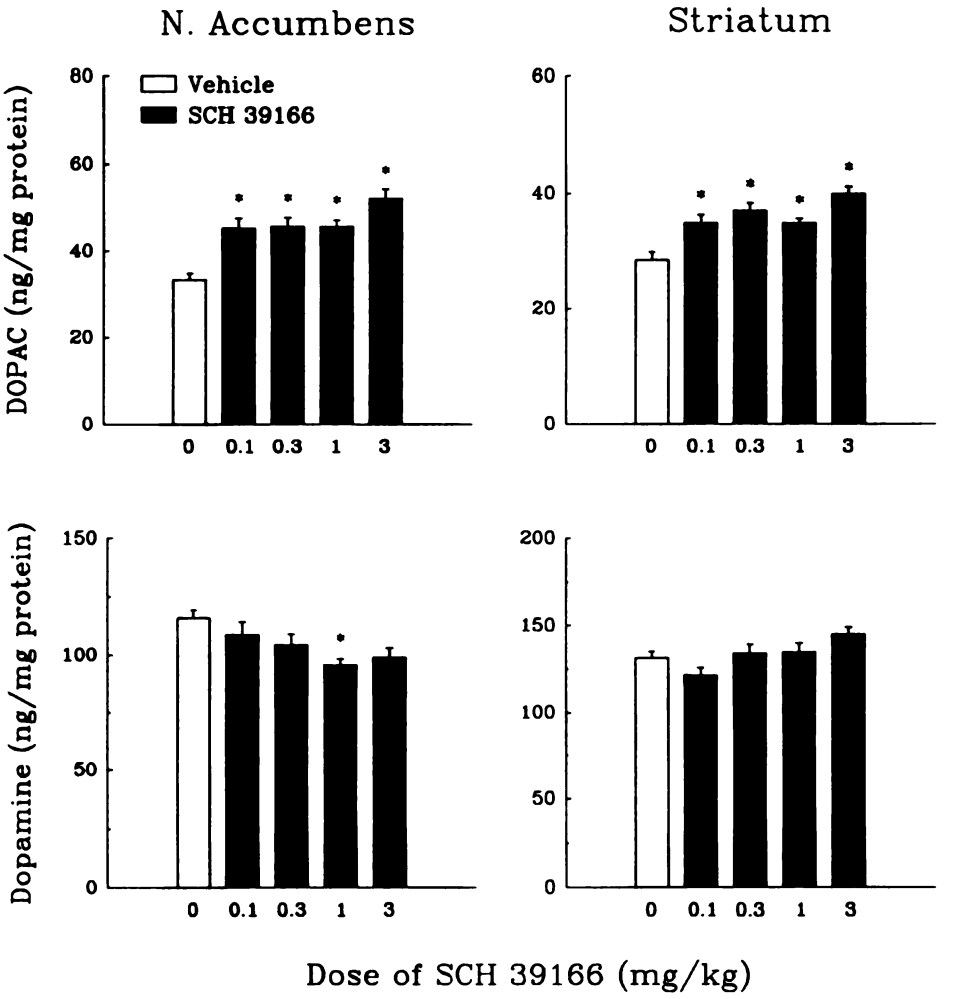

Figure 5.9. Dose-response effects of SCH 39166 on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected s.c. with SCH 39166 .1, .3, 1 and 3 mg/kg or its 50% ethanol vehicle 1 ml/kg and killed 1 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for SCH 39166-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

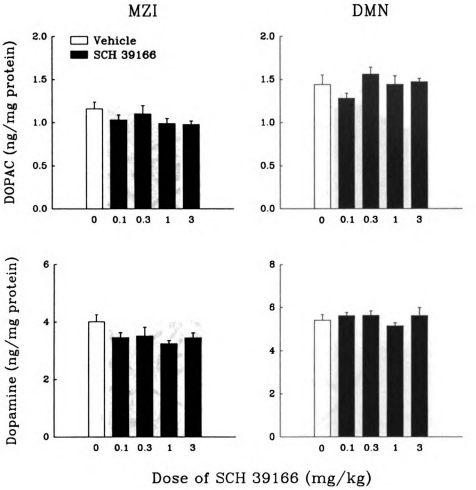

Figure 5.10. Dose-response effects of SCH 39166 on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected s.c. with SCH 39166 .1, .3, 1 and 3 mg/kg or its 50% ethanol vehicle 1 ml/kg and killed 1 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations.

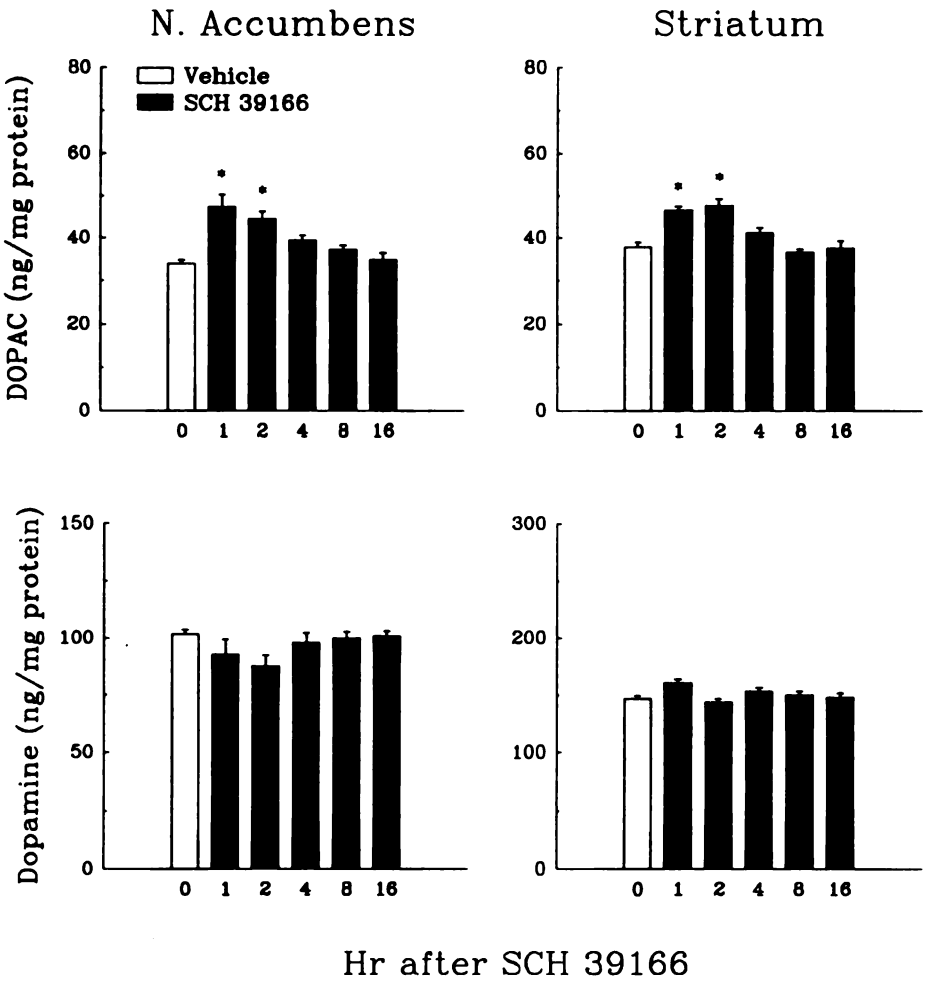

Figure 5.11. The time-course effects of SCH 39166 on DOPAC and dopamine concentrations in the nucleus accumbens and striatum. Male rats were injected with SCH 39166 (3 mg/kg; s.c.) and killed 1, 2, 4, 8 or 16 hr later. Rats received 50% ethanol vehicle (1 ml/kg; s.c.) injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values for SCH 39166-treated rats that are significantly different ($p<0.05$) from zero-time controls.

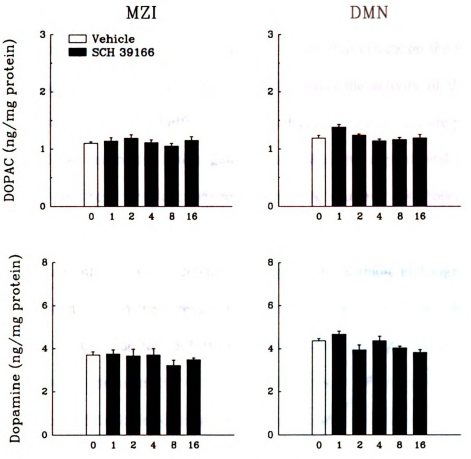

Figure 5.12. The time-course effects of SCH 39166 on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with SCH 39166 (3 mg/kg; s.c.) and killed 1, 2, 4, 8 or 16 hr later. Rats received 50% ethanol vehicle (1 ml/kg; s.c.) injection and killed 1 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations.

D1 receptors and, therefore, as D2 receptor antagonists, in rats, they increase dopamine metabolism (Magnusson et al., 1987, 1988) and antagonize the D2 receptor agonist reversal of GBL-induced increase of dopamine synthesis in striatum, nucleus accumbens and olfactory tubercle (Magnusson et al., 1988). Results presented in this chapter indicate that raclopride and remoxipride display differential effects on the activity of incertohypothalamic DA neurons. Raclopride increases the activity of these neurons whereas remoxipride is without effect. These results indicate that there are probably two groups of D2-like receptors; both regulate mesolimbic and nigrostriatal DA neurons whereas only one regulates incertohypothalamic DA neurons. Raclopride binds to receptors that regulate mesolimbic, nigrostriatal and incertohypothalamic DA neurons, and remoxipride only binds to receptors that regulate mesolimbic and nigrostriatal DA neurons. Consistent with these results are the findings by Eaton et al. (in press) that raclopride and remoxipride have differential effects on α-melanocyte stimulating hormone secretion from the melanotrophs in the intermediate lobe of the pituitary but not on prolactin secretion from lactotrophs in the anterior pituitary. Secretions of both prolactin and α-melanocyte stimulating hormone are under inhibitory controls through "D2" receptors (for review see Holzbauer and Racké, 1985). Therefore, raclopride and remoxipride appear to block different populations of "D2" receptors. Additionally, in contrast to the effect of "D2" receptor antagonist haloperidol, chronic treatment with remoxipride does not increase the concentration of neurotensin (Levant et al., 1991), the amount of D2 receptors, or the preproenkephalin mRNA (Köhler et al., 1991) in the striatum or nucleus accumbens. These reports along with the results presented in this

chapter indicate that remoxipride is different from a "typical" D2 antagonist in that "typical" D2 receptor antagonists (e.g. haloperidol and raclopride) probably bind to all "D2" receptors whereas remoxipride binds only a sub-population of these receptors. The discovery of novel dopamine receptor subtypes (for review see Van Tol et al., 1991) may provide clues as to why raclopride and remoxipride, both proposed to be selective "D2" antagonists, have differential effects on both peripheral and central DA systems. Unfortunately, at present time neither enough information regarding nor appropriate tools to study these novel dopamine receptors are available. The implication from the finding that raclopride and remoxipride have differential effect on central DA neurons is that it may be possible to selectively manipulate one DA systems without affecting others.

The D1 receptor has been proposed to play a role in regulation of the activity of the nigrostriatal DA system. Systemic injection of the selective D1 agonist SKF 38393 reduces dopamine release in the striatum, and this inhibitory effect is prevented by pretreatment with D1 antagonist SCH 23390 (Zetterström et al., 1986). Like D1 receptor antagonists SCH 23390 (Imperato et al., 1987), SCH 39166 activates nigrostriatal DA neurons as indicated in this study by increasing the metabolism of dopamine in the striatum, although to a much less extent than that induced by raclopride. Since D1 receptors are not located on membranes of DA nerve terminals in the striatum (Seeman, 1980) nor on DA cell bodies in the substantia nigra (Seeman, 1980; Kebabian et al., 1986) but on non-DA neurons in these two regions, it has been hypothesized that D1 receptors may mediate regulation of DA neurons by two different mechanisms: 1) activation of postsynaptic D1 receptors on neurons in the striatum that comprise negative

feedback loops inhibits DA neurons (striatonigral feedback loop; Carlsson and Lindquist, 1963), 2) activation of D1 receptors located on GABA or other non-DA neurons in the substantia nigra indirectly inhibit DA neurons (Imperato et al., 1987).

In contrast to the effect on mesotelencephalic DA systems, SCH 39166 is totally ineffective on incertohypothalamic DA neurons. Consequently, it is unlikely that D1 receptors play any important role in regulating these neurons.

In conclusion, although the activity of incertohypothalamic DA neurons is regulated through dopamine receptor-mediated mechanisms, these mechanisms are somewhat different from those that regulate nigrostriatal or mesolimbic DA neurons. Only a sub-group of "D2" or a novel dopamine receptors yet to be discovered modulate the activity of incertohypothalamic DA neurons and this provides the basis for the differential effects of raclopride and remoxipride on these neurons. In contrast, D1 receptors play a minor role in regulating mesotelencephalic DA neurons, and this DA receptor subtype does not play a role in regulating incertohypothalamic DA neurons.

