DIFFERENTIAL DRUG EFFECTS ON DOPAMINERGIC NEURONS OF THE RAT BRAIN

Dissertation for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

GARY A. GUDELSKY

1977

This is to certify that the

thesis entitled

DIFFERENTIAL DRUG EFFECTS ON DOPAMINERGIC NEURONS OF THE RAT BRAIN

presented by

Gary A. Gudelsky

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pharmacology

K. E. Moore

Major professor

Date June 29, 1977

ABSTRACT

DIFFERENTIAL DRUG EFFECTS ON DOPAMINERGIC
NEURONS OF THE RAT BRAIN

By

Gary A. Gudelsky

The responses of tuberoinfundibular neurons were compared with those of nigrostriatal and mesolimbic dopaminergic neurons to a number of pharmacological agents and endocrinological manipulations. A sensitive radioenzymatic procedure was used to quantify changes in dopamine concentrations and rates of turnover in the median eminence, corpus striatum and olfactory tubercle, regions containing the terminals of tuberoinfundibular, nigrostriatal and mesolimbic neurons, respectively.

Systemic administration of γ-butyrolactone and baclofen produced a large increase in the dopamine concentration in the striatum and olfactory tubercle; no change was observed in the median eminence. In contrast, the median eminence dopamine concentration was selectively increased 4 weeks after ovariectomy. This effect of ovariectomy was reversed by estradiol administration. These results indicate that regional differences of drug action exist with respect to central dopaminergic neurons. Moreover, these results demonstrate possible hormonal influences on tuberoinfundibular neurons.

Dopamine turnover studies were performed in order to examine possible differences in the regulatory mechanisms governing the

activity of tuberoinfundibular, nigrostriatal and mesolimbic dopaminergic neurons. Haloperidol (0.5 mg/kg, i.p.) increased and piribedil (30 mg/kg, i.p.) decreased dopamine turnover in the striatum and olfactory tubercle within 2 hours, as determined from the α-methyltyrosine-induced decline of the dopamine concentrations. Neither drug altered dopamine turnover in the median eminence. The actions of dopaminergic antagonists and agonists on striatal dopamine metabolism are generally thought to be mediated by a neuronal feedback loop. Therefore, such a neuronal feedback mechanism may not influence the activity of tuberoinfundibular neurons.

Dopamine turnover was increased only in the median eminence 16 and 24 hours after a single subcutaneous injection of haloperidol, 2.5 mg/kg. This action of haloperidol appears to be hormonally mediated since the effect was blocked by hypophysectomy.

Hormonal modulation of the activity of tuberoinfundibular neurons was examined further by observing the effects of estradiol benzoate administration. Three and five daily injections of estradiol benzoate (25 μg/kg) selectively increased dopamine turnover in the median eminence. Similar to the actions of haloperidol, this effect was blocked by hypophysectomy, indicating that the action of estradiol is indirect.

The abilities of haloperidol and estradiol to increase dopamine turnover in the median eminence may be mediated by elevated serum prolactin concentrations, since administration of exogenous prolactin (5 mg/kg, s.c.) also increased dopamine turnover in this brain region. The prolactin-induced increase in the turnover of dopamine in tuberoinfundibular neurons not only represents a hormonal-neuronal feedback

modulation of these neurons but also represents a mechanism by which prolactin may regulate its own secretion.

A hypothalamic island was made with a modified Halász knife in order to determine whether this prolactin mediated feedback mechanism involves extra- or intrahypothalamic neuronal pathways. Norepinephrine concentrations were reduced 50% and dopamine concentrations were unaltered in the median eminence and hypothalamic island 16-33 days after hypothalamic deafferentation. More importantly, hypothalamic deafferentation did not alter the ability of haloperidol to increase dopamine turnover in the median eminence. Thus, prolactin mediated increases in the activity of tuberoinfundibular neurons may result from a direct action of the hormone on these neurons.

In summary, whereas the activity of nigrostriatal and mesolimbic dopamine neurons appears to be modulated, in part, by a rapid neuronal feedback loop, the activity of tuberoinfundibular dopamine neurons appears to be influenced by a sluggish hormonal-neuronal feedback mechanism.

DIFFERENTIAL DRUG EFFECTS ON DOPAMINERGIC NEURONS OF THE RAT BRAIN

By

Gary A. Gudelsky

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology

1977

to Judy, for her love and patience

ACKNOWLEDGEMENTS

The author takes this opportunity to express his gratitude to Dr. Kenneth E. Moore for his guidance in the development of this dissertation.

He acknowledges the constructive criticism offered him by the other members of his graduate committee, Dr. T. M. Brody, Dr. J. L. Bennett, and Dr. J. Meites.

He appreciates the help of Mrs. Mirdza Gramantins and Nan Friedle and sincerely recognizes and thanks Mrs. Sue Stahl for her time, effort and patience.

He also wishes to thank Dr. D. B. Goodale for his advice during the author's graduate training.

The collaborative efforts of several others are also acknowledged: Dr. Lucio Annunziato, for hypothalamic deafferentations and prolactin determinations, and Alan Ravitz and Jim Simpkins, for prolactin and LH and FSH analyses, respectively.

TABLE OF CONTENTS

		Page
INTRODUCTION		1
I.	Neuroanatomy	2
II.	Functional Roles of Dopaminergic Pathways	7
	A. Nigrostriatal and mesolimbic dopaminergic neurons	7
	B. Tuberoinfundibular dopaminergic neurons	13
III.	Regulation of Dopamine Synthesis in the Central Nervous System	17
STATEMENT OF PURPOSE		23
MATERIALS AND METHODS		24
I.	Animals and Blood Collection	24
II.	Lesions	24
III.	Biochemical Procedures	25
	A. Dissections	25
	B. Radioenzymatic assays	28
	C. COMT preparation	30
	D. Estimation of dopamine turnover	31
	E. Radioimmunoassays	32
	F. Drugs	32
	G. Statistics	33
RESULTS		34
I.	Steady State Dopamine Concentrations	34
	A. Dopamine concentrations in striatum, olfactory tubercle and median eminence	34
	B. Effects of γ-butyrolactone and baclofen on dopamine concentrations in the corpus striatum, olfactory tubercle and median eminence	36
	C. Median eminence dopamine concentrations in female rats	38
II.	Dopamine Turnover Studies	40
	A. Differential effects of acute agonist and antagonist administration on dopamine turnover in the striatum, olfactory tubercle and median eminence	40
	B. Time course for the effects of haloperidol on dopamine turnover in the striatum, olfactory tubercle and median eminence	45
	C. Haloperidol in hypophysectomized rats	53

Page

D. Selective actions of prolactin administration on dopamine turnover. 61
E. Effects of estradiol benzoate on dopamine turnover and serum prolactin concentrations. . 68
F. Effects of hypothalamic deafferentation on drug-induced changes in serum prolactin concentrations and dopamine turnover in the median eminence. 76
G. Effects of subacute piribedil administration on dopamine turnover in the median eminence. . 92

DISCUSSION. 98

I. Determination of Steady State Dopamine Concentrations 98
A. Effects of γ-butyrolactone and baclofen on steady state dopamine concentrations 99
B. Hormonal influences on the dopamine concentration in the median eminence 101
II. Determination of Dopamine Turnover 103
A. Methodological considerations. 103
B. Comparison of the synthesis rates for dopamine in the striatum, olfactory tubercle and median eminence 104
C. Acute effects of haloperidol and piribedil administration 105
D. Selective actions of prolactin and estrogen on tuberoinfundibular dopamine neurons 110
E. Lag time in response of tuberoinfundibular dopamine neurons 113
F. Prolactin-induced changes in dopamine turnover in the median eminence of rats with an isolated hypothalamic island 114

