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AMINO ACID NEUROTRANSMITTER
REGULATION OF HYPOTHALAMIC
DOPAMINERGIC NEURONS
IN THE RAT

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Edward J. Wagner

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Major professor

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AMINO ACID NEUROTRANSMITTER REGULATION OF HYPOTHALAMIC DOPAMINERGIC NEURONS IN THE RAT

By

Edward John Wagner

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ABSTRACT

AMINO ACID NEUROTRANSMITTER REGULATION OF HYPOTHALAMIC DOPAMINERGIC NEURONS IN THE RAT

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Edward J. Wagner

The purpose of the present studies was to characterize the regulation of tuberoinfundibular dopaminergic (TIDA) and periventricular-hypophysial dopaminergic (PHDA) neurons by endogenous amino acid neurotransmitters, and to determine sexual differences therein. To this end, the effects of N-methyl-D-aspartate (NMDA), non-NMDA, γ-aminobutyric acid $(GABA)_A$ and $GABA_B$ receptor blockade and activation on these neurons were evaluated in intact and gonadectomized male and female rats using potent and selective agonists and antagonists at these receptors. The activity of TIDA and PHDA neurons was estimated by measuring either the accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 min after administration of the decarboxylase inhibitor NSD-1015, or the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), in the median eminence and intermediate lobe, respectively.

There is a sexual difference in NMDA receptor-mediated regulation of TIDA neurons by endogenous excitatory amino acid (EAA) neurotransmitters. EAA neurotransmitters acting at these receptors tonically stimulate TIDA neurons in female but

not male rats, and this effect is dependent on the presence of estrogen acting via a prolactin-independent mechanism. By contrast, EAA neurotransmitters acting at non-NMDA receptors tonically inhibit TIDA neurons in male and female rats. This action is mediated exclusively via the α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor and not the kainate receptor. On the other hand, EAA neurotransmitters tonically inhibit PHDA neurons in male and female rats by acting at both NMDA and AMPA receptors.

γ-Aminobutyric acid (GABA) tonically inhibits TIDA but not PHDA neurons in male and female rats through an action at $GABA_A$ rather than $GABA_B$ receptors. GABA acting at $GABA_A$ receptors is responsible for the AMPA receptor-mediated tonic inhibition of TIDA neurons by EAA neurotransmitters, whereas endogenous κ-opioids are partially responsible for the AMPA receptor-mediated tonic inhibition of PHDA neurons by EAA neurotransmitters. Taken together, these results indicate multiple roles for EAA neurotransmitters in the regulation hypothalamic dopaminergic neurons: an estrogen-dependent NMDA receptor-mediated tonic stimulation of TIDA neurons, a NMDA receptor-mediated tonic inhibition of PHDA neurons and an indirect AMPA receptor-mediated tonic inhibition of TIDA and PHDA neurons.

To my loving wife, Carol, and to my family.

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my mentor, Dr. Keith J. Lookingland, for allowing me the opportunity to pursue my career goals (as a neuropharmacologist if not as a ballplayer) in his laboratory. Thanks to him, I have gained the ability to plan and perform experiments, and to express subsequent results and implications in writing. It also was an honor to work with the distinguished professor and chairman, Dr. Kenneth E. Moore. His knowledge of the pharmacology of catecholaminergic neurons served as a nice compliment to Keith's expertise in neuroendocrinology, providing me with the ideal setting for the study of hypothalamic dopaminergic neurons.

Together with Drs. Peter Cobbett, M. Duff Davis and James J. Galligan, this committee of five provided invaluable advice on the interpretation of results and the direction of future experiments. I gratefully extend my thanks for their help in keeping me focused and my dissertation project circumscribed.

It was a pleasure to have collaborated with Drs. John L. Goudreau and Jorge Manzanares. Not only were the collaborative efforts fruitful but the friendships developed no doubt will provide me with lifelong memories of my time here in Michigan.

I also wish to acknowledge the clerical and technical support of Marty Burns, Dr. Misty J. Eaton, Gretchen Gardner, Robert Kranich, Carol Loviska, Christine Stevens, Mickie Vanderlip and Michael Woolhiser. Their superb assistance helped immeasurably in allowing my research to proceed smoothly.

Once again, thanks to <u>all</u> of you for helping me get this far!!

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LIST OF ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
α-MSH	α-melanocyte-stimulating hormone
ANOVA	analysis of variance
DOPAC	3,4-dihydroxyphenylacetic acid
DOPA	3,4-dihydroxyphenylalanine
EAA	excitatory amino acid
GABA	γ-aminobutyric acid
HPLC	high performance liquid chromatography
NSD-1015	3-hydroxybenzylhydrazine
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
i.v.	intravenous
LSD	Least Significant Difference
NBQX	6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3(1H,4H)-dione
NMDA	N-methyl-D-aspartate
PHDA	periventricular-hypophysial dopaminergic
s.c.	subcutaneous
TIDA	tuberoinfundibular dopaminergic

1. INTRODUCTION

A. Anatomy and function of central dopaminergic neuronal systems

The anatomy of catecholaminergic neurons is classified by an alphanumeric system developed by Dahlström and Fuxe (1964). Figure 1.1 illustrates the anatomical organization of catecholaminergic cell groups. Dopaminergic cell groups (A_{8-16}) have a more rostral orientation than do noradrenergic cell groups (A_{1-7}), which are located in the pons and medulla.

The best understood dopaminergic systems are the ascending neuronal systems, namely the nigrostriatal, mesolimbic and mesocortical dopaminergic neurons. Nigrostriatal dopaminergic neurons, comprised of the A_8 and A_9 cell groups, have cell bodies located in the pars compacta of the substantia nigra and project to the corpus striatum (Björklund and Lindvall, 1978). Mesolimbic and mesocortical dopaminergic neurons, comprised of the the A_{10} cell group, have cell bodies located in the ventral tegmental area and project to various limbic and cortical structures such as the nucleus accumbens, olfactory tubercle and prefrontal cortex (Björklund and Lindvall, 1978). Collectively, these dopaminergic neuronal systems modulate motor and behavioral output (Costall et al., 1977; Mogenson et al., 1980; Beringer, 1983), and dysfunctions in these systems have been implicated in the pathogenesis of disorders such as Parkinson's disease (Zigmond

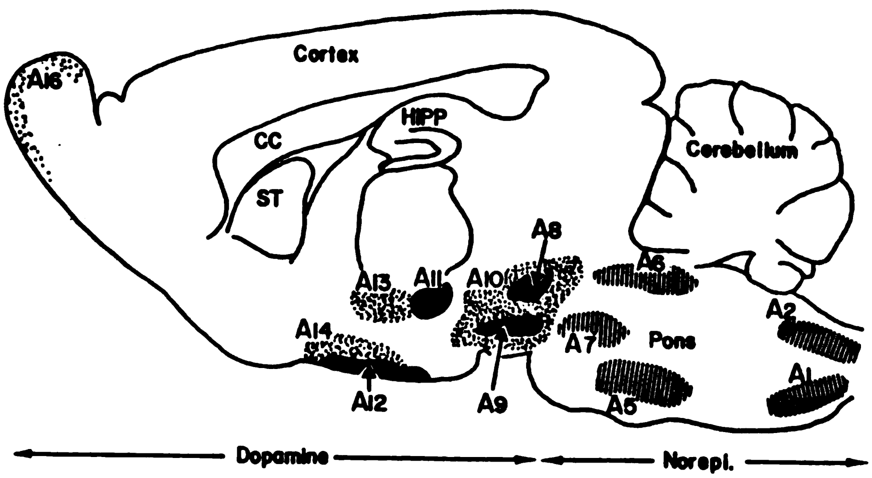

Figure 1.1 Sagittal view of the rat brain illustrating the distribution of catecholaminergic cell groups (Moore, 1987). CC = corpus callosum; HIPP = hippocampus; Norepi. = norepinephrine; ST = striatum

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et al., 1990) and schizophrenia (Snyder et al., 1974; Matthyssee, 1980).

Another group of dopaminergic neurons, known as the incertohypothalamic dopaminergic neurons, have cell bodies located within various parts of the diencephalon, including the medial zona incerta (the A_{13} cell group; Björklund and Nobin, 1973) and the rostral periventricular nucleus (the A_{14} cell group; Björklund and Nobin, 1973). Relatively little is known about the regulation, function or anatomical projections of this neuronal system. It has been purported, however, to play a stimulatory role in the release of luteinizing hormone releasing hormone from the median eminence (Wilkes et al., 1979), the secretion of luteinizing hormone (MacKenzie et al., 1984), and in ovulation (MacKenzie et al., 1984) and sexual behavior (Bitran et al., 1988). This is supported anatomically in a report demonstrating that incertohypothalamic dopaminergic neurons originating in the anterior periventricular nucleus make synaptic contact with perikarya of luteinizing hormone releasing hormone neurons in the medial preoptic area (Horvath et al., 1993).

Cell bodies of tuberohypophysial dopaminergic neurons projecting through the median eminence and infundibular stalk to the intermediate lobe of the posterior pituitary were proposed originally to reside in the arcuate nucleus of the mediobasal hypothalamus as part of the A_{12} cell group (Björklund et al., 1973). Subsequent anatomical studies have demonstrated that these neurons originate from the A_{14} cell gr

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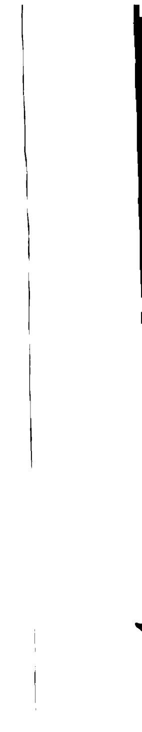
group in the periventricular nucleus rather than the A_{12} cell group in the arcuate nucleus (Luppi et al., 1986; Kawano and Daikoku, 1987). Recent evidence indicates that tuberohypophysial dopaminergic neurons originating in or passing through the periventricular nucleus and projecting to the intermediate lobe subserve the purported dopaminergic function (Goudreau et al., 1992), namely the dopamine receptor-mediated tonic inhibiton of the secretion of pro-opiomelanocortin-derived peptide hormones such as α-melanocyte-stimulating hormone (α-MSH) from melanotrophs (Millington and Chronwall, 1988). Accordingly, it has been suggested that these neurons currently be referred to as periventricular-hypophysial dopaminergic (PHDA) neurons (Goudreau et al., 1992).

Tuberoinfundibular dopaminergic (TIDA) neurons, comprised of the A_{12} cell group, have cell bodies located throughout the rostral-caudal extent of the arcuate nucleus and project to the external layer of the median eminence (Björklund and Nobin, 1973; Björklund et al., 1973). The processes of TIDA neurons, replete with varicosities (Ajika and Hökfelt, 1973; Loose et al., 1990), form a dense plexus in the external layer of the median eminence, and are found in close proximity to precapillary spaces of hypophysial portal vessels (Hökfelt, 1973; Ajika and Hökfelt, 1973). Dopamine released from these neurons is carried via the hypophysial portal vasculature to the anterior pituitary where it activates D2 dopamine receptors on lactotrophs to tonically inhibit the secretion of prolactin (Ben-Jonathan, 1985). A schematic representation of the anatomy and function of the two dopaminergic systems originating in the mediobasal hypothalamus, namely the PHDA and TIDA neuronal systems, is depicted in Figure 1.2.



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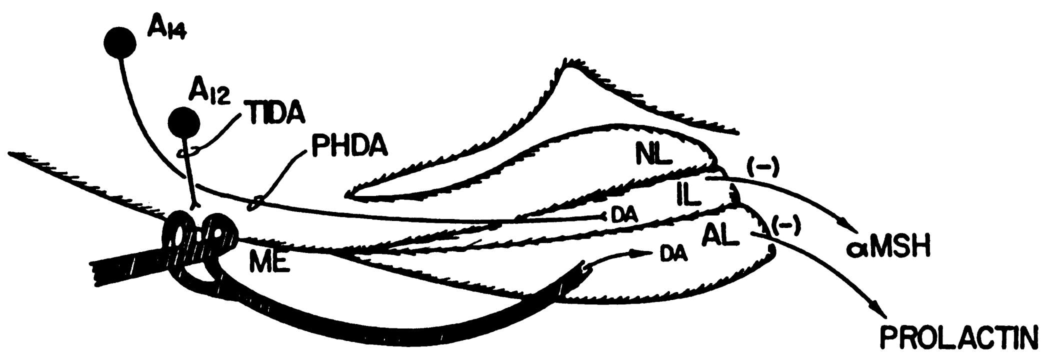

Figure 1.2 Mid-sagittal view of the rat mediobasal hypothalamus depicting the location of TIDA (A_{12}) and PHDA (A_{14}) neurons (Goudreau et al., 1992). AL = anterior lobe; DA = dopamine; IL = intermediate lobe; ME = median eminence; NL = neural lobe

B. Biochemistry of dopaminergic neurons

The first step in the biosynthesis of dopamine is the active uptake of the dietary amino acid precursor tyrosine (Guroff et al., 1961). Tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by the rate-limiting enzyme tyrosine hydroxylase. DOPA is rapidly decarboxylated by the enzyme aromatic-L-amino acid decarboxylase to form dopamine (Nagatsu, 1973), which can be either packaged into synaptic vesicles or oxidatively deaminated by mitochondrial monoamine oxidase to form 3,4-dihydroxyphenylacetic acid (DOPAC). Dopamine, presumably of cytosolic origin, also decreases the enzymatic activity of tyrosine hydroxylase by end-product inhibition.

Upon the arrival of an action potential at the nerve terminal dopamine is released, thereby relieving the inhibition of tyrosine hydroxylase by dopamine. Dopamine released from ascending and incertohypothalamic neurons can act at postsynaptic receptors and presynaptic autoreceptors in the forebrain, whereas that released from TIDA and PHDA neurons can act at posteffector receptors in the pituitary. Activation of presynaptic autoreceptors inhibits the synthesis and release of dopamine (Christiansen and Squires, 1974; Roth et al., 1975). Dopamine is removed from the synaptic cleft primarily by the presynaptic nerve terminal via a high affinity uptake system. Once inside the nerve terminal, dopamine either can feedback to inhibit tyrosine hydroxylase, be repackaged into synaptic vesicles or be acted on by mi

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mitochondrial monoamine oxidase to form DOPAC. In both hypothalamic and mesolimbic dopaminergic nerve terminals, DOPAC can exist as a free acid or as a conjugate (Nagatsu, 1973) and is rapidly cleared from the brain by a probenecid-sensitive acid transport mechanism (Karoum et al., 1977; Umezu and Moore, 1979). Within nigrostriatal dopaminergic nerve terminals, however, acid transport does not appear to be a prevalent mechanism for DOPAC disposition (Karoum et al., 1977; Umezu and Moore, 1979). Instead, DOPAC is converted to homovanillic acid by the extraneuronal enzyme catechol-O-methyltransferase following its diffusion out of the cell (Westerink, 1985).

The vast majority of biochemical events that occur within a dopaminergic nerve terminal are common to all dopaminergic systems. TIDA neurons, however, differ from other dopaminergic neurons (e.g., nigrostriatal dopaminergic neurons) in two important respects. Although released dopamine is capable of being taken up by TIDA nerve terminals (Demarest and Moore, 1979a; Annunziato et al., 1980; Anderson and Mitchell, 1985), its affinity for the uptake sites on these nerve terminals is considerably lower than for other dopaminergic neurons (Demarest and Moore, 1979a; Annunziato et al., 1980). In view of the fact that TIDA neurons terminate in the vicinity of perivascular spaces in the external layer of the median eminence (Ajika and Hökfelt, 1973), affording any released dopamine easy access to the hypophysial portal vasculature, the uptake sites on TIDA nerve terminals most l

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likely do not play a relevant role in the removal of dopamine from the median eminence. Indeed, basal concentrations of DOPAC in the median eminence result from the metabolism of newly synthesized rather than recaptured dopamine (Lookingland et al., 1987b). In addition, neither the synthesis of dopamine in, nor the release of dopamine from, TIDA nerve terminals is regulated by pre-effector autoreceptors (Fuxe et al., 1975; Gudelsky and Moore, 1976; Demarest and Moore, 1979b; Lookingland and Moore, 1984). The differences between biochemical events occurring in TIDA nerve terminals and those occurring within other dopaminergic nerve terminals are illustrated in Figure 1.3.

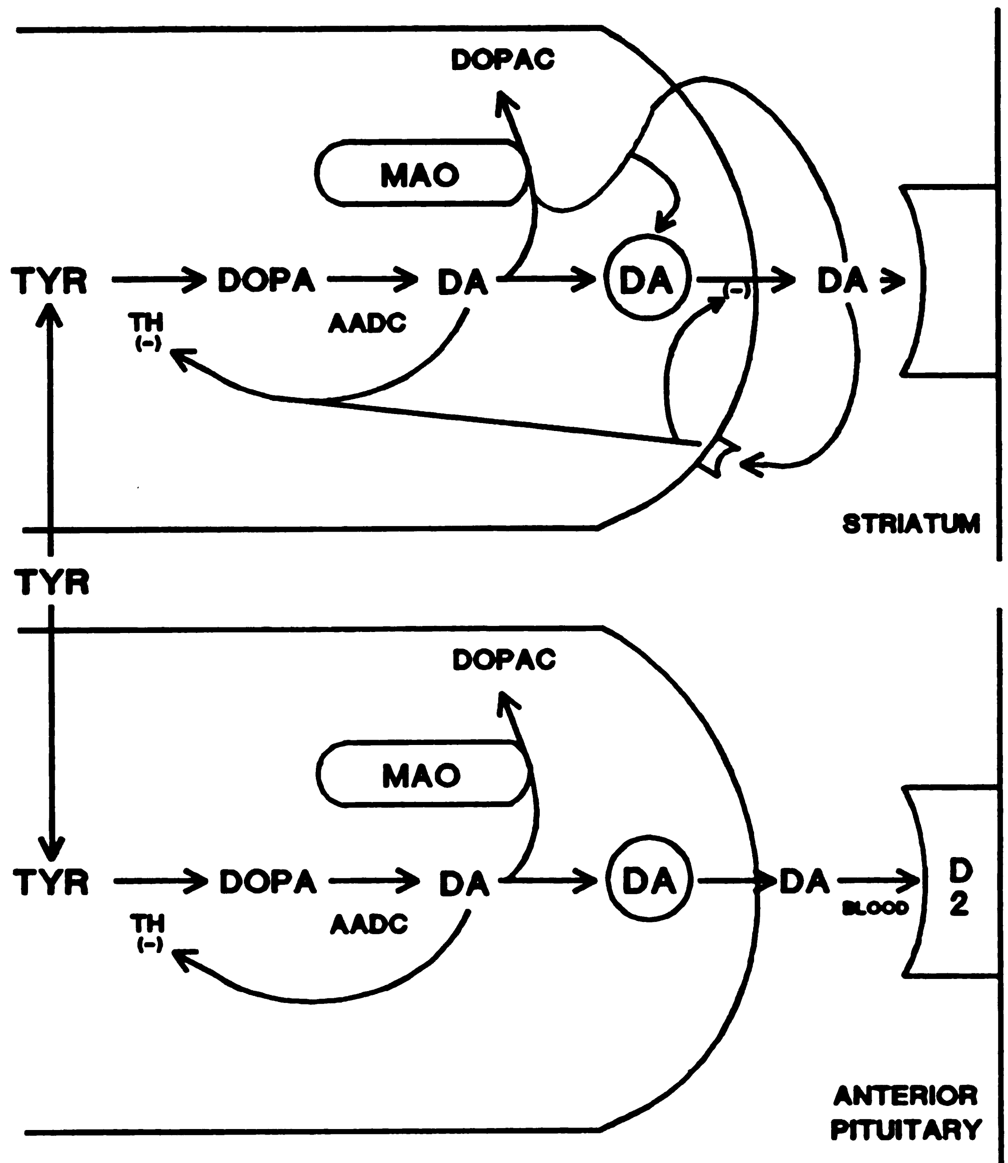

Figure 1.3 Schematic representation of differences in some of the biochemical events occurring in and around nigrostriatal dopaminergic (top) and TIDA (bottom) nerve terminals. AADC = aromatic L-amino acid decarboxylase; DA = dopamine; D2 = D2 dopamine receptor; MAO = monoamine oxidase; TH = tyrosine hydroxylase; TYR = tyrosine

C. Neurochemical indices of dopaminergic neuronal activity

End-product inhibition of tyrosine hydroxylase by cytosolic dopamine, and activation of presynaptic autoreceptors by released dopamine, serve to couple the synthesis and metabolism of the amine with its release. This enables the steady-state levels of dopamine to remain fairly constant despite alterations in neuronal activity (Moore, 1987).

Neurochemical estimates of neuronal activity have been made following manipulations of dopamine synthesis. α-Methyltyrosine, an inhibitor of tyrosine hydroxylase, causes an exponential decline in the concentration of dopamine in regions containing terminals of dopaminergic neurons at a rate proportional to the level of neuronal activity (Brodie et al., 1966; Fuxe et al., 1969; Löfström et al., 1976; Lookingland and Moore, 1984). 3-Hydroxybenzylhydrazine (NSD-1015), a decarboxylase inhibitor, causes an accumulation of DOPA (Carlsson et al. 1972) in regions containing terminals of dopaminergic neurons, at a linear rate that is proportional to the level of neuronal activity (Murrin and Roth, 1976; Demarest et al., 1979; Demarest and Moore, 1980).

These indices provide, in most instances, accurate and reliable estimates of neuronal activity. They require, however, a considerable amount of time (≥30 min) to observe either an appreciable fall in the concentration of dopamine following the administration of α-methyltyrosine, or an appreciable accumulation of DOPA following the administration

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of NSD-1015. This prohibits the use of these indices to measure transient changes in the activity of dopaminergic neurons. Due to the fact that newly synthesized dopamine is preferentially released (Gudelsky and Porter, 1979), disruption of dopamine synthesis in the terminals of TIDA neurons also increases basal levels of prolactin (Carr et al., 1975; Demarest et al., 1984). This precludes the concomitant measurement of TIDA neuronal activity and physiologically relevant prolactin secretion.

Regions containing incertohypothalamic dopaminergic neurons have a greater density of noradrenergic nerve terminals than of dopaminergic nerve terminals. Coupled with the fact that conversion of tyrosine to DOPA is common to the synthesis of dopamine and norepinephrine, it is difficult to determine if changes in DOPA accumulation in these regions are due to changes in the activity of incertohypothalamic dopaminergic neurons or to noradrenergic neurons. DOPA accumulation, therefore, is not a valid index of activity of incertohypothalamic dopaminergic neurons (Moore, 1987). In the median eminence and intermediate lobe, however, concentrations and turnover rates for dopamine exceed those determined for norepinephrine (Demarest et al., 1979; Lookingland and Moore, 1984). The use of DOPA accumulation in estimating the activity of TIDA and PHDA neurons, therefore, does not pose a problem.

Alternatively, the metabolism of dopamine can be used to estimate neuronal activity by measuring DOPAC concentrations in regions containing terminals of dopaminergic neurons (Roth et al., 1976; Lookingland et al., 1987b; Lindley et al., 1988; Tian et al., 1991). This does not require any pharmacological manipulation, and therefore does not alter basal levels of prolactin and α-MSH. Accordingly, this index enables the measurement of rapid, transient changes in the activity of dopaminergic neurons, as well as the concurrent measurement of activity of TIDA and PHDA neurons, and secretion of prolactin and α-MSH.

D. Regulation of TIDA and PHDA neurons

PHDA neurons respond acutely to the administration of "classical" D2 dopamine receptor ligands: agonists such as apomorphine decrease, and antagonists such as haloperidol increase, the activity of these neurons (Lookingland et al., 1985). This indicates that PHDA neurons are regulated by a dopamine receptor-mediated mechanism. While D2 dopamine receptors are thought to mediate the dopaminergic regulation of, and the tonic inhibition of α-MSH secretion by, PHDA neurons (Lookingland et al., 1985; Millington and Chronwall, 1988), recent evidence using atypical neuroleptics suggests that this occurs via a subtype of D2 dopamine receptor, or a member of the D2 dopamine receptor family, which differs from that mediating either the autoreceptor-mediated regulation of other dopaminergic neurons (e.g., nigrostriatal dopaminergic neurons) or the tonic inhibition of prolactin by TIDA neurons (Eaton et al., 1992; personal communication). On the other hand, TIDA neurons do not respond acutely to "classical" D2 dopamine receptor agonists and antagonists (Gudelsky and Moore, 1976; Demarest and Moore, 1979), indicating a lack of pre-effector D2 dopamine autoreceptor-mediated regulation of these neurons. Interestingly, the administration of D2/D3 dopamine receptor agonists such as quinelorane increases the activity of TIDA but not PHDA neurons, an effect which is blocked by D2 dopamine receptor antagonists (Eaton et al., 1993). It is evident that D2 dopamine receptor-mediated regulation of PHDA neurons differs markedly from that of TIDA neurons.