6. 5-HYDROXYTRYPTAMINE NEURONS DO NOT TONICALLY REGULATE THE ACTIVITY OF INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS

Introduction

The hypothalamus contains a high density of 5HT nerve terminals and all the nuclei in the hypothalamus contain at least some 5HT nerve fibers (Steinbusch and Nieuwenhuys, 1981; Steinbusch, 1981). These 5HT fibers are believed to originate from cell bodies located in the midbrain raphé nuclei. 5HT neurons interact with DA neurons in the central nervous system. Anatomical evidence indicates that 5HT nerve terminals make direct contact with DA cells in the ventral tegmental area (Chesselet, 1984; Hervé et al., 1987), which provide morphological basis for functional interactions between these two neuronal systems. Both inhibitory and stimulatory effects of 5HT on DA neurons have been reported (Williams and Davies, 1983; Ahlenius et al., 1989, 1990; Guan and McBride, 1989). Some recent studies indicate that $5HT_3$ receptors mediate facilitation of dopamine release from mesotelencephalic neurons (Imperato and Angelucci, 1989; Blandina et al., 1988). In the diencephalon, 5HT nerve fibers terminate in the arcuate nucleus of the hypothalamus and MZI, where they make direct contact with tuberoinfundibular and incertohypothalamic DA neurons respectively (Kiss and Halasz, 1986; Bosler et al., 1984).

5HT has been considered to be involved in stimulation of prolactin secretion from the lactotrophs in the anterior pituitary. Increasing 5HT neuronal transmission increases, whereas increasing tuberoinfundibular DA neuronal activity decreases the release of prolactin (for review see Ben-Jonathan et al., 1989). Therefore, DA and 5HT systems

are reciprocally involved in the control of prolactin secretion. Little is known the exact mechanism by which 5HT stimulates prolactin release, but two mechanisms have been proposed. 5HT may stimulate the release of prolactin releasing factors or inhibit tuberoinfundibular DA neurons. Acute morphine treatment increases serum prolactin concentrations, at least partially by inhibiting tuberoinfundibular DA neurons (Demarest and Moore, 1981). Disruption of 5HT neuronal function abolishes the morphine-induced decrease in the activity of tuberoinfundibular DA neurons (Demarest and Moore, 1981). Stress decreases the activity of tuberoinfundibular DA neurons and increases serum prolactin levels (Demarest et al., 1985b; Lookingland et al., 1990). 5HT neurons also play an important role in the stress-induced neuroendocrine changes (Demarest et al., 1985a; Goudreau et al., 1991). Therefore, consistent with anatomical findings, 5HT neuronal systems interact with tuberoinfundibular DA neurons functionally.

The function of 5HT terminals in the MZI is unknown. Eaton and Moss (1989) reported that in the brain slices 5HT inhibits firing of some of the neurons located in the MZI, possibly incertohypothalamic DA neurons. Therefore, 5HT neurons may play a role in regulation or modulation of the activity of incertohypothalamic DA neurons.

The primary objective of the experiments described in this chapter was to investigate possible regulation of incertohypothalamic DA neurons by 5HT neuronal system by using the experimental protocols developed in the previous chapters. 8-OH-DPAT is a selective $5HT_{1A}$ receptor agonist, and activation of these receptors decreases the activity of 5HT neurons through an autoreceptor-mediated mechanism (Hjorth and Magnusson, 1988; Sotelo et al., 1990). In this study, 8-OH-DPAT was employed to

suppress the activity of 5HT neurons.

Results

The activity of 5HT neurons was estimated by measuring the synthesis, as estimated from the accumulation of 5HTP 30 min after injection of an AAAD inhibitor, NSD 1015 (100 mg/kg; i.p.), and metabolism of 5HT, as estimated from the measurement of the concentration of 5HIAA, a major metabolite of 5HT. Figure 6.1 summarizes the dose response effects of 8-OH-DPAT on 5HTP accumulation, and the time course effects of this compound on 5HIAA concentrations in the nucleus accumbens and DMN. Thirty min after s.c. injections of 300 μg/kg 8-OH-DPAT, 5HTP accumulation in the nucleus accumbens and DMN was decreased. This dose of 8-OH-DPAT reduced 5HIAA concentrations in both nucleus accumbens and DMN 30 min after injection, and this inhibitory effect lasted at least 120 min. These data are consistent with reports that 8-OH-DPAT is a $5HT_{1A}$ receptor agonist and activation of these receptors decreases the activity of 5HT neurons projecting to these areas.

Injection of 8-OH-DPAT (300 μg/kg) had no effect on the DOPAC concentration in the nucleus accumbens at any time, suggesting a lack of effect of 8-OH-DPAT on DA neurons of the mesolimbic system (Figure 6.2). In contrast, the DOPAC concentration in the DMN was elevated 30 min after an injection of 8-OH-DPAT and the stimulatory effect of 8-OH-DPAT lasted up to 60 min after injection, indicating a possible activation of incertohypothalamic DA neurons. The effect of 8-OH-DPAT on incertohypothalamic DA neurons were re-examined utilizing the optimal dose and time of injection determined from the experiments depicted in Figure 6.1. Subcutaneous injection of 8-OH-DPAT (300

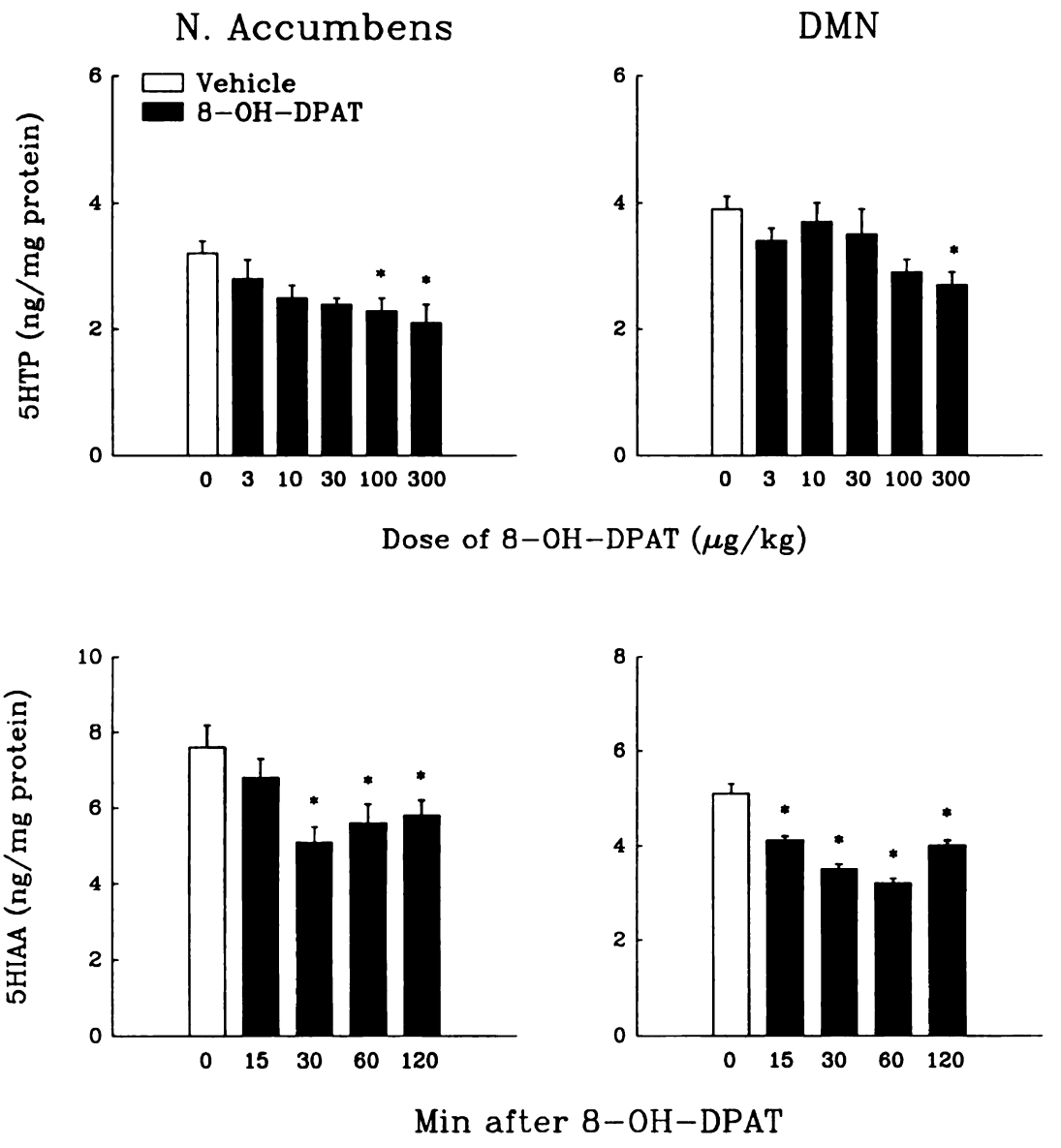

Figure 6.1. Effects of 8-OH-DPAT on 5HTP accumulation and 5HIAA concentrations in nucleus accumbens and DMN. In the dose-response study, male rats were injected s.c. with 8-OH-DPAT from 3 to 300 μg/kg or its water vehicle (1 ml/kg) and killed 30 min later. In the time-course study, male rats were injected with 8-OH-DPAT (300 μg/kg; s.c.) and killed 15, 30, 60 and 120 min later. Animals injected with water vehicle (1 ml/kg; s.c.) were killed 30 min later and used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 8-OH-DPAT treated rats that are significantly different ($p < 0.05$) from controls.

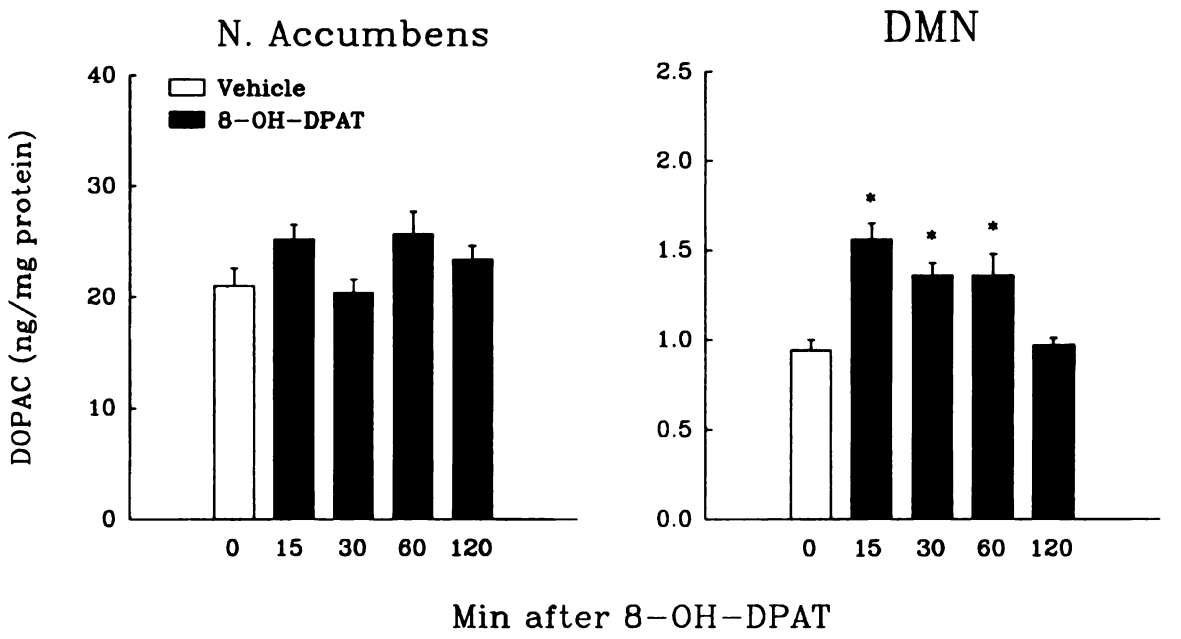

Figure 6.2. Effects of 8-OH-DPAT on DOPAC concentrations in nucleus accumbens and DMN. Male rats were injected with 8-OH-DPAT (300 μg/kg; s.c.) and killed 15, 30, 60 and 120 min later. Animals injected with water vehicle (1 ml/kg; s.c.) were killed 30 min later and used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 8-OH-DPAT treated rats that are significantly different ($p < 0.05$) from zero-time controls.