SUMMARY AND CONCLUSION. 116

BIBLIOGRAPHY. 121

LIST OF TABLES

Table		Page
1	Dopamine contents of striatum, olfactory tubercle and median eminence in rat brain	35
2	Effects of γ-butyrolactone (GBL) and baclofen on dopamine concentrations in various brain regions	37
3	Median eminence and hypothalamic catecholamine concentrations in the female rat.	39
4	Effects of a dopaminergic agonist and antagonist on dopamine synthesis rates in various brain regions. . . .	44
5	Effects of haloperidol on dopamine synthesis rates in the median eminence and striatum.	56
6	Effects of thioridazine and clozapine on the α-methyltyrosine induced depletion of dopamine in the median eminence	60
7	Effects of prolactin (PRL) administration on serum LH and FSH concentrations of female ovariectomized rats	67
8	Effects of estradiol benzoate and α-methyltyrosine on serum concentrations of prolactin	72
9	Effects of anesthesia on median eminence and hypothalamic concentrations of dopamine and norepinephrine .	84
10	Time course for the reduction of estradiol-elevated serum prolactin concentrations by piribedil.	93
11	Effect of piribedil on the α-methyltyrosine-induced depletion of dopamine in the median eminence	94
12	Effects of piribedil on the α-methyltyrosine-induced depletion of dopamine in various brain regions	96

LIST OF FIGURES

Figure		Page
1	Major dopaminergic pathways in the rat brain	3
2	Schematic representation of the median eminence in sagittal (upper) and frontal (lower) views	6
3	Regulation of prolactin secretion from the anterior pituitary.	15
4	Feedback regulation of nigrostriatal neurons indicating the involvement of (A) postsynaptic neuronal feedback mechanism and (B) presynaptic receptors.	21
5	Sagittal and frontal sections of the rat brain indicating median eminence fragment.	27
6	The effects of a dopaminergic agonist (piribedil) and antagonist (haloperidol) on the rate of decline of the dopamine concentration in the corpus striatum, olfactory tubercle and median eminence after i.p. injection of α-methyltyrosine (250 mg/kg).	43
7	The effect of haloperidol on the α-methyltyrosine-induced depletion of dopamine in the striatum.	48
8	The effect of haloperidol on the α-methyltyrosine-induced depletion of dopamine in the olfactory tubercle	50
9	The effect of haloperidol on the α-methyltyrosine-induced depletion of dopamine in the median eminence . .	52
10	The α-methyltyrosine-induced decline of the dopamine concentration in the median eminence of (A) unoperated control and (B) hypophysectomized rats 16 hours after the subcutaneous administration of vehicle (open circles) or haloperidol, 2.5 mg/kg (solid circles) . . .	55
11	The α-methyltyrosine-induced decline of the dopamine concentration in the striata of (A) unoperated controls and (B) hypophysectomized rats 16 hours after the subcutaneous administration of vehicle (open circles) or haloperidol, 2.5 mg/kg (solid circles).	59

Figure Page

12 Effects of prolactin (PRL) administration on the α-methyltyrosine-induced depletion of dopamine in the median eminence. 63

13 Effects of prolactin (PRL) administration on the α-methyltyrosine-induced depletion of dopamine in the corpus striatum. 66

14 Effects of estrogen on the α-methyltyrosine-induced reduction of dopamine concentrations in the (A) median eminence and (B) striatum. 71

15 Effects of estrogen on the α-methyltyrosine-induced reduction of dopamine concentrations in the median eminence of control and hypophysectomized rats 75

16 Relationship between median eminence, hypothalamic island and remaining hypothalamus. 77

17 Sagittal view of the rat brain depicting the brain regions examined after hypothalamic deafferentation. . . 79

18 Effects of hypothalamic deafferentation on the concentration of dopamine and norepinephrine in the median eminence, hypothalamic island and remaining hypothalamus 82

19 Serum prolactin concentrations in deafferented male rats 86

20 Effects of haloperidol on the α-methyltyrosine-induced decline of the dopamine concentration in the median eminence, hypothalamic island and remaining hypothalamus 89

21 Effect of haloperidol on the α-methyltyrosine-induced decline of the dopamine concentration in the median eminence of normal unoperated control and deafferented male rats 91

22 Prolactin-mediated feedback regulation of tuberoinfundibular dopamine neurons indicating possible sites of action of prolactin 119

INTRODUCTION

Evidence for the existence of catecholamines in the central nervous system came from the biochemical demonstration of the regional distribution of norepinephrine (Vogt, 1954) and dopamine (Montagu, 1957; Carlsson et al., 1958) in the brain. Although the results of these early studies suggested that monoamines were contained in nonvascular elements in the brain, the use of the Falck-Hillarp histochemical technique provided proof for a cellular localization of catecholamines (Falck and Hillarp, 1959; Carlsson et al., 1962a,b). Subsequent histofluorescent studies localized catecholamine-containing cell bodies and nerve terminals in the central nervous system (Dahlström and Fuxe, 1964; Fuxe, 1965). This work has been extended in the past several years, such that the major monoaminergic pathways of the brain have been mapped using a combination of histofluorescent, biochemical and specific lesioning techniques (Ungerstedt, 1971a; Palkovits and Jacobowitz, 1974).

The demonstration of a significant dopamine concentration in the corpus striatum, in the absence of measurable norepinephrine (Bertler and Rosengren, 1959), provided an early indication that dopamine may function as a neurotransmitter, in contrast to the prior contention of its role as a precursor for norepinephrine. Studies during the past twenty years support a role for dopamine as a neurotransmitter in the central nervous system, particularly in the basal ganglia and mesolimbic areas of the brain and, more recently, in the medial basal

hypothalamus. The demonstration of several distinct dopaminergic neuronal pathways in the aforementioned areas has led to the study of the dynamics of dopaminergic neurotransmission (e.g., synthesis, storage and release of the putative neurotransmitter), particularly as it may relate to the neural control of a wide variety of functions.

The present studies represent a comparative neuropharmacological examination of three of these dopaminergic neuronal systems, the prominent nigrostriatal pathway and the mesolimbic and tuberoinfundibular pathways.

I. Neuroanatomy

The neuroanatomical aspects of the nigrostriatal, mesolimbic and tuberoinfundibular pathways are depicted in Figure 1. Nigrostriatal neurons originate from the dopaminergic cell group in the zona compacta of the substantia nigra and dopamine-containing cell bodies dorsomedial to this nucleus, designated areas A8 and A9 in the rat brain, respectively (Dahlström and Fuxe, 1964). From the substantia nigra the axons of these neurons ascend through the lateral hypothalamus and crus cerebri. They then enter the internal capsule, fan out in the globus pallidus and terminate in the striatum (caudate-putamen) (Ungerstedt, 1971a).

Dopamine-containing cell bodies dorsal to the interpeduncular nucleus in the ventral tegmentum, the A10 cell group, give rise to neurons of the mesolimbic pathway. Axons of these neurons ascend together with nigrostriatal axons in the medial forebrain bundle. At the anterior commissure the mesolimbic pathway divides such that one branch terminates in the nucleus accumbens and nucleus interstitial striae terminalis, while the other branch terminates in the

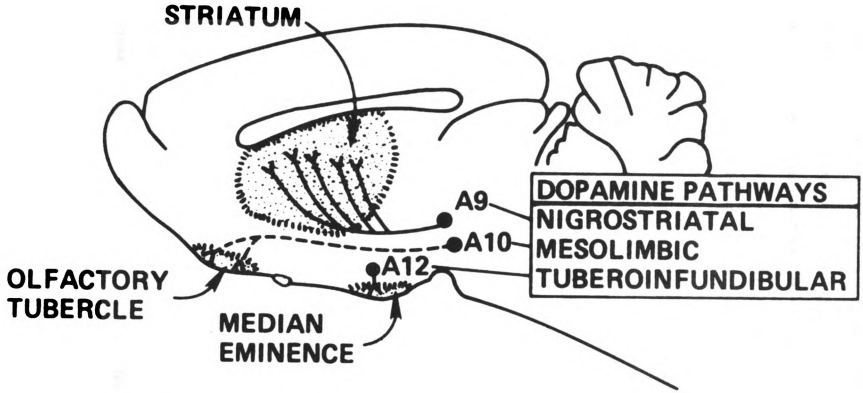


Figure 1. Major dopaminergic pathways in the rat brain.

olfactory tubercle. Recent studies have also identified mesolimbic neurons terminating in some regions of the limbic cortex (frontal, cingulate and entorhinal cortex) (Lindvall et al., 1974).