TIDA neurons are subject to stimulatory feedback regulation by the hormone whose release they tonically inhibit, namely prolactin (Annunziato and Moore, 1979; Demarest et al., 1984). In female rats prolactin feeds back to tonically stimulate these neurons (Demarest et al., 1984; Toney et al., 1992b). The mechanism underlying stimulatory prolactin feedback regulation of TIDA neurons currently is unknown. There is an appreciable density of prolactin receptors in the hypothalamus of rats (Muccioli et al., 1991; Chiu et al., 1992) and humans (DiCarlo et al., 1992). In addition, prolactin binding sites have been localized specifically in the rat median eminence (Barton et al., 1989b). It is possible that the stimulatory feedback effects of prolactin could be due to a direct action on TIDA neurons. This contention is supported by the observation that complete and retrochiasmatic deafferentation of the mediobasal hypothalamus in female rats does not alter the responsiveness

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of TIDA neurons to the stimulatory effects of prolactin (Barton et al., 1988; Barton et al., 1989a). In contrast, there is no feedback regulation of PHDA neurons by α-MSH (Lindley et al., 1990a).

The basal activity of TIDA and PHDA neurons is reported to be higher in female than in male rats (Gudelsky and Porter, 1981; Demarest et al., 1985b; Gunnett et al., 1986; Manzanares et al., 1992a). The sexual difference in the basal activity of TIDA neurons can be attributed, in large part, to the influence of gonadal steroid hormones since castration reverses the sexual difference in the basal activity of TIDA neurons (Gunnett et al., 1986). Although reported to have an inhibitory effect on the transcription (Blum et al., 1987) and activity (Pasqualini et al., 1993) of tyrosine hydroxylase in the periarcuate region of female rats, estrogen, due in part to its ability to directly stimulate the synthesis and secretion of prolactin from the anterior pituitary (Shull and Gorski, 1984; Shull and Gorski, 1989), exerts a net stimulatory effect on TIDA neurons. Estrogen also negatively modulates κ-opioid receptor-mediated inhibition of TIDA neurons, which is independent of its effects on prolactin secretion (Wagner et al., 1994). In male rats androgens have an inhibitory effect on TIDA neurons (Demarest et al., 1981; Aguila-Mansilla et al., 1991; Toney et al., 1991). On the other hand, the reported sexual difference in the basal activity of PHDA neurons is not dependent on the gonadal steroid milieu (Manzanares et al., 1992a).

It has been shown previously that κ-opioid agonists injected systemically or directly into the mediobasal hypothalamus increase the secretion of both prolactin and α-MSH (Kapoor and Willoughby, 1990; Manzanares et al., 1990; Manzanares et al., 1993). Nerve terminals containing the endogenous κ-opioid ligand dynorphin also have been reported to make synaptic contact with both TIDA and PHDA neuronal perikarya (Fitzsimmons et al., 1992). Blockade of κ-opioid receptors or immunoneutralization of dynorphin activates both TIDA and PHDA neurons (Manzanares et al., 1991; Manzanares et al., 1992b; Manzanares et al., 1992c), indicating that these neurons are subject to a κ-opioid receptor-mediated tonic inhibition. The regulation of TIDA neurons by endogenous κ-opioids is sexually differentiated. That is, endogenous κ-opioids tonically inhibit TIDA neurons in male but not female rats (Manzanares et al., 1992b), and estrogen suppresses κ-opioid receptor-mediated tonic inhibition of these neurons in female rats (Wagner et al., 1994). Testosterone has been shown to increase the release of dynorphin *in vitro* from hypothalamic slices (Almeida et al., 1987), suggesting that gonadal steroids modulate κ-opioid receptor-mediated inhibition of TIDA neurons by altering the release of this endogenous κ-opioid peptide.

Both TIDA and PHDA neurons are regulated by afferent mechanisms which are activated under certain physiological conditions. With regard to TIDA neurons, these physiological conditions are inherent almost exclusively to the female rat.

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For example, TIDA neurons are inhibited on the afternoon of proestrus during the preovulatory surge of prolactin (Ahren et al., 1971). In addition, TIDA neurons are inhibited, and prolactin secretion is stimulated, by suckling in the lactating rat (Demarest et al., 1983). It also has been reported that these neurons are inhibited by stress in both female and orchidectomized male rats (Demarest et al., 1985b; Toney et al., 1991). PHDA neurons also are responsive to the inhibitory effects of stress (Lookingland and Moore, 1988; Lookingland et al., 1991). There is evidence to suggest that 5-hydroxytryptamine is an important mediator of the inhibitory effects of stress on TIDA and PHDA neurons (Demarest et al., 1985a; Goudreau et al., 1993a).

E. Amino acid neurotransmitter regulation of dopaminergic neurons

Excitatory amino acids (EAAs) are considered as the primary mediators of excitatory synaptic transmission in the mammalian central nervous system (Watkins and Evans, 1981; Cotman et al., 1987). Although L-glutamate is thought to be the principal EAA neurotransmitter, L-aspartate and potent sulfur-containing amino acids (e.g., D-homocysteine sulfinic acid) mimic many of the actions of L-glutamate and also are considered as neurotransmitter candidates (Curtis and Watkins, 1963; Mewett et al., 1983; Collingridge and Lester, 1989). EAA neurotransmitters interact with several subtypes of receptor, namely N-methyl-D-aspartate (NMDA) receptors, non-NMDA receptors which include both α-amino-3-hydroxy-5-methyl-

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4-isoxazole proprionic acid (AMPA) and kainate receptors, and metabotropic receptors. NMDA and non-NMDA receptors are ionotropic ligand-gated cation channels, whose activation depolarizes receptor-bearing excitable cells by allowing the movement of sodium, potassium and calcium across the cell membrane (Collingridge and Lester, 1989). The metabotropic receptor is coupled to a guanine nucleotide-binding protein, and the activation of this receptor is thought to elicit phosphoinositide hydrolysis (Monaghan et al., 1989). The pharmacology and function of the metabotropic receptor is not particularly well understood.

The amino acid neurotransmitter γ-aminobutryic acid (GABA) is considered the principal inhibitory neurotransmitter in the mammalian central nervous system (Haefely, 1990; Costa, 1991; Olsen, 1991). GABA interacts with two subtypes of receptors, namely $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1987). The $GABA_A$ receptor is a ligand-gated chloride channel whose activation hyperpolarizes neurons and glia by allowing an influx of the chloride anion (Haefely and Polc, 1986; Barnard et al., 1987). The $GABA_B$ receptor is coupled to a guanine nucleotide-binding protein, and activation of these receptors can either hyperpolarize cells by increasing outward potassium conductance at postsynaptic sites or decrease neurotransmitter release by decreasing inward calcium conductance at presynaptic sites (Bowery, 1989; Wojcik and Holopainen, 1992).

Studies aimed at investigating amino acid neurotransmitter regulation of dopaminergic neuronal activity have focused almost exclusively on nigrostriatal and mesolimbic dopaminergic neurons. L-Glutamate and non-NMDA receptor agonists have been shown to directly increase the firing rate of, and the release of dopamine from, nigrostriatal and mesolimbic dopaminergic neurons (Cheramy et al., 1986; Leviel et al., 1990; Carrozza et al., 1991; Suaud-Chagny et al., 1992). While several investigators have reported similar findings with NMDA receptor activation following local agonist application to either the cell body or terminal regions of nigrostriatal and mesolimbic dopaminergic neurons (Carrozza et al., 1992; Overton and Clark, 1992; Suaud-Chagny et al., 1992; Westerink et al., 1992), there is evidence for an indirect NMDA receptor-mediated tonic inhibition of these neurons by endogenous EAA neurotransmitters (Cheramy et al., 1986; French and Ceci, 1990; Leviel et al., 1990; Moghaddam and Gruen, 1991).

The NMDA receptor-mediated tonic inhibition of nigrostriatal and mesolimbic dopaminergic neurons by endogenous EAA neurotransmitters appears to arise from a stimulation of GABAergic interneurons via corticofugal pathways which, in turn, tonically inhibit these neurons through an action at $GABA_A$ receptors (Cheramy et al., 1986; Cotman et al., 1987; Leviel et al., 1990; Gruen et al., 1992). Indeed, NMDA receptor antagonists decrease the release of [^{3}H]GABA from striatal neurons *in vitro* (Jouanen et al., 1991). Finally, $GABA_B$ receptor activation hyperpolarizes nigrostriatal dopaminergic neurons (Dray and Straughn, 1976), thereby inhibiting impulse flow through these neurons, and decreases the release of dopamine from the striatum (Bowery et al., 1980). This leads to a marked accumulation of dopamine, its precursors and metabolites in the terminals of nigrostriatal dopaminergic neurons (Roth et al., 1976; Demarest and Moore, 1979b), which has been attributed to decreased occupation of presynaptic autoreceptors by dopamine, and a resultant decrease in the inhibitory control of the synthesis of dopamine provided by these receptors (Christiansen and Squires, 1974; Roth et al., 1975; Demarest and Moore, 1979b). The cessation of impulse flow through nigrostriatal dopaminergic neurons following $GABA_B$ receptor activation, therefore, uncouples the synthesis and metabolism of dopamine with its release.

F. Amino acid regulation of pituitary hormone secretion

There is evidence that EAAs play a prevalent role in the regulation of the hypothalamic-pituitary axis (van den Pol et al., 1990; Brann and Mahesh, 1992). For example, EAAs increase the secretion of luteinizing hormone from the anterior pituitary (Bourguignon et al., 1989; Pohl et al., 1989; Donoso et al., 1990; Abbud and Smith, 1991), and do so by stimulating the release of luteinizing hormone-releasing hormone from the mediobasal hypothalamus (Bourguignon et al., 1989; Donoso et al., 1990). While both NMDA and non-NMDA receptors are involved in the stimulatory effects of EAAs on luteinizing hormone-releasing hormone, the potency of non-NMDA receptor agonists ranks higher than that for NMDA receptor agonists (Donoso et al., 1990; López et al., 1992). The stimulatory effect of NMDA receptor activation on the expression of luteinizing hormone-releasing hormone, and on the secretion of luteinizing hormone is positively modulated by estrogen (Estienne et al., 1990; Reyes et al., 1990; Liaw and Barraclough, 1993).

Evidence also exists for a role of GABA in regulating the secretion of luteinizing hormone. In female rats endogenous GABA mediates the negative feedback effects of estrogen on luteinizing hormone secretion (Akema and Kimura, 1991). In addition, both activation of GABA receptors and inhibition of GABA metabolism abolish the stimulation of luteinizing hormone secretion observed either following blockade of opioid receptors (Masotto and Negro-Vilar, 1987; Brann et al., 1992), or during the preovulatory luteinizing hormone surge (Adler and Crowley, 1986). GABAergic nerve terminals form synapses with luteinizing hormone-releasing hormone neurons in the medial preoptic area (Leranth et al., 1985), and it would appear that these effects are due to an inhibition of luteinizing hormone-releasing hormone rather than a direct action at the level of the pituitary. In contrast, other reports demonstrate that activation of $GABA_A$ receptors increases the basal release of both luteinizing hormone-releasing hormone (Donoso et al., 1992) and luteinizing hormone (Brann et al., 1992).

NMDA receptor activation with L-glutamate and its analogue N-methyl-D,L-aspartate increases prolactin secretion, both *in vivo* (Pohl et al., 1989; Abbud and Smith, 1991; Arslan et al., 1991a; Arslan et al., 1991b) and *in vitro* (Login, 1990). This latter finding is suggestive of a direct effect at the level of the anterior pituitary. NMDA receptor activation by endogenous EAA neurotransmitters also has been implicated in the preovulatory surge of prolactin (Brann and Mahesh, 1991), and may be involved in the suckling-induced increase in prolactin secretion during lactation (Pohl et al., 1989). Although a stimulatory effect of EAAs on the secretion of α-MSH from melanotrophs in the posterior pituitary has yet to be demonstrated, NMDA receptor activation increases the release of α-MSH derived from pro-opiomelanocortin-synthesizing neurons in the hypothalamus (Wayman and Wilson, 1991).

Like dopamine, GABA is considered a prolactin inhibiting factor (Schally et al., 1977; Locatelli et al., 1978; Casanueva et al., 1981). This is evident by the observations that systemic administration of $GABA_A$ receptor agonists and inhibition of GABA metabolism decrease the secretion of prolactin (Locatelli et al., 1978; Racagni et al., 1979; Casanueva et al., 1981). $GABA_A$ receptors have been identified on lactotrophs (Grandison and Guidotti, 1979), which indicates that these effects are due to a direct effect at the level of the anterior pituitary. This is substantiated by reports demonstrating that GABA and $GABA_A$ receptor agonists exert an inhibitory effect on prolactin release *in vitro* (Grandison and

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Guidotti, 1979; Locatelli et al., 1979; Grossmann et al., 1981; Anderson and Mitchell, 1986). On the other hand, central administration of GABA and GABA agonists, as well as systemic administration of $GABA_B$ receptor agonists, have been shown to increase the secretion of prolactin (Mioduszewski et al., 1975; Ravitz and Moore, 1977; Vijayan and McCann, 1978; Casanueva et al., 1981), suggesting a central role of GABA in the regulation of prolactin secretion.

G. Thesis objective

Excitatory and inhibitory amino acids play a relevant regulatory role in the secretion of prolactin. While some of the effects on prolactin secretion can be attributed to a direct action at the level of the anterior pituitary, it is unknown if these are accompanied by alterations in the basal activity of TIDA neurons, and thus the degree of tonic inhibition of prolactin secretion. In view of the fact that amino acid neurotransmitters are involved in the regulation of nigrostriatal and mesolimbic dopaminergic neurons, this possibility is not easily dismissed. Much of the information concerning the effects of excitatory and inhibitory amino acids on prolactin secretion was obtained through the use of exogenously administered agonists. It is of primary interest, therefore, to determine the role of endogenous amino acid neurotransmitters in regulating the basal activity of TIDA neurons and the secretion of prolactin.

The purpose of the studies comprising this dissertation was to examine amino acid neurotransmitter regulation of TIDA and,

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and, for comparison, PHDA neuronal activity. To this end, the effects of acute NMDA, non-NMDA, $GABA_A$ and $GABA_B$ receptor blockade and activation were evaluated in intact and gonadectomized male and female rats. The activity of TIDA and PHDA neurons was estimated by measuring the accumulation of DOPA 30 min after the administration of the decarboxylase inhibitor NSD-1015 or the concentration of DOPAC in the median eminence and intermediate lobe, respectively.

2. MATERIALS AND METHODS

A. Animals

Male and female Long-Evans rats (200-225g) were purchased from Harlan Breeding Laboratories (Indianapolis, IN), and kept under conditions of constant temperature (21±1°C) and light (lights on between 05:00-19:00h). Rats of the same gender were housed 4 per cage, and provided food and water *ad libitum*. In experiments involving intact female rats, estrous cycles were monitored by taking daily vaginal lavages, and only those regularly cycling animals displaying an epithelial morphology consistent with the first day of diestrus were used.

B. Drugs

NSD-1015 dihydrochloride (Sigma Chemical Company, St. Louis, MO), D,L-(tetrazol-5-yl) glycine hydrate (kindly provided by Dr. William H.W. Lunn of Lilly Research Laboratories, Indianapolis, IN), dizocilpine maleate ((+)-MK-801), 2-carboxy-4-(1-methylethenyl)-3-pyrrolidinacetic acid (kainic acid), 1,2,5,6-tetrahydroisonicotinic acid hydrochloride (isoguvacine) and 2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)-pyridazinium bromide (SR 95531) were dissolved in distilled water. AMPA hydrobromide was dissolved in 0.9% saline. *cis*-4-Phosphonomethyl-2-piperidine-carboxylic acid (CGS-19755; kindly provided by Dr. Richard A. Lovell of The CIBA GEIGY Corporation, Summit, NJ) was placed in distilled water and brought into solution with 3 drops of 5N

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NaOH. 6-Nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3(1H,4H)-dione (NBQX; kindly provided by Dr. Lars Nordholm of Novo Nordisk A/S, Målφv, Denmark and M. Duff Davis of The Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI) and 3-amino-2-(4-chlorophenyl)-2-hydroxypropane sulfonic acid (2-hydroxysaclofen) were dissolved in 0.1N NaOH and diluted to the appropriate volume with 0.2N HCl. (±)-ß-(Aminomethyl)-4-chlorobenzenepropanoic acid ((±)-baclofen) was dissolved in 1.0N NaOH and diluted to the appropriate volume with 0.2N HCl. 17ß-Estradiol benzoate (Sigma) was dissolved in corn oil. 17ß-Hydroxy-3-oxo-4-androstene (testosterone; Sigma) was used in its crystalline form. Unless otherwise indicated, all drugs were purchased from Research Biochemicals Inc. (Natick, MA). Doses of drugs refer to either the salt (NSD-1015, D,L-(tetrazol-5-yl) glycine, MK-801, isoguvacine, SR 95531, AMPA and 17ß-estradiol benzoate), the acid (kainic acid, CGS-19755, NBQX, 2-hydroxysaclofen and baclofen) or the neutral compound (testosterone).

Serum containing antibodies to rat prolactin was generated in rabbits as described previously (Toney et al., 1992b). These prolactin antibodies do not detect up to 100 ng/tube of rat growth hormone or 1 μg/tube of either rat luteinizing hormone or rat thyroid-stimulating hormone. Drugs and prolactin antibody were administered as indicated in the legends to the appropriate figures.

C. Surgical procedures

Gonadectomies were performed under diethylether anes

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anesthesia 7 days prior to the experiment. In female rats, ovaries were removed by making bilateral incisions through the skin and muscle layers. The ovaries were externalized, ligated with suture thread and severed. Wounds were closed using suture thread and wound clips. In male rats, testicles were removed by making a single incision along the midline of the lower abdomen through the skin and muscle layers. The testicles were externalized, ligated with suture thread and severed. Wounds were closed with suture thread and wound clips. Silastic capsules (30 mm in length; I.D. 1.57 mm; O.D. 3.8 mm) were constructed as previously described (Wise et al., 1981; Roselli et al., 1987; Clark et al., 1988), and contained either 17ß-estradiol benzoate (37.5 μg/ml), its corn oil vehicle, or crystalline testosterone. Silastic capsules were implanted subcutaneously (s.c.) in gonadectomized rats under diethylether anesthesia 3 days prior to the experiment. In ovariectomized female rats estradiol-containing capsules have been shown to produce circulating concentrations of estrogen similar to those observed in gonadally-intact diestrous females (Wise et al., 1981). In orchidectomized male rats testosterone-containing capsules produce serum testosterone concentrations approximating those observed in gonadally intact males (Kalra and Kalra, 1982).

Cannulations of the right lateral ventricle were performed in male rats anesthetized by intraperitoneal (i.p.) injection of Equithesin (3 ml/kg; 3.24 mg/kg pentobarbital & 14.17 mg/kg chloral hydrate), and in female rats anesthetized by i.

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by i.p. injection of a ketamine (44 mg/kg)/xylazine (10 mg/kg) mixture. In gonadally-intact female rats the barbiturate component of the Equithesin mixture disrupts the estrus cycle (Kaneko, 1980), which prohibits the use of this anesthetic. Rats were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set 2.4 mm below the horizontal plane (König and Klippel, 1963). A 23-gauge stainless steel cannula, 10 mm in length, was implanted 1.4 mm lateral to bregma and 3.2 mm below the dura (König and Klippel, 1963), and secured to the skull with stainless steel anchoring screws and dental cement (Repair Material, Dentsply, York, PA). Following surgery, animals received a 0.2-ml intramuscular bolus of Combiotic (200,00 U procaine penicillin & dihydrostreptomycin per ml; Pfizer Inc., New York, NY). The animals were allowed to recover 3-5 days prior to the experiment. Drugs and either vehicle or 0.9% saline were injected in a volume of 5 µl with a 10 µl Hamilton syringe connected to a 30-gauge needle which protruded 1 mm beyond the tip of the guide cannula and into the right lateral ventricle. Cannula placement was verified post-mortem by examining the position of the cannula tract viewed from the appropriate brain section under a dissecting microscope. Only those animals with the tip of the cannula tract in the right lateral ventricle were included in the studies.

D. Tissue and blood processing

Following appropriate treatments, animals were decapitated, and their trunk blood collected in heparinized, glass test tubes containing 150 μg bacitracin and kept cold on ice. Brains and pituitaries were rapidly removed from the skull and immediately frozen over dry ice. Trunk blood was centrifuged (2000 rpm) for 20 min at 4°C. The plasma supernatant was removed and stored at -20°C until assayed. Frontal brain sections (600 μm) were prepared with the aid of a cryostat (-9°C), and the median eminence was dissected according to a modification (Lookingland and Moore, 1984) of the method of Palkovits (1973). The intermediate lobe of the pituitary was separated from the neural and anterior lobes as described previously (Lookingland et al., 1985). Tissue samples were placed in 60μl of 0.1 M phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at -20°C until assayed.

E. Assays

On the day of the assay tissue samples were thawed, sonicated for 3 s (Sonicator Cell Disruptor, Heat Systems-Ultrasonic, Plainview, NY) and centrifuged for 30 s in a Beckman Microfuge B. DOPA, DOPAC and dopamine concentrations in the supernatants of the tissue extracts were measured by high performance liquid chromatography (HPLC) coupled to electrochemical detection as previously described (Chapin et al., 1986). Briefly, 50 μl of sample 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) preceded by a protective precolumn cartridge filter (5 μm spheres; 30 X 4.6 mm). Following separation, DOPA, DOPAC and dopa

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dopamine signals in median eminence samples were determined using a electrochemical detector (LC4A; Bioanalytical Systems) equipped with a TL-5 glassy carbon electrode set at +0.75 V relative to a Ag/AgCl reference electrode. DOPA, DOPAC and dopamine signals in intermediate lobe samples were determined with 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, and the analytical electrodes were set at +0.12 V and -0.40 V, respectively, relative to the Ag/AgCl reference electrode. The HPLC mobile phase consisted of a 0.1 M phosphate/citrate buffer (pH 2.65) containing 0.1 mM ethylenediaminetetraacetic acid, 0.03% sodium octylsulfate and 20-25% methanol. The content of DOPA, DOPAC and dopamine in the samples was quantified by comparing peak heights (recorded using a Hewlett Packard Integrator, Model 3393A, Avondale, PA) 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).

Prolactin was measured in plasma by a double-antibody radioimmunoassay utilizing the reagents and procedures of the NIDDK assay kit (kindly supplied by Drs. A.F. Parlow and S. Raiti, NIDDK National Hormone and Pituitary Program). NIDDK rat prolactin (RP-3) was used as the standard. Using a 100 μl aliqout of plasma, the lower limit of sensitivity for prolactin was 1 ng/ml.

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α-MSH was measured in plasma by a double-antibody radioimmunoassay modified (Lindley et al., 1990b) from a procedure originally described by Penny and Thody (1978). Antisera to α-MSH were kindly supplied by Dr. G. Mueller (Uniformed Services University for the Health Sciences, Bethesda, MD). These antisera cross-react on a equimolar basis with des-acetyl-α-MSH and diacetyl-α-MSH, but do not detect up to 30 ng/tube of any of the following: deaminated α-MSH, ß-MSH, ACTH, ACTH 1-10, ACTH 1-13 or ACTH 1-24, ß-endorphin peptides or ß-lipotropin. Using an aliquot of 250 μl, the lower limit of sensitivity for α-MSH was approximately 35 pg/ml.

F. Statistical analyses

The data were analyzed using either a one- or two-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test (Steel and Torrie, 1979). Differences were considered statistically significant if the probability of error was less than 5%.

3. NMDA RECEPTOR-MEDIATED REGULATION OF TIDA AND PHDA NEURONS

Introduction

Compelling evidence exists for a stimulatory role of EAAs in the secretion of prolactin. The putative EAA neurotransmitter glutamate and its analogue N-methyl-D,L-aspartate stimulate prolactin release both *in vitro* (Login, 1990) and *in vivo* (Arslan et al., 1991a; Arslan et al., 1991b; Pohl et al., 1989). EAAs have been implicated in the preovulatory surge of prolactin in the female rat (Brann and Mahesh, 1991), as well as in the suckling-induced surge of prolactin in the lactating female rat (Pohl et al., 1989). Although *in vitro* data (Login, 1990) demonstrate a direct effect of EAAs at the level of the lactotroph, it is not known whether these changes in prolactin secretion are accompanied by alterations in the activity of TIDA neurons.

The purpose of the experiments presented in this chapter was to examine the role of NMDA receptors in regulating TIDA and, for comparison, PHDA neuronal activity. To this end, the effect of the NMDA receptor antagonists MK-801 (Wong et al., 1986) and CGS-19755 (Lehmann et al., 1988; Bennett et al., 1989), and agonist D,L-(tetrazol-5-yl) glycine (Schoepp et al., 1991) were evaluated on TIDA and PHDA neuronal activity in intact and gonadectomized male and female rats.