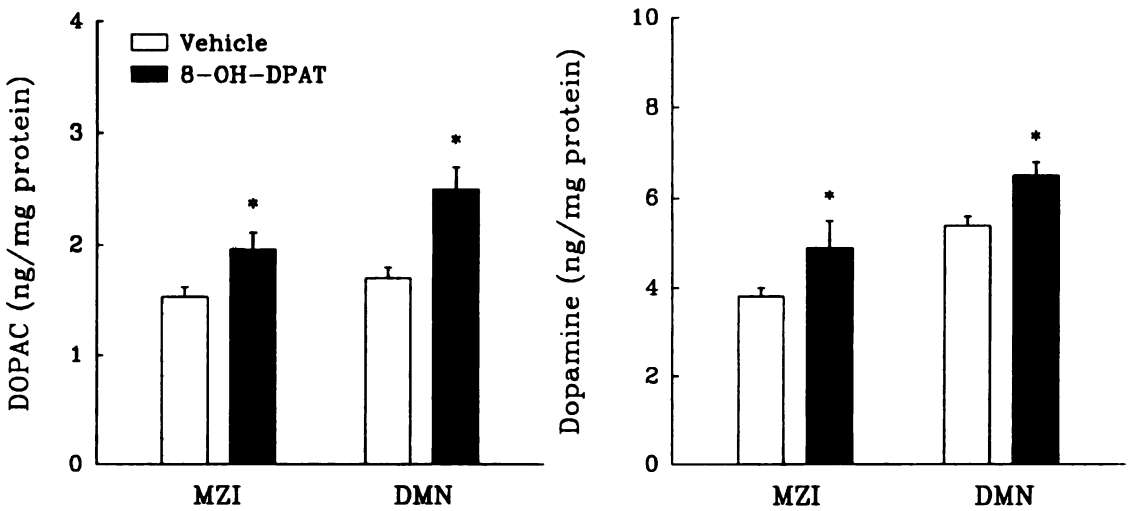

Figure 6.3. Effects of 8-OH-DPAT on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with 8-OH-DPAT (300 μg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 8-OH-DPAT-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

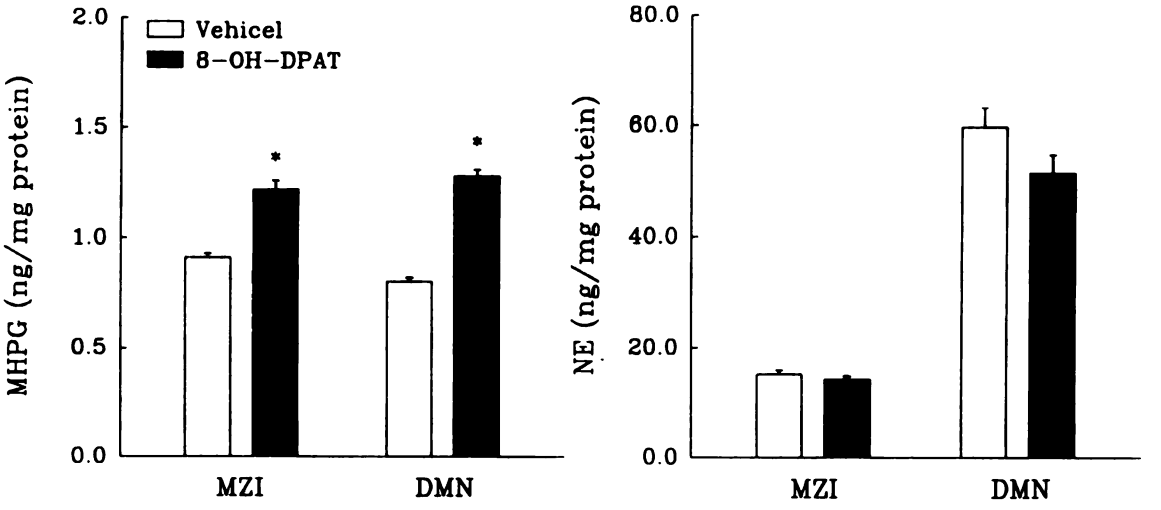

Figure 6.4. Effects of 8-OH-DPAT on MHPG and NE concentrations in the MZI and DMN. Male rats were injected with either 8-OH-DPAT (300 μg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for 8-OH-DPAT-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

μg/kg; 60 min) increased both DOPAC and dopamine concentrations in the MZI and DMN (Figure 6.3). As noted in Chapter 4, elevation of DOPAC concentrations accompanied with an increase in dopamine concentrations in brain regions containing dense NE fibers could indicate an activation of NE neurons. Accordingly, the effects of 8-OH-DPAT on the activity of NE neurons were examined by measuring the MHPG and NE concentrations in the MZI and DMN (Figure 6.4). 8-OH-DPAT increased concentrations of MHPG but not NE, indicating that NE neurons projecting to the MZI and DMN are activated.

To determine if incertohypothalamic DA neurons were also activated by 8-OH-DPAT, the effects of 8-OH-DPAT on DOPAC and dopamine concentrations in the MZI and DMN were determined in animals whose NE neurons projecting to the hypothalamus had been destroyed with 5-ADMP (Jarry et al., 1986). One week after bilateral injections of 5-ADMP into ventral NE bundles, the NE content in the MZI and DMN was depleted about 80% (from 63.9±1.6 and 16.6±.9 in intact animals to 11.2±.7 and 4.3±.2 in 5-ADMP-treated animals in the DMN and MZI respectively). Nevertheless, 5-ADMP treatment did not alter basal concentrations of dopamine, DOPAC, 5HT or 5HIAA in the MZI (open columns in Figure 6.5). In addition, destruction of NE neurons projecting to the MZI and DMN with 5-ADMP did not alter the ability of 8-OH-DPAT to decrease 5HIAA concentrations in these regions. Although the ventral NE bundle lesion did not alter the ability of 8-OH-DPAT to inhibit 5HT neurons, it did prevent the ability of this compound to increase DOPAC and dopamine concentrations in MZI (Figure 6.5). Identical changes to those seen in MZI (Figure 6.5) were also

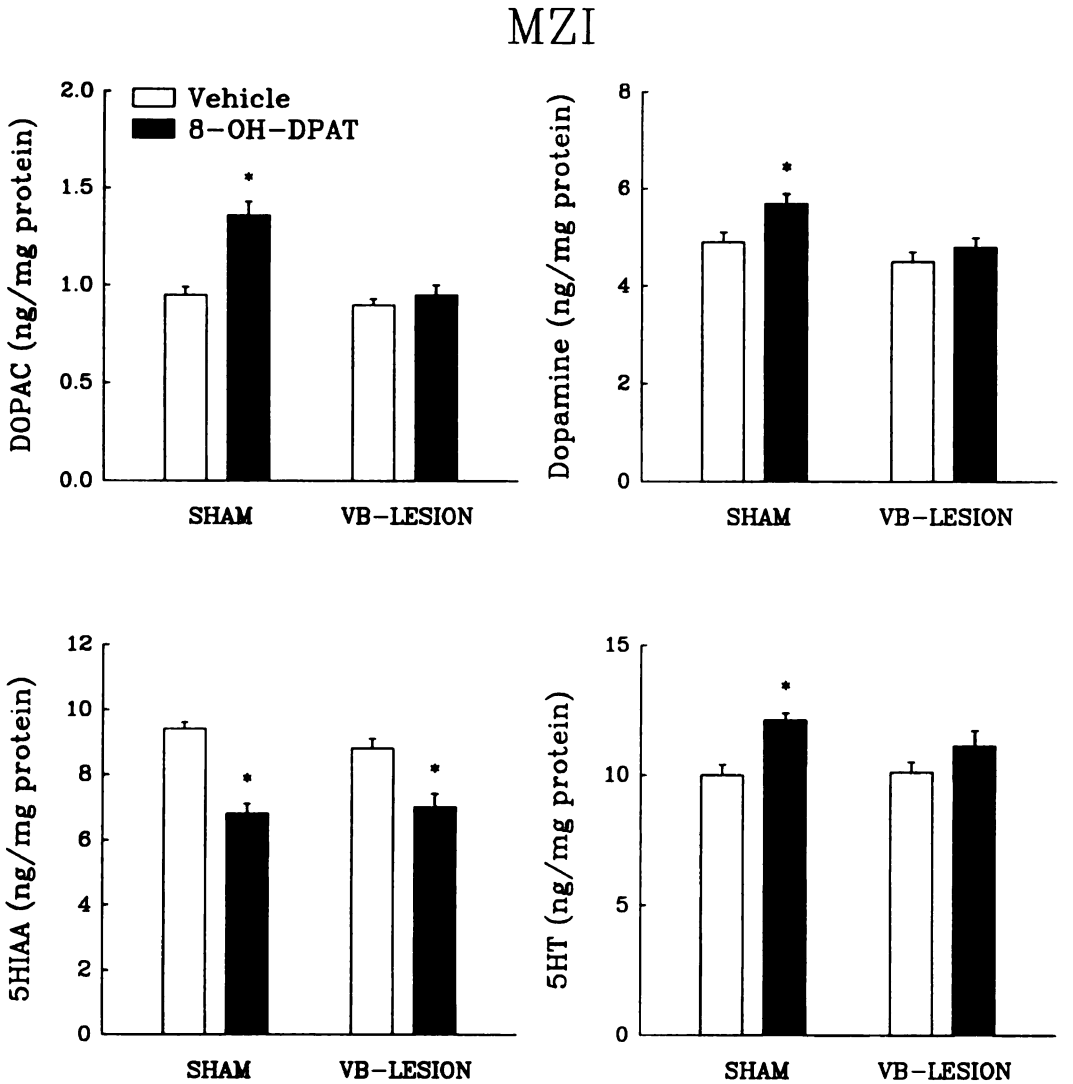

Figure 6.5. Comparison of the effects of 8-OH-DPAT on dopamine, DOPAC, 5HT and 5HIAA concentrations in the MZI of vehicle- or 5-ADMP-treated rats. Seven days after bilaterally injections of 5-ADMP (8 μg free base/side; i.c.; VB-lesion) or its saline with 0.1% ascorbic acid vehicle (SHAM) into ventral NE bundles, male rats were injected with 8-OH-DPAT (0.3 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 8-9 determinations. *, values for 8-OH-DPAT-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

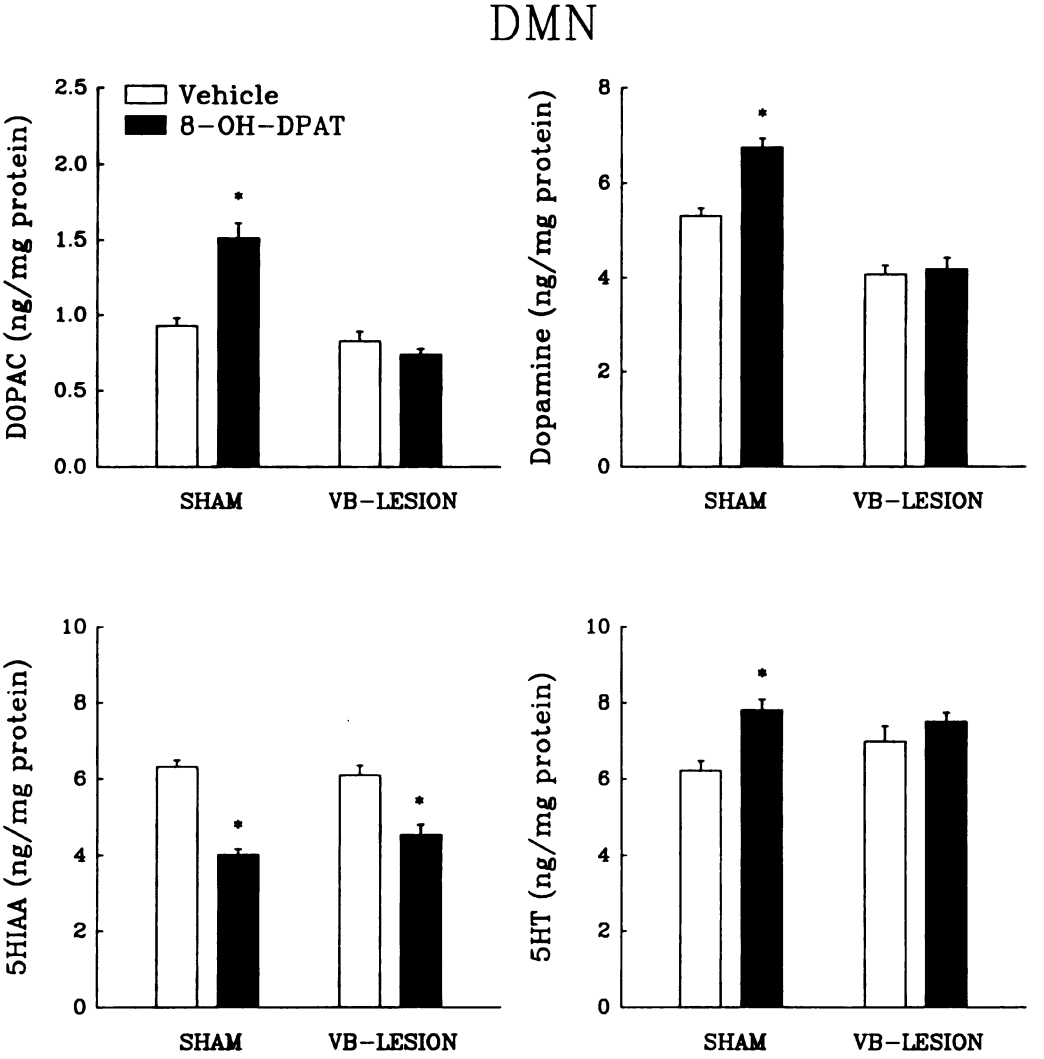

Figure 6.6. Comparison of the effects of 8-OH-DPAT on dopamine, DOPAC, 5HT and 5HIAA concentrations in the DMN of vehicle- or 5-ADMP-treated rats. Seven days after bilaterally injections of 5-ADMP (8 μg free base/side; i.c.; VB-lesion) or its saline with 0.1% ascorbic acid vehicle (SHAM) into ventral NE bundles, male rats were injected with 8-OH-DPAT (0.3 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 8-9 determinations. *, values for 8-OH-DPAT-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

observed in the DMN (Figure 6.6). These data indicate that increases in concentrations of DOPAC and dopamine in the MZI and DMN caused by 8-OH-DPAT in intact animals (Figure 6.3) are due to the activation of NE neurons projecting to these brain regions and not to the activation of incertohypothalamic DA neurons located in these regions.