The neuroanatomical identification and characterization of the tuberoinfundibular dopaminergic pathway has been difficult due to the complex pattern of catecholamine terminals within the hypothalamus. In the first histochemical studies, Carlsson et al. (1962a) observed a zone of diffuse green fluorescence in the infundibular region of the rat. Since this initial observation several investigators have described the organization of catecholaminergic neuronal systems in the medial basal hypothalamus (Jonsson et al., 1972; Björklund and Nobin, 1973; Björklund et al., 1973). Dopaminergic nerve terminals in the median eminence-pituitary region arise from dopaminergic cell bodies in the arcuate and periventricular nuclei and constitute a tuberohypophysial system which can be separated into three components. One component originates in the most rostral portion of the arcuate nucleus and innervates the entire pars intermedia. The second is a small group of cells immediately caudal to the first which gives rise to neurons innervating the neural lobe. The third system, the tuberoinfundibular pathway, consists of short axons which originate in the A12 cell group in the arcuate and periventricular nuclei and terminate in the median eminence (Fuxe, 1963; Dahlström and Fuxe, 1964; Fuxe and Hökfelt, 1966; Björklund et al., 1973).

The regional distribution of the terminals of tuberoinfundibular dopaminergic neurons within the median eminence is of interest in that it provides a morphological basis for the possible interaction with other neurosecretory elements. Pharmacological analysis of the distribution of dopaminergic and noradrenergic nerve terminals in the

median eminence indicates that the external layer of the median eminence contains dopaminergic terminals of the tuberoinfundibular pathway, exclusively. The evidence for dopaminergic, rather than noradrenergic, innervation of the external layer of the median eminence includes the observation that catecholamine fluorescence in the external layer of the median eminence is unaffected by hypothalamic deafferentation, indicating that the terminals do not belong to noradrenergic neurons which originate in the brain stem (Jonsson et al., 1972; Björklund et al., 1973; Löfström et al., 1976a). Recently, monoamine synthesizing enzymes have been localized immunohistochemically in various regions of the median eminence (Hökfelt et al., 1976). The external layer of the median eminence was shown to contain tyrosine hydroxylase and dopa decarboxylase but not dopamine-β-hydroxylase positive cells. Thus, only those enzymes necessary for the synthesis of dopamine were found in the external layer of the median eminence. Löfström et al. (1976a) have examined the regional distribution of dopamine and norepinephrine nerve terminals within the median eminence. In view of the complex pattern of nerve terminals in this area of the brain, the median eminence was divided anatomically into a rostral, central and caudal region (Figure 2). Microfluorometric analysis of dopamine and norepinephrine revealed that the vast majority of catecholamine terminals in the subependymal layer were noradrenergic. The lateral palisade zone contained dopamine terminals almost exclusively while the medial palisade zone contained both monoamines, dopamine accounting for 50-75%. Thus, from histofluorescent and pharmacological approaches it seems that the short tuberoinfundibular dopaminergic neurons with cell bodies in the arcuate and periventricular nuclei give rise to a

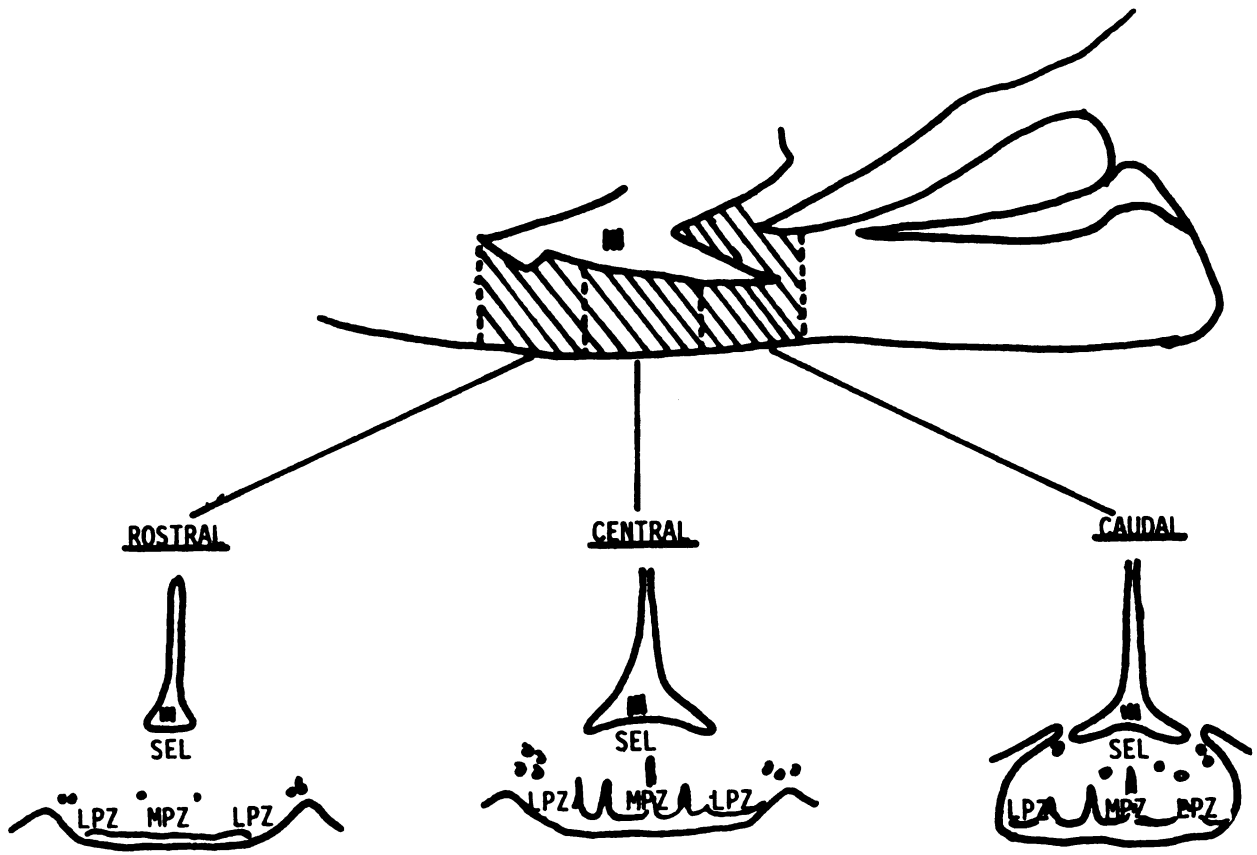


Figure 2. Schematic representation of the median eminence in sagittal (upper) and frontal (lower) views. SEL, subependymal layer; LPZ, lateral palisade zone; MPZ, medial palisade zone; III, third ventricle. The lateral and medial palisade zones make up what is generally referred to as the external layer of the median eminence.

large part of the nerve terminals in the medial and lateral palisade zones, generally referred to, collectively, as the external layer of the median eminence.