Results

As shown in Figure 3.1, administration of the non-

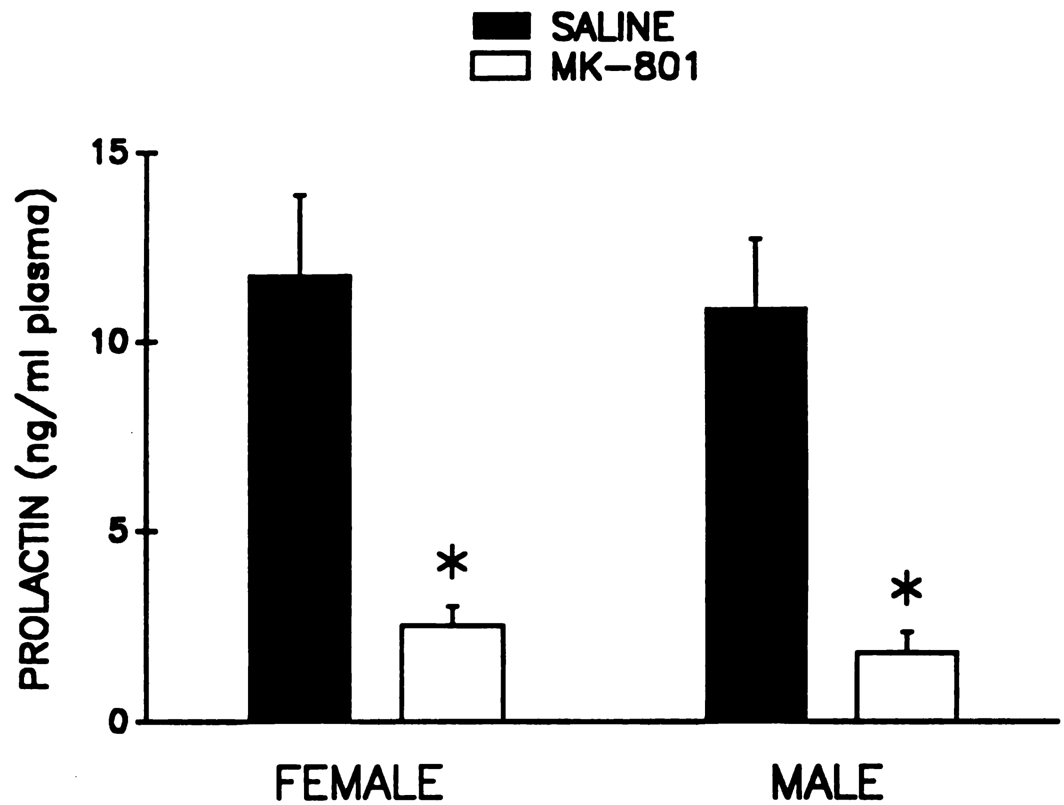

Figure 3.1 Comparison of the effects of MK-801 on plasma prolactin concentrations in female and male rats. Rats were injected with either MK-801 (1 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) and killed by decapitation 60 min later. Columns represent the means and vertical lines 1 S.E.M. of plasma prolactin concentrations from 6-9 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (solid columns).

competitive NMDA receptor antagonist MK-801 markedly decreased plasma prolactin concentrations in both male and female rats. To determine if this decrease in prolactin secretion was mediated through an increase in the activity of TIDA neurons, the effects of MK-801 on DOPA accumulation in the median eminence were examined. As shown in Figure 3.2, MK-801 produced a dose-dependent decrease in DOPA accumulation in the median eminence of female, but not male rats. This inhibitory effect of MK-801 on TIDA neurons in female rats was long-lasting, producing decreases in DOPAC concentrations in the median eminence after a latent period of 1 hr which were sustained at least 24 hr after administration (Figure 3.3). These results indicate that MK-801-induced decrease in prolactin secretion is not due to an activation of TIDA neurons.

That MK-801 was able to decrease both plasma prolactin concentrations and TIDA neuronal activity raised the possibility that the inhibitory effect of MK-801 on TIDA neurons in female rats was due to removal of the tonic stimulatory effects of prolactin on these neurons. If this were true then immunoneutralization of endogenous prolactin, a procedure known to effectively remove the tonic stimulatory effects of prolactin on TIDA neurons (Toney et al., 1992b), should abolish the inhibitory effects of MK-801

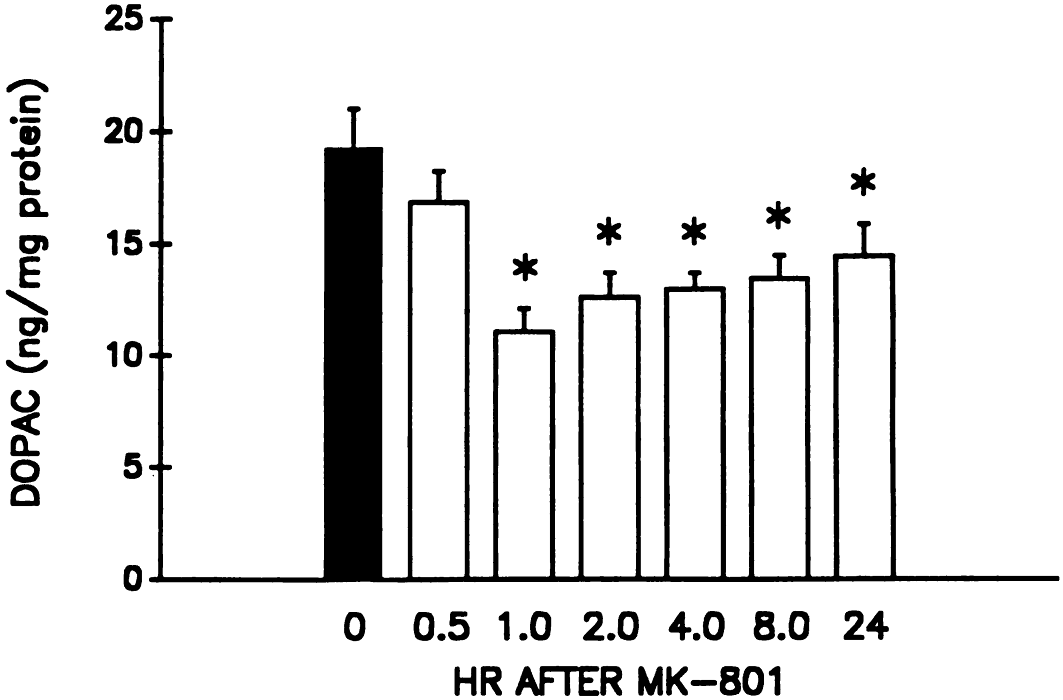

Figure 3.3 Time course of the effects of MK-801 on DOPAC concentrations in the median eminence of female rats. Rats were injected with MK-801 (3 mg/kg; s.c.) and killed by decapitation either 0.5, 1, 2, 4, 8 or 24 hr later. Zero time control rats were injected with 0.9% saline (1 ml/kg; s.c.) 1 hr prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence of 7-8 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (solid columns).

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on these neurons. As depicted in Figure 3.4, intravenous (i.v.) prolactin antibody pre-treatment decreased DOPA accumulation in the median eminence. MK-801 decreased DOPA accumulation in the median eminence of both normal rabbit serum and prolactin antibody pre-treated animals. This indicates that the inhibitory effect of MK-801 on TIDA neurons in female rats is independent of its ability to decrease prolactin secretion.

To determine whether the gender-related differential responsiveness of TIDA neurons to MK-801 was due to the presence of circulating gonadal steroids, the effect of MK-801 was examined in gonadectomized female and male rats. Figure 3.5 shows the effects of MK-801 on TIDA neurons in intact and ovariectomized female rats. MK-801 decreased DOPA accumulation in the median eminence of intact female rats. Likewise, ovariectomy also decreased DOPA accumulation in this region. In ovariectomized female rats, however, MK-801 had no effect on DOPA accumulation in the median eminence. Estrogen replacement, which increases DOPA accumulation in the median eminence of ovariectomized female rats (Figure 3.6), restored the responsiveness of TIDA neurons to the inhibitory effects of MK-801 even in ovariectomized female rats treated with prolactin antiserum. These results indicate that estrogen alters the responsiveness of TIDA neurons to MK-801 in female rats by a prolactin-independent mechanism. MK-801 had no effect on DOPAC concentrations in the median eminence of

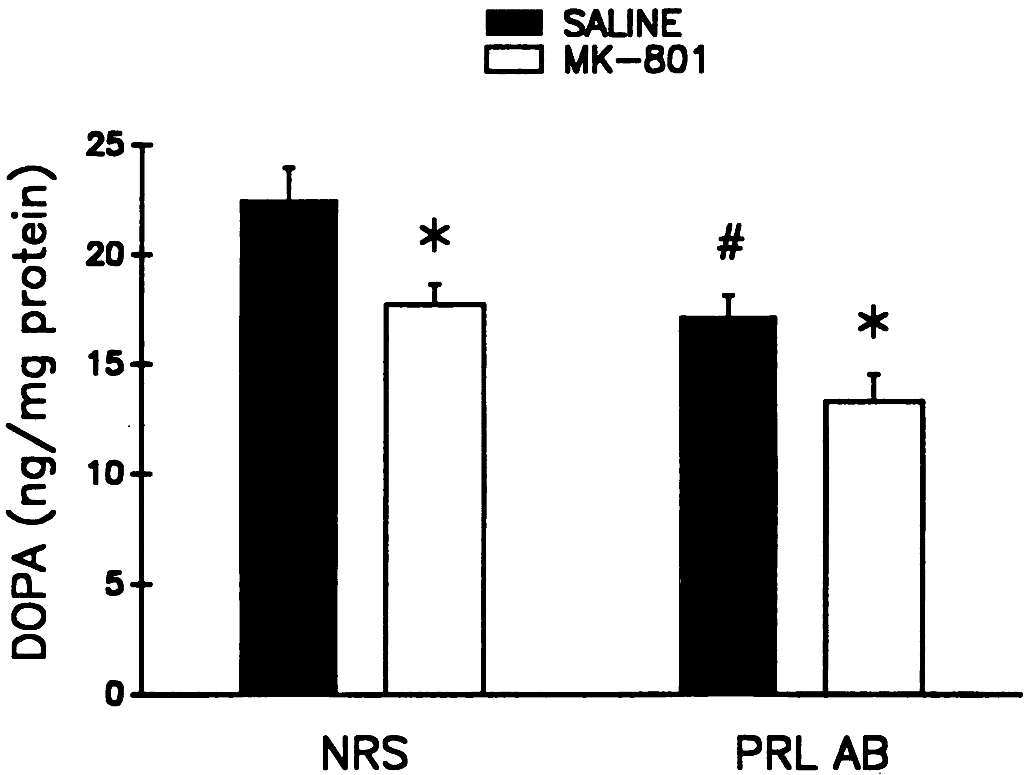

Figure 3.4 Effects of MK-801 on DOPA accumulation in the median eminence of female rats pre-treated with prolactin antibody. Rats were injected with either prolactin antibody (PRL AB; 200 μl/rat; i.v.) or its normal rabbit serum vehicle (NRS; 200 μl/rat; i.v.) 120 min prior to decapitation, and with either MK-801 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. All 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 DOPA concentrations in the median eminence of 6-8 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (solid columns). #, Values from saline-treated PRL AB rats which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated NRS controls.

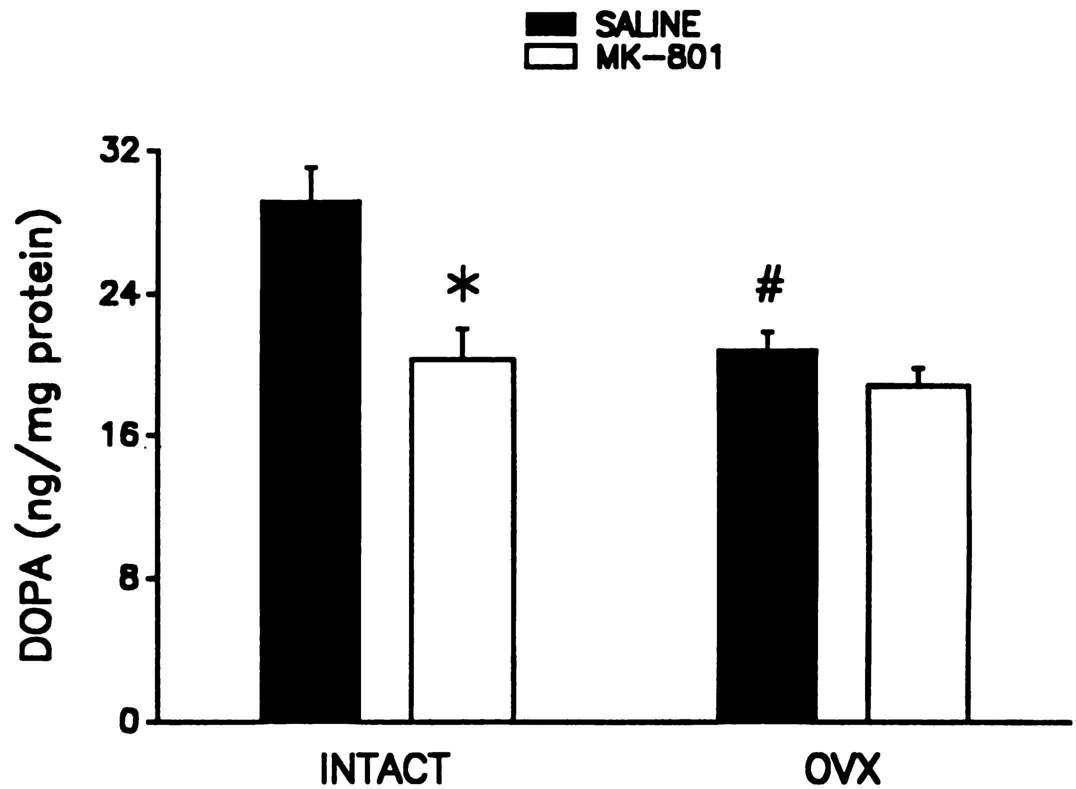

Figure 3.5 Effects of MK-801 on DOPA accumulation in the median eminence of ovariectomized female rats. Intact (INTACT) and ovariectomized (OVX) rats were injected with either MK-801 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. All rats were injected with NSD-1015 (100 mg/kg; i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPA concentrations in the median eminence of 8-9 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (solid columns). #, Values from saline-treated OVX rats which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated INTACT controls.

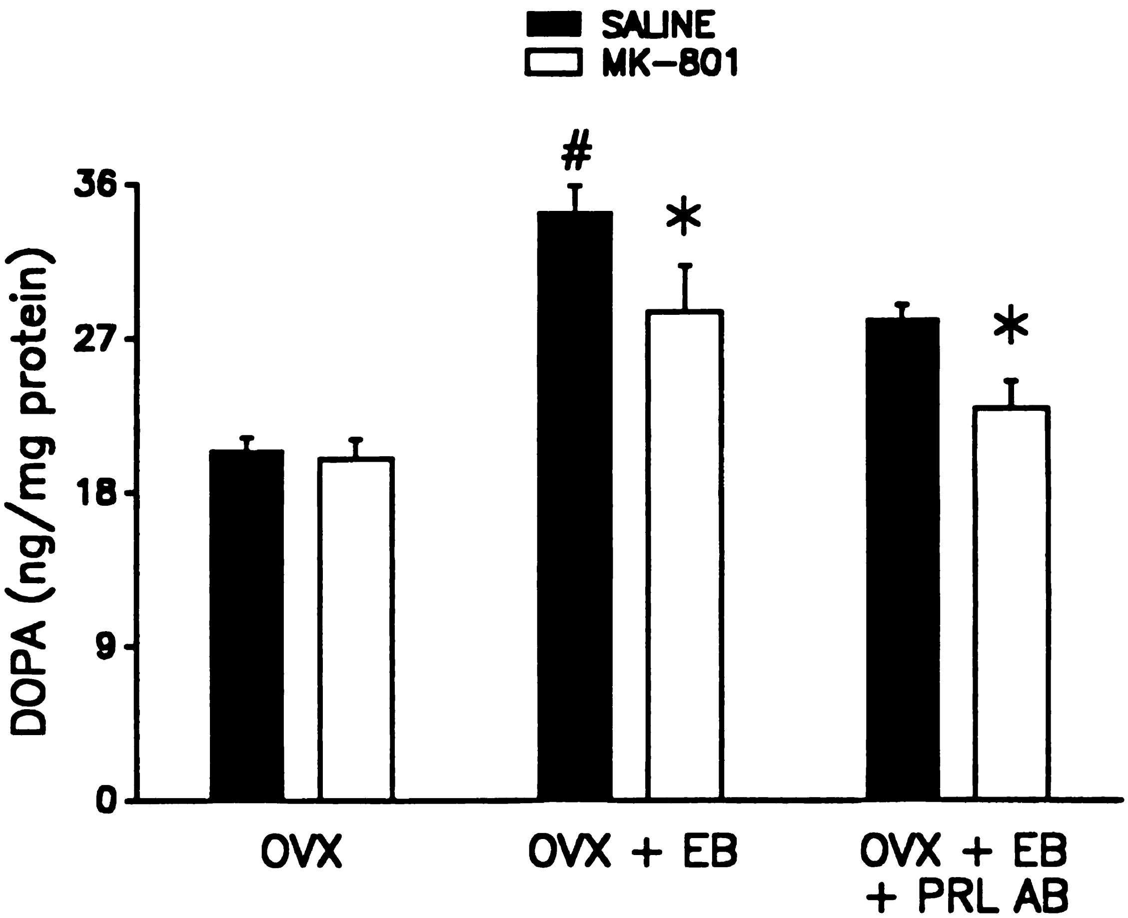

Figure 3.6 Effects of MK-801 on DOPA accumulation in the median eminence of estrogen- and estrogen plus prolactin antibody-treated ovariectomized female rats. Ovariectomized female rats were implanted s.c. with Silastic capsules containing either 17β-estradiol benzoate (OVX + EB; 37.5 μg/ml) or its corn oil vehicle (OVX) 3 days prior to decapitation. Rats were injected with either prolactin antibody (PRL AB; 200 μl/rat; i.v.) or its normal rabbit serum vehicle (200 μl/rat; i.v.) 2 h prior to decapitation. Rats were injected with either MK-801 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. All 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 DOPA concentrations in the median eminence of 5-10 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (two-way ANOVA/LSD; p<.05) from saline-treated controls (solid columns). #, Values from saline-treated OVX + EB rats which are significantly different (two-way ANOVA/LSD; p<.05) from either OVX or OVX + EB + PRL AB, saline-treated controls.

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orchidectomized male rats, despite the removal of testosterone and its inhibitory effects on TIDA neurons (Figure 3.7). This indicates that the lack of effect of MK-801 on TIDA neurons in male rats is independent of the presence of circulating androgens.

Although MK-801 is best known as a highly potent, non-competitive NMDA receptor antagonist, it also blocks other excitatory receptor-gated ion channels (Galligan and North, 1990) as well as voltage-gated ion channels (Wamil and McLean, 1992), and has adverse psychotomimetic effects (Collingridge and Lester, 1989). If the inhibitory effect of MK-801 on TIDA neurons in female rats was due to NMDA receptor blockade, rather than a non-specific blockade of other ion channels, then this effect should be mimicked by a competitive antagonist. As shown in Figure 3.8, the competitive NMDA receptor antagonist CGS-19755 produced a dose-dependent decrease in DOPA accumulation in the median eminence of female rats. This inhibitory effect of CGS-19755, as illustrated in Figure 3.9 by a decrease in DOPAC concentrations in the median eminence, was prevented in a dose-dependent fashion by the NMDA receptor agonist D,L-(tetrazol-5-yl) glycine. Interestingly, CGS-19755 increased rather than decreased prolactin concentrations in the plasma, an effect that also was prevented by administration of the agonist. The ability of both MK-801 and CGS-19755 to inhibit TIDA neurons in female rats suggests that these effects are mediated via a blockade

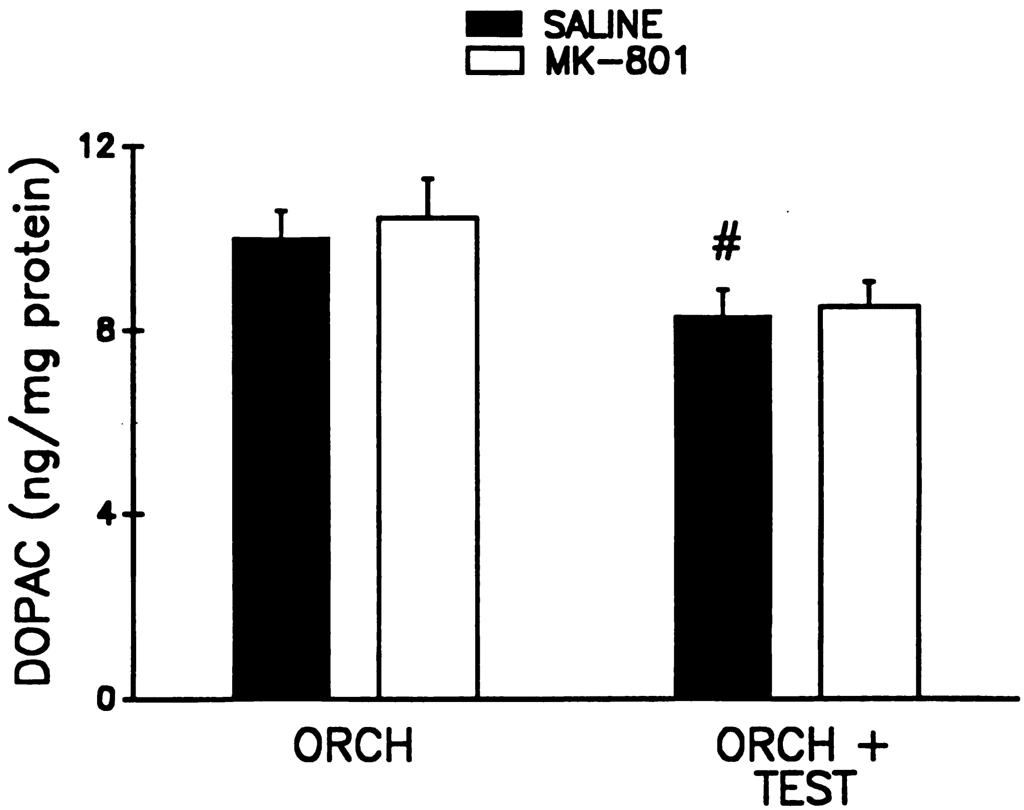

Figure 3.7 Effects of MK-801 on DOPAC concentrations in the median eminence of orchidectomized and orchidectomized, testosterone-treated male rats. Orchidectomized rats were implanted s.c. with either a testosterone-filled (ORCH + TEST) or an empty (ORCH) Silastic capsule. Three days later, rats were injected with either MK-801 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) and decapitated 60 min later. Columns represent the means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence of 6-8 rats. #, Values from saline-treated ORCH + TEST rats which are significantly different (two-way ANOVA/LSD; p<.05) from saline-treated ORCH controls.

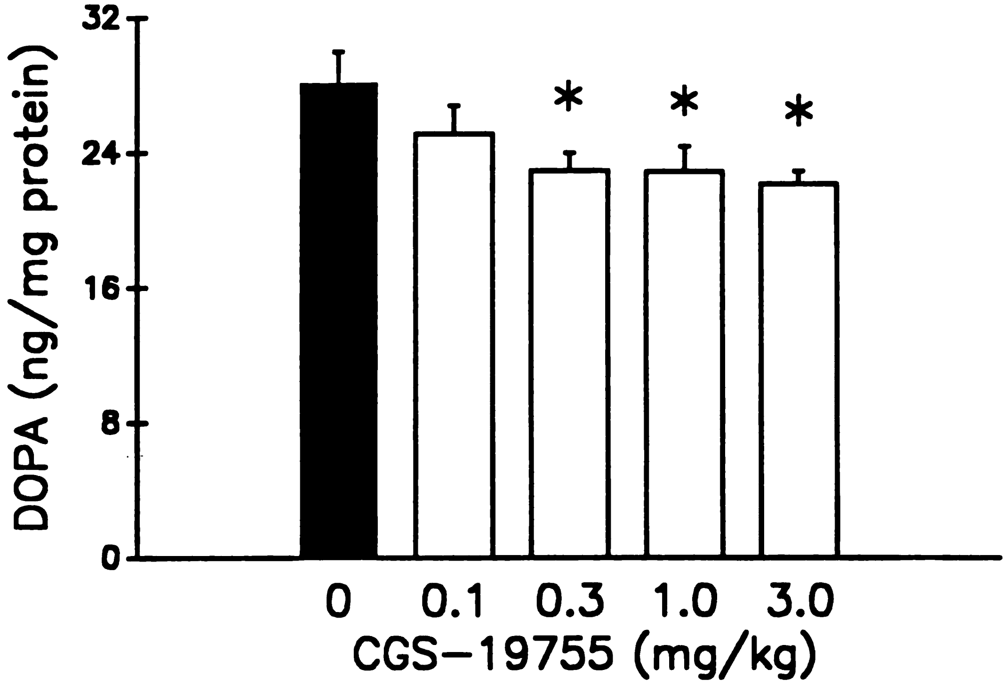

Figure 3.8 Dose response effects of CGS-19755 on DOPA accumulation in the median eminence of female rats. Rats were injected with either CGS-19755 (0.1, 0.3, 1.0 or 3.0 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. All rats were injected with NSD-1015 (100 mg/kg; i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPA concentrations in the median eminence of 7-8 rats. *, Values from rats treated with CGS-19755 (open columns) which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls (solid columns).