Experiments were carried one step further to examine the possible mechanisms mediating the stimulatory effect of 8-OH-DPAT on NE neurons. A relatively selective neurotoxin for 5HT neurons, 5,7-DHT (200 μg; i.c.v.), was used to destroy 5HT neurons; to prevent destruction of NE neurons desipramine (25 mg/kg; i.p.), a NE uptake inhibitor, was administered 45 min prior to the 5,7-DHT injection. One week following the 5,7-DHT injection 5HT and 5HIAA contents were depleted to less than 0.5 pg (sensitivity of assay) or more than 95% (Table 6.1). After the destruction of 5HT neurons there was an increase in the MHPG concentrations in the MZI and DMN (Figure 6.7), which was similar to the increase caused by 8-OH-DPAT (Figure 6.4).

Discussion

$5HT_{1A}$ receptors located on membranes of cell bodies and dendrites of 5HT neurons control the activity of these neurons (Sotelo et al., 1990). Activation of these "autoreceptors" with 8-OH-DPAT (Dourish et al., 1986; Hjorth and Magnusson, 1988; Middlemiss and Fozard, 1983) decreases the activity of 5HT neurons. This is reflected in Figure 6.1 by the decreases in synthesis and metabolism of 5HT in both the nucleus accumbens and DMN. Despite the effect of 8-OH-DPAT on 5HT neurons it has no effect on the activity of mesolimbic DA neurons projecting to the nucleus accumbens, suggesting that 8-OH-DPAT does not have dopamine receptor agonist or antagonist

Table 6.1. 5,7-DHT depletion of 5HIAA and 5HT in the MZI and DMN.

	5HIAA		5HT	
	SHAM	LESION	SHAM	LESION
MZI	10.8 ± .6	n.d.	10.0 ± .6	n.d.
DMN	7.3 ± .4	n.d.	8.2 ± .4	n.d.

Seven days after an injection of 5,7-DHT (200 μg; i.c.v.) or its 0.3% saline with 0.1% ascorbic acid vehicle (5 μl; i.c.v.), rats were killed, and 5HIAA and 5HT contents were analyzed with HPLC coupled to electrochemical detection. Data represent the means ± 1 S.E.M. of 7-8 determinations. n.d.: not detectable.

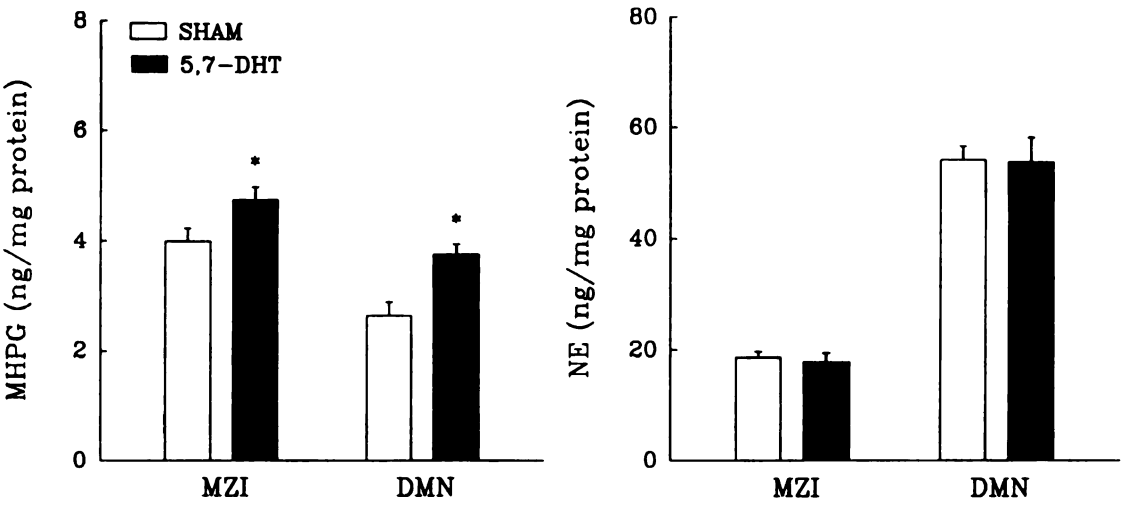

Figure 6.7. Effects of 5,7-DHT on MHPG and NE concentrations in the MZI and DMN. Diestrous female rats were injected with desipramine (25 mg/kg; i.p.) 45 min prior to an injection of either 5,7-DHT (200 μg; i.c.v.) or its 0.3% saline with 0.1% ascorbic acid vehicle (SHAM). Animals were killed 6-8 days later at first day of diestrous. Columns represent the means and vertical lines 1 S.E.M. of 8-9 determinations. *, values for 5,7-DHT-treated rats that are significantly different ($p < 0.05$) from vehicle-treated SHAM animals.

properties *per se*. Interestingly, 8-OH-DPAT consistently increases DOPAC and dopamine concentrations in the MZI and DMN. It would appear, therefore, that the 8-OH-DPAT-induced increase in DOPAC and dopamine concentrations in the MZI and DMN reflects activations of DA and/or NE neurons in these brain regions.

Further study of the effect of 8-OH-DPAT on MHPG concentrations indicate that the activity of NE neurons projecting to the MZI and DMN increased after administration of 8-OH-DPAT. Therefore, the effect of 8-OH-DPAT on incertohypothalamic DA neurons was examined in animals in which NE neurons projecting to these brain regions had been destroyed. Removal of NE fibers to the hypothalamus by bilateral injection of 5-ADMP into the ventral NE bundle does not alter basal activities of DA and 5HT neurons in the MZI and DMN, as evidenced by the lack of change in concentrations of DOPAC, dopamine, 5HIAA and 5HT. This is consistent with the idea that under basal conditions, DOPAC is mainly formed within DA neurons (chapter 4). After destruction of NE neurons 8-OH-DPAT is still able to reduce 5HIAA concentrations, indicating that 5HT neurons projecting to the MZI and DMN are intact and functional. In these animals whose NE neurons have been destroyed 8-OH-DPAT totally lost its ability to increase DOPAC and dopamine concentrations in the MZI and DMN, indicating that suppression of 5HT neuronal activity with 8-OH-DPAT has no effect on incertohypothalamic DA neurons. Therefore, results presented in this chapter suggest that under basal condition 5HT neurons do not tonically regulate the activity of incertohypothalamic DA neurons. In this respect, incertohypothalamic DA neurons resemble the tuberoinfundibular DA neurons which also are not tonically regulated by 5HT neurons (Demarest and Moore,

1981).

It has been reported that 5HT neurons originating from the raphé nuclei and NE neurons originating from the locus ceruleus, which contains perikarya of the major ascending NE system, reciprocally innervate each other (Baraban and Aghajanian, 1981; Pickel et al., 1977). Activation of $5HT_3$ receptors has been reported to facilitate the release of [^{3}H]NE from rabbit hippocampus (Feuerstein and Hertting, 1986). In the rat hypothalamus 5HT inhibits K^+-induced release of NE (Blandina et al. 1991). Results presented in this chapter show that the increase in the metabolism of dopamine in the MZI and DMN after the 8-OH-DPAT treatment reflects increase in the activity of NE neurons projecting to these brain regions. Suppression of the activity of 5HT neurons with 8-OH-DPAT results in an activation of NE neurons projecting to the MZI and DMN. It is proposed, therefore, that 5HT neurons tonically inhibit NE neurons projecting to these brain regions. Activation of $5\text{-}HT_{1A}$ receptors by 8-OH-DPAT inhibits 5HT neurons, and thereby reduces the inhibitory influence of these neurons on NE neurons.

8-OH-DPAT has been reported to have α_2-adrenergic receptor antagonist property in peripheral tissues (Crist and Surprenant, 1987). It is possible that 8-OH-DPAT may activate NE neurons by blocking α_2-adrenergic receptors directly. Therefore, a second method for eliminating 5HT activity was examined. Destruction of central 5HT neurons also results in an activation of NE neurons projecting to the MZI and DMN. These findings are consistent with the report by Pujol et al., (1978) that lesions of 5HT neurons by either destroying 5HT cell bodies in the raphe nucleus or terminals with 5,6-DHT

increase TH and DBH activities in the locus ceruleus. Accordingly, results presented in this chapter provide evidence that regulation of NE neurons by 5HT systems also occurs in the rat hypothalamus. Interrupting 5HT neuronal transmission with 8-OH-DPAT or destroying these neurons with 5,7-DHT causes activation of NE neurons projecting to the MZI and DMN. Accordingly, these results indicate that 5HT tonically inhibits NE neurons projecting to the hypothalamus. Although the possibility that 8-OH-DPAT activates NE neurons via blocking α_2-adrenergic receptors directly has not been excluded, results presented in this chapter indicate that 8-OH-DPAT increases the activity of NE neurons by inhibiting 5HT neurons.

In summary, inhibition of 5HT neurons with the selective $5HT_{1A}$ receptor agonist 8-OH-DPAT increases the activity of NE neurons projecting to the MZI and DMN without affecting incertohypothalamic DA neurons located in these regions. Destruction of 5HT neurons with 5,7-DHT also elevates the activity of NE neurons projecting to these regions. Thus, under basal conditions 5HT neurons do not regulate the activity of incertohypothalamic DA neurons but tonically inhibit NE neurons projecting to the MZI and DMN.

7. EFFECT OF ACUTE MORPHINE TREATMENT ON INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS: THE ROLE OF 5-HYDROXYTRYPTAMINE NEURONS

Introduction

Systemic administration of morphine increases the activity of nigrostriatal and mesolimbic DA neurons (Di Chiara and Imperato, 1988), but inhibits the hypothalamic tuberoinfundibular DA neurons (Gudelsky and Porter, 1979; Alper et al, 1980a; Haskins et al, 1981; for review see Moore and Demarest, 1982; Ben-Jonathan et al., 1989). The turnover rate of dopamine in the MZI and DMN is increased following acute morphine treatment (Lookingland and Moore, 1985), suggesting the drug activates incertohypothalamic DA neurons; in this respect these neurons resemble the mesotelencephalic DA neurons rather than the hypothalamic tuberoinfundibular DA neurons.

As discussed in the chapter 6, 5HT neurons interact with tuberoinfundibular DA neurons in the hypothalamus as well as mesotelencephalic DA neurons in the forebrain. Anatomical evidence that 5HT nerve terminals make direct contact with DA neurons in the MZI provides a morphological basis for potential interactions of 5HT neurons with incertohypothalamic DA neurons. Although results from the previous chapter indicate that under basal conditions 5HT neurons do not regulate incertohypothalamic DA neurons, it is possible that they play a role in regulation of incertohypothalamic DA neurons when these neurons are undergoing a dynamic change in their activity. This has been shown to be true for the tuberoinfundibular DA neurons. For example, under basal conditions interruption of 5HT neuronal transmission does not affect the activity of

tuberoinfundibular DA neurons, indicating that 5-HT neurons do not tonically regulate the activity of these neurons (Demarest and Moore, 1981). Nevertheless, when tuberoinfundibular DA neurons are inhibited by morphine or stress, interruption of 5HT neuronal transmission prevents this inhibition (Demarest and Moore, 1981; Goudreau et al., 1991). Since morphine increases the activity of incertohypothalamic DA neurons and concurrently increases the activity of 5HT neurons projecting to the hypothalamus (Johnston and Moore, 1983), one might speculate that there is a causal relationship between these two actions of morphine.

Accordingly, efforts were made to investigate the possibility that the activation of incertohypothalamic DA neurons by morphine is mediated by 5HT neurons. Comparisons were made between the responses of incertohypothalamic and tuberoinfundibular DA neurons to morphine in intact animals and in animals whose 5HT neurons had been destroyed by treatment with a selective neurotoxin.

Results

Thirty min after injection of morphine at doses from 2.5 to 20 mg/kg DOPAC concentrations in the MZI and DMN increased in a dose-dependent fashion (Figure 7.1). The time course effect of morphine on DOPAC concentrations in MZI and DMN is depicted in the Figure 7.2. Morphine (10 mg/kg; s.c.) increased the DOPAC concentrations in MZI and DMN with a maximal effect at 60 min. These results are consistent with the idea that morphine increases the activity of incertohypothalamic DA neurons (Lookingland and Moore, 1985). The results of these studies revealed that a maximal increase in the activity of incertohypothalamic DA neurons was obtained 60 min

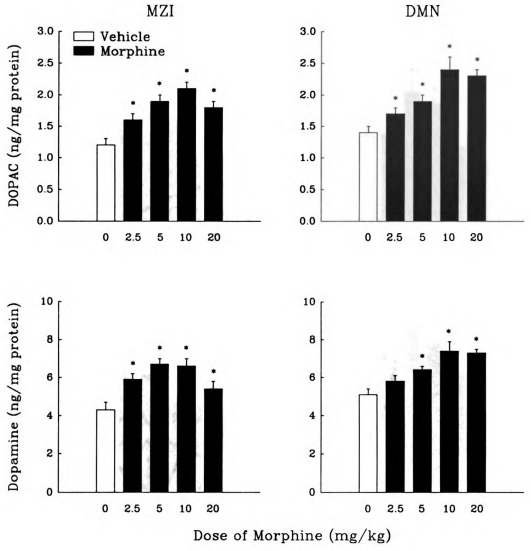

Figure 7.1. The dose response effect of morphine on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with morphine (2.5, 5, 10 and 20 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) 1 hr before they were decapitated. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values that are significantly different ($p < 0.05$) from vehicle-treated controls.