II. Functional Roles of Dopaminergic Pathways

A. Nigrostriatal and mesolimbic dopaminergic neurons

The development of the present concepts of central catecholamines and motor function evolved, in large part, from the study of Parkinson's disease. Parkinson's disease is a chronic and progressive degenerative disease of the central nervous system. The cardinal symptoms, akinesia, rigidity and tremor, are primarily those of motor dysfunction. The most specific neuropathological lesion found in Parkinson's disease is the degeneration of the melanin-containing neurons in the zona compacta of the substantia nigra. The neurochemical correlate associated with this degeneration is a marked decrease in the dopamine concentration of the striatum, putamen and substantia nigra (Bernheimer et al., 1973; Lloyd et al., 1973). In addition to the loss of dopamine in these brain regions, there is a parallel deficiency in its synthetic enzymes, tyrosine hydroxylase and dopa decarboxylase (Lloyd et al., 1973) and a reduction in dopamine uptake and the concentration of homovanillic acid (HVA) (see Hornykiewicz, 1966). From these findings it was suggested that the destruction of the nigrostriatal dopamine pathway was the neuropathological event responsible for the clinical symptoms of Parkinson's disease. This contention has been supported by several observations. First, the degree of dopamine deficiency has been correlated with the degree of cell loss in the substantia nigra (Bernheimer et al., 1973). Secondly, in hemi-parkinsonian patients, there is a greater reduction

of striatal dopamine in the side contralateral to the side of the symptoms. Moreover, administration of L-dopa to parkinsonian patients has been found to be effective in the treatment of akinesia and rigidity and the clinical improvement is correlated with an elevated striatal dopamine concentration (see Lloyd and Hornykiewicz, 1975). Thus, Parkinson's disease is often viewed as a striatal dopamine deficiency syndrome.

The nigrostriatal dopamine pathway appears also to be involved in other dyskinetic syndromes. Although L-dopa administration ameliorates the akinesia and rigidity in Parkinson's disease, it has other effects on motor function in these patients. L-dopa-induced dyskinesias consist mostly of oral-buccal-facial dyskinesias. These choreoathetoic movements are generally believed to result from an overstimulation of striatal dopamine receptors.

The chronic use of antipsychotic drugs may also result in oral-facial-buccal dyskinesias. One explanation for the development of these dyskinesias is that they reflect an increased sensitivity of striatal dopamine receptors, a consequence of prolonged receptor blockade. Involvement of the nigrostriatal dopamine pathway in tardive dyskinesias is suggested from the resemblance between tardive dyskinesias and L-dopa-induced dyskinesias. Furthermore, in patients with parkinsonism and tardive dyskinesias there is an inverse relationship between the severity of the two sets of symptoms (i.e., treatments which improve the parkinsonism exacerbate the tardive dyskinesias and vice versa) (Crane, 1972).

Aside from the probable involvement of dopamine in motor function, increasing interest has been focused on central catecholamines as a possible biochemical correlate of schizophrenia. Involvement of

dopaminergic mechanisms in the pathophysiology of schizophrenia is based, primarily, upon two observations. One is that effective antipsychotic agents block central dopamine receptors. Secondly, there is a close resemblance between amphetamine psychosis and schizophrenia. This second observation is of interest, with respect to dopamine, in that some of the behavioral effects of amphetamine result from a drug-induced enhancement of dopaminergic neurotransmission (Randrup and Scheel-Krüger, 1966; Scheel-Krüger and Randrup, 1967).

However, there have been conceptual difficulties associating both extrapyramidal motor dysfunctions and complex behavioral abnormalities with nigrostriatal dopamine neurons. The localization of dopamine neurons in subcortical limbic nuclei (Ungerstadt, 1971a and many others) and limbic forebrain cortical areas (Thierry et al., 1972, 1973; Hökfelt et al., 1974) has led to the suggestion that mesolimbic and/or mesocortical dopamine neurons might provide a more reasonable neuroanatomical substrate for the symptoms of schizophrenia. Indeed, it has been hypothesized that the extrapyramidal side effects of neuroleptics result from dopaminergic receptor blockade in the striatum whereas the antipsychotic actions of these drugs result from the blockade of mesolimbic dopamine receptors.

Subsequent to dopaminergic receptor blockade by neuroleptics, the activity of dopamine neurons is increased (Bunney et al., 1973). This is reflected biochemically by an increased turnover rate of dopamine (Andén et al., 1964; Neff and Costa, 1966; Nybäck et al., 1967). The concentration of the dopamine metabolites (HVA and 3,4-dihydroxyphenylacetic acid [DOPAC]) have often been used as indices of dopamine turnover. Neuroleptic-induced changes in the

concentrations of these metabolites in the striatum and limbic areas of the brain have been used to examine the relationship of these brain regions to the extrapyramidal and antipsychotic effects of these agents.

Andén and Stock (1973a) reported that clozapine, a neuroleptic associated with little or no extrapyramidal side effects, increased HVA levels in the limbic areas of the rabbit brain to a greater extent than in the corpus striatum. In contrast, the haloperidol-induced increase of HVA was equivalent in the two brain regions. Although Bartholini et al. (1975) were unable to confirm the observation of Andén and Stock in the rat, Bartholini (1976) has reported that clozapine increases the release of dopamine from the nucleus accumbens to a much greater extent than from the striatum. Although it has not been clearly demonstrated that those neuroleptics which produce few extrapyramidal side effects alter dopamine metabolism in limbic areas preferentially, there is some evidence to suggest that the converse may be true. That is, neuroleptics which are associated with a high incidence of extrapyramidal side effects may alter dopamine metabolism in the striatum to a greater extent than in limbic regions. Chlorpromazine and haloperidol-induced increases in HVA concentrations, for example, are greater in the striatum than in the limbic system of rats, whereas clozapine-induced increases are equivalent in the two systems (Bartholini et al., 1975). In further support of this hypothesis, Carlsson (1975) observed that pimozide, haloperidol and chlorpromazine stimulate dopamine synthesis more markedly in the striatum than in limbic regions; the effects of clozapine and thioridazine were less marked between the two areas. Zivkovic et al. (1975a) have determined the potencies of several

antipsychotics to change the kinetics of tyrosine hydroxylase in the striatum and nucleus accumbens. They found that pimozide and haloperidol were more potent in the striatum than in the nucleus accumbens In contrast, clozapine and thioridazine were more potent in the nucleus accumbens. It has also been suggested that the incidence of extrapyramidal side effects with haloperidol may be associated with the differential time course of its effects on striatal and mesolimbic dopamine metabolism (Wilk et al., 1975).

Despite the number of observations demonstrating differential sensitivity of mesolimbic and striatal dopamine receptors for various antipsychotics, the evidence for such a hypothesis is not unequivocal. There are an equally impressive number of studies which examine dose-response relationships and provide little support for the contention that various antipsychotic agents alter mesolimbic and striatal dopamine systems differentially (Waldmeier and Maître, 1976; Westerink and Korf, 1975; Wiesel and Sedvall, 1975; Wilk and Glick, 1976; Stawarz et al., 1975).