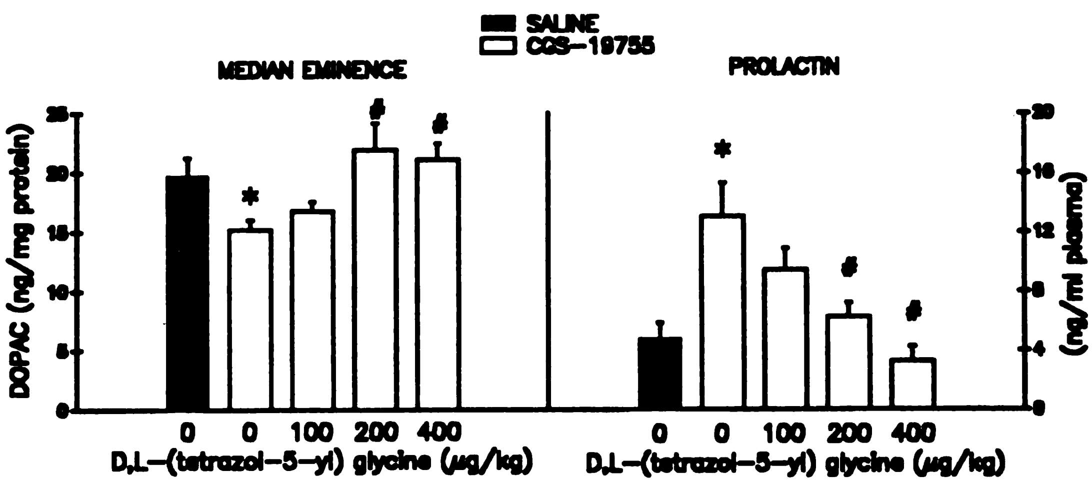

Figure 3.9 Dose response effects of D,L-(tetrazol-5-yl) glycine on DOPAC concentrations in the median eminence and on prolactin concentrations in plasma of CGS-19755 pre-treated female rats. Rats were injected with CGS-19775 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation, and with either D,L-(tetrazol-5-yl) glycine (100, 200 or 400 µg/kg; i.p.) or its 0.9% saline vehicle (1 ml/kg; i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence, and of prolactin concentrations in plasma, of 6-9 rats. *, Values from rats treated with CGS-19755 (open columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (solid columns). #, Values from rats treated with D,L-(tetrazol-5-yl) glycine which are significantly different (one-way ANOVA/LSD; p<.05) from rats treated with CGS-19755 alone.

of tonically activated NMDA receptors.

In contrast to the effects of NMDA receptor blockade on TIDA neurons, MK-801 increased DOPAC concentrations in the intermediate lobe of both male and female rats (Figure 3.10). This was associated with a decrease in α-MSH concentrations in plasma of both male and female rats. Similarly, CGS-19755 increased DOPA accumulation in the intermediate lobe of female rats (Figure 3.11). Conversely, D,L-(tetrazol-5-yl) glycine produced a dose-dependent decrease in DOPAC concentrations in the intermediate lobe of CGS-19755 pre-treated female rats (Figure 3.12). Although this dose of CGS-19755 (3 mg/kg) had no effect on DOPAC concentrations in the intermediate lobe *per se*, it decreased α-MSH concentrations in plasma. This effect was counteracted in a dose-dependent fashion by administration of the agonist.

Discussion

The results of these experiments reveal that endogenous EAA neurotransmitters acting at NMDA receptors tonically activate TIDA neurons in female, but not male rats. This conclusion is based on the observation that in female rats the NMDA receptor antagonists MK-801 and CGS-19755 decrease both the synthesis and metabolism of dopamine in the median eminence, the brain region containing the axon terminals of these neurons. That the administration of a NMDA receptor antagonist alone elicits an inhibitory effect on TIDA neurons implies that these compounds are blocking the actions of

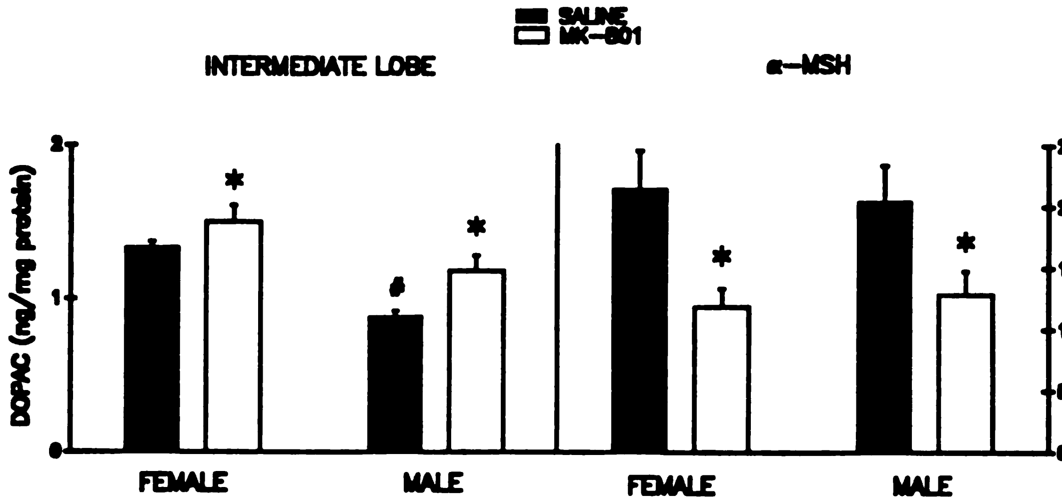

Figure 3.10 Comparison of the effects of MK-801 on intermediate lobe DOPAC concentrations and on plasma α-MSH concentrations in female and male rats. Rats were injected with either MK-801 (1 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) and killed by decapitation 60 min later. Columns represent the means and vertical lines 1 S.E.M. of intermediate lobe DOPAC concentrations, and of plasma α-MSH concentrations, from 7-10 rats. *, Values from rats treated with MK-801 (open columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (solid columns). #, Values from saline-treated male rats which are significantly different (two-way ANOVA/LSD; $P<.05$) from saline-treated female rats.

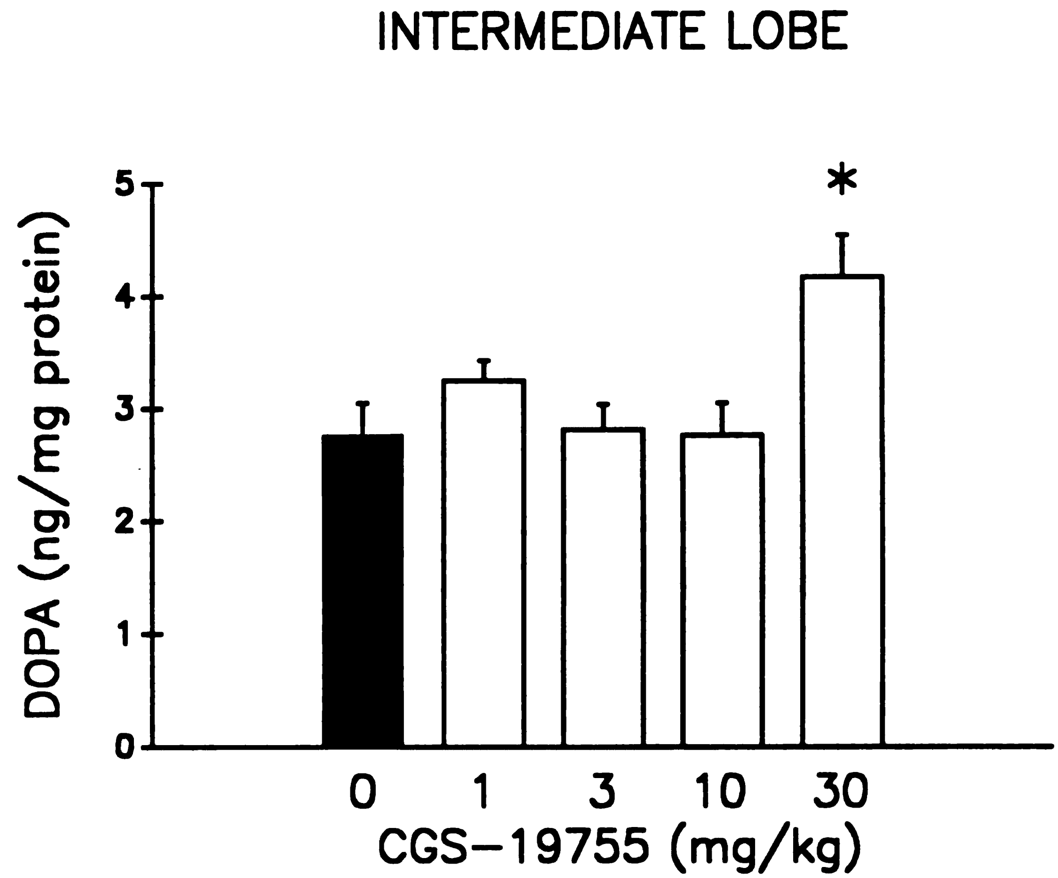

Figure 3.11 Dose response effects of CGS-19755 on DOPA accumulation in the intermediate lobe of female rats. Rats were injected with either CGS-19755 (1, 3, 10 or 30 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation. All rats were injected with NSD-1015 (100 mg/kg; i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPA concentrations in the intermediate lobe of 6-8 rats. *, Values from rats treated with CGS-19755 (open columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (solid columns).

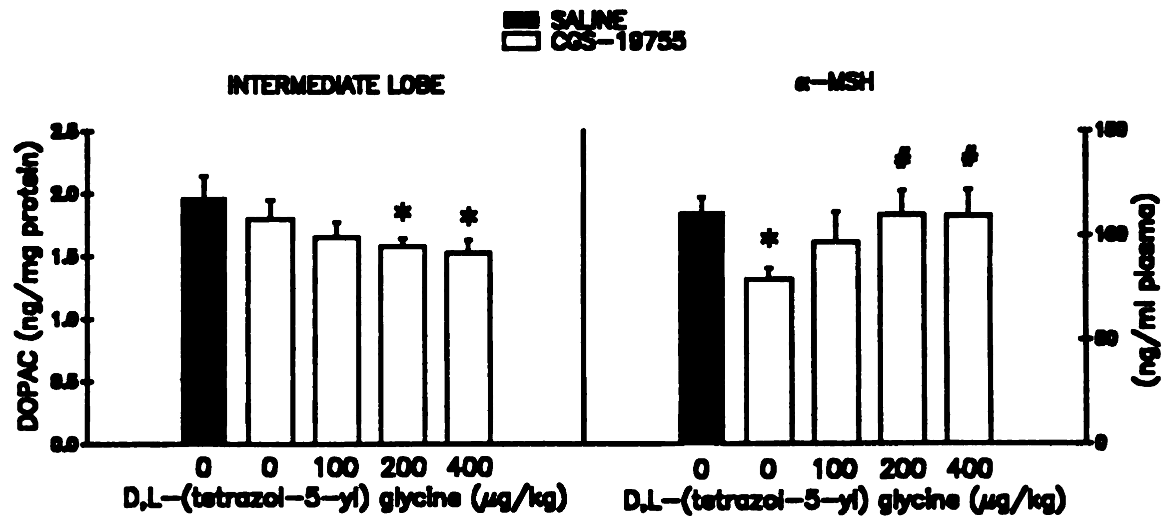

Figure 3.12 Dose response effects of D,L-(tetrazol-5-yl) glycine on DOPAC concentrations in the intermediate lobe and on α-MSH concentrations in plasma of CGS-19755 pre-treated female rats. Rats were injected with CGS-19775 (3 mg/kg; s.c.) or its 0.9% saline vehicle (1 ml/kg; s.c.) 60 min prior to decapitation, and with either D,L-(tetrazol-5-yl) glycine (100, 200 or 400 μg/kg; i.p.) or its 0.9% saline vehicle (1 ml/kg; i.p.) 30 min prior to decapitation. Columns represent the means and vertical lines 1 S.E.M. of DOPAC concentrations in the intermediate lobe, and of α-MSH concentrations in plasma, of 5-8 rats. *, Values from rats treated with CGS-19755 (open columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (solid columns). #, Values from rats treated with D,L-(tetrazol-5-yl) glycine which are significantly different (one-way ANOVA/LSD; p<.05) from rats treated with CGS-19755 alone.

endogenous EAA neurotransmitters at these receptors.

MK-801 markedly decreased basal prolactin secretion in both female and male rats. These results are consistent with a previous report demonstrating that MK-801 decreases basal prolactin release from dispersed anterior pituitary cells (Login, 1990), suggesting a direct inhibitory effect of MK-801 at the level of the lactotroph. The additional inhibitory effect of MK-801 on TIDA neurons is surprising, however, because decreases in prolactin are usually associated with increases in TIDA neuronal activity (Lookingland et al., 1987a). On the other hand, removal of the tonic stimulatory effects of endogenous prolactin in female rats decreases TIDA neuronal activity (Toney et al., 1992b), and this may account for the observed effect of MK-801 on these neurons. That MK-801 was able to decrease TIDA neuronal activity in the absence of the tonic stimulatory effect of prolactin following immunoneutralization of endogenous prolactin, however, suggests that the inhibitory effect of MK-801 on TIDA neurons occurs independently of its inhibitory effect on prolactin secretion.

In contrast to MK-801, the competitive NMDA receptor antagonist CGS-19755 increases rather than decreases prolactin secretion in female rats. This is consistent with another report demonstrating that the competitive NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid increases prolactin secretion in male rats (Arslan et al., 1991a). This disparity in the effect of NMDA receptor antagonists on prolactin secretion could be attributed to the ability of MK-801 to block voltage-gated ion channels (Wamil and McLean, 1992), thereby disrupting stimulus-secretion coupling in the lactotroph. A comparable decrease in the activity of TIDA neurons is seen, however, with the competitive NMDA receptor antagonist CGS-19755, an effect which is overcome in a dose-dependent manner by administration of the NMDA receptor agonist D,L-(tetrazol-5-yl) glycine. These observations reinforce the idea that the effect of MK-801 on TIDA neurons is due to blockade of NMDA receptors.

The lack of responsiveness of TIDA neurons to MK-801 in ovariectomized female rats, and the restoration of the inhibitory effect of MK-801 following estrogen replacement is consistent with previous reports demonstrating estrogen's ability to modulate the activity of TIDA neurons via a prolactin-independent mechanism. For example, estrogen has been shown to block the stimulatory effects of the peptide bombesin on TIDA neurons (Toney et al., 1992a), and to prevent the activation of these neurons either by the kappa opioid receptor antagonist nor-binaltorphimine or immunoneutralization of dynorphin (Wagner et al., in press). Moreover, the stimulatory action of N-methyl-D,L-aspartate on luteinizing hormone release is reversed in the ovariectomized rhesus monkey (Reyes et al., 1990), and estrogen reinstates the stimulatory effects of N-methyl-D,L-aspartate on luteinizing hormone secretion in the ovariectomized ewe (Estienne et al., 1990), supporting the contention that estrogen plays a role in modulating NMDA receptor-mediated regulation of the hypothalamic-pituitary axis in female rats.

By contrast, the lack of effect of MK-801 on TIDA neurons in male rats is independent of the presence of gonadal androgens. This finding is similar a report demonstrating that androgens do not influence the stimulatory effect of bombesin on TIDA neurons in male rats (Toney et al., 1992a). Although testicular steroids positively modulate prolactin responsiveness to acute N-methyl-D,L-aspartic acid stimulation in the adult male rhesus monkey (Arslan et al., 1991b), the ability of testosterone to inhibit TIDA neurons in orchidectomized male rats occurs without any concomitant change in prolactin secretion (Toney et al., 1991). Therefore, androgen modulation of NMDA receptor-mediated regulation of prolactin secretion in the male (Arslan et al., 1991b) appears to be separate from the inhibitory actions of androgens on TIDA neurons.

The identity of the neurotransmitter(s) responsible for the putative, tonic stimulatory effect on TIDA neurons in female rats is not evident from this study. L-Glutamate is thought to be the principal neurotransmitter acting at EAA receptors, and in the presence of glycine it is a NMDA receptor agonist in neuronal cell cultures (Johnson and Ascher, 1987). The effects of L-glutamate, however, are somewhat resistant to blockade with relatively selective NMDA receptor antagonists (Davies et al., 1981; Collingridge et al., 1983). On the other hand, L-aspartate and potent sulfur-containing amino acids such as D-homocysteine sulfinic acid are quite susceptible to NMDA receptor antagonism (Curtis and Watkins, 1963; Mewett et al., 1983; Collingridge and Lester, 1989).

Also uncertain is the precise location of these receptors. Radioligand binding studies show that NMDA receptor density in the hypothalamus is considerably lower than that observed in the neocortex and hippocampus (Monaghan and Cotman, 1985; Maragos et al., 1988; Gibson et al., 1992), and that there is a greater abundance of non-NMDA receptors than of NMDA receptors in the hypothalamus (Unnerstall and Wamsley, 1983; May et al., 1989; Petralia and Wenthold, 1992). This would suggest that NMDA receptor activation tonically stimulates an extrahypothalamic excitatory afferent pathway which regulates the activity of TIDA neurons in the female rat. On the other hand, NMDA stimulates luteinizing hormone-releasing hormone from the mediobasal hypothalamus *in vitro* (Bourguignon et al., 1989; Donoso et al., 1990). Furthermore, neonatal administration of monosodium glutamate produces an excitotoxic lesion of the arcuate nucleus (Olney, 1969), which is accompanied by a depletion of dopamine from the mediobasal hypothalamus (Nemeroff et al., 1977). This excitotoxic effect is mimicked by N-methyl aspartate in the adult rodent (Olney and Price, 1983). These findings argue in favor of a direct stimulatory effect of NMDA receptor activation by endogenous EAA neurotransmitters on TIDA neurons in female rats.

By contrast, endogenous EAA neurotransmitters acting at

NMDA receptors tonically inhibit PHDA neurons in both male and female rats. This conclusion is based on the observation that blockade of NMDA receptors with either MK-801 or CGS-19755 increases the synthesis and metabolism of dopamine in the intermediate lobe of the pituitary, which contains the terminals of these neurons. Support for an inhibitory effect of EAA neurotransmitters on PHDA neurons stems from the demonstration that NMDA receptor activation with D,L-(tetrazol-5-yl) glycine decreases the metabolism of dopamine in the intermediate lobe of CGS-19755 pre-treated rats. The concept of NMDA receptor-mediated tonic inhibition of dopaminergic neurons is not unprecedented, as there is appreciable evidence that endogenous EAAs tonically inhibit nigrostriatal and mesolimbic dopaminergic neurons by a NMDA receptor-mediated mechanism (Cheramy et al., 1986; French and Ceci, 1990; Leviel et al., 1990; Moghaddam and Gruen, 1991).

The dose of CGS-19755 necessary to activate PHDA neurons (30 mg/kg) is 100-fold greater than that required to inhibit TIDA neurons (0.3 mg/kg). Although this suggests that PHDA neurons are less sensitive to NMDA receptor antagonism than are TIDA neurons, no such disparity exists between the potency of MK-801 in inhibiting TIDA neurons and that observed to activate PHDA neurons. Perhaps this reflects a relative inability of CGS-19755 to penetrate brain regions other than circumventricular organs such as the median eminence. While the increase in PHDA neuronal activity following blockade of NMDA receptors with MK-801 is associated with a decrease in α-

MSH secretion, it is noted that CGS-19755 affects a decrease in α-MSH secretion at a lower dose than is necessary to increase PHDA neuronal activity. It is possible that in addition to tonically inhibiting PHDA neurons, endogenous EAA neurotransmitters acting at NMDA receptors tonically stimulate α-MSH secretion via a releasing factor such as 5-hydroxytryptamine (Randle et al., 1983). Alternatively, the NMDA receptor-mediated tonic stimulation of α-MSH secretion by endogenous EAAs could be due to a combination of a direct stimulatory effect at the level of the melanotroph and a tonic inhibition of PHDA neurons.

In summary, these experiments provide evidence for a sexual difference in NMDA receptor-mediated regulation of TIDA neurons, but not prolactin secretion, and indicate that estrogen facilitates the tonic stimulation of these neurons by endogenous EAA neurotransmitters (i.e., L-glutamate, L-aspartate) in the female rat through a prolactin-independent mechanism. In contrast, endogenous EAA neurotransmitters acting at NMDA receptors tonically inhibit PHDA neurons in a manner that is not sexually differentiated.

4. NON-NMDA RECEPTOR-MEDIATED REGULATION OF TIDA AND PHDA NEURONS

Introduction

It is clear that EAAs have a prominent role in neuroendocrine regulation (Brann and Mahesh, 1992). While much of this evidence focuses on the effects of EAAs via NMDA receptors, there are recent reports implicating ionotropic non-NMDA receptors as the primary mediators of the neuroendocrine actions of EAAs, such as the stimulation of luteinizing hormone-releasing hormone release (Donoso et al., 1990; López et al., 1992). Indeed, there is an appreciable density of both kainate and AMPA receptors in the mediobasal hypothalamus (Unnerstall and Wamsley, 1983; Petralia and Wenthold, 1992), and glutamatergic nerve terminals make synaptic contact with neurons in the arcuate nucleus (Decavel and van den Pol, 1992). Given the abundance of EAA neurotransmitter and receptors in the mediobasal hypothalamus, it is not surprising that EAA agonists stimulate both prolactin and α-MSH secretion (Login, 1990; Arslan et al., 1991a; Abbud and Smith, 1991; Wayman and Wilson, 1991). It is not known, however, if these effects of EAAs acting at non-NMDA receptors are due to alterations in the tonic inhibition of hormone secretion by TIDA and PHDA neurons.

The purpose of the experiments presented in this chapter was to examine the role of non-NMDA receptors in regulating TIDA and PHDA neuronal activity. To this end, the effect of the non-NMDA receptor antagonist NBQX (Sheardown et al., 1990) and agonists kainic acid and AMPA (Collingridge and Lester, 1989) were evaluated in male and female rats.

Results

As shown in Figure 4.1, NBQX increased DOPAC concentrations in the median eminence and decreased prolactin concentrations in the plasma of both male and female rats in a dose-dependent fashion. Similarly, NBQX produced a dose-dependent increase in DOPAC concentrations in the intermediate lobe and decrease in α-MSH concentrations in the plasma of both male and female rats (Figure 4.2). These data reveal that the responsiveness of these DA neurons to the effects of NBQX is not sexually differentiated. Therefore, all subsequent experiments were performed solely on male rats.