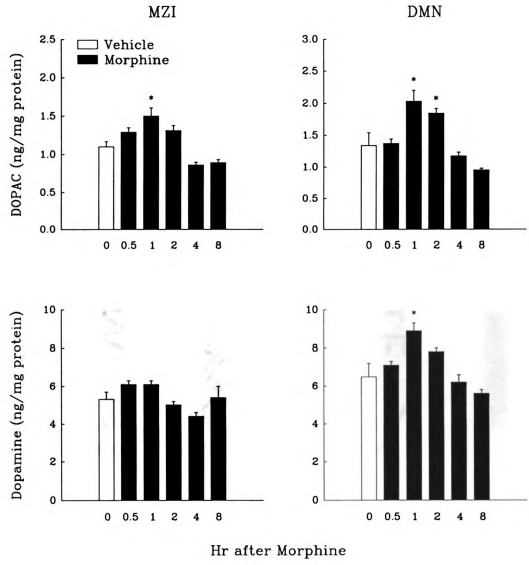

Figure 7.2. The time course effect of morphine on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with morphine (10 mg/kg; s.c.) and killed 0.5, 1, 2, 4 and 8 hr later. Rats receiving water injection (1 ml/kg; s.c.) were killed 1 hr after the injection and used as the zero-time control. Columns represent the means and vertical lines 1 S.E.M. of 5-8 determinations. *, values that are significantly different ($p < 0.05$) from zero-time controls.

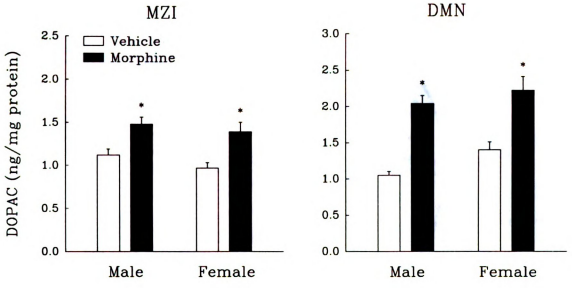

Figure 7.3. Comparison of the effect of morphine on DOPAC concentrations in the MZI and DMN of male and female rats. Male and fist day diestrous female rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 1 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8-9 determinations. *, values for morphine-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

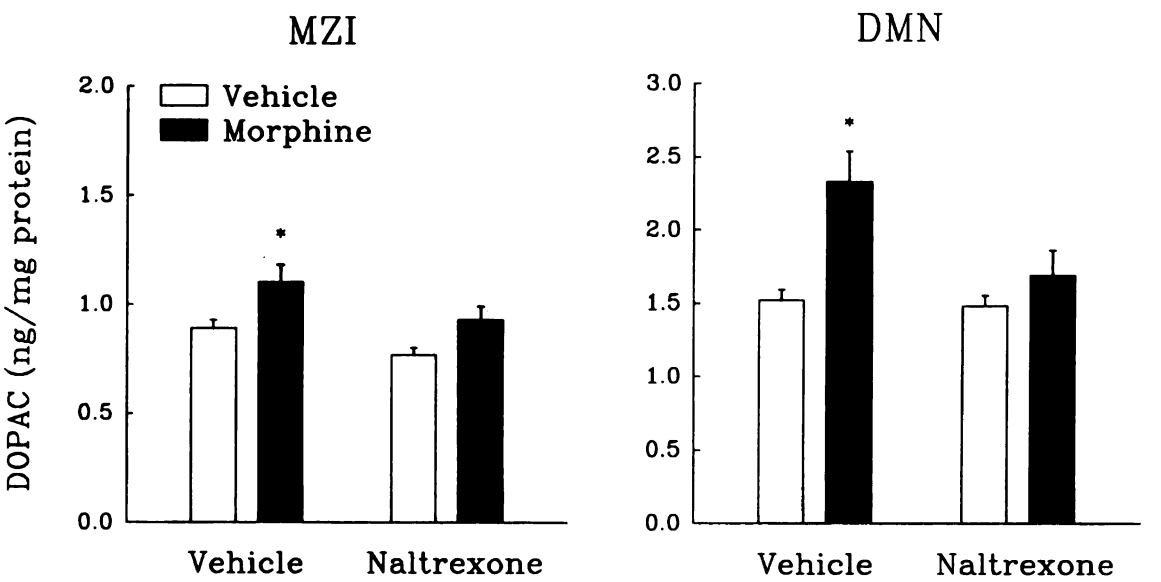

Figure 7.4. Effects of morphine on DOPAC concentrations in the MZI and DMN of naltrexone- or vehicle-treated rats. Thirty min after an injection of naltrexone (2 mg/kg; i.p.) or its water vehicle (1 ml/kg; i.p.) male rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for morphine-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

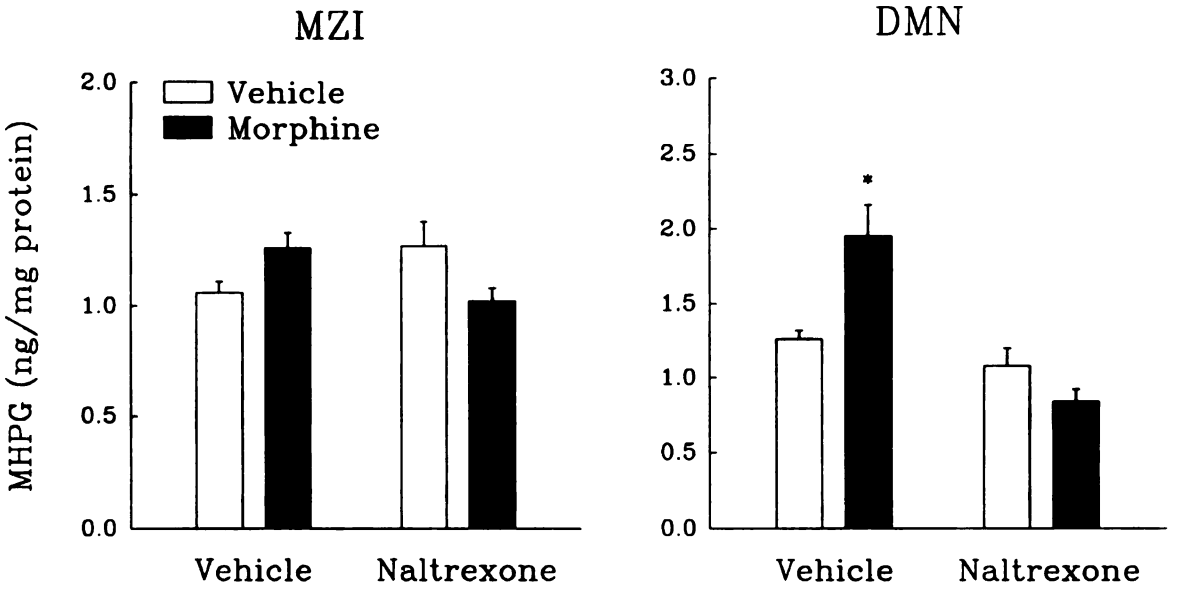

Figure 7.5. Effects of morphine on MHPG concentrations in the MZI and DMN of naltrexone- or vehicle-treated rats. Thirty min after an injection of naltrexone (2 mg/kg; i.p.) or its water vehicle (1 ml/kg; i.p.) male rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for morphine-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

after injection of morphine, therefore, morphine was administered at this dose and time in subsequent studies designed to characterize some neurochemical effects of this drug using DOPAC as an index of the activity of incertohypothalamic DA neurons. It was noted that morphine also caused a dose-dependent and time-related increase in dopamine concentrations in the MZI and DMN, which might indicate an activation of NE neurons projecting to these two brain regions (Chapter 4).

Similar concentrations of DOPAC were found in the MZI and DMN of both male and diestrous female rats, and morphine produced a comparable increase in DOPAC concentrations in the MZI and DMN of both male and female rats (Figure 7.3). As depicted in Figure 7.4, a selective μ opioid receptor antagonist, naltrexone, administered 30 min prior to morphine did not alter basal DOPAC concentrations in the MZI and DMN but prevented the morphine-induced increase in DOPAC concentration in both brain regions. Examination of the effect of morphine on NE neurons revealed that 60 min after morphine (10 mg/kg; s.c.) administration MHPG concentration increased in the DMN (Figure 7.5). Pretreatment with naltrexone had no effect on basal MHPG concentrations in the MZI and DMN, but abolished the morphine-induced increase of MHPG in the DMN. The effects of morphine on incertohypothalamic DA neurons were examined in animals in which NE neurons had been destroyed (Table 7.1). Injection of 5-ADMP into ventral NE bundles depleted about 80% of NE concentrations in the MZI and DMN (from 58.4 ± 3.2 and $15.0\pm.7$ in intact animals to $9.5\pm.5$ and $3.8\pm.6$ in 5-ADMP-treated animals in the DMN and MZI respectively). In 5-ADMP-treated animals although morphine-induced increases in DOPAC concentrations in the MZI and DMN

were attenuated there was still an increase in DOPAC concentrations in the DMN after morphine (compare with Figure 7.3). In the 5-ADMP-treated animals morphine lost its ability to increase dopamine concentrations in both MZI and DMN (Table 7.1).

Sixty min after injection of morphine there was a small but consistent increase in 5HIAA concentration in the MZI and DMN (Figure 7.6). This morphine-induced increase in 5HIAA concentrations was lost by pretreatment with naltrexone 30 min prior to morphine.

Table 7.1. Effects of morphine on DOPAC and dopamine concentrations of ventral NE bundle-lesioned rats

	MZI		DMN	
Treatment	Vehicle	Morphine	Vehicle	Morphine
DOPAC	.93±.10	.92±.08	.87±.07	1.23±.13*
Dopamine	5.8±.5	4.7±.3	4.7±.2	5.6±.5

Seven days after bilateral injections of 5-ADMP (8 μg free base/side; i.c.) into ventral NE bundles, male rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Data represent the means ± 1 S.E.M. of 6-8 determinations. *, values for morphine-treated rats that are significantly different ($p < 0.05$) from vehicle-treated

Seven days after an i.c.v. injection of 5,7-DHT, which destroys central 5HT neurons (Chapter 6) there was no change in DOPAC concentrations in the median eminence, MZI and DMN of vehicle-treated animals (Figure 7.7). In SHAM-treated animals morphine decreased DOPAC concentration in the median eminence and increased DOPAC concentrations in the MZI and DMN. In 5,7-DHT-lesioned animals, morphine

lost its ability to decrease DOPAC in the median eminence, whereas the stimulatory effects of morphine on DOPAC concentrations in the MZI and DMN were not altered.

Discussion

Incertohypothalamic and tuberoinfundibular DA neuronal systems, although located anatomically close to each other, respond differently to acute morphine treatment. Tuberoinfundibular DA neurons are inhibited by morphine as evidenced by decreases in the turnover rate of dopamine in their terminals in the median eminence (Alper et al, 1980a) and by reduction of dopamine concentrations in the portal blood (Gudelsky and Porter, 1979). In contrast, acute morphine treatment activates incertohypothalamic DA neurons as evidenced by increases in the rate of dopamine turnover in the MZI and DMN (Lookingland and Moore 1985). Results presented in this chapter, using DOPAC concentrations to estimate the activity of DA neurons, are consistent with the report of Lookingland and Moore (1985). Morphine increases DOPAC concentrations in the MZI and DMN in a dose-dependent and time-related manner, indicating an activation of incertohypothalamic DA neurons. Pretreatment with the μ opioid receptor antagonist naltrexone abolishes morphine-induced increases in DOPAC concentrations, showing that morphine stimulates incertohypothalamic DA neurons via a μ opioid receptor-mediated mechanism.

Results presented in this chapter indicate that there is no difference in the basal activity of incertohypothalamic DA neurons between male and diestrous female rats. This is consistent with results of a study by Gunnet et al. (1986) who measured the rates of dopamine turnover in the MZI and DMN of male and female rats. Furthermore,

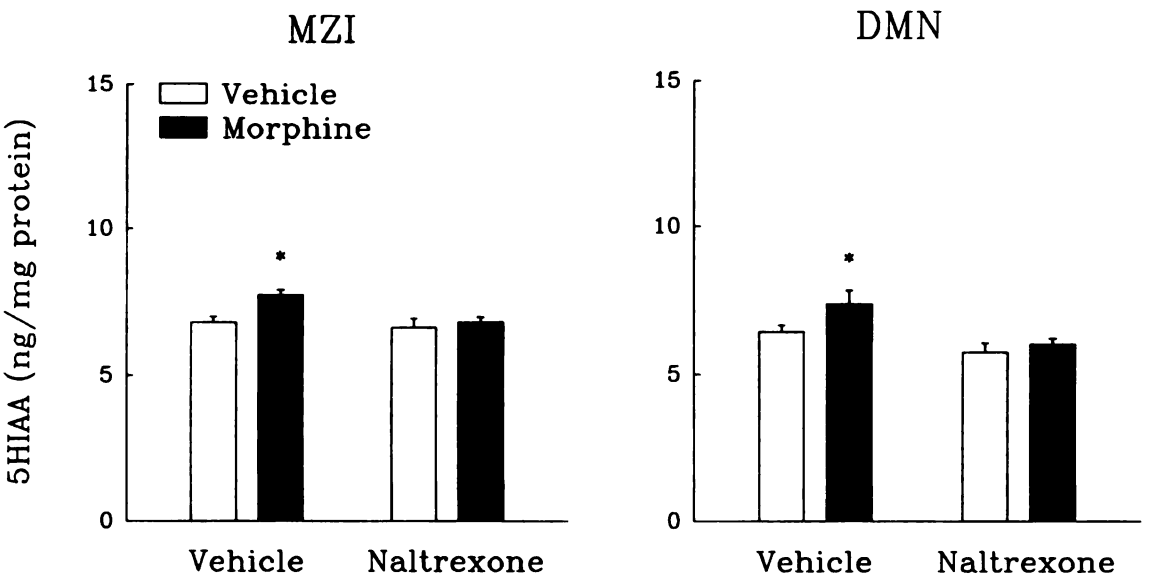

Figure 7.6. Effects of morphine on 5HIAA concentrations in the MZI and DMN of naltrexone- or vehicle-treated rats. Thirty min after an injection of naltrexone (2 mg/kg; i.p.) or its water vehicle (1 ml/kg; i.p.) male rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for morphine-treated rats that are significantly different ($p<0.05$) from vehicle-treated controls.