Although the biochemical responses of mesolimbic and nigrostriatal dopamine neurons to drugs appear to be similar, it has been possible to differentiate these two pathways on the basis of drug-induced behaviors. The stimulation of locomotor activity after the administration of *d*-amphetamine and other dopaminergic agonists appears to be mediated through mesolimbic dopamine neurons. Thus, 6-hydroxydopamine (6OHDA)-induced lesions of the nucleus accumbens septa have been shown to block the *d*-amphetamine-induced stimulation of locomotor activity in rats (Kelly et al., 1975; Iversen and Kelly, 1975; Kelly and Iversen, 1976). The ability of dopaminergic agonists (e.g., ergometrine, ADTN, apomorphine, etc.) to increase locomotor activity is enhanced

in animals with electrolytic or 6OHDA-induced lesions of the nucleus accumbens, resulting, most likely, from the development of denervation supersensitivity (Kelly et al., 1975; Woodruff et al., 1976). 6OHDA-induced lesions of the substantia nigra (Creese and Iversen, 1972; Fibiger et al., 1973) or the caudate nucleus do not alter the *d*-amphetamine-induced stimulation of locomotor activity. In addition to lesion experiments, studies utilizing intracerebral injections of drugs support the contention that mesolimbic dopamine neurons are involved with drug-induced motor activity. Pijnenburg et al. (1973) reported that the injection of ergometrine into the nucleus accumbens, but not the caudate nucleus, stimulated locomotor activity. This observation was confirmed when it was reported that the injection of dopamine, apomorphine and amphetamine into the nucleus accumbens also increased locomotor activity (Pijnenburg and Van Rossum, 1973; Costall and Haylor, 1975; Pijnenburg et al., 1976). Moreover, bilateral injection of haloperidol into the nucleus accumbens antagonized *d*-amphetamine-induced motor activity (Pijnenburg et al., 1975a).

d-Amphetamine, in addition to increasing locomotor activity, produces stereotyped behavior, which consists of sniffing, licking and gnawing. While it appears that moesolimbic dopamine neurons mediate drug-induced increases in locomotor activity, nigrostriatal dopamine neurons may be responsible for the mediation of drug-induced stereotyped behavior. Electrolytic and 6OHDA-induced lesions, electrical stimulation and intracerebral injections of drugs have been used to investigate the role of the corpus striatum in stereotyped behaviors. The injection of 6OHDA into the substantia nigra has been reported to block *d*-amphetamine-induced stereotypy (Creese and Iversen, 1972;

Fibiger et al., 1973; Creese and Iversen, 1975), whereas electrical stimulation of this region promotes stereotypy (Anlezark et al., 1971). Selective electrolytic (Fog et al., 1970; Fuxe and Ungerstedt, 1970; Naylor and Olley, 1972) or 6OHDA-induced (Asher and Aghajanian, 1974; Creese and Iversen, 1974; Kelly et al., 1975) lesions of the caudate nucleus antagonize *d*-amphetamine-induced stereotyped behavior. Furthermore, stereotyped behavior can be elicited by direct injection of *d*-amphetamine or dopamine into the striatum (Fog et al., 1967; Fog et al., 1971). On the other hand, injection of neuroleptics into the caudate nucleus reduces apomorphine and *d*-amphetamine-induced stereotyped behavior (Pijnenburg et al., 1975b).

Thus, the involvement of dopaminergic neuronal pathways in drug-induced motor behaviors in the rat may be twofold. The mesolimbic pathway may mediate changes in locomotor activity, whereas nigrostriatal neurons may have a role in the elicitation of stereotypy.

B. Tuberoinfundibular dopaminergic neurons

The predominant action of the hypothalamus on prolactin secretion is one of inhibition. This has been demonstrated by a number of different experimental approaches. Transplantation of the pituitary into the kidney capsule (Everett, 1954) or pituitary stalk section (Everett and Nikitovitch-Winer, 1963) results in a marked elevation of plasma prolactin concentrations. Meites and his co-workers were among the first to demonstrate that the pituitary releases large amounts of prolactin, but negligible amounts of other hormones, when incubated *in vitro* (Meites et al., 1963; Meites and Nicoll, 1966). In addition, plasma prolactin concentrations are increased following electrolytic lesions in the median eminence

(Chen et al., 1970; Bishop et al., 1971; Donoso et al., 1973). Talwalker et al. (1963) demonstrated that crude hypothalamic extracts were capable of inhibiting the release of prolactin from pituitaries incubated *in vitro*, and suggested that the hypothalamus contained a "prolactin inhibiting factor" (PIF).

Reports over many years have suggested that biogenic amines, particularly catecholamines, influence the release of pituitary hormones. Administration of reserpine or α-methyltyrosine has been shown to increase pituitary prolactin release *in vitro* (MacLeod and Abad, 1968). Lu et al. (1970) demonstrated that reserpine, α-methyltyrosine and chlorpromazine elevated serum prolactin concentrations, whereas the administration of L-dopa reduced prolactin levels (Lu and Meites, 1971; Donoso et al., 1971). Thus, interference with central catecholamine function resulted in increased serum prolactin concentrations, whereas enhancement of catecholamine neurotransmission reduced prolactin levels. The predominant catecholamine involved is most likely dopamine since dopaminergic agonists decrease (Mueller et al., 1976) and dopaminergic antagonists increase (Dickerman et al., 1972; Clemens et al., 1974) serum prolactin concentrations.

Although the existence of a hypothalamic PIF has been generally accepted, it is not certain whether dopamine enhances the release of a peptidergic PIF or acts directly as a PIF on the pituitary (Figure 3). The demonstration that tuberoinfundibular dopaminergic neurons terminate in the median eminence in close proximity to hypophysial portal vessels (Hökfelt, 1967) provided a morphological basis for the contention of Van Maanen and Smelik (1968) that tuberoinfundibular monoaminergic neurons may regulate prolactin secretion through the release of an inhibitory neurotransmitter directly into portal

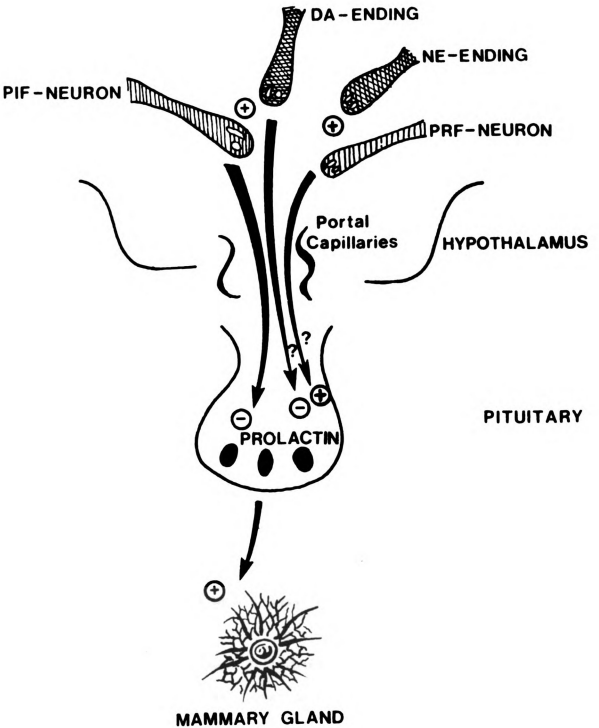


Figure 3. Regulation of prolactin secretion from the anterior pituitary. DA, dopamine; NE, norepinephrine; PIF, prolactin inhibiting factor; PRF, prolactin releasing factor.

vessels. Evidence for the direct inhibition of prolactin secretion by dopamine was first provided by MacLeod (1969) and Birge et al. (1970), who observed that dopamine suppressed pituitary prolactin release *in vitro*. Shaar and Clemens (1974) reported that the endogenous dopamine content of the hypothalamus could account for the total PIF activity of hypothalamic extracts. Moreover, the PIF activity of hypothalamic extracts could be removed by prior incubation with a monoamine oxidase preparation or adsorption onto alumina. Donoso et al. (1973, 1974) also concluded that dopamine acts directly to inhibit prolactin secretion since administration of L-dopa to rats bearing pituitaries transplanted under the kidney capsule reduced serum prolactin concentrations, an effect which was blocked by inhibiting the conversion of L-dopa to dopamine. Moreover, L-dopa was capable of reducing prolactin concentrations in rats with electrolytic lesions in the median eminence. Direct infusion of a hypophysial portal vessel with dopamine has been shown to reduce prolactin levels (Takahara et al., 1974). In further support for the argument that dopamine may be PIF are the recent reports of the identification of endogenous dopamine in portal blood (Ben-Jonathan et al., 1977) and the existence of dopamine receptors in the pituitary and the lack of such receptors in the medial basal hypothalamus (Brown et al., 1976).