Following the administration of NBQX, comparable time-related alterations in the neurochemical indices of TIDA and PHDA neuronal activity were also observed. The onset of the NBQX-induced increase in median eminence DOPAC concentrations and the corresponding decrease in plasma prolactin concentrations occurred by 30 min, and these effects were no longer present by 240 min after its administration (Figure 4.3). The onset of the NBQX-induced increase in intermediate lobe DOPAC concentrations and the corresponding decrease in plasma α-MSH concentrations occurred by 60 min, and again

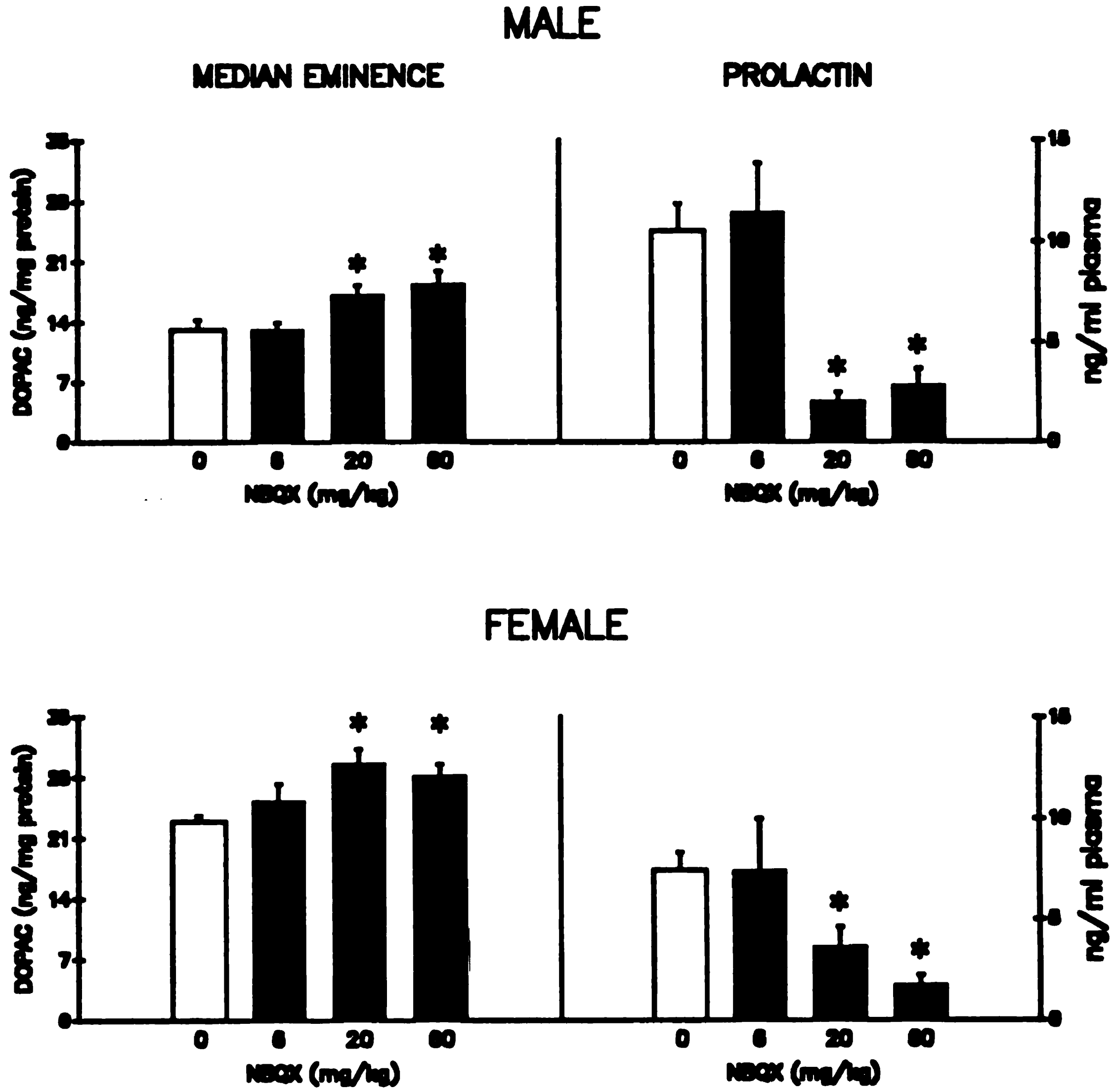

Figure 4.1 Dose response effects of NBQX on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of male and female rats. Rats were injected with either NBQX (6, 20 or 60 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) and killed by decapitation 60 min later. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in the plasma from 5-9 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

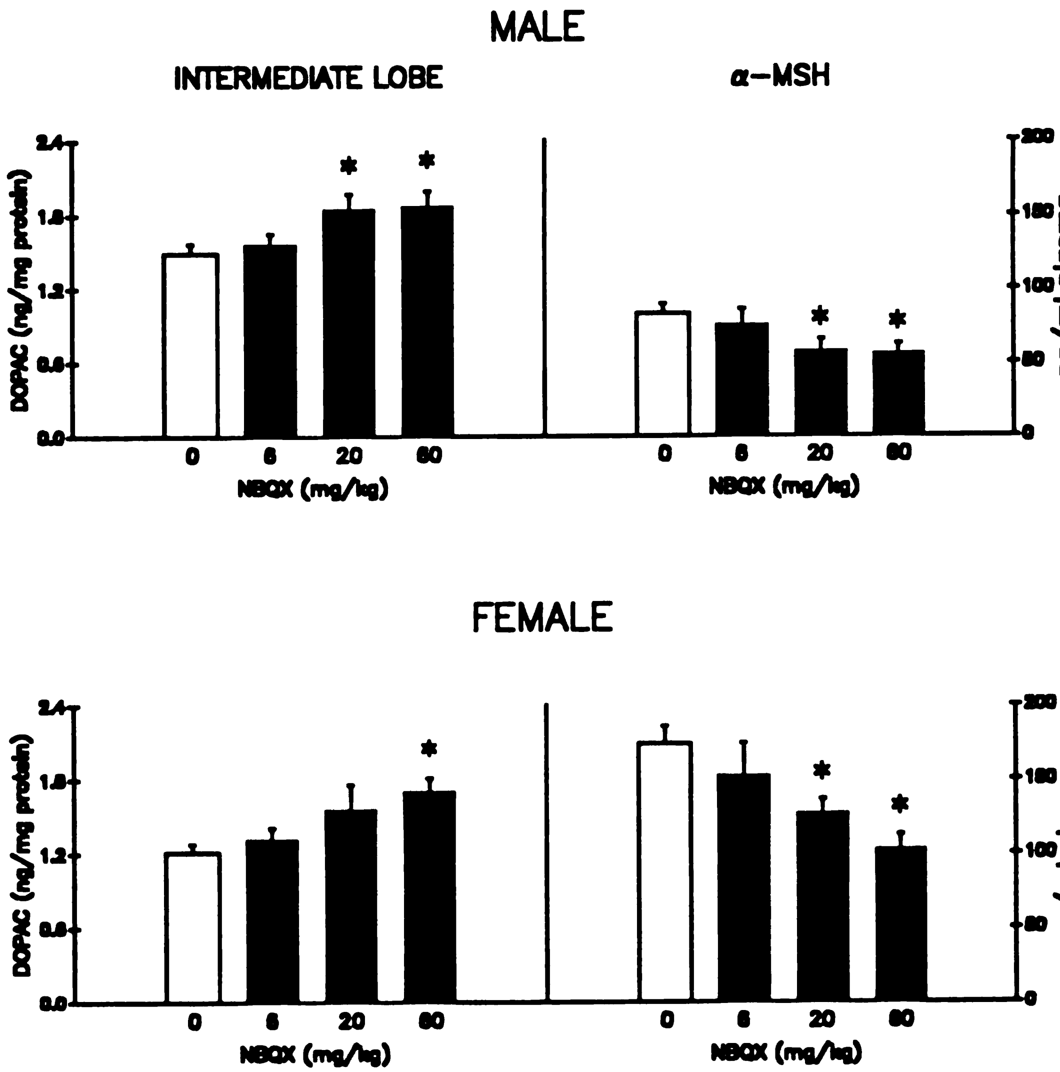

Figure 4.2 Dose response effects of NBQX on DOPAC concentrations in the intermediate lobe and α-MSH concentrations in the plasma of male and female rats. Rats were injected with either NBQX (6, 20 or 60 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the intermediate lobe and of α-MSH concentrations in the plasma from 6-9 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns).

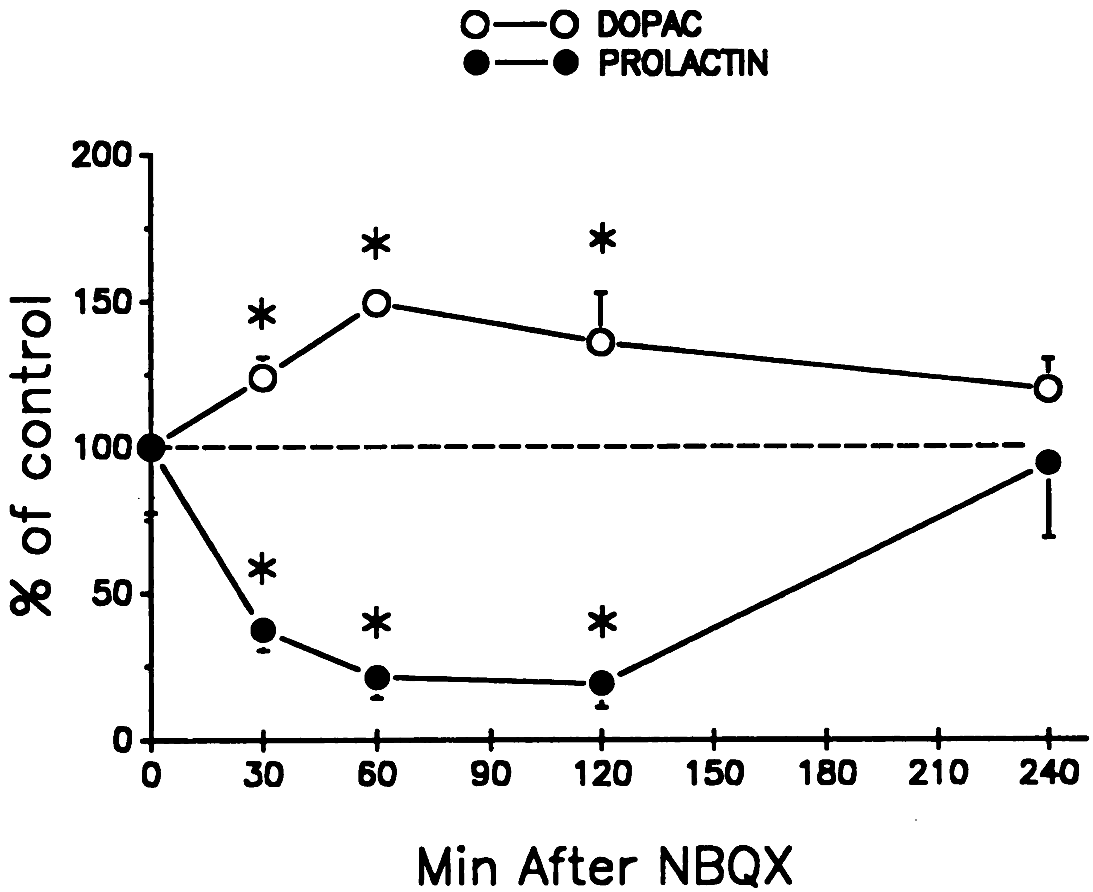

Figure 4.3 Time course of the effects of NBQX on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of male rats. Rats were injected with NBQX (20 mg/kg; i.p.) and killed by decapitation either 30, 60, 120 or 240 min later. Zero time control rats were injected with 0.9% saline (2.5 ml/kg; i.p.) 60 min prior to decapitation. Symbols represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence (open circles) and of prolactin concentrations in plasma (solid circles) from 5-10 rats. Control values for median eminence DOPAC and plasma prolactin concentrations were 10.57±0.59 ng/mg protein and 12.74±2.86 ng/ml plasma, respectively. *, Values from rats treated with NBQX which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls.

these effects were no longer present by 240 min following its administration (Figure 4.4).

Although NBQX is considered a non-NMDA receptor antagonist selective for the AMPA receptor (Sheardown et al., 1990), its affinity for the AMPA receptor is only 32 times greater than that for the kainate receptor (Watkins, 1991) Accordingly, these effects of NBQX on TIDA and PHDA neurons could be due to blockade of AMPA and/or kainate receptors. To distinguish between the two receptor types, the effects of AMPA and kainic acid on TIDA and PHDA neurons were evaluated in NBQX-treated rats. As depicted in Figure 4.5, the NBQX-induced increase in median eminence DOPAC concentrations and the associated decrease in plasma prolactin concentrations were counteracted by intracerebroventricular (i.c.v.) administration of AMPA, as were the NBQX-induced increase in intermediate lobe concentrations and the associated decrease in plasma α-MSH concentrations (Figure 4.6). On the other hand, kainic acid did not counteract either the NBQX-induced increase in median eminence DOPAC concentrations and the associated decrease in plasma prolactin concentrations (Figure 4.7), or the NBQX-induced increase in intermediate lobe DOPAC concentrations and the associated decrease in plasma α-MSH concentrations (Figure 4.8). Taken together, these data indicate that NBQX activates TIDA and PHDA neurons by blocking the actions of endogenous EAAs at AMPA rather than kainate receptors.

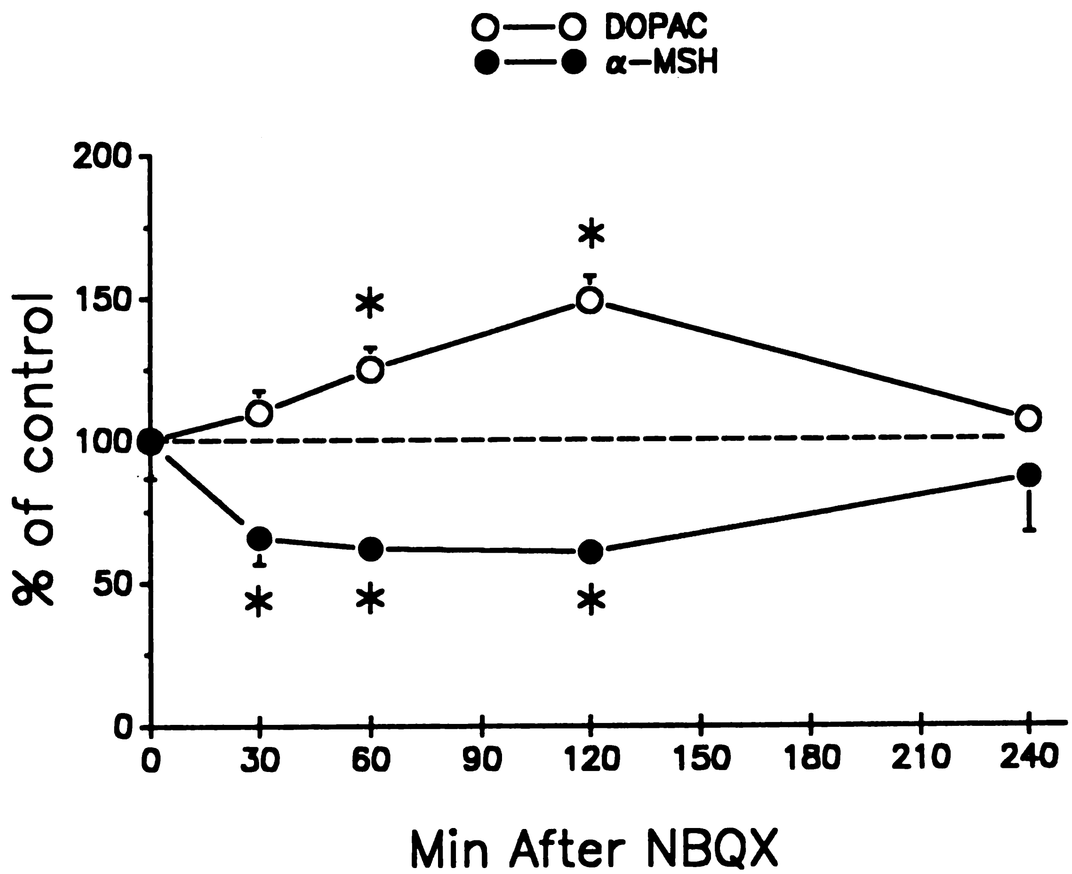

Figure 4.4 Time course of the effects of NBQX on DOPAC concentrations in the intermediate lobe and α-MSH concentrations in the plasma of male rats. Rats were injected with NBQX (20 mg/kg; i.p.) and killed by decapitation either 30, 60, 120 or 240 min later. Zero time control rats were injected with 0.9% saline (2.5 ml/kg; i.p.) 60 min prior to decapitation. Symbols represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the intermediate lobe (open circles) and of α-MSH concentrations in the plasma (solid circles) from 6-9 rats. Control values for intermediate lobe DOPAC and plasma α-MSH concentrations were 0.92±0.05 ng/mg protein and 108.7±14.4 pg/ml plasma, respectively. *, Values from rats treated with NBQX which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls.

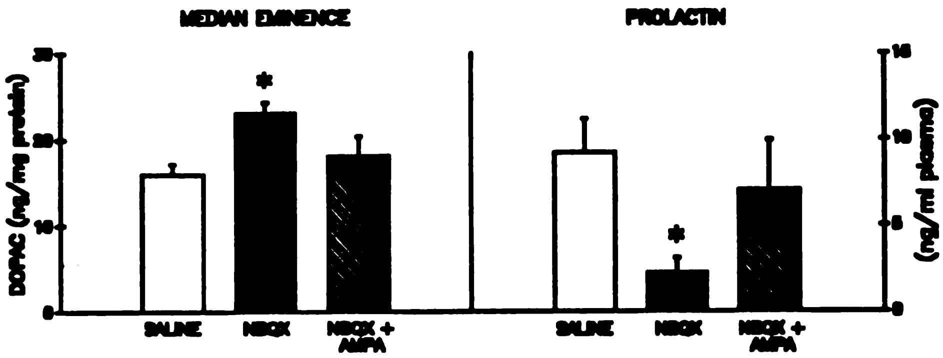

Figure 4.5 Effects of AMPA on median eminence DOPAC concentrations and plasma prolactin concentrations in NBQX-treated male rats. Rats were injected with NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with AMPA (40 μg/rat; i.c.v.) or its 0.9% saline vehicle (5 μl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of median eminence DOPAC concentrations and of plasma prolactin concentrations from 5-8 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

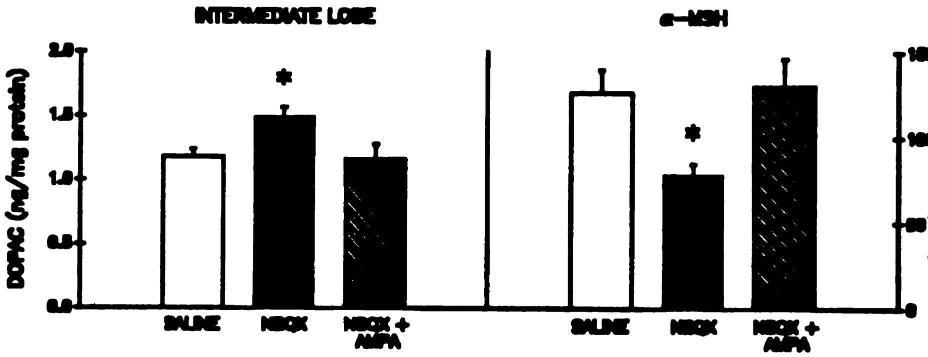

Figure 4.6 Effects of AMPA on intermediate lobe DOPAC concentrations and plasma α-MSH concentrations in NBQX-treated male rats. Rats were injected with NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with AMPA (40 μg/rat; i.c.v.) or its 0.9% saline vehicle (5 μl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of intermediate lobe DOPAC concentrations and of plasma α-MSH concentrations from 6-7 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

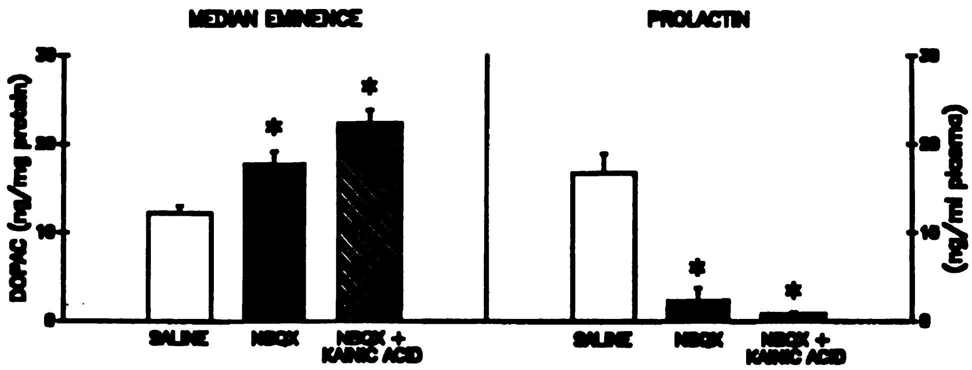

Figure 4.7 Effects of kainic acid on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of NBQX-treated male rats. Rats were injected with NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with kainic acid (10 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in the plasma from 6-7 rats. *, Values from rats treated with NBQX (solid columns), and with NBQX + KAINIC ACID (diagonally-hatched columns), which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

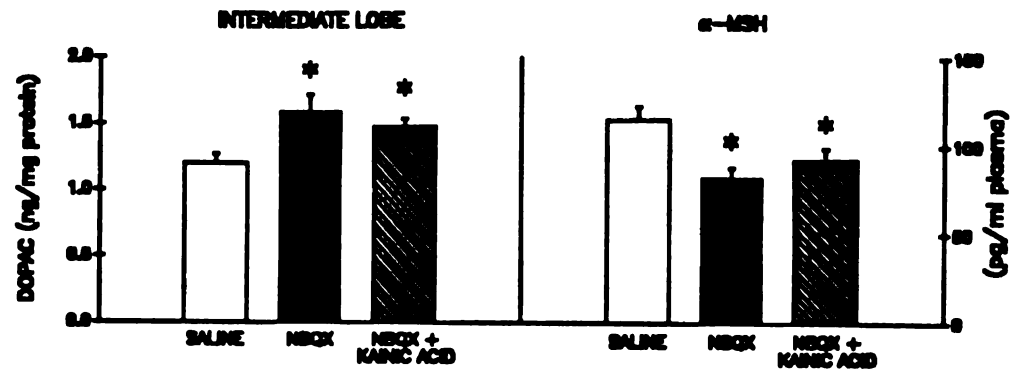

Figure 4.8 Effects of kainic acid on DOPAC concentrations in the intermediate lobe and on α-MSH concentrations in the plasma of NBQX-treated male rats. Rats were injected with NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with kainic acid (10 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the intermediate lobe and of α-MSH concentrations in the plasma from 6-7 rats. *, Values from rats treated with NBQX (solid columns), and with NBQX + KAINIC ACID (diagonally-hatched columns), which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns).

Discussion

The results of these experiments reveal that endogenous EAA neurotransmitters acting at AMPA receptors tonically inhibit TIDA and PHDA neurons in male and female rats. This conclusion is based on the observation that the non-NMDA receptor antagonist NBQX increases the metabolism of dopamine in the median eminence and intermediate lobe, the regions containing the axon terminals of these neurons. That the administration of AMPA prevents the stimulatory effect of NBQX on TIDA and PHDA neurons, as well as the concomitant decrease in prolactin and α-MSH secretion, implies that the antagonist is blocking the inhibitory action of endogenous EAA neurotransmitters on these neurons which is mediated through AMPA receptors. In view of reports describing NMDA receptor-mediated tonic inhibition of nigrostriatal dopaminergic neurons by endogenous EAA neurotransmitters (Cheramy et al., 1986; Leviel et al., 1990), the concept of EAA-mediated inhibition certainly is plausible.

Although previous reports have shown that the peripherally administered dose of kainic acid used in this study (10 mg/kg) is sufficient to activate central kainate receptors in rats (Lason et al, 1989; Pennypacker et al., 1993), kainic acid did not affect the NBQX-induced increase in TIDA and PHDA neuronal activity. Consistent with this finding is a report that, unlike NMDA, kainic acid does not stimulate prolactin secretion (Abbud and Smith, 1991). Evidently, activation of these neurons by NBQX is not mediated via a blockade of kainate receptors.

Despite reports of sexual differences in the basal activity of TIDA (Demarest et al., 1981; Gudelsky and Porter, 1981; Gunnett et al.; 1986) and PHDA (Manzanares et al., 1992a) neurons, the responsiveness of these neurons to the stimulatory effect of NBQX is not sexually differentiated. This indicates that the actions of endogenous EAA neurotransmitters at AMPA receptors do not contribute to the sexual difference in the basal activity of these neurons. Instead, they provide an integral inhibitory component which serves to dampen the basal activity of these neurons, thereby preventing them from firing unabated.

It is apparent that the AMPA receptor-mediated inhibition of TIDA and PHDA neurons underlies, in part, the stimulatory effect of EAAs on the secretion of prolactin and α-MSH in both male and female rats. NMDA receptor-mediated stimulation of prolactin and α-MSH secretion has been extensively documented (Login, 1990; Arslan et al., 1991a; Abbud and Smith, 1991; Wayman and Wilson, 1991) and with regard to prolactin it is due to a direct action at the level of the anterior pituitary (Login, 1990). The results of these experiments reveal another regulatory pathway through which EAAs can stimulate the secretion of prolactin and α-MSH, namely the alteration in the extent of dopaminergic inhibition of hormone secretion.

Although the site of action of NBQX in these experiments is not specifically addressed, there is a significant AMPA receptor density in the arcuate and periventricular hypothalamic nuclei (Petralia and Wenthold, 1992). It is unlikely, however, that these receptors reside directly on the soma/dendritic regions of TIDA and PHDA neurons. Glutamatergic nerve terminals have been shown to make synaptic contact with identified GABAergic neurons in the arcuate nucleus (Decavel and van den Pol, 1992). It is possible that endogenous EAA neurotransmitters elicit an AMPA receptor-mediated tonic stimulation of intrinsic inhibitory interneurons, which in turn tonically inhibit TIDA and PHDA neurons.

In summary, these experiments provide evidence for an activation of TIDA and PHDA neurons following non-NMDA receptor antagonism, and indicate that endogenous EAAs tonically inhibit these neurons through an AMPA receptor-mediated mechanism.