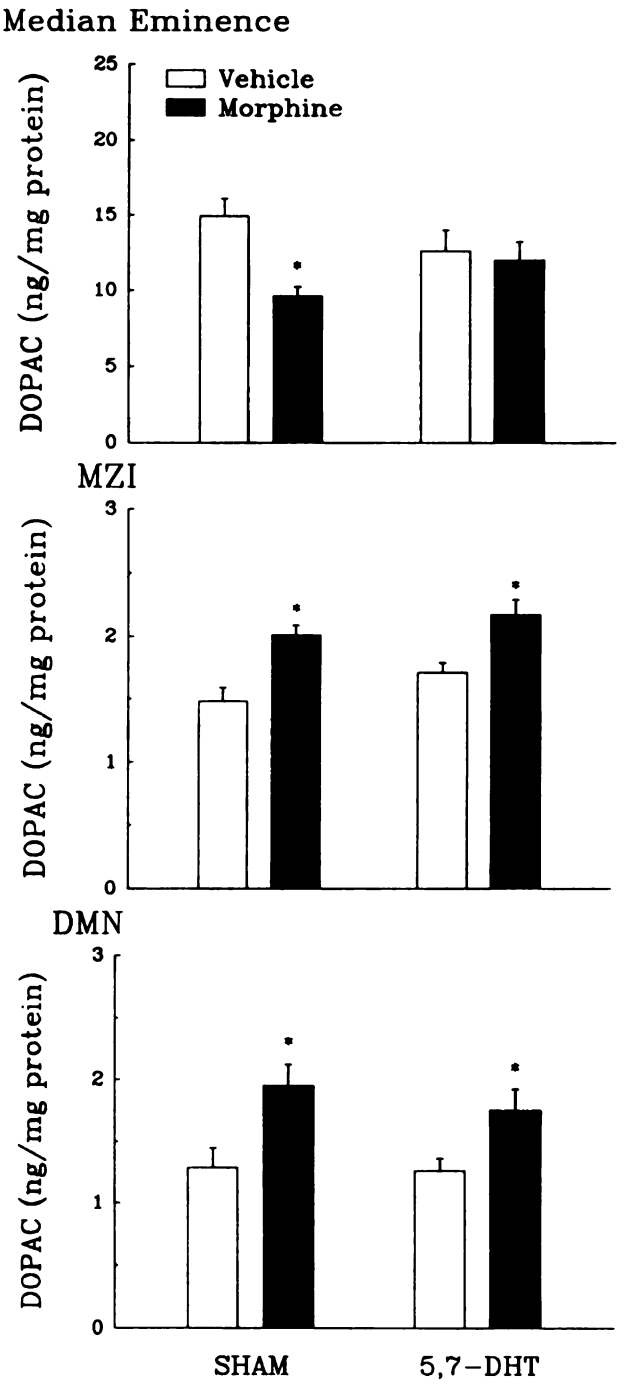

Figure 7.7. Comparison of the morphine effect on DOPAC concentrations in the median eminence, MZI and DMN of 5,7-DHT- and vehicle-treated male rats. Seven days after injection of 5,7-DHT (200 μg; i.c.v.) or its vehicle (5 μl saline containing 0.1% ascorbic acid; SHAM) into the lateral ventricle, rats were injected with morphine (10 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values for morphine-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

results present here indicated that there is no gender difference in response of incertohypothalamic DA neurons to acute morphine treatment.

Since morphine treatment also increased dopamine concentrations in the MZI and DMN, MHPG concentration was measured in these brain regions. Increases in MHPG concentrations in the DMN indicate that morphine also activates NE neurons projecting to the hypothalamus. After depletion of NE in the MZI and DMN, morphine is still able to increase DOPAC concentrations in the DMN, indicating an activation of incertohypothalamic DA neurons. This is consistent with the report that morphine activates incertohypothalamic DA neurons as evidenced by increases in the turnover rate of dopamine in the MZI and DMN (Lookingland and Moore, 1985).

Little is known about the mechanisms by which morphine stimulates incertohypothalamic DA neurons. Direct contact of 5HT nerve terminals with DA neurons in the arcuate nucleus provides a morphological basis for 5HT neurons to mediate the morphine-induced inhibition of tuberoinfundibular DA neurons (Kiss and Halasz, 1986; Bosler et al., 1984; Demarest and Moore, 1981). Inasmuch as 5HT nerve terminals make direct contact with DA cells in the MZI (Bosler et al., 1984; Frankfurt and Beaudet, 1988), there is potential for 5HT neurons to interact with incertohypothalamic DA neurons. Although in the Chapter 6 it was demonstrated that 5HT neurons do not regulate incertohypothalamic DA neurons under basal conditions, 5HT neurons may play a role in regulation of dynamic changes in the activity of these neurons. Morphine increased 5HIAA concentrations in the MZI and DMN, suggesting that it activates 5HT neurons projecting to these regions which may be responsible for

increasing the activity of incertohypothalamic DA neurons. Possible involvement of 5HT neurons in the morphine-induced activation of incertohypothalamic DA neurons were studied by examining effects of morphine on DOPAC concentrations in 5HT-depleted animals, using tuberoinfundibular DA neurons as a experimental control. Results of this study were consistent with the report by Demarest and Moore (1981) that morphine inhibits tuberoinfundibular DA neurons, and while destruction of 5HT neurons with 5,7-DHT does not alter the basal activity of tuberoinfundibular DA neurons it prevents the morphine-induced inhibition of these neurons. These data confirm that the inhibitory effect of morphine on tuberoinfundibular DA neurons is mediated through 5HT neurons. In contrast, results presented in this chapter indicate that destruction of 5HT neurons does not alter the basal concentrations of DOPAC or the ability of morphine to increase DOPAC concentrations in the MZI and DMN. Since the increase in DOPAC concentration in the MZI and DMN represents the activation of incertohypothalamic DA neurons as well as NE neurons projecting to these brain regions, data presented in this chapter indicate that the stimulatory effect of morphine on incertohypothalamic DA neurons is not mediated through 5HT neurons. Furthermore, since NE neurons also contribute to DOPAC concentrations in the MZI and DMN in morphine-treated animals, the lack of effect of 5,7-DHT treatment on DOPAC concentrations in these brain regions after morphine indicates that 5HT neurons do not mediate morphine-induced activation of NE neurons as well.

In summary, results presented in this chapter indicate that morphine-induced activation of incertohypothalamic DA neurons is not mediated through 5HT neurons.

Furthermore, there is no gender difference in the basal activity of incertohypothalamic DA neurons or in the response of these neurons to acute morphine treatment.

8. INFLUENCE OF NEUROTENSIN ON INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS

Introduction

Many neuropeptides appear to act as neurotransmitters or neuromodulators in the central nervous system. The discovery that two or more transmitters can occur in the same nerve terminal greatly changed the concept that one neuron synthesizes and releases only one neurotransmitter. It has been discovered that many neuropeptides coexist with each other or with classical monoamine transmitters within same neurons. For example, substance P colocalizes with 5HT, cholecystokinin with dopamine, neuropeptide Y with NE and neurotensin with dopamine (Hökfelt et al., 1980). The physiological significance of the colocalization of two putative neurotransmitters within the same neuron seems to be the great complexity and flexibility that a limited number of neurotransmitters can generate. Of importance is that coexistence of putative transmitters does not follow a single pattern. Colocalized neurotransmitters can regulate each other's release (Bartfai et al., 1988) or modulate each other's effect on postsynaptic cells (Kow and Pfaff, 1988) by various mechanisms.

The hypothalamus contains high density of neuropeptides. Among others, neurotensin has been shown to be located in the hypothalamus and to interact with DA neurons (Ibata et al., 1983; Gudelsky et al., 1989). In this chapter the influence of neurotensin on incertohypothalamic DA neurons was investigated.

Neurotensin is a tridecapeptide, originally isolated from bovine hypothalamus (Carraway and Leeman, 1973). It distributes widely throughout the central nervous

system (for reviews see: Kahn et al., 1982) and fulfills most of the major criteria required for a substance to be classified as a neural transmitter or a modulator. For example, it is present in nerve cell bodies, terminals and fibers, throughout the brain and spinal cord (Uhl, 1982), and can be released by depolarization of cell membranes in a calcium-dependent mechanism (Iversen, et al., 1978). Neurotensin affects the activity of neurons in many brain regions and elicits certain behaviors when injected i.c.v. (Nemeroff, et al., 1982). It binds to specific receptors (Uhl, 1982; Kitabgi et al., 1985) and is inactivated by peptidase (Kitabgi et al., 1986). It has been postulated that neurotensin interacts with other neuronal systems in the brain (Kitabgi, 1989; Nemeroff, 1986; Gudelsky et al, 1989).

Of primary interest is that neurotensin interacts with DA neurons (Haubrich et al., 1982; Nemeroff, 1986; Ervin and Nemeroff, 1988; Bissette and Nemeroff, 1988). Intracerebroventricular injection of neurotensin activates mesotelencephalic DA neurons (Stowe and Nemeroff, 1991; Faggin et al., 1990) and elicits a number of effects which are similar to that elicited by neuroleptics. For example, it induces hypothermia, suppresses spontaneous locomotor activity and causes muscle relaxation. Therefore, it has been hypothesized to act as an endogenous neuroleptic (Nemeroff et al., 1982; Nemeroff, 1986).

Neurotensin is present in many brain regions where DA neurons are located, e.g., regions containing cell bodies and terminals of mesotelencephalic DA neurons, such as substantia nigra, ventral tegmental area, nucleus accumbens, striatum and prefrontal cortex (for review see Kitabgi, 1989). In some regions neurotensin is colocalized with

dopamine (Bean et al., 1989; Studler et al., 1988). Autoradiographic studies demonstrate a high density of neurotensin binding sites, presumably neurotensin receptors, in the substantia nigra, ventral tegmental area, nucleus accumbens, striatum and prefrontal cortex (Schotte and Leysen, 1989; Uhl, 1982; Kitabgi et al., 1985). Selective lesions of mesotelencephalic DA neurons lead to a decrease in neurotensin receptors in their cell bodies regions (Palacios and Kuhar, 1981; Quirion et al., 1985; Masuo, et al., 1990) and terminal regions (Schotte et al., 1988; Quirion et al., 1985; Masuo, et al., 1990), indicating that the neurotensin receptor is located on DA neurons. Combination of autoradiographic and immunohistochemical techniques reveals that neurotensin receptors are selectively associated with 95% of TH-positive neurons in the substantia nigra and 90% in the ventral tegmental area (Szigethy et al., 1989). Therefore, in these brain regions a large proportion of the neurotensin receptors are located on DA terminals. Neurotensin modulates release of dopamine and the postsynaptic response of target cells to this amine. Accordingly, neurotensin is an important modulator of DA neurotransmission, at least in the major ascending DA neurons (Kasckow and Nemeroff, 1991; Kitabgi, 1989). Conversely, neurotensin synthesis, and possibly release, is under control of dopamine receptors (Masuo et al., 1990; Williams, et al., 1990; Kilts et al., 1988; Govoni, et al., 1980; Uhl and Kuhar, 1984).

In the hypothalamus, neurotensin-like immunoreactivity is detected in the bed nucleus of the stria terminalis, the medial preoptic area, the periventricular nucleus, the paraventricular nucleus, arcuate nucleus and median eminence (Kahn et al., 1982). In the arcuate nucleus and periventricular nucleus neurotensin-like immunoreactivity

colocalizes with dopamine (Meister, et al., 1989; Ibata et al., 1983). Neurotensin has been proposed to play roles in neuroendocrine control of hormone release from the anterior pituitary (Vijayan, et al., 1988). For instance, central administration of neurotensin decreases prolactin secretion (Maeda and Frohman, 1978; Vijayan and McCann, 1979), whereas immunoneutralization with neurotensin-antibodies increases prolactin secretion (Vijayan et al., 1988). The inhibitory effect of neurotensin on prolactin secretion seems to be, at least partially, mediated by tuberoinfundibular DA neurons (Fuxe, et al., 1984; Tojo et al., 1986). In the MZI and the dorsal portion of the DMN, neurotensin-like immunoreactive cell bodies and fibers have also been detected by Kahn and coworkers (1982). It is not known, however, if neurotensin colocalizes with dopamine in these brain regions, but specific binding sites for the neurotensin have been identified in the zona incerta and DMN (Meister et al., 1989). If these neurotensin binding sites are neurotensin receptors, what are the physiological functions of these receptors? Do they have any regulatory function on incertohypothalamic DA neurons?