However, several groups of investigators maintain that a PIF, other than dopamine, exists. Quijada et al. (1973) found that haloperidol could block the ability of dopamine to suppress pituitary prolactin release *in vitro*, but had no effect on the ability of hypothalamic fragments to inhibit prolactin release. Systemic administration of hypothalamic extracts has been reported to lower prolactin

levels *in vivo* (Amenomori and Meites, 1970; Watson et al., 1971). However, systemic administration of dopamine in amounts greater than those found in the hypothalamus has not been found to alter serum prolactin concentrations (Lu et al., 1970). The dopaminergic antagonist, pimozide, increased serum prolactin concentrations to a greater extent when implanted into the median eminence-arcuate region than when implanted into the pituitary directly (Ojeda et al., 1974). Thus, it appeared that the median eminence was the primary site of action of pimozide and that the pimozide-induced elevation of serum prolactin resulted from the blockade of dopaminergic receptors located on neurosecretory elements containing PIF. More recently, Enjalbert et al. (1977) have demonstrated the existence of a dopamine-free PIF in rat hypothalamic extracts.

Despite the uncertainty of the chemical nature of PIF, the tonic inhibition of prolactin secretion most likely results from a direct or indirect action of dopamine released from tuberoinfundibular neurons.

III. Regulation of Dopamine Synthesis in the Central Nervous System

The endogenous dopamine concentration of the brain is a steady state reflection of the dynamic processes of synthesis and release. Since only minor changes occur in the endogenous dopamine concentration under a wide variety of pharmacological and physiological conditions, regulatory mechanisms must exist to insure that the release of the neurotransmitter does not greatly exceed its synthesis. Tyrosine hydroxylase is the rate limiting enzyme in the biosynthesis of catecholamine neurotransmitters. Hence, changes in the synthesis

rates of dopamine usually involve changes in the activity of tyrosine hydroxylase.

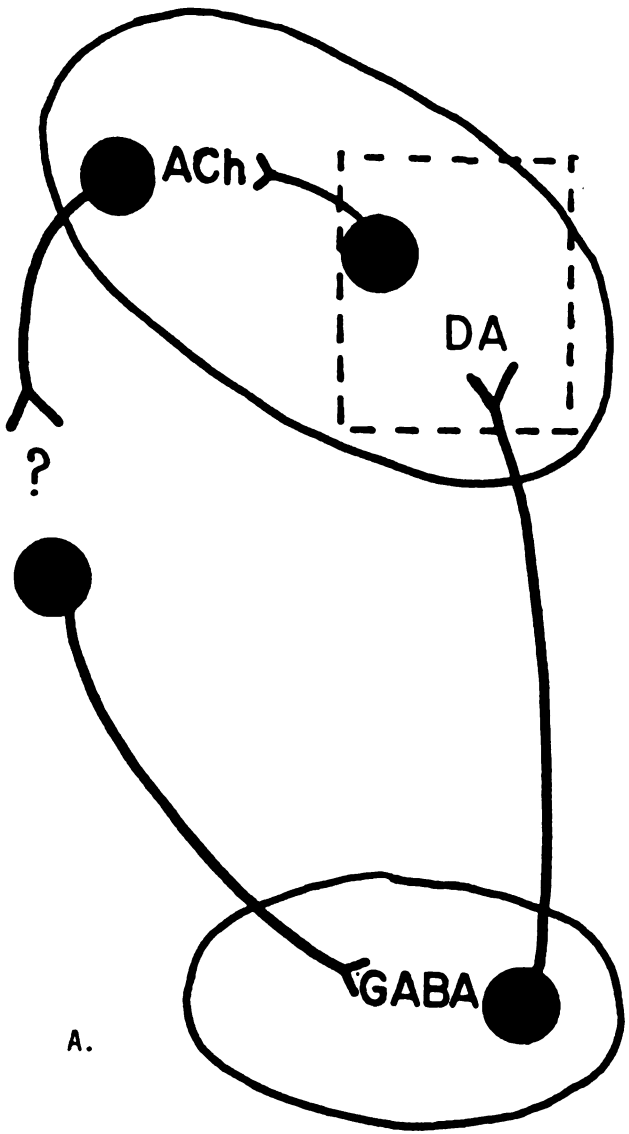
Carlsson et al. (1960) were the first to suggest that catecholamine synthesis could be inhibited by elevated monoamine levels. Several years later, it was shown that dopamine and other catechols could inhibit tyrosine hydroxylase (Nagatsu et al., 1964). Thus, it appeared that this rate limiting enzyme was subject to end product inhibition. The *in vivo* importance of end product inhibition in the control of tyrosine hydroxylase came from the demonstration that monoamine oxidase inhibitors increased catecholamine concentrations and decreased catecholamine synthesis (Costa and Neff, 1966; Spector et al., 1967). It was hypothesized, therefore, that decreased nerve impulse flow resulted in decreased release of the neurotransmitter. Decreased release elevated intraneuronal catecholamine concentrations which inhibited tyrosine hydroxylase via end product inhibition. Increased nerve impulse flow produced opposite effects and led to an increased synthesis rate.

However, a mechanism of feedback regulation of tyrosine hydroxylase different from end product inhibition appears also to occur in dopaminergic neurons in the central nervous system. Chlorpromazine and haloperidol were found to increase the concentrations of dopamine metabolites without altering the concentration of dopamine (Carlsson and Lindqvist, 1963). Thus, it appeared that the synthesis and release of dopamine had increased as a result of these neuroleptics. Carlsson and Lindqvist (1963) proposed that chlorpromazine blocked dopamine receptors in the striatum and, subsequent to receptor blockade, a neuronal feedback loop mediated an increased firing rate of dopaminergic neurons. Increased neuronal activity, then, led to an

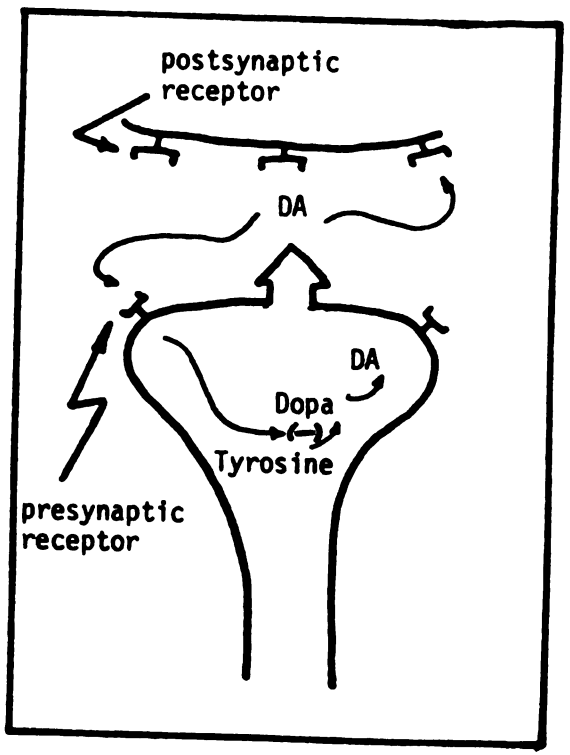
increased release of dopamine in an attempt to overcome the receptor blockade. Indeed, neuroleptics have been shown to increase the firing rate of dopamine neurons (Bunney et al., 1973) and the turnover rate and synthesis rate of dopamine in the brain (Andén et al., 1964; Neff and Costa, 1966; Nybäck et al., 1967). Costa et al. (1974) reported that the neuroleptic-induced increase in dopamine synthesis appears to result from a drug-induced change in the kinetic state of tyrosine hydroxylase such that its affinity for the pterin cofactor is increased (Costa et al., 1974; Zivkovic et al., 1975a). Central hemisection was shown to block the ability of haloperidol to activate tyrosine hydroxylase, indicating that a neuronal feedback loop is required for this action of the neuroleptic (Zivkovic et al., 1975b). A model for neuronal feedback modulation of nigrostriatal dopamine neurons is presented in Figure 4A.