5. GABAERGIC REGULATION OF TIDA NEURONS IN THE MALE RAT

Introduction

The prolactin inhibiting factors dopamine (Ben Jonathan, 1985) and GABA (Schally et al., 1977) are released from tuberoinfundibular neurons terminating in the median eminence of the hypothalamus (Björklund and Nobin, 1973; Everitt et al., 1984; Schimchowitsch et al., 1991). In mammals, GABA is found in virtually all tuberoinfundibular dopaminergic (TIDA) neurons, and there is a subpopulation of tuberoinfundibular GABAergic neurons which do not contain dopamine (Schimchowitsch et al., 1991). GABAergic nerve terminals also make synaptic contact with neurons in the arcuate nucleus, the site of TIDA perikarya (Decavel and van den Pol, 1992).

GABA receptor activation by exogenous agonists inhibits dopamine release from TIDA neurons, both *in vivo* and *in vitro* (Demarest and Moore, 1979b; Casanueva et al., 1981; Anderson and Mitchell, 1985). Tonic activity of GABAergic neurons in the mediobasal hypothalamus has been demonstrated (Loose et al., 1991), but the identity of the postsynaptic target has not been established. Despite the anatomical basis and pharmacological evidence for an interaction between GABA and TIDA neurons, it is unknown if endogenous GABA tonically regulates the activity of TIDA neurons.

The purpose of the experiments presented in this chapter was to determine the effects of acute $GABA_A$ and $GABA_B$ receptor activation and blockade on TIDA neurons. To this end, the effects of the $GABA_A$ receptor agonist isoguvacine (Krogsgaard-Larsen et al., 1977) and antagonist SR 95531 (Wermuth and Bizière, 1986), as well as the $GABA_B$ receptor agonist baclofen (Bowery, 1989) and antagonist 2-hydroxysaclofen (Kerr et al., 1989) were evaluated in male rats.

Results

In preliminary experiments it was determined that systemic administration of the $GABA_A$ receptor antagonist SR 95531 produced lethal seizures at 10 but not at 3 mg/kg. The latter dose, but not lower doses (0.3 and 1.0 mg/kg) increased DOPAC concentrations in the median eminence and lowered prolactin concentrations in plasma when measured 15 min after subcutaneous injection (Figure 5.1). As depicted in Figure 5.2, SR 95531 caused a rapid (within 15 min) but transient increase in DOPAC concentrations in the median eminence, and an equally prompt but slightly more prolonged (at least for 30 min) decrease in prolactin concentrations in plasma. In contrast, the $GABA_A$ receptor agonist isoguvacine had no effect on DOPAC concentrations in the median eminence at any dose or time point examined (Tables 5.1 & 5.2). Isoguvacine did elicit a delayed decrease in plasma prolactin concentrations 120 min following its administration (Table 5.2). The increase in DOPAC concentrations in the median eminence produced by SR 95531 was prevented by isoguvacine pre-treatment (Figure 5.3). On the other hand, neither SR 95531

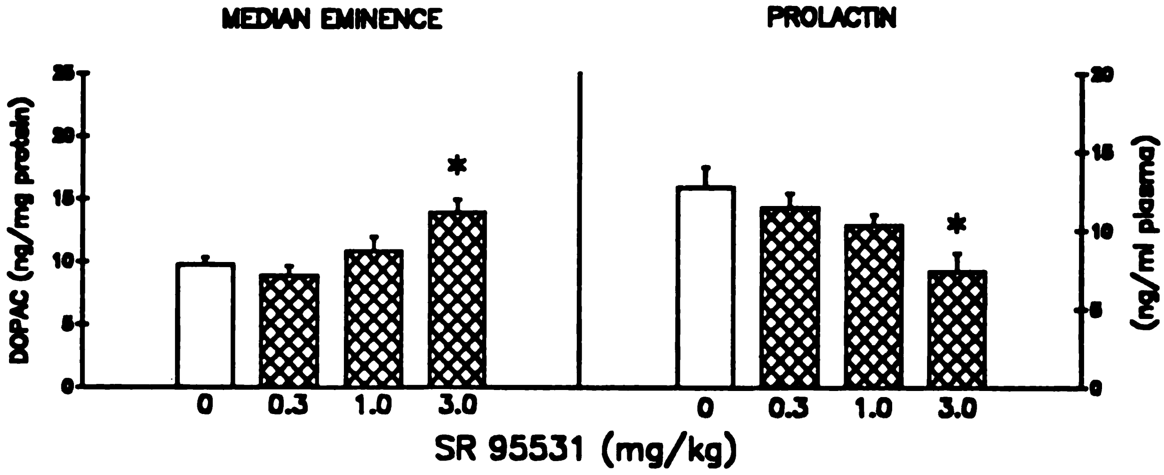

Figure 5.1 Dose response effects of the $GABA_A$ receptor antagonist SR 95531 on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of male rats. Rats were injected with either SR 95531 (0.3, 1.0 or 3.0 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 15 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in the plasma from 7-9 rats. *, Values from rats treated with SR 95531 (cross-hatched columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

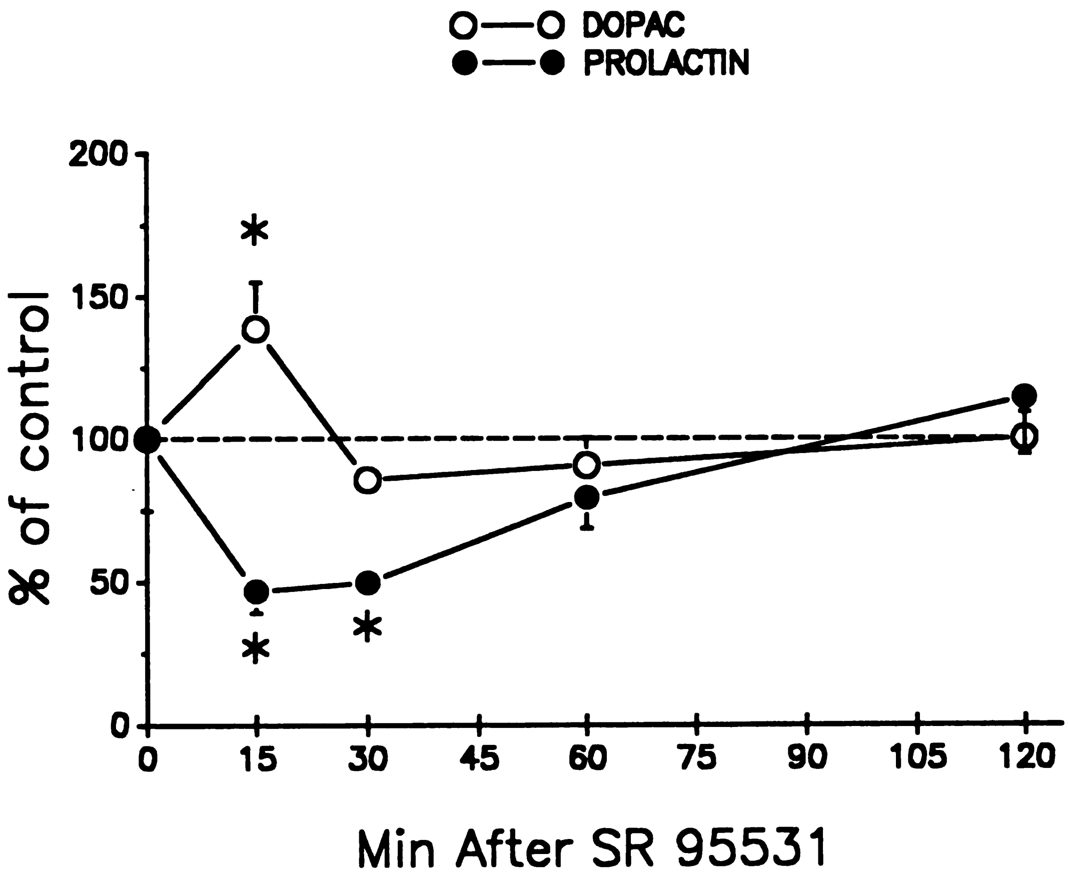

Figure 5.2 Time course of the effects of SR 95531 on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of male rats. Rats were injected with SR 95531 (3 mg/kg; s.c.) 15, 30, 60 or 120 min prior to decapitation. Zero time values were determined in rats injected with 0.9% saline (1 ml/kg; s.c.) 60 min prior to decapitation. Symbols represent the means and vertical lines 1 S.E.M. of the concentrations of DOPAC in the median eminence (open circles) and of prolactin concentrations in the plasma (solid circles) from 6-9 rats. Control values for DOPAC concentrations in the median eminence were 12.78±0.71 ng/mg protein, and those for prolactin concentrations in the plasma were 16.33±4.13 ng/ml. *, Values from rats treated with SR 95531 which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls.

Table 5.1 Lack of effect of the $GABA_A$ receptor agonist isoguvacine on DOPAC concentrations in the median eminence of male rats.

Isoguvacine (μg/rat)	DOPAC (ng/mg protein)
0	11.63±0.97
3	14.08±1.15
10	12.38±0.77
30	11.40±0.57

Rats were injected with either isoguvacine (3, 10 or 30 μg/rat; i.c.v.) or 0.9% saline (5 μl/rat; i.c.v.) and killed by decapitation 30 min later. Values represent means ± 1 S.E.M. of DOPAC concentrations in the median eminence from 7-8 rats.

Table 5.2 Isoguvacine produces a delayed decrease in plasma prolactin concentrations without any concomitant change in median eminence DOPAC concentrations in male rats.

Min after isoguvacine	Prolactin	DOPAC
0	12.47±2.22	16.17±1.22
15	15.51±4.80	14.92±1.19
30	6.98±3.06	16.65±1.24
60	7.09±1.34	13.78±1.04
120	2.79±0.39*	15.53±1.29

Rats were injected with isoguvacine (30 μg/rat; i.c.v.) 15, 30, 60 or 120 min prior to decapitation. Zero time values were obtained from rats injected with 0.9% saline (5 μl/rat; i.c.v.) 30 min prior to decapitation. Values represent means ± 1 S.E.M. of prolactin concentrations in the plasma (ng/ml) and of DOPAC concentrations in the median eminence (ng/mg protein) from 6-8 rats. *, Values from rats treated with isoguvacine which are significantly different (one-way ANOVA/LSD; $p<.05$) from saline-treated controls.

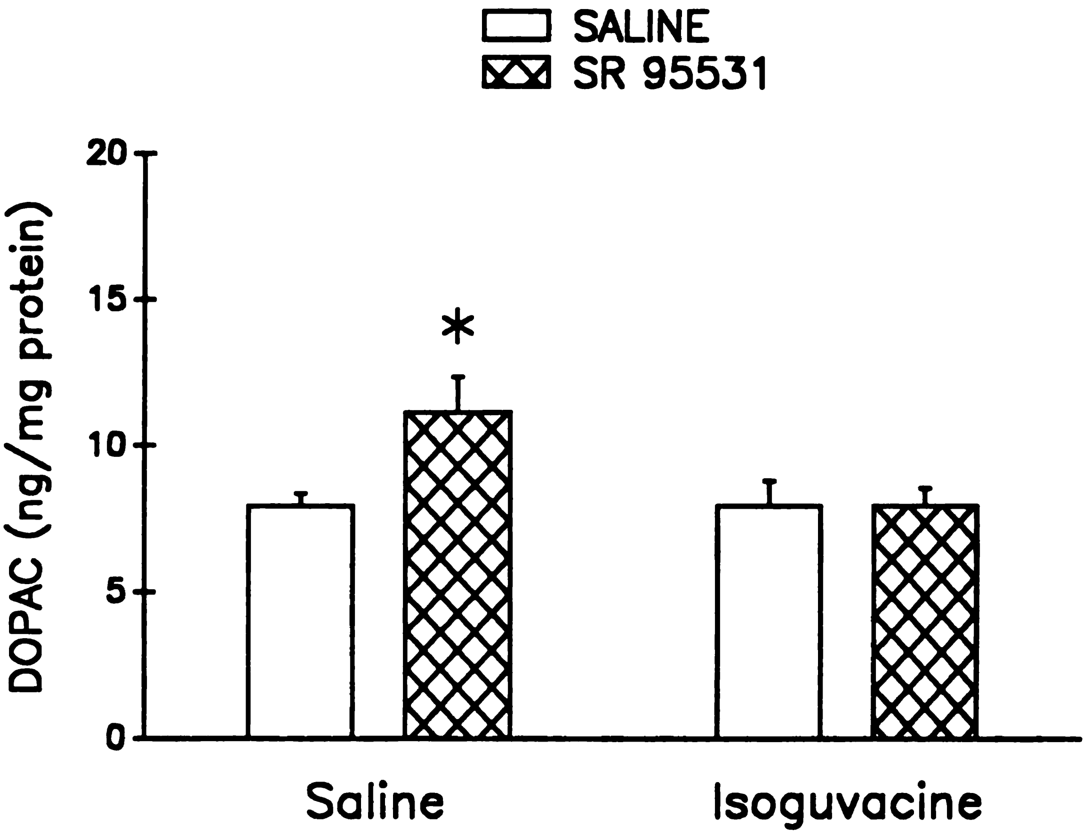

Figure 5.3 The effects of SR 95531 on DOPAC concentrations in the median eminence of isoguvacine pre-treated male rats. Rats were injected with either isoguvacine (10 µg/rat; i.c.v.) or 0.9% saline (5 µl/rat; i.c.v.) 30 min, and with either SR 95531 (3 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 15 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence from 6-9 rats. *, Values from rats treated with SR 95531 (cross-hatched columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns).

nor isoguvacine had any effect on either DOPAC concentrations in the intermediate lobe or α-MSH concentrations in plasma (Tables 5.3 & 5.4).

As shown in Figure 5.4, the $GABA_B$ receptor agonist baclofen decreased DOPAC concentrations in the median eminence, and increased plasma prolactin concentrations, in a dose-dependent manner. The $GABA_B$ receptor antagonist 2-hydroxysaclofen had no effect on DOPAC concentrations in the median eminence (Table 5.5), but did block the baclofen-induced decrease in DOPAC concentrations in the median eminence and the corresponding increase in prolactin concentrations in plasma (Figure 5.5).

Discussion

Taken together, the results of these experiments indicate that endogenous GABA tonically inhibits TIDA but not PHDA neurons in male rats through an action at $GABA_A$ rather than $GABA_B$ receptors. This conclusion is based on the observations that blockade of $GABA_A$ receptors with SR 95531 increases DOPAC concentrations in the median eminence but not the intermediate lobe, and this effect is counteracted by pre-treatment with the $GABA_A$ receptor agonist isoguvacine. No such increase in median eminence DOPAC concentrations is elicited by administration of the $GABA_B$ receptor antagonist 2-hydroxysaclofen.

Over the range of doses employed in the present study (3-30 μg/rat), $GABA_A$ receptor activation by central

Table 5.3 Lack of effect of SR 95531 on DOPAC concentrations in the intermediate lobe and on α-MSH concentrations in plasma of male rats.

SR 95531 (mg/kg)	DOPAC	α-MSH
0	1.25±0.03	121.5±22.7
0.3	1.10±0.06	138.4±12.3
1.0	1.37±0.09	142.1±15.3
3.0	1.11±0.09	116.2±14.1

Rats were injected with either SR 95531 (0.3, 1.0 or 3.0 mg/kg; i.p.) or 0.9% saline (1 ml/kg; i.p.) and killed by decapitation 15 min later. Values represent means ± 1 S.E.M. of DOPAC concentrations in the intermediate lobe, and of α-MSH concentrations in plasma, from 6-9 rats.

Table 5.4 Lack of effect of isoguvacine on DOPAC concentrations in the intermediate lobe and on α-MSH concentrations in plasma of male rats.

Isoguvacine (μg/rat)	DOPAC	α-MSH
0	1.35±0.06	137.8±20.3
3	1.39±0.10	137.8±23.3
10	1.49±0.13	196.7±20.2
30	1.37±0.13	199.7±26.5

Rats were injected with either isoguvacine (3, 10 or 30 μg/rat; i.c.v.) or 0.9% saline (5 μl/rat; i.c.v.) and killed by decapitation 30 min later. Values represent means ± 1 S.E.M. of DOPAC concentrations in the intermediate lobe, and of α-MSH concentrations in plasma, from 7-8 rats.

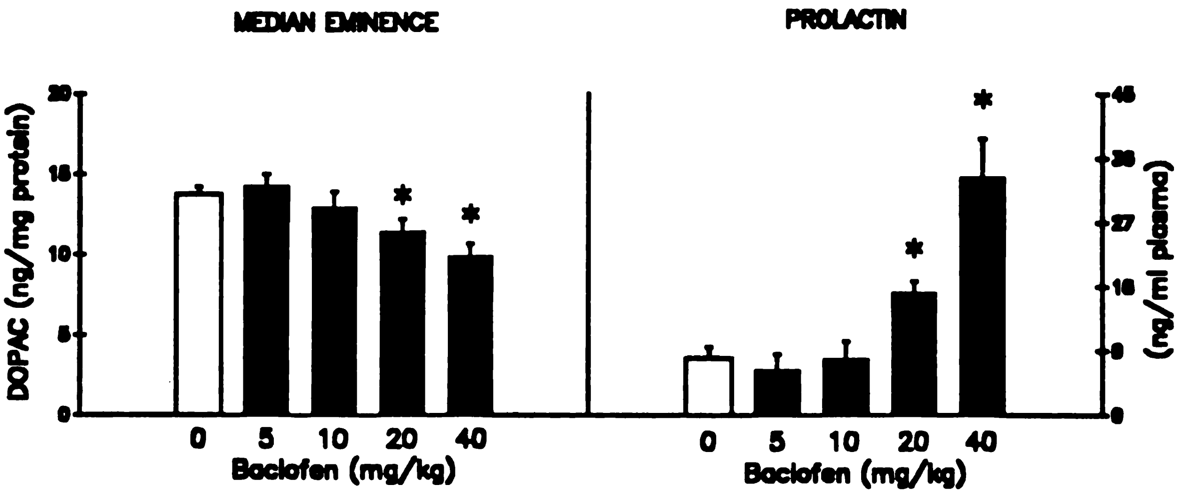

Figure 5.4 Dose-response effects of the $GABA_B$ receptor agonist baclofen on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of male rats. Rats were injected with either baclofen (5, 10, 20 or 40 mg/kg; i.p.) or 0.9% saline (2 ml/kg; i.p.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in the plasma from 6-8 rats. *, Values from rats treated with baclofen (solid columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

Table 5.5 Lack of effect of the $GABA_B$ receptor antagonist 2-hydroxysaclofen on DOPAC concentrations in the median eminence in male rats.

2-Hydroxysaclofen (μg/rat)	DOPAC (ng/mg protein)
0	11.70±0.89
10	9.96±0.79
30	11.77±0.78
100	13.77±1.01

Rats were injected with either 2-hydroxysaclofen (10, 30 or 100 μg/rat; i.c.v.) or its neutralized 0.1N NaOH vehicle (5 μl/rat; i.c.v.) and killed by decapitation 30 min later. Values represent means ± 1 S.E.M. of DOPAC concentrations in the median eminence from 7-8 rats.

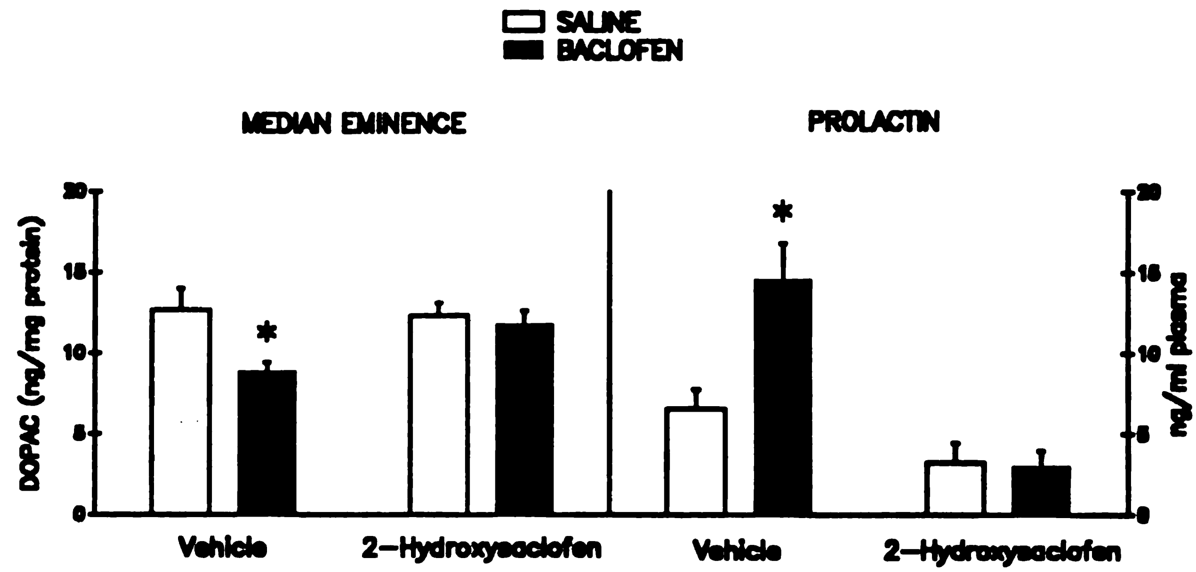

Figure 5.5 Effects of baclofen on DOPAC concentrations in the median eminence and prolactin concentrations in the plasma of 2-hydroxysaclofen-treated male rats. Rats were injected with either baclofen (20 mg/kg; i.p.) or 0.9% saline (2 ml/kg; i.p.) 60 min, and with either 2-hydroxysaclofen (100 μg/rat; i.c.v.) or its neutralized 0.1N NaOH vehicle (5 μl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in the plasma from 7-8 rats. *, Values from baclofen-treated rats (solid columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns).

administration of isoguvacine has no effect on TIDA neurons. A comparable dose of centrally administered isoguvacine (10 μg/rat) has been shown previously to produce a $GABA_A$ receptor-mediated hypothermia (Zarrindast and Oveissi, 1988). That the administration of exogenous agonist cannot inhibit TIDA neurons to an appreciable extent suggests that the $GABA_A$ receptor-mediated tonic inhibition of these neurons by endogenous GABA is maximal.

Consistent with observations that GABA and $GABA_A$ receptor agonists function as prolactin inhibiting factors (Schally et al., 1977; Locatelli et al., 1978; Casanueva et al., 1981; Anderson and Mitchell, 1986), $GABA_A$ receptor activation following central administration of isoguvacine produces a delayed decrease in prolactin secretion. This may be due to redistribution of the agonist from the brain to the peripheral circulation, where it eventually would have access to $GABA_A$ receptors on lactotrophs (Grandison and Guidotti, 1979) and thereby directly inhibit the secretion of prolactin. Indeed, previous reports have demonstrated an inhibitory effect of $GABA_A$ receptor activation on prolactin secretion from the anterior pituitary *in vitro* (Grandison and Guidotti, 1979; Locatelli et al., 1979; Grossmann et al., 1981; Anderson and Mitchell, 1986).

On the other hand, inhibition of prolactin secretion following $GABA_A$ receptor blockade with SR 95531 most likely is due to disruption of the $GABA_A$ receptor-mediated tonic inhibition of TIDA neurons by endogenous GABA. In view of the fact that prolactin secretion is not increased following the $GABA_A$ receptor blockade, endogenous GABA acting at $GABA_A$ receptors does not appear to tonically inhibit the secretion of prolactin. If this were the case, then the inhibitory effect of increased TIDA neuronal activity on prolactin secretion should be overcome by blocking the tonic inhibitory effects of endogenous GABA at the anterior pituitary. SR 95531 also might activate tuberoinfundibular GABAergic neurons, providing further inhibitory input to the lactotroph. While these neurons are responsive to stimuli such as prolactin (Kolbinger et al., 1992), dopamine is released preferentially over GABA in response to prolactin and other stimuli such as high potassium (Felman and Tappaz, 1990). It would appear, therefore, that dopamine released from TIDA neurons plays the predominant role in tonically inhibiting the secretion of prolactin. Moreover, endogenous GABA tonically stimulates rather than inhibits the secretion of prolactin through a $GABA_A$ receptor-mediated tonic inhibition of TIDA neurons.

Although in the present study the site of action of SR 95531 is not addressed specifically, several lines of evidence suggest that it might be acting at the level of the mediobasal hypothalamus. GABAergic nerve terminals make synaptic contact with neuronal perikarya in the arcuate nucleus, including those identified as GABAergic (Decavel and van den Pol, 1992), which also might very well contain dopamine considering the extensive colocalization of the two neurotransmitters in tuberoinfundibular neurons (Everitt et al., 1984; Schimchowitsch et al., 1991). Furthermore, in the hypothalamic slice preparation the $GABA_A$ receptor antagonist bicuculline abolishes spontaneous postsynaptic potentials in whole cell recordings from neurons in the arcuate nucleus (Loose et al., 1991). These findings suggest that the $GABA_A$ receptors activated by endogenous GABA to tonically inhibit TIDA neurons, and blocked by SR 95531 to activate TIDA neurons, might be located in the soma/dendritic regions of these neurons. On the other hand, $GABA_A$ receptor activation inhibits potassium-stimulated ^{3}[H]-dopamine release from median eminence synaptosomes (Anderson and Mitchell, 1985), suggesting the presence of functional $GABA_A$ receptors in the terminal regions of TIDA neurons. While endogenous GABA does not appear to tonically inhibit the secretion of prolactin, it is possible that GABA is released into the median eminence to tonically inhibit the release of dopamine from TIDA nerve terminals.