In this chapter, possible regulation of incertohypothalamic DA neurons by neurotensin was investigated by examining the effect of a putative neurotensin agonist, Sub-NT_{8-13}. The C-terminal hexapeptide fragment of neurotensin has similar effects as neurotensin (Gilbert et al., 1989; Stowe and Nemeroff, 1991). The compound Sub-NT_{8-13}, modified from this fragment has been proposed to be a peripherally active neurotensin agonist (Yutaka et al., 1989). The structures of neurotensin and Sub-NT_{8-13} are shown in the Figure 8.1.

Figure 8.1. Chemical structures of neurotensin and Sub-NT_{8-13}

	1	2	3	4	5	6	7	8	9	10	11	12	13
Neurotensin	**Glu**	**Leu**	**Tyr**	**Glu**	**Asn**	**Lys**	**Pro**	**Arg**	**Arg**	**Pro**	**Tyr**	**Ile**	**Leu**
Sub-NT_{8-13}								**me-Arg**	**Lys**	**Pro**	**Tyr**	**(3-me)Val**	**Leu**

Results

Direct comparison of effects of i.c.v. injections of neurotensin and Sub-NT_{8-13} on different DA systems revealed that both peptides increased DOPAC concentrations and were without effect on dopamine concentrations in the nucleus accumbens, striatum and median eminence (Figure 8.2). These results indicate the activation of mesolimbic, nigrostriatal and tuberoinfundibular DA neurons by neurotensin and its modified C-terminal fragment, confirming the agonist property of Sub-NT_{8-13}. Both neurotensin and Sub-NT_{8-13} increased DOPAC concentrations in the MZI and DMN and dopamine concentration in the DMN (Figure 8.3). As discussed in previous chapters, increase in dopamine concentrations indicates a possible activation of NE neurons.

Since Sub-NT_{8-13} has been suggested as a peripherally active neurotensin agonist (Yutaka et al., 1989) the effects of peripherally injected Sub-NT_{8-13} were examined. Two hr after a s.c. injection of Sub-NT_{8-13}, DOPAC concentrations increased in the median eminence at the dose of 0.1 mg/kg or higher (Figure 8.4); concurrently, the plasma prolactin concentration was reduced. Similarly, two hr after a s.c. injection of Sub-NT_{8-13} the DOPAC concentration in the MZI and DMN were elevated in a dose-related manner; the dopamine concentrations in the MZI and DMN were also increased (Figure 8.5). A time course of the effect of Sub-NT_{8-13} on tuberoinfundibular DA neurons is depicted on Figure 8.6. Sub-NT_{8-13} increased DOPAC concentrations in the median eminence one hr after injection and lasted for at least 2 hr; plasma prolactin concentrations were decreased for at least 4 hr. In the MZI and DMN, Sub-NT_{8-13} increased DOPAC and dopamine concentrations one hr after injection and this

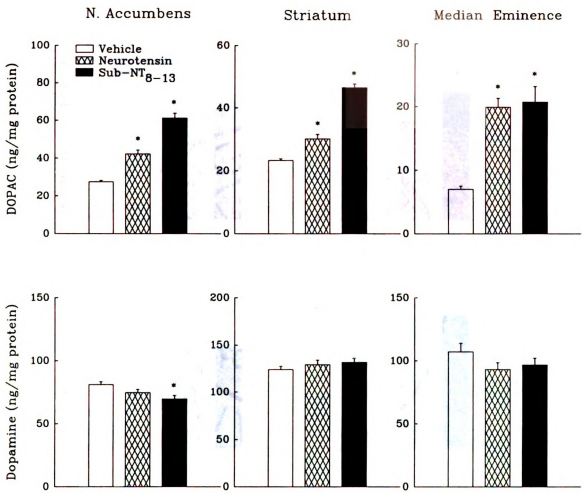

Figure 8.2. Comparison of the effects of neurotensin and Sub-NT_{8-13} on DOPAC and dopamine concentrations in the nucleus accumbens, striatum and median eminence. Male rats were injected with neurotensin (12 nmole/rat; i.c.v.) or Sub-NT_{8-13} (17 nmole/rat; i.c.v.), or their 0.1% ascorbic acid-saline vehicle (3 μl/rat; i.c.v.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values from neurotensin- or Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

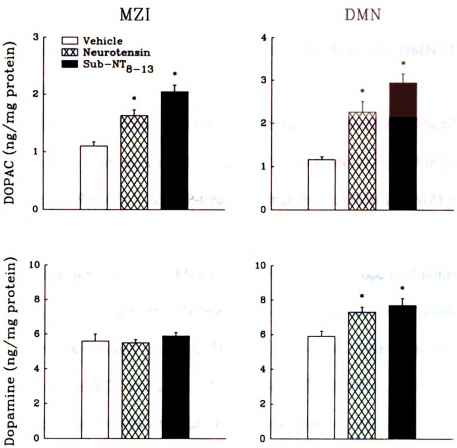

Figure 8.3. Comparison of the effects of neurotensin and Sub-NT_{8-13} on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with neurotensin (12 nmole/rat; i.c.v.) or Sub-NT_{8-13} (17 nmole/rat; i.c.v.) or their 0.1% ascorbic acid-saline vehicle (3 μl/rat; i.c.v.) and killed 60 min later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values from neurotensin- or Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

stimulatory effect lasted for at least two hr (Figure 8.7).

Since an increase in both dopamine and DOPAC concentrations in the MZI and DMN generally indicates an increase in the activity of NE neurons, the effect of Sub-NT_{8-13} on MHPG concentrations was examined. Sub-NT_{8-13} increased MHPG concentrations and decreased NE concentrations in the MZI and DMN (Figure 8.8), indicating an activation of NE neurons.

One week after bilateral injections of 5-ADMP into ventral NE bundles, the NE concentrations were depleted about 80% (from 85.4 ± 4.0 and 21.9 ± 2.3 in intact animals to 13.9 ± 1.5 and $4.9 \pm .6$ in 5-ADMP-treated animals in the DMN and MZI respectively) in the MZI and DMN. Destruction of ventral NE bundles did not alter basal DOPAC or dopamine concentrations in the MZI and DMN (Figure 8.9, open columns). Although the Sub-NT_{8-13}-induced increase in DOPAC concentrations was greatly attenuated in the ventral NE bundle-lesioned animals, Sub-NT_{8-13} was still able to increase DOPAC concentrations in the MZI and DMN of these rats. The Sub-NT_{8-13}-induced increase in dopamine concentrations was abolished by the destruction of ventral NE bundle in both regions.

Discussion

Neurotensin increases the activity of nigrostriatal, mesolimbic and mesocortical DA neurons (Nemeroff, 1986; Nemeroff et al., 1982; Stowe and Nemeroff, 1991; Blaha et al., 1988, 1990) and, in turn, dopamine seems to regulate neurotensin synthesis (Williams et al., 1990) and neurotensin receptor density (Hervé, et al., 1986). This has been evidenced by the following observations: following AAAD inhibition, central

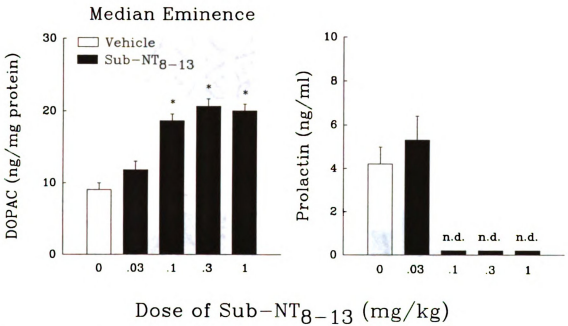

Figure 8.4. Dose-response effects of Sub-NT_{8-13} on DOPAC concentrations in the median eminence and plasma prolactin level. Male rats were injected s.c. with Sub-NT_{8-13} .03, .1, .3 and 1 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

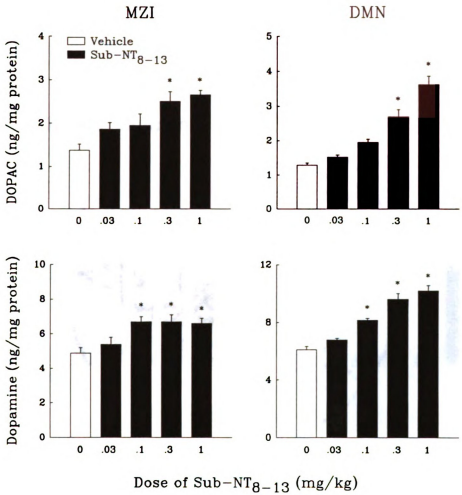

Figure 8.5. Dose-response effects of Sub-NT_{8-13} on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected s.c. with Sub-NT_{8-13} .03, .1, .3 and 1 mg/kg or its water vehicle and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

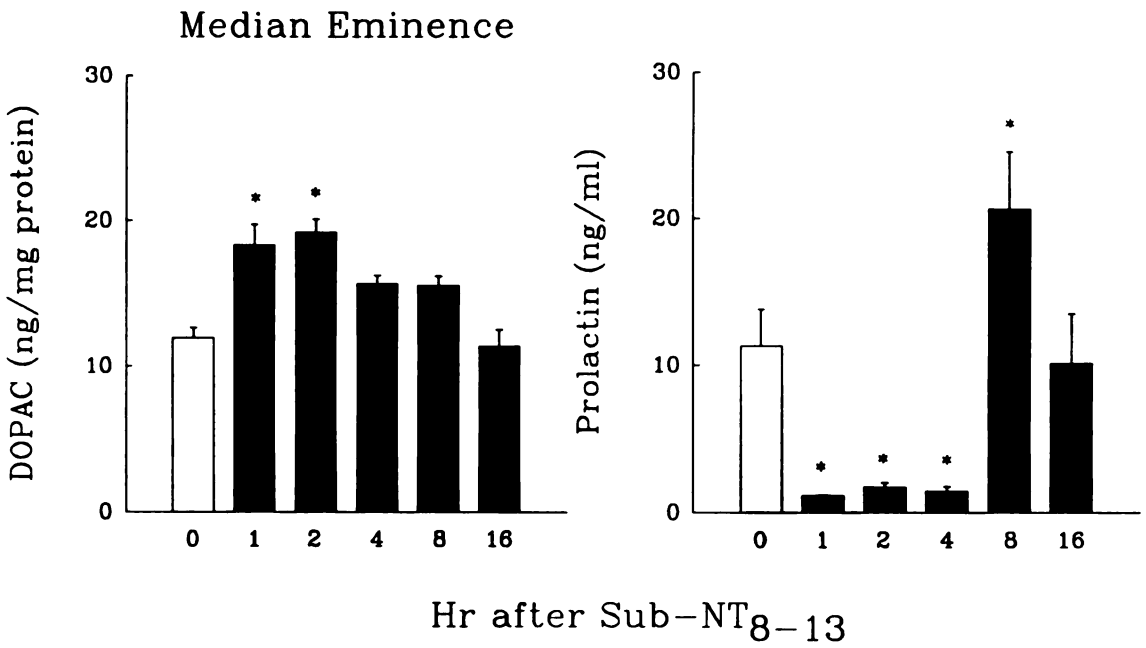

Figure 8.6. The time-course effects of Sub-NT_{8-13} on DOPAC concentrations in the median eminence and plasma prolactin level. Male rats were injected with Sub-NT_{8-13} (1 mg/kg; s.c.) and killed 1, 2, 4, 8 or 16 hr later. Rats received water injection (1 ml/kg; s.c.) and killed 2 hr later were used as zero time control. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p<0.05$) from zero-time controls.

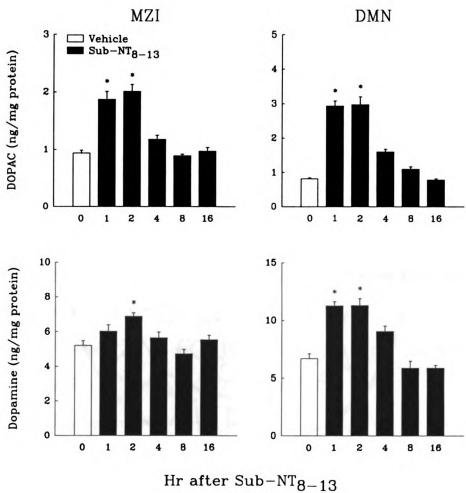

Figure 8.7. The time-course effects of Sub-NT_{8-13} on DOPAC and dopamine concentrations in the MZI and DMN. Male rats were injected with Sub-NT_{8-13} (1 mg/kg; s.c.) and killed 1, 2, 4, 8 or 16 hr later. Rats which received a water injection (1 ml/kg; s.c.) and killed 2 hr later were used as zero time controls. Columns represent the means and vertical lines 1 S.E.M. of 8 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from zero-time controls.

injection of neurotensin increases DOPA accumulation in terminal regions of the nigrostriatal and mesolimbic DA systems, indicating an increase in dopamine synthesis (Widerlöv et al., 1982); intracisternally or i.c.v. injection of neurotensin increases metabolism of dopamine, i.e. increases DOPAC and HVA concentrations, in striatum, nucleus accumbens as well as other brain regions including hypothalamus (Widerlöv et al., 1982; Reche, et al., 1983; Drumheller, et al., 1990). Direct application of neurotensin increases the cell firing rate of putative DA neurons in the substantia nigra and ventral tegmental area (Stowe and Nemeroff, 1991). Chronic administration of neuroleptics which block central D2 receptors or lesions of DA neurons in the ventral tegmental area increase neurotensin binding sites in the prefrontal cortex and nucleus accumbens (Hervé, et al., 1986). Studies on interactions between neurotensin and incertohypothalamic DA neurons, however, have not been reported.