It has generally been assumed that dopamine synthesis is positively correlated with nerve impulse flow. However, there is one notable exception. Inhibition of impulse flow in nigrostriatal neurons induced by axotomy or γ-butyrolactone results in a marked increase in the concentration and synthesis of dopamine in nerve terminal regions (Gessa et al., 1966; Walters and Roth, 1972; Carlsson, 1975). This observation has led to the hypothesis that presynaptic dopamine receptors or autoreceptors are involved in the short term regulation of dopamine synthesis (see Carlsson, 1975; Roth et al., 1975). According to this model, as depicted in Figure 4B, dopamine released by nerve impulse flow activates presynaptic receptors and decreases the synthesis and further release of dopamine. Indeed, dopaminergic agonists antagonize the increase in dopamine concentration and synthesis rate following γ-butyrolactone-induced inhibition

Figure 4. Feedback regulation of nigrostriatal neurons indicating the involvement of (A) postsynaptic neuronal feedback mechanism and (B) presynaptic receptors. ACh, acetylcholine; DA, dopamine; GABA, gamma-aminobutyric acid; Dopa, dihydroxyphenylalanine.


ACh
DA
?
GABA

A.


postsynaptic
receptor
DA
DA
Dopa
Tyrosine
presynaptic
receptor

B.

Figure 4

of impulse flow (Walters and Roth, 1974; Gianutsos et al., 1976). Thus, activation of presynaptic receptors appears to have a damping effect, opposing any large change in dopamine synthesis resulting from a decrease or an increase in nerve impulse flow.

Pre- and postsynaptic receptors, therefore, appear to be involved in short and long loop feedback mechanisms, respectively, which function to regulate the activity of central dopaminergic neurons.

STATEMENT OF PURPOSE

Nigrostriatal dopamine neurons have been used, almost exclusively, as the model for dopaminergic neurotransmission in the central nervous system. To some extent mesolimbic dopamine neurons have been examined and have been found to respond to pharmacological agents in a manner similar to nigrostriatal neurons. Until recently, it has not been possible to measure dopamine concentrations or dopamine metabolites in brain regions other than the striatum.

The purpose of the present studies is to examine regional drug effects on central dopaminergic neurons. More specificially, the following experiments were designed to investigate the regulatory mechanisms governing the activity of nigrostriatal, mesolimbic and, in particular, tuberoinfundibular dopamine neurons.

MATERIALS AND METHODS

I. Animals and Blood Collection

Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 200-300 g were maintained in air conditioned rooms under alternate 12 hour periods of light and dark. Female Sprague-Dawley rats weighing 175-275 g were obtained from the same source, ovariectomized and maintained in a similar manner. Hypophysectomized male Sprague-Dawley rats and their unoperated controls were obtained from Hormone Assay Laboratories, Inc. (Chicago, IL). The animals had free access to food and water. Hypophysectomized rats received crushed food and orange slices in addition to regular laboratory rat chow. Hypophysectomized animals were used 3-8 days following surgery.

All blood samples were collected by decapitation between 0800 and 1300 hours. The serum was separated after centrifugation and stored at -20°C until assayed for prolactin, LH and FSH.

II. Lesions

Hypothalamic islands were made under Equithesin anesthesia with a modified Halász knife (diameter 1.5 mm; height 2.0 mm). The rostral border of the island was at the level of the retrochiasmatic area and the posterior border at the level of the premamillary area. The animals were sacrificed 16-32 days after surgery.

III. Biochemical Procedures

A. Dissections

After decapitation rat brains were rapidly removed and placed with the dorsal surface on a glass plate placed over ice. The median eminence was removed from the hypothalamus with the aid of a dissecting microscope and fine scissors (Cuello et al., 1973b). The dissection roughly corresponds to the "superficial hypothalamic region" of Kavanagh and Weisz (1974). The posterior portion of the median eminence was grasped with fine forceps and cuts made along the tuberoinfundibular sulcus on both sides to the rostral pole of the median eminence. The dissected tissue was composed primarily of the floor of the third ventricle (Figure 5) and measured approximately 1.7 x 0.6 mm. Bilateral samples of olfactory tubercle and corpus striatum were also taken. In rats with hypothalamic deafferentations, the median eminence was removed from the hypothalamic island, which was then removed from the remaining hypothalamus with the aid of a dissecting microscope and fine scissors.

Median eminence samples were homogenized in micro tissue grinders (Little Smoothies, Micro-Metric Instrument Co.) in 20 µl of 0.4N perchloric acid containing 10 mg% EDTA. The resulting homogenates were transferred to microcentrifuge tubes and centrifuged for 20 seconds in a Beckman Microfuge. Striatal and olfactory tubercle samples were weighed and then homogenized in 20-40 volumes of 0.4N perchloric acid containing 10 mg% EDTA. One hundred microliter aliquots of these homogenates were also centrifuged in the Beckman Microfuge. In rats with hypothalamic deafferentations, the median eminence samples were homogenized in 30 µl and hypothalamic islands

Figure 5. Sagittal and frontal sections of the rat brain indicating median eminence fragment. AN, arcuate nucleus; MB, mamillary body; ME, median eminence; MFB, medial forebrain bundle; NVM, ventromedial nucleus; PD, pars distalis; PI, pars intermedia; PN, pars nervosa.