In contrast to isoguvacine, the $GABA_B$ receptor agonist baclofen inhibits TIDA neurons, and thereby increases the secretion of prolactin. This is consistent with previous reports demonstrating that baclofen reduces the α-methyltyrosine-induced decline of dopamine in the median eminence (Demarest and Moore, 1979b), hyperpolarizes neurons in electrophysiological recordings from the arcuate nucleus (Loose et al., 1991; Lin et al., 1993), and increases prolactin secretion (Ravitz and Moore, 1977). This indicates the presence of functional $GABA_B$ receptors in the mediobasal hypothalamus. The capacity for $GABA_B$ receptors to inhibit TIDA neurons may provide the basis for the stimulatory effects of central administration of both GABA and the mixed GABA receptor agonist muscimol (Bowery, 1993) on prolactin secretion (Mioduszewski et al., 1975; Vijayan and McCann, 1978; Casanueva et al., 1981). At doses ranging from 10-100 μg/rat, administration of 2-hydroxysaclofen does not activate TIDA neurons, however, suggesting that these receptors are not tonically activated by endogenous GABA. A 100 μg/rat dose of 2-hydroxysaclofen, which has been shown previously to stimulate luteinizing hormone secretion under negative feedback conditions in ovariectomized, estrogen-primed female rats (Akema and Kimura, 1991), is sufficient to block the inhibitory effects of baclofen on TIDA neurons, thus demonstrating its effectiveness as a $GABA_B$ receptor antagonist in this paradigm. These effects of $GABA_B$ receptor activation and blockade on TIDA neurons are identical to those observed for PHDA neurons (Goudreau et al., 1993b). The lack of tonic regulation of TIDA neurons under basal conditions by endogenous GABA acting through $GABA_B$ receptors is consistent with the contention that $GABA_B$ receptors assume a modulatory rather than a tonic role in regulating many physiological (e.g., control of spinal reflexes) and pathological states (e.g., spasticity, stroke, epilepsy; Bowery, 1989; Wojcik and Holopainen, 1992).

In summary, these experiments provide evidence that while

$GABA_B$ receptors have the capacity to inhibit TIDA neurons, these neurons are tonically inhibited by endogenous GABA acting via $GABA_A$ but not $GABA_B$ receptors.

6. EVIDENCE THAT THE AMPA RECEPTOR-MEDIATED TONIC INHIBITION OF TIDA AND PHDA NEURONS OCCURS VIA INHIBITORY INTERMEDIARIES

Introduction

In Chapter 4, it was demonstrated that blockade of AMPA receptors with the antagonist NBQX (Sheardown et al., 1990) activates TIDA and PHDA neurons in both male and female rats. This suggests that endogenous EAA neurotransmitters acting at AMPA receptors tonically inhibit these neurons. Indeed, there is an appreciable density of AMPA receptors in arcuate and periventricular nuclei (Petralia and Wenthold, 1992). While glutamatergic nerve terminals make synaptic contact with neuronal perikarya in the arcuate nucleus (Decavel and van den Pol, 1992), it is unlikely that glutamatergic neurons which tonically inhibit TIDA and PHDA neurons synapse directly on these neurons. Rather, the AMPA receptor-mediated tonic inhibition of TIDA and PHDA neurons most likely occurs via inhibitory intermediaries released from presumptive interneurons.

Two such candidates are GABA and the endogenous κ-opioid peptide dynorphin. GABAergic neurons make synaptic contact with neuronal perikarya in the arcuate nucleus (Decavel and van den Pol, 1992), and endogenous GABA acting at $GABA_A$ receptors tonically inhibits TIDA but not PHDA neurons in male rats (see Chapter 5). Similarly, dynorphin-containing nerve terminals make direct synaptic contact with TIDA and PHDA neuronal perikarya (Fitzsimmons et al., 1992), and endogenous dynorphin tonically inhibits TIDA neurons in male but not female rats (Manzanares et al., 1992c; Wagner et al., 1994), and PHDA neurons in male rats (Manzanares et al., 1992c).

The purpose of the present study was to address the hypothesis that the AMPA receptor-mediated tonic inhibition of TIDA and PHDA neurons by endogenous EAA neurotransmitters occurs through inhibitory interneurons. To this end, the ability of the $GABA_A$ receptor agonist isoguvacine (Krogsgaard-Larsen et al., 1977) to prevent the NBQX-induced activation of TIDA and PHDA neurons was evaluated in male and female rats. Likewise, the ability of the κ-opioid receptor agonist U-50,488 (Von Voigtlander et al., 1983) to prevent the NBQX-induced activation of these neurons was evaluated in male rats.

Results

As shown in Figure 6.1, administration of the $GABA_A$ receptor agonist isoguvacine prevented the NBQX-induced increase in median eminence DOPAC concentrations, and the corresponding decrease in plasma prolactin concentrations, in male rats. In contrast, isoguvacine did not prevent the NBQX-induced increase in intermediate lobe DOPAC concentrations (Table 6.1). On the other hand, the κ-opioid receptor agonist U-50,488 had no effect on the NBQX-induced increase in median eminence DOPAC concentrations or the decrease in plasma

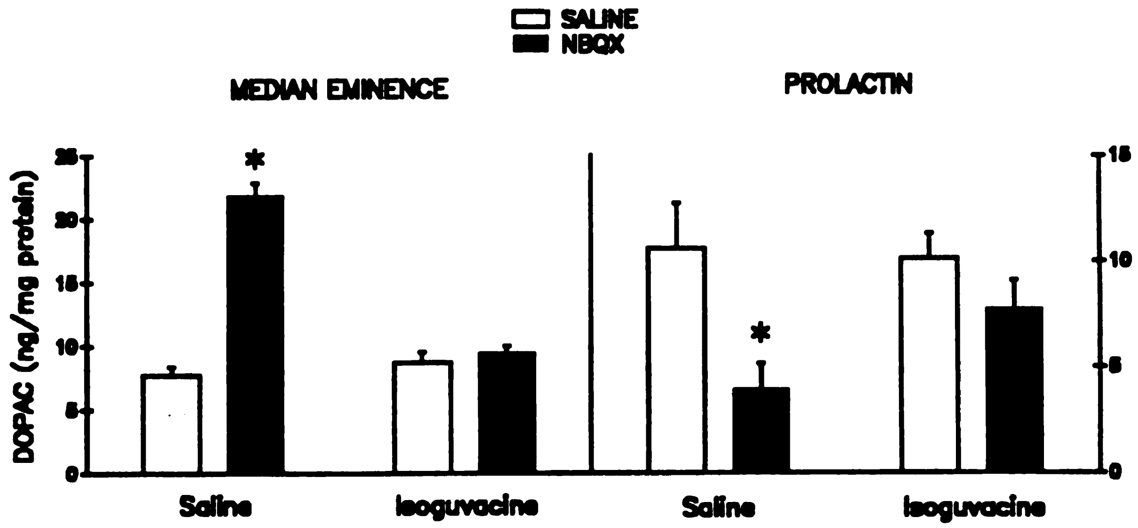

Figure 6.1 Effects of the $GABA_A$ receptor agonist isoguvacine on median eminence DOPAC concentrations and on plasma prolactin concentrations in male rats treated with the AMPA-selective antagonist NBQX. Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with either isoguvacine (10 µg/rat; i.c.v.) or 0.9% saline (5 µl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of median eminence DOPAC concentrations and of plasma prolactin concentrations from 5-8 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (two-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

Table 6.1 Lack of effect of isoguvacine on the NBQX-induced increase in intermediate lobe DOPAC concentrations in male rats.

GROUP	DOPAC (ng/mg protein)
SALINE + SALINE	1.24±0.07
SALINE + ISOGUVACINE	1.26±0.05
NBQX + SALINE	1.46±0.09*
NBQX + ISOGUVACINE	1.42±0.05*

Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with either isoguvacine (10 μg/rat; i.c.v.) or 0.9% saline (5 μl/rat; i.c.v.) 30 min prior to decapitation. Values represent means ± 1 S.E.M. of intermediate lobe DOPAC concentrations from 7-8 rats. *, Values from rats treated with NBQX which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls.

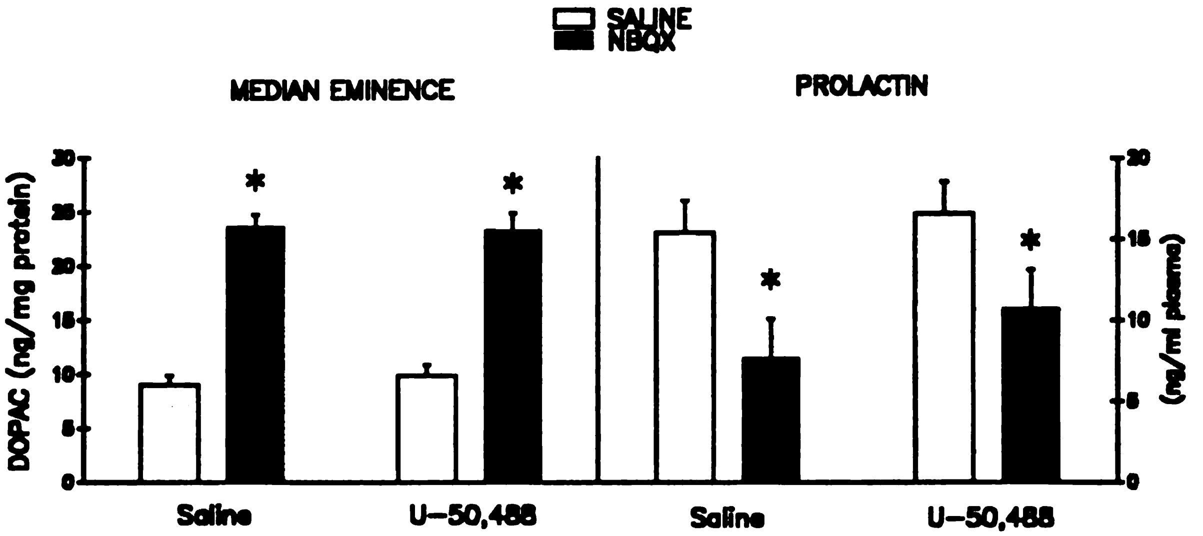

Figure 6.2 Effects of the κ-opioid receptor agonist U-50,488 on median eminence DOPAC concentrations and on plasma prolactin concentrations in NBQX-treated male rats. Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.), and with either U-50,488 (5 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence and of prolactin concentrations in plasma from 6-9 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns).

prolactin concentrations (Figure 6.2), but did attenuate the NBQX-induced increase in intermediate lobe DOPAC concentrations and the decrease in plasma α-MSH concentrations (Figure 6.3).

While endogenous GABA tonically inhibits TIDA, but not PHDA neurons in male rats through an action at $GABA_A$ receptors (see Chapter 5), this effect has yet to be established in female rats. It was deemed worthwhile to examine the dose-response effects of the $GABA_A$ receptor antagonist SR 95531 (Wermuth and Bizière, 1986) on TIDA and PHDA neurons in female rats. SR 95531 produced a dose-dependent increase in DOPAC concentrations in the median eminence (Figure 6.4) but not in the intermediate lobe (Table 6.2), indicating that endogenous GABA acting at $GABA_A$ receptors tonically inhibits TIDA but not PHDA neurons in female rats.

NBQX activates TIDA neurons in both male and female rats. In view of numerous reports of sexual differences in the regulation and basal activity of these neurons (Gudelsky and Porter, 1981; Demarest et al., 1985b; Gunnet et al., 1986; Manzanares et al., 1992b), it was necessary to confirm expermentally if isoguvacine could also prevent the NBQX-induced activation of TIDA neurons in female rats. As illustrated in Figure 6.5, the same dose of isoguvacine that was used in the male rat (10 μg/rat) attenuated the NBQX-induced increase in median eminence DOPAC concentrations in female rats. As was true for male rats, isoguvacine had no

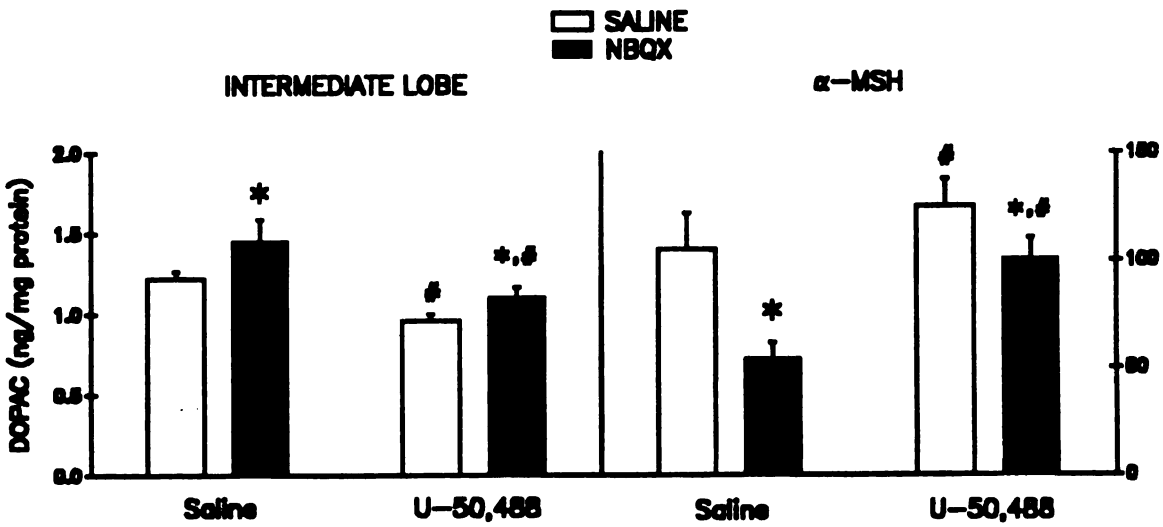

Figure 6.3 Effects of U-50,488 on intermediate lobe DOPAC concentrations and on plasma α-MSH concentrations in NBQX-treated male rats. Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.), and with either U-50,488 (5 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 60 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the intermediate lobe and of α-MSH concentrations in plasma from 7-9 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns). #, Values from rats treated with U-50,488 which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls.

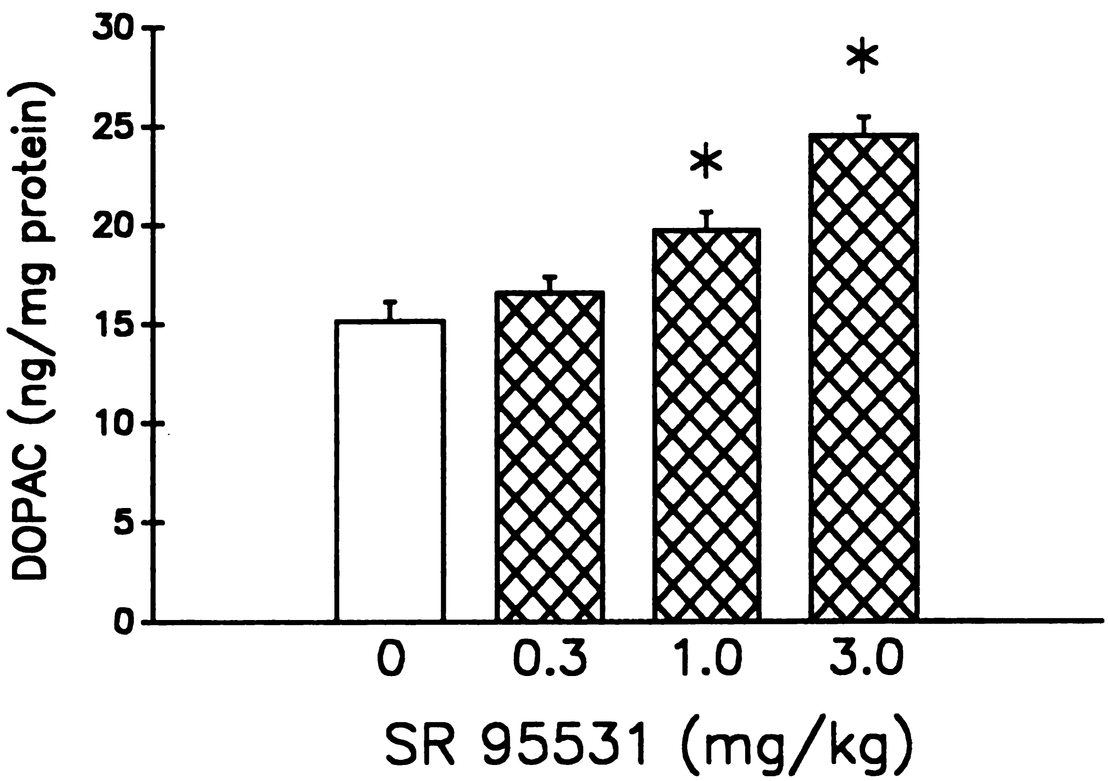

Figure 6.4 Dose-response effects of the $GABA_A$ receptor antagonist SR 95531 on median eminence DOPAC concentrations in female rats. Rats were injected with either SR 95531 (0.3, 1.0 or 3.0 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 15 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence from 8-9 rats. *, Values from rats treated with SR 95531 (cross-hatched columns) which are significantly different (one-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

Table 6.2 Lack of effect of SR 95531 on intermediate lobe DOPAC concentrations in female rats.

SR 95531 (mg/kg)	DOPAC (ng/mg protein)
0	1.43±0.11
0.3	1.53±0.11
1.0	1.43±0.11
3.0	1.42±0.12

Rats were injected with either SR 95531 (0.3, 1.0 or 3.0 mg/kg; s.c.) or 0.9% saline (1 ml/kg; s.c.) 15 min prior to decapitation. Values represent means ± 1 S.E.M. of DOPAC concentrations in the intermediate lobe from 7-9 rats.

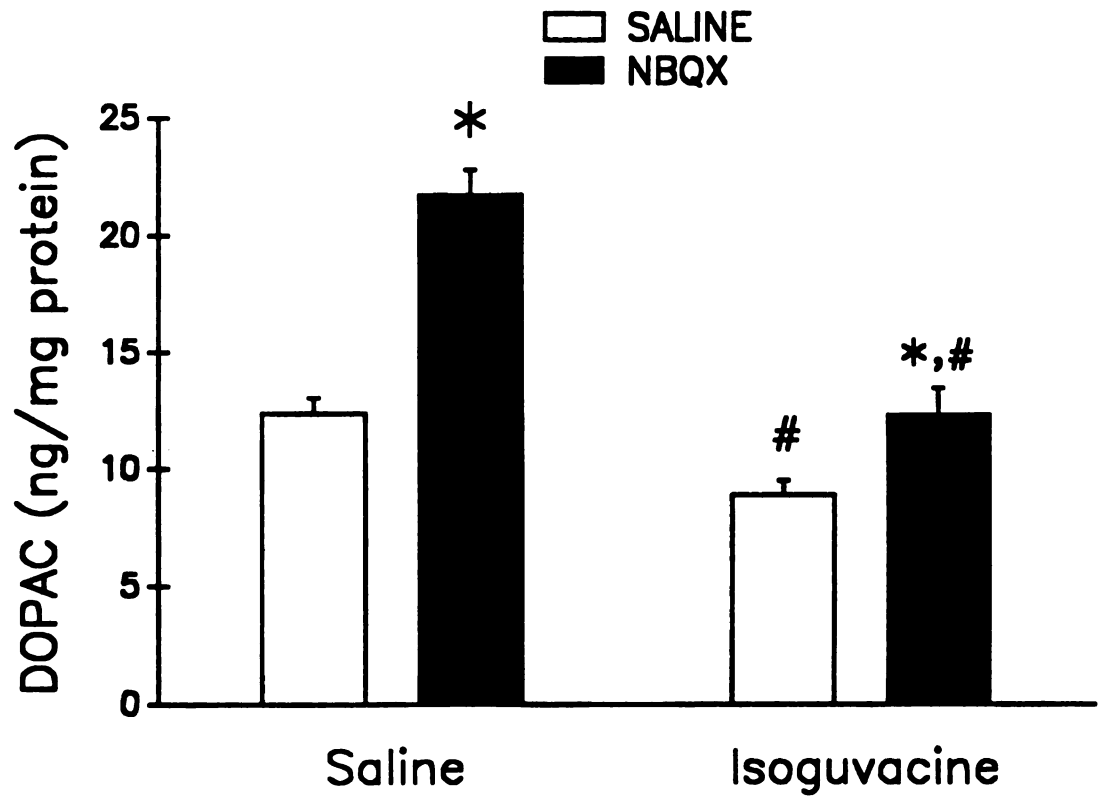

Figure 6.5 Effects of isoguvacine (10 µg/rat) on median eminence DOPAC concentrations in NBQX-treated female rats. Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with either isoguvacine (i.c.v.) or 0.9 saline (5 µl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence from 8-9 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls (open columns). #, Values from rats treated with isoguvacine which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls.

effect on the NBQX-induced increase in intermediate lobe DOPAC concentrations in female rats (Table 6.3).

The inability of isoguvacine to prevent completely the NBQX-induced activation of TIDA neurons in female rats could be attributed to an insufficient dose of the agonist. This could be rectified simply by increasing the dose of isoguvacine. Alternatively, there might be an additional inhibitory mechanism involved in the female rat, in which case increasing the dose of isoguvacine should not dampen further the NBQX-induced activation of TIDA neurons. To address these possibilities, the ability of a 30 μg/rat dose of isoguvacine to counteract the NBQX-induced activation of TIDA was evaluated in female rats. As depicted in Figure 6.6, high-dose isoguvacine counteracted fully the NBQX-induced increase in median eminence DOPAC concentrations.

Discussion

The results of this study indicate that activation of TIDA and PHDA neurons following blockade of AMPA receptors in male and female rats arises from a disinhibition of these neurons. That is, AMPA receptor antagonism disrupts the tonic stimulatory effects of endogenous EAA neurotransmitters on GABAergic interneurons impinging on TIDA neurons, thereby disrupting the $GABA_A$ receptor-mediated tonic inhibition which, in effect, activates these neurons. This conclusion is based on the observations that NBQX increases DOPAC concentrations in the median eminence of male and female rats, the region

Table 6.3 Lack of effect of isoguvacine on the NBQX-induced increase in intermediate lobe DOPAC concentrations in female rats.

GROUP	DOPAC (ng/mg protein)
SALINE + SALINE	1.45±0.10
SALINE + ISOGUVACINE	1.22±0.08
NBQX + SALINE	1.76±0.14*
NBQX + ISOGUVACINE	1.66±0.15*

Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with either isoguvacine (10 µg/rat; i.c.v.) or 0.9% saline (5 µl/rat; i.c.v.) 30 min prior to decapitation. Values represent means ± 1 S.E.M. of intermediate lobe DOPAC concentrations from 8-9 rats. *, Values from rats treated with NBQX which are significantly different (two-way ANOVA/LSD; $p<.05$) from saline-treated controls.