In this chapter, it is demonstrated that Sub-NT_{8-13} activates incertohypothalamic DA neurons as well as NE neurons projecting to the MZI and DMN.

Direct comparison of the effects of centrally administered neurotensin with Sub-NT_{8-13} on major ascending DA neurons and tuberoinfundibular DA neurons reveals that these two peptides produce similar pharmacological profiles. Both neurotensin and Sub-NT_{8-13} increase the activity of DA neurons of the nigrostriatal, mesolimbic and tuberoinfundibular systems, confirming that Sub-NT_{8-13} is an agonist at neurotensin receptors. Both peptides increase DOPAC and dopamine concentrations in the MZI and DMN similarly, indicating that Sub-NT_{8-13} is a useful tool to study neurotensin regulation of incertohypothalamic DA neurons.

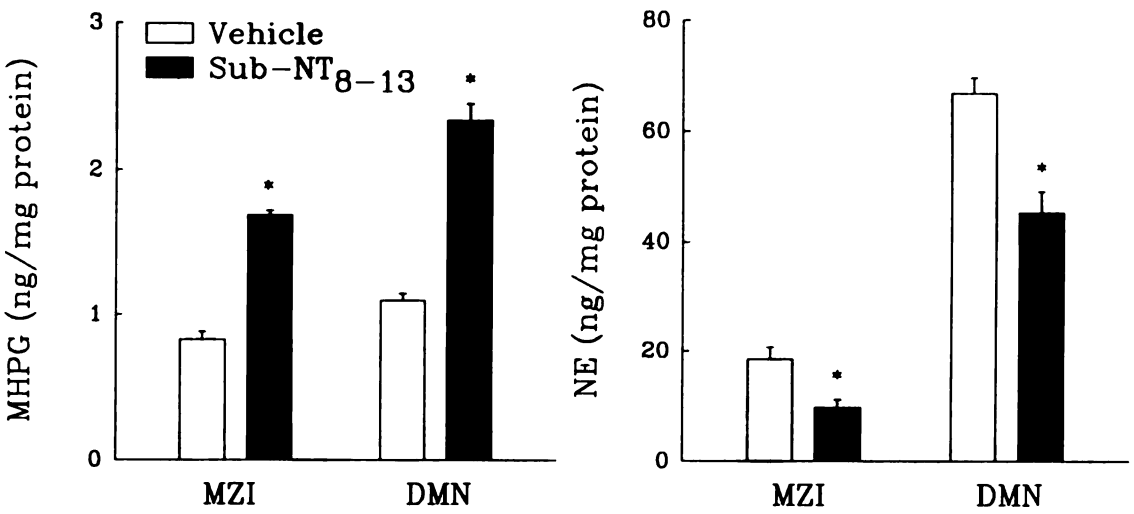

Figure 8.8. Effects of Sub-NT_{8-13} on MHPG and NE concentrations in the MZI and DMN. Male rats were injected with Sub-NT_{8-13} (1 mg/kg; s.c.) or its water vehicle (1 ml/kg; s.c.) and killed 2 hr later. Columns represent the means and vertical lines 1 S.E.M. of 7-8 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

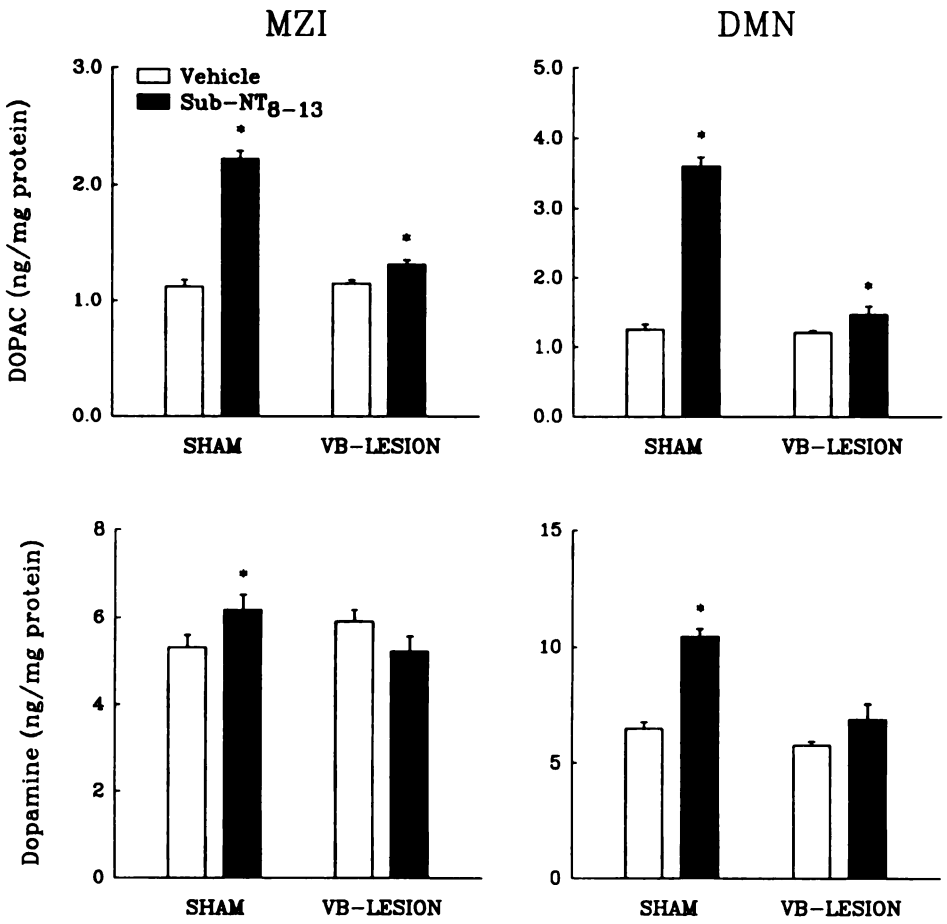

Figure 8.9. Comparison of the effects of Sub-NT_{8-13} on DOPAC and dopamine concentrations in the MZI and DMN of 5-ADMP- and vehicle-treated rats. Seven days after bilaterally injections of 5-ADMP (8 μg free base/side; i.c.; VB-lesion) or its 0.1% ascorbic acid-saline vehicle (0.3 μl/site; i.c.; SHAM) into the ventral NE bundle, male rats were injected with Sub-NT_{8-13} (17 nmole/rat; i.c.v.) or its 0.1% ascorbic acid-saline vehicle (3 μl/rat; i.c.v.) and killed 1 hr later. Columns represent the means and vertical lines 1 S.E.M. of 8-9 determinations. *, values from Sub-NT_{8-13}-treated rats that are significantly different ($p < 0.05$) from vehicle-treated controls.

Since Sub-NT_{8-13} is peripherally active the effects of s.c. injected Sub-NT_{8-13} on incertohypothalamic DA neurons were examined and compared with those on tuberoinfundibular DA neurons. Consistent with the report that neurotensin increases DA neuronal activities (Stowe and Nemeroff, 1991; Faggin et al., 1990), Sub-NT_{8-13} activates tuberoinfundibular DA neurons as evidenced by an increase in DOPAC concentrations in the median eminence and a decrease in plasma prolactin concentration. The increased MHPG concentrations and the decreased NE concentrations in the MZI and DMN indicates a stimulatory effect of Sub-NT_{8-13} on NE neurons as well.

Although Stowe and Nemeroff (1991) reported that neurotensin does not increase the firing of NE neurons in the locus ceruleus different groups of NE neurons may be regulated differently. It has been reported that neurotensin-like immunoreactivity is detected in the brainstem medulla oblongata and colocalized with NE in the A_2 cell group (Hökfelt et al., 1984b). A_1 and A_2 cell groups give rise to the major ascending NE neurons to the hypothalamus (Moore and Bloom, 1979). It is not surprising, therefore, to see the activation by neurotensin of NE neurons projecting to the hypothalamus.

To determine if neurotensin also activates incertohypothalamic DA neurons the effect of Sub-NT_{8-13} on DOPAC concentrations was examined in the MZI and DMN in rats whose NE neurons projecting to these regions had been destroyed. In ventral NE bundle-lesioned animals Sub-NT_{8-13} still caused a small but consistent increase in DOPAC concentrations in the MZI and DMN. Since under same condition, idazoxan does not increase DOPAC concentration in the MZI and DMN (Chapter 4, Figure 4.5), the Sub-NT_{8-13}-induced increase in DOPAC concentrations in these regions suggests the activation

of incertohypothalamic DA neurons. These data indicate, therefore, that Sub-NT_{8-13} has a stimulatory effect on both incertohypothalamic DA neurons and NE neurons projecting to the MZI and DMN.

In conclusion, neurotensin has a stimulatory effect on incertohypothalamic DA neurons and on NE neurons projecting to the MZI and DMN.

SUMMARY AND CONCLUSIONS

Incertohypothalamic DA neurons have cell bodies in the MZI and project to many brain regions including the DMN. Both MZI and DMN receive dense innervation from NE neurons, which may confound interpretation of results when neurochemical techniques are used to study incertohypothalamic DA neurons.

When animals are injected with an AAAD inhibitor, NSD 1015, DOPA accumulates in both DA and NE neurons. Therefore, measurement of DOPA accumulation cannot be employed to study incertohypothalamic DA neurons selectively.

When NE neurons are not activated, DOPAC in the MZI and DMN arises mainly from DA neurons and its concentration reflects the activity of incertohypothalamic DA neurons. Once NE neurons are activated, dopamine starts to accumulate and a significant amount of DOPAC can be generated within NE neurons. Therefore, measuring DOPAC concentrations alone cannot estimate the activity of incertohypothalamic DA neurons. In combination with other neurochemical techniques (e.g. measuring MHPG concentrations to estimate the activity of NE neurons and lesioning NE neurons projecting to the hypothalamus), DOPAC can be employed to study DA neurons selectively. Consequently, an experimental protocol has been developed to study incertohypothalamic DA neurons, which eliminates the interference from NE neurons. Using this protocol, responses of incertohypothalamic DA neurons to a variety of pharmacological manipulations was studied.

Incertohypothalamic DA neurons are regulated by dopamine receptor-mediated

mechanisms. Raclopride increases the activity of these neurons whereas remoxipride does not. Therefore, unlike the mesotelencephalic DA neurons which are activated by both raclopride and remoxipride, only a subgroup of D2 receptors or a novel dopamine receptor yet to be defined, regulate incertohypothalamic DA neurons. D1 receptors play a role in regulating the activity of mesotelencephalic DA neurons. In contrast, incertohypothalamic DA neurons are not responsive to the D1 receptor antagonist SCH 39166. Therefore, the activity of incertohypothalamic DA neurons, like mesotelencephalic DA neurons, is regulated by dopamine receptors, but the precise mechanisms by which these neurons are regulated differ.

5HT neurons do not tonically regulate incertohypothalamic DA neurons but they do inhibit NE neurons projecting to the MZI and DMN. As a result, when animals are treated with 8-OH-DPAT to inhibit or with 5,7-DHT to destroy 5HT neurons the activity of NE neurons projecting to these brain regions increases.

The basal activities of incertohypothalamic DA neurons and responses of these neurons to acute morphine treatment are similar in male and diestrous female rats. Morphine has a stimulatory effect on these neurons through a μ opioid receptor-mediated mechanism. Morphine also increases the activity of 5HT neurons projecting to the MZI and DMN through a similar mechanism. Unlike tuberoinfundibular DA neurons whose activity is reduced by morphine via a 5HT-mediated mechanism, the stimulatory effect of morphine on incertohypothalamic DA neurons is not modulated by 5HT neurons. Consequently, morphine increases DOPAC concentrations by a similar mechanism in both intact animals and in animals in which 5HT neurons have been destroyed.

Table 8.1 Summary of the effects of various drugs on the activity of incertohypothalamic dopaminergic neurons

COMPOUNDS	EFFECT
Dopamine receptor antagonists	
Raclopride (D_2)	+
Remoxipride (D_2)	o
SCH 39166 (D_1)	o
Neuropeptides	
Sub-NT_{8-13} (NT agonist)	+
5-HT receptor agonist	
8-OH-DPAT	o
Opioid receptor agonists	
Morphine (mu)	+

+ increase; o no change.

Neurotensin increases the activity of tuberoinfundibular DA neurons and thereby inhibits the release of prolactin from the anterior pituitary. It also stimulates incertohypothalamic DA neurons as evidenced by the stimulatory effect of the neurotensin agonist Sub-NT_{8-13} on these neurons. In addition, neurotensin also activates NE neurons projecting to the MZI and DMN.

In summary, incertohypothalamic DA neurons are regulated by mechanisms substantially different from those regulating mesotelencephalic or tuberoinfundibular DA neurons. The major contribution of studies reported in this dissertation is the development of a neurochemical protocol by which incertohypothalamic DA neurons can be studied. Using this protocol, it was possible to characterize the effects of drug on incertohypothalamic DA neurons and on NE neurons that project to the MZI and DMN. More importantly, the neurochemical technique developed in this dissertation provides a logical approach to study other hypothalamic DA neurons. The results described in this dissertation provide a start toward understanding the regulation of incertohypothalamic DA neurons. By understanding the regulation of this group of DA neurons one may be able to determine the physiological functions of these incertohypothalamic DA neurons. Although this dissertation provides only a beginning of studies of incertohypothalamic DA neurons, it provides a base for investigators who are interested in further exploring the characteristics of these DA neurons.

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