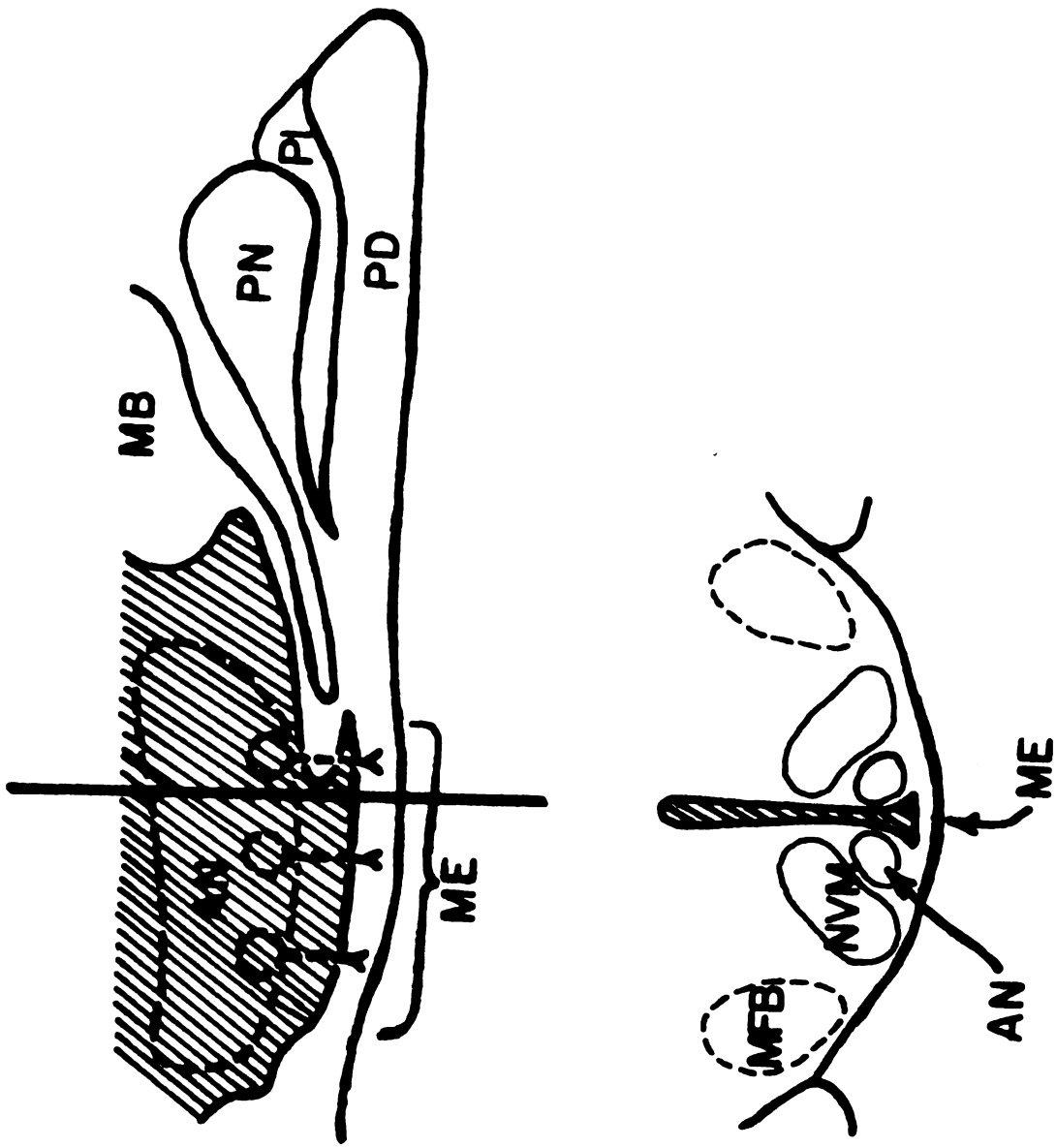


Figure 5

and remaining hypothalamic fragments in 15-20 volumes of 0.4N perchloric acid containing 10 mg% EGTA. The entire median eminence homogenate, 30 µl of the hypothalamic island homogenate and 50 µl of homogenate of the remaining hypothalamus were transferred to microcentrifuge tubes and centrifuged for 20 seconds in the Microfuge.

B. Radioenzymatic assays

Supernatants from tissue homogenates were analyzed for dopamine and norepinephrine by one of two modifications of the radiochemical enzymatic method of Cuello et al. (1973a). In those experiments in which supernatants were analyzed only for dopamine, a radioenzymatic assay as described by Moore and Phillipson (1975) was employed. Ten microliter samples of the supernatants were added to centrifuge tubes on ice. Twenty-five microliters of a freshly prepared incubation mix were then added. The incubator mix (sufficient for 40 samples) consisted of 67 µl of 20 mM EGTA sodium salt (pH 7.2); 332 µl of the catechol-O-methyltransferase (COMT) preparation; 200 µl of [^{3}H-methyl]S-adenosyl methionine (New England Nuclear) diluted to 0.1 mCi/ml; 67 µl of pargyline (16 mg/ml) in 10% β-mercaptoethanol; and 432 µl of 1M Tris-HCl buffer (pH 10.4) containing 3 mM $MgCl_2$. After a 40 minute incubation period at 37°C, the methylation reaction was stopped by the addition of 30 µl of a 5:1 mixture of 0.45M borate buffer (pH 10) and 3-methoxytyramine (10 mg/ml in 0.1% sodium metabisulfite). The O-methylated products were extracted into 0.5 ml of toluene/isoamyl alcohol (3:2). Following agitation on a vortex mixer and centrifugation, the organic phase was transferred to conical centrifuge tubes with pointed tips containing 25 µl of 0.1M hydrochloric acid. The tubes were again agitated and centrifuged and the

organic phase removed by aspiration. Fifteen microliters of the acid phase (3 x 5 µl) were applied to Whatman #1 chromatographic paper which had been spotted previously with 5 µl of non-radiolabelled 3-methoxytyramine (10 mg/ml in 0.1% sodium metabisulfite). 3-Methoxytyramine was isolated by descending paper chromatography using a solvent system of t-amyl alcohol/methylamine (4:1). The product was visualized under ultraviolet light. The spots were cut out, placed in scintillation vials and eluted with 3 ml of a solution of ethanol/ammonium hydroxide (100:22) for at least 8 hours. After adding 10 ml of scintillation fluid (0.5% PPO in toluene/ethanol, 7:3), the radioactivity was determined in a Beckman LS-100 liquid scintillation spectrometer. The sensitivity of this assay (i.e., that quantity which gave values twice the value of the blank) for dopamine was approximately 200 pg.

The sensitivity of this assay, however, was not great enough to allow for the determination of the norepinephrine content of a single median eminence. Thus, in those experiments in which supernatants were analyzed for both dopamine and norepinephrine, a modification of the procedure of Ben-Jonathan and Porter (1976) was employed. Ten microliter samples of the supernatants were placed in tubes to which 140 µl of 0.1N perchloric acid was added. The reaction was initiated by the addition of 50 µl of a freshly prepared incubation mix. The incubation mix (sufficient for 40 samples) consisted of 14 mg dithiotreitol; 100 µl of 1M $MgCl_2$; 1480 µl of 2M Tris-HCl buffer, pH 8.6; 100 µl of 0.05M EGTA; 400 µl of the COMT preparation; and 80 µl (20 mCi) of [^{3}H-methyl]S-adenosyl methionine (New England Nuclear). The tubes were incubated for 1 hour at 37°C. The reaction was stopped by the addition of 0.3 ml of 0.5M borate buffer, pH 10. A mixture

containing 10 µg of normetanephrine and 3-methoxytyramine in 25 µl of 0.1M acetic acid was added to each tube. Fifty microliters of a 1.5% solution of tetraphenylborate were also added. The O-methylated products were extracted into 3.5 ml of toluene/isoamyl alcohol (4:1). After mixing and centrifugation, the organic phase was transferred to tubes containing 0.3 ml of 0.5M borate buffer, pH 10. The tubes were vortexed and centrifuged and the organic phase was transferred to another set of tubes containing 200 µl of 0.1M hydrochloric acid. After extraction into the acid, the samples were lyophilized and resuspended in 55 µl of methanol/0.001M hydrochloric acid. Forty microliters were then spotted on thin layer chromatographic plates (Quantum Industries), which were prespotted with 20 µg of carrier O-methylated amines. The plates were developed in a solvent system of chloroform/ethanol/ethylamine (16:3:2). The plates were dried in an oven in the presence of paraformaldehyde vapor. The spots were localized under UV light and scraped into scintillation vials and eluted for 30 minutes with 1 ml 0.5M hydrochloric acid. For liquid scintillation spectrometry, 12 ml of a scintillation fluid made up of 0.25% PPO and 0.01% POPOP in toluene/Triton X-100 (2:1) were added. The sensitivity of this assay was 30 pg for dopamine and 100 pg for norepinephrine.

The homogenate pellet of each sample was analyzed for protein by the method of Lowry et al. (1951).

C. COMT preparation

All steps of COMT purification were performed at 0-4°C. Rat livers (70 g) were homogenized in 40 volumes of 1.1% KCl and centrifuged at 100,000 x g for 1 hour. The resulting supernatant was

filtered through glass wool and adjusted to pH 5.3 with 1M acetic acid. After centrifugation at 14,000 x g for 10 minutes, the supernatant was adjusted to pH 6.8 with 0.5M phosphate buffer, pH 7.0, and fractionated with ammonium sulfate (enzyme grade) as described by Nikodejevic et al. (1970). The final precipitate was dissolved in 8 ml of 1 mM phosphate buffer (pH 7.0) containing 10^{-4}M dithiothreitol and dialyzed overnight against 4 liters of this buffer.

D. Estimation of dopamine turnover

Dopamine turnover in the present study was estimated using the nonsteady state method of observing the decline of the endogenous dopamine concentration after synthesis inhibition with α-methyltyrosine. Although this method of estimating turnover has some disadvantages (see Weiner, 1974), it is currently the only method sensitive enough for the analysis of catecholamine turnover in small brain regions, such as the median eminence. The method used in these studies involves the assumption that the inhibition is the same for the various brain regions examined and is not markedly altered by the administration of drugs. The validity of such an assumption is supported by the fact that the same rate of catecholamine turnover in the hypothalamus was