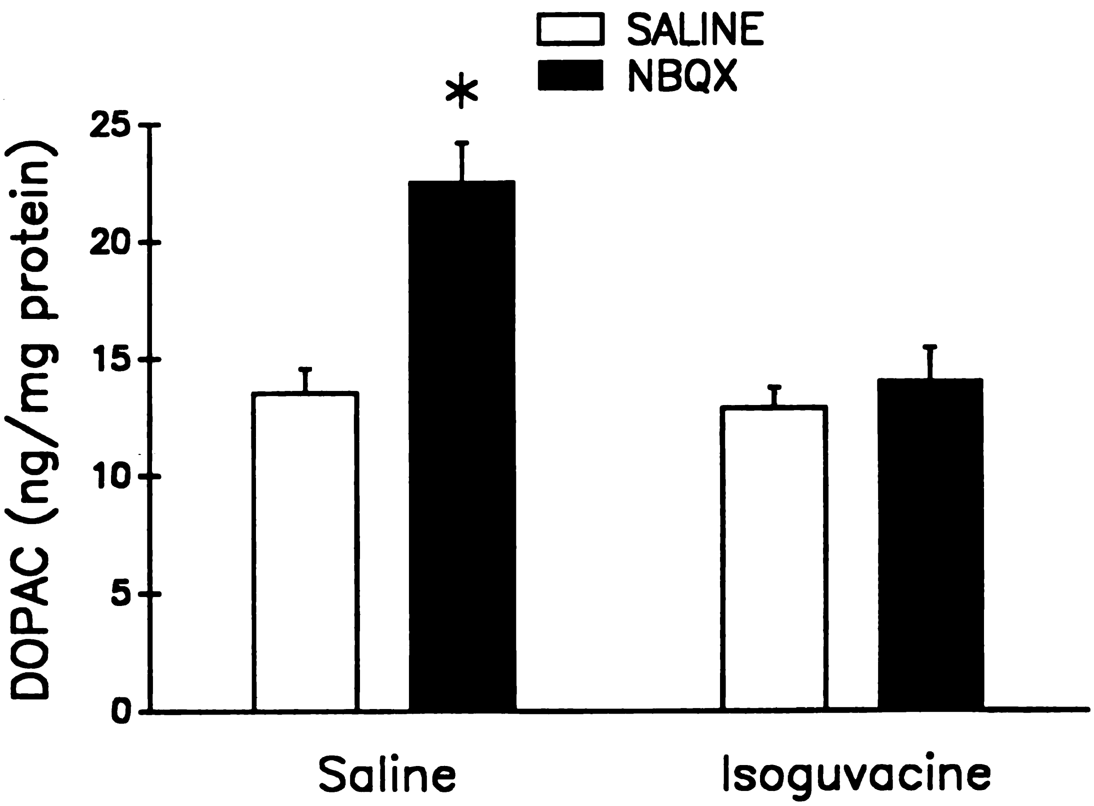

Figure 6.6 Effects of isoguvacine (30 μg/rat) on median eminence DOPAC concentrations in NBQX-treated female rats. Rats were injected with either NBQX (20 mg/kg; i.p.) or 0.9% saline (2.5 ml/kg; i.p.) 60 min, and with either isoguvacine (i.c.v.) or 0.9 saline (5 μl/rat; i.c.v.) 30 min prior to decapitation. Columns represent means and vertical lines 1 S.E.M. of DOPAC concentrations in the median eminence from 7-8 rats. *, Values from rats treated with NBQX (solid columns) which are significantly different (two-way ANOVA/LSD; p<.05) from saline-treated controls (open columns).

containing the nerve terminals of TIDA neurons, and this effect is counteracted by activation of $GABA_A$ receptors with isoguvacine.

The ability of EAAs to elicit GABA release in other neuronal systems has been documented. For example, *in vitro* studies have shown that AMPA and NMDA evoke the release of GABA from rat cortical neurons (Erdo et al., 1993), and NMDA evokes the release of GABA from mouse striatal neurons (Jouanen et al., 1991). Stimulation of GABA release from striatal interneurons by endogenous EAA neurotransmitters is thought to play a role in the proposed NMDA receptor-mediated tonic inhibition of nigrostriatal dopaminergic neurons (Cheramy et al., 1986; Leviel et al., 1990).

This evidence for a putative hierarchal circuit by which endogenous EAA neurotransmitters activate AMPA receptors to tonically stimulate GABAergic interneurons that, in turn, tonically inhibit TIDA neurons is supported both by anatomical and functional studies. In the arcuate nucleus, a significant AMPA receptor density has been observed (Petralia and Wenthold, 1992), and glutamatergic nerve terminals have been shown to make synaptic contact with GABAergic neuronal perikarya (Decavel and van den Pol, 1992). GABAergic nerve terminals also make synaptic contact with GABAergic neuronal perikarya in the arcuate nucleus (Decavel and van den Pol, 1992), which may serve as a marker for TIDA neuronal perikarya considering the extensive colocalization of GABA and dopamine in tuberoinfundibular neurons (Everitt et al., 1984;

Schimchowitsch et al., 1991).

Furthermore, endogenous GABA acting at $GABA_A$ receptors is purported to tonically inhibit TIDA neurons in male rats (see Chapter 5). In the present study this has been substantiated by the observed stimulatory effect of $GABA_A$ receptor blockade with SR 95531 on these neurons in female rats, and demonstrates that the $GABA_A$ receptor-mediated tonic inhibition of TIDA neurons is not sexually differentiated. A higher dose of isoguvacine, however, is required in female rats to negate completely the NBQX-induced increase in TIDA neuronal activity. It is possible that subtle differences in the sensitivity of TIDA neurons to the tonic inhibition by this putative hierarchal pathway exist between male and female rats.

Although activation of $GABA_B$ receptors with the agonist baclofen inhibits TIDA neurons, endogenous GABA does not activate these receptors to tonically inhibit TIDA neurons (see Chapter 5). This suggests that $GABA_B$ receptors have the capacity to inhibit TIDA neurons, and is consistent with the proposed neuromodulatory role of these receptors (Wojcik and Holopainen, 1992). Activation of $GABA_B$ receptors by endogenous GABA may play a relevant role in inhibiting TIDA neurons under certain physiological conditions such as stress (Demarest et al., 1985b). Indeed, endogenous GABA acting at $GABA_B$ receptors is an important mediator of the inhibitory effect of stress on PHDA neurons (Goudreau et al., submitted). GABA is believed to be equipotent in its affinity for $GABA_A$

and $GABA_B$ receptors (Bowery, 1993). It is likely, therefore, that $GABA_A$ receptor-mediated tonic inhibition of TIDA neurons, and $GABA_B$ receptor-mediated modulation of these neurons, arise from distinct subpopulations of GABAergic neurons as has been proposed for other extrahypothalamic neuronal systems in the lateral amygdala and the ventral tegmental area (Sugita et al., 1992).

On the other hand, dynorphin-containing nerve terminals make direct synaptic contact with TIDA neuronal perikarya (Fitzsimmons et al., 1992). This tonic inhibition of TIDA neurons, however, is sexually differentiated in that dynorphin inhibits these neurons in male but not female rats (Manzanares et al., 1992c; Wagner et al., 1994). In view of the fact that the AMPA receptor-mediated tonic inhibition of TIDA is present in both male and female rats, it is improbable that stimulation of dynorphin-containing neurons by endogenous EAA neurotransmitters would account for this phenomenon. This is consistent with the lack of effect of κ-opioid receptor activation with U-50,488 on the NBQX-induced stimulation of TIDA neurons observed in the present study.

In contrast to TIDA neurons, the tonic inhibition of PHDA neurons by endogenous EAA neurotransmitters acting at AMPA receptors is mediated through dynorphinergic rather than GABAergic interneurons. This conclusion is based on the observation that NBQX increases DOPAC concentrations in the intermediate lobe, which contains the nerve terminals of PHDA neurons, and this effect is attenuated by activation of κ-

opioid receptors with U-50,488. These results are in agreement with reports demonstrating that in the periventricular nucleus, the site of origin of PHDA neurons, there is a prominent AMPA receptor density (Petralia and Wenthold, 1992), and dynorphin-containing nerve terminals make synaptic contact with PHDA neuronal perikarya (Fitzsimmons et al., 1992). There is no evidence to suggest a sexual difference in the κ-opioid receptor-mediated tonic inhibition of PHDA neurons (Manzanares et al., 1993), and it follows that this putative hierarchal circuit likely plays a relevant role in the inhibitory regulation of these neurons in both male and female rats. It has been demonstrated previously that endogenous GABA does not tonically inhibit PHDA neurons in the male rat (see Chapter 5), and in the present study this is extended and confirmed both by the observed lack of effect of $GABA_A$ receptor blockade with SR 95531 on these neurons in female rats, and by the inability of $GABA_A$ receptor activation with isoguvacine to prevent the NBQX-induced stimulation of these neurons in male and female rats.

The tonic stimulation of dynorphin-containing interneurons by endogenous EAA neurotransmitters only partially accounts for the AMPA receptor-mediated tonic inhibition of PHDA neurons. The synaptic contact rate of dynorphin-containing nerve terminals with PHDA neuronal perikarya is relatively low (15% as compared with 70% observed for TIDA neuronal perikarya; Fitzsimmons et al., 1992), and this may account for the inhibitory effects of post-synaptic

κ-opioid receptor activation with exogenously administered U-50,488 *per se* on these neurons. The dose of U-50,488 employed in the present study (5 mg/kg) has been shown previously to produce the maximal inhibitory effect of the agonist on PHDA neurons (Manzanares et al., 1990). It is unlikely, therefore, that increasing the dose of U-50,488 would attenuate further the NBQX-induced activation of these neurons. It is plausible that endogenous EAA neurotransmitters also act via additional, as-yet-to-be-identified inhibitory intermediary(ies) which, in combination with dynorphin-containing interneurons, fully account for the AMPA receptor-mediated tonic inhibition of PHDA neurons.

In summary, the present study provides evidence that the AMPA receptor-mediated tonic inhibition of hypothalamic dopaminergic neurons occurs ultimately through inhibitory intermediaries. Endogenous EAA neurotransmitters acting at AMPA receptors tonically stimulate GABAergic interneurons, and GABA released from these neurons activates $GABA_A$ receptors to tonically inhibit TIDA neurons. In contrast, endogenous EAA neurotransmitters tonically stimulate κ-opioid-containing interneurons, and dynorphin released from these neurons accounts, in part, for the AMPA receptor-mediated tonic inhibition of PHDA neurons.

7. SUMMARY AND CONCLUSIONS

The studies described in this dissertation provide evidence for an integral role of endogenous amino acid neurotransmitters in regulating the basal activity of hypothalamic dopaminergic neurons. The salient features of this work are summarized as follows.

First, EAA neurotransmitters (i.e., L-glutamate, L-aspartate, D-homocysteine sulfinic acid) act at NMDA receptors to tonically stimulate TIDA neurons in female but not male rats. NMDA receptor-mediated regulation of these neurons is facilitated by estrogen acting in a prolactin-independent fashion. This scenario is illustrated schematically in Figure 7.1. In addition, EAA neurotransmitters acting at these receptors tonically inhibit PHDA neurons in both male and female rats.

Second, EAA neurotransmitters act at non-NMDA receptors to tonically inhibit both TIDA and PHDA neurons in male and female rats. This tonic inhibitory effect is due to the action of EAA neurotransmitters at AMPA rather than kainate receptors.

Third, endogenous GABA tonically inhibits TIDA but not PHDA neurons in both male and female rats. The tonic inhibitory effect of GABA on TIDA neurons is due to its action at $GABA_A$ but not $GABA_B$ receptors.

Finally, the AMPA receptor-mediated tonic inhibition of

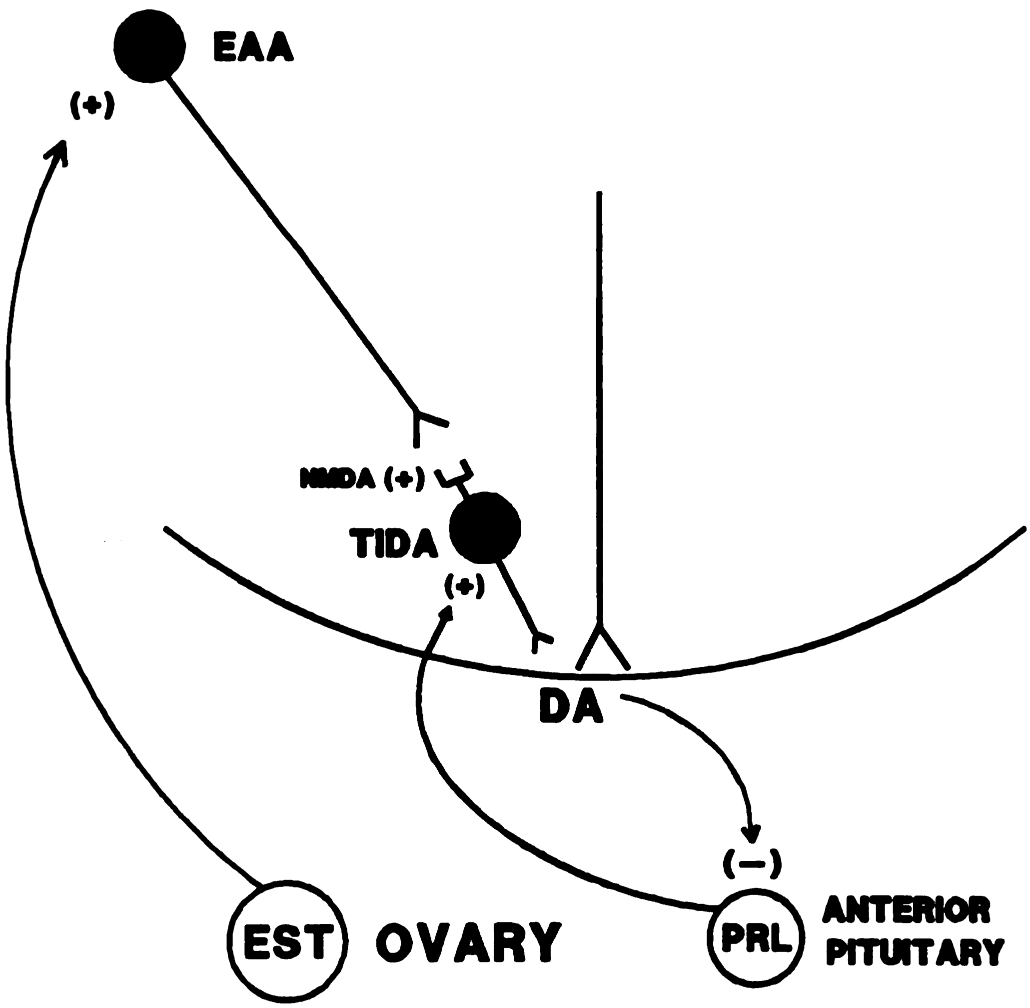

Figure 7.1 Schematic diagram of a coronal section of the hypothalamus illustrating NMDA receptor-mediated regulation of TIDA neurons by EAA neurotransmitters in female rats. DA = dopamine; EST = estrogen; PRL = prolactin

TIDA and PHDA neurons is the result of a tonic stimulation of inhibitory interneurons. These interneurons, in turn, tonically inhibit TIDA and PHDA neurons. As represented schematically in Figure 7.2., EAA neurotransmitters activate AMPA receptors to tonically stimulate GABAergic neurons. GABA released from these neurons activates $GABA_A$ receptors to tonically inhibit TIDA neurons. By contrast, the intermediary responsible, in part, for the AMPA receptor-mediated tonic inhibition of PHDA neurons by EAA neurotransmitters is the endogenous κ-opioid peptide dynorphin and not GABA (Figure 7.3).

In conclusion, these data indicate a dual role of EAA neurotransmitters in regulating TIDA neurons: a sexually differentiated, NMDA receptor-mediated tonic stimulation and an indirect AMPA receptor-mediated tonic inhibition. A dual function of EAA neurotransmitters has been described for the regulation of nigrostriatal dopaminergic neurons (Cheramy et al., 1986; Leviel et al., 1990), but differs from EAA neurotransmitter regulation of TIDA neurons in the following respects: nigrostriatal dopaminergic neurons undergo a NMDA receptor-mediated tonic inhibition (Cheramy et al., 1986; Leviel et al., 1990; Moghaddam and Gruen, 1991) and a AMPA receptor-mediated phasic activation (Leviel et al., 1990).

It would appear that the NMDA receptor-mediated regulation of TIDA neurons by EAA neurotransmitters is due to a direct effect on these neurons. This conclusion is based on

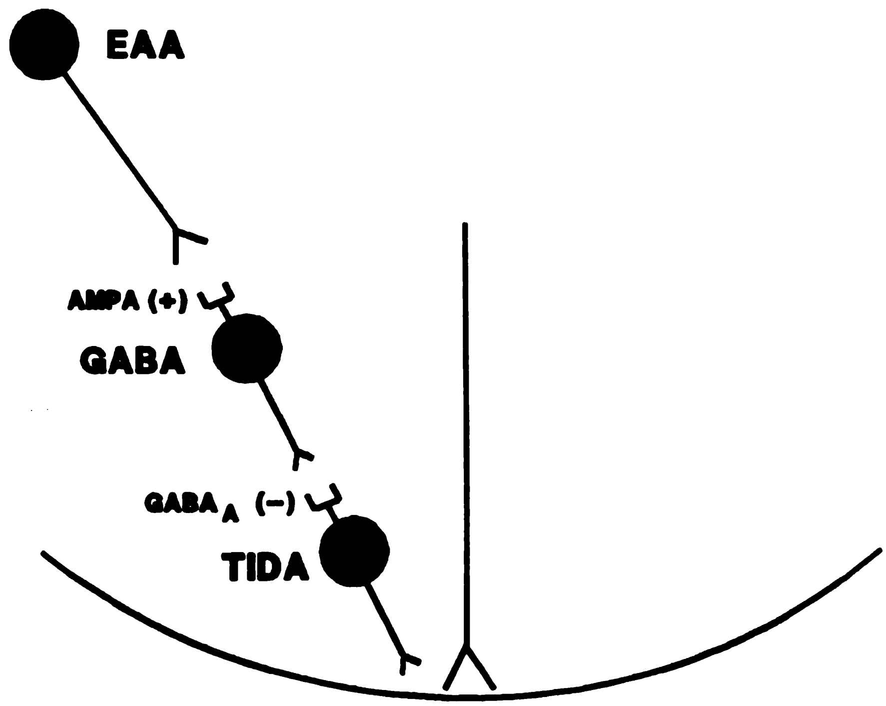

Figure 7.2 Schematic diagram of a coronal section of the hypothalamus representing the pathway involved in AMPA receptor-mediated regulation of TIDA neurons by EAA neurotransmitters in male and female rats.

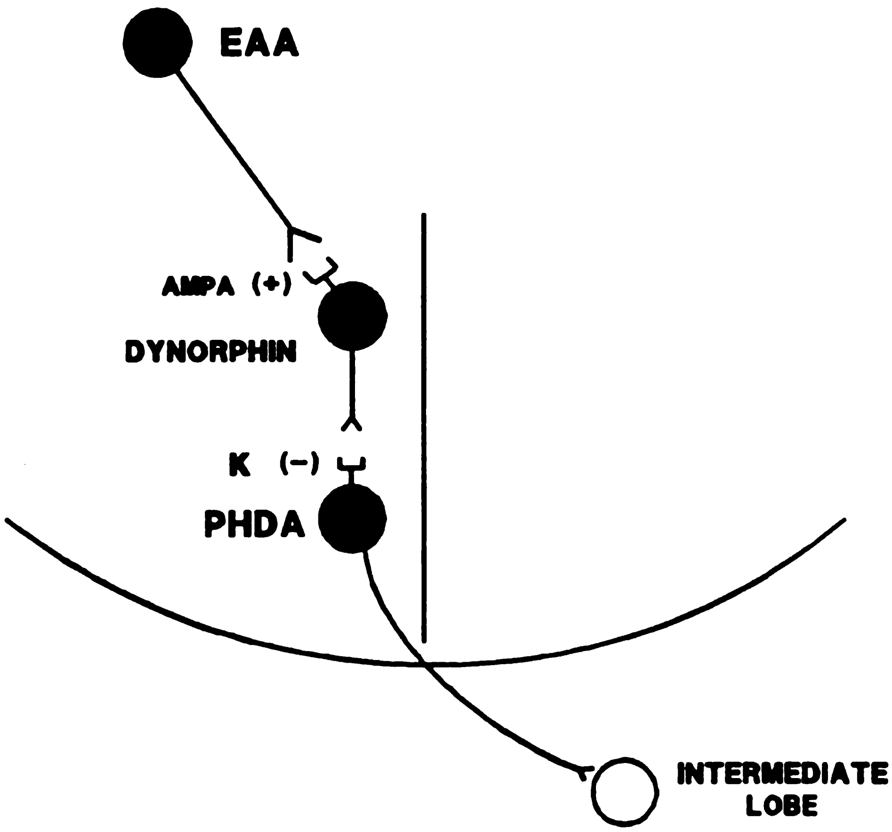

Figure 7.3 Schematic representation of a coronal section through the hypothalamus illustrating the partial pathway involved in AMPA receptor-mediated regulation of PHDA neurons by EAA neurotransmitters in male and female rats. κ = κ-opioid receptor

reports that, despite a modest NMDA receptor density in the mediobasal hypothalamus (Monaghan and Cotman, 1985; Maragos et al., 1988; Gibson et al., 1992), administration of high-dose monosodium glutamate to neonatal rodents or N-methyl aspartate to adult rodents produces an excitotoxic lesion of the arcuate nucleus (Olney, 1969; Nemeroff et al., 1977; Olney and Price, 1983). In addition, NMDA elicits neurosecretion from the mediobasal hypothalamus *in vitro* (Bourguignon et al., 1989; Donoso et al., 1990). These findings demonstrate the presence of functionally relevant NMDA receptors in the mediobasal hypothalamus, which arguably mediate the tonic stimulation of TIDA neurons by EAA neurotransmitters.

NMDA receptors are subject to voltage-dependent channel blockade by Mg^{2+} ions, which becomes prominent with increasingly negative neuronal membrane potentials (Collingridge and Lester, 1989). In the hypothalamic slice preparation, TIDA neurons have a resting membrane potential of -60 mV (Loose et al., 1990). If, under *in vivo* conditions, the resting membrane potential of these neurons approximates that observed *in vitro*, then the Mg^{2+} blockade of the NMDA receptor channel should be substantial. It follows that in order for NMDA receptor activation by endogenous EAA neurotransmitters to tonically stimulate TIDA neurons, there must be additional tonic stimulatory components which depolarize TIDA neurons and relieve the NMDA receptor channel of the Mg^{2+} blockade. The inhibitory effect of NMDA receptor blockade on TIDA neurons is not dependent on the tonic stimulatory effects of endogenous prolactin, suggesting that prolactin does not facilitate the NMDA receptor-mediated tonic stimulation of these neurons. On the other hand, estrogen does facilitate the NMDA receptor-mediated regulation of TIDA neurons. In addition, estrogen antagonizes the potent stimulatory effects of exogenously administered bombesin on TIDA neurons in female rats (Toney et al., 1992a), which may be related to a potential permissive role of estrogen in enabling endogenous mammalian analogues of bombesin (e.g., gastrin-releasing peptide; Brown et al., 1980) within the brain to tonically stimulate these neurons. Bombesin has been shown to excite neurons of the arcuate nucleus observed during electrophysiological recording from a brain slice preparation (Pan et al., 1992). Perhaps gastrin-releasing peptide provides the depolarization of TIDA neurons necessary to relieve the Mg^{2+} blockade of the NMDA receptor ionophore, thereby allowing the NMDA receptor-mediated tonic stimulation of these neurons.

The tonic inhibition of TIDA neurons by EAA neurotransmitters acting at AMPA receptors is mediated via a tonic stimulation of GABAergic neurons. The GABA released from these neurons activates $GABA_A$ receptors to tonically inhibit TIDA neurons. While the interpretation offered thus far contends that the GABAergic neurons are intrinsic interneurons, it cannot be ruled out that these neurons might be tuberoinfundibular neurons in which GABA colocalizes with dopamine (Everitt et al., 1984; Schimchowitsch et al., 1991).

Dopamine release from tuberoinfundibular neurons exhibits differential sensitivity to various stimuli as compared with GABA release from these neurons (Felman and Tappaz, 1990). It is conceivable that EAA neurotransmitters tonically stimulate the release of GABA from tuberoinfundibular neurons, which leads to an activation of $GABA_A$ receptors on nerve terminals in the median eminence (Anderson and Mitchell, 1985) and a resultant tonic inhibition of TIDA neurons. It is tempting to speculate that in lieu of pre-effector dopamine autoreceptors, $GABA_A$ receptors may serve as autoreceptors, more appropriately termed heteroreceptors, which provide inhibitory control over dopamine release at the level of the nerve terminal.

Although EAAs are capable of activating NMDA receptors to directly stimulate the secretion of prolactin from the anterior pituitary (Login, 1990), the studies presented in this dissertation provide evidence for additional mechanisms by which EAA neurotransmitters can affect the secretion of prolactin. By virtue of their ability to tonically stimulate TIDA neurons via a NMDA receptor-mediated mechanism, EAA neurotransmitters exert an inhibitory effect on prolactin secretion. These studies also identify another mechanism by which EAA neurotransmitters stimulate prolactin secretion, namely the AMPA receptor-mediated tonic inhibition of TIDA neurons. Thus, not only can EAAs directly stimulate prolactin secretion, but they also modify prolactin secretion by altering the degree of its tonic inhibition by dopamine.

GABA has long been regarded as a prolactin-inhibiting factor (Schally et al., 1977; Locatelli et al., 1978; Casanueva et al., 1981). These studies indicate that the inhibitory effect of endogenous GABA on prolactin secretion is not tonically active. Moreover, endogenous GABA actually affects a tonic stimulation of prolactin secretion, which is attributed to the $GABA_A$ receptor-mediated tonic inhibition of TIDA neurons driven by the AMPA receptor-mediated tonic stimulation of GABAergic neurons. Under certain physiological situations, GABA released from a distinct subpopulation of GABAergic neurons comprising part of a modulatory circuit may also increase prolactin secretion by virtue of its ability to activate $GABA_B$ receptors and thereby inhibit TIDA neurons.

Finally, EAA neurotransmitters play but a single role in regulating PHDA neurons. They tonically inhibit PHDA neurons, and do so by activating both NMDA and AMPA receptors. Studies presented in this dissertation aimed at investigating the mechanism of the AMPA receptor-mediated tonic inhibition of PHDA neurons indicate that it is indirect, and that endogenous κ-opioids are responsible, in part, for this effect.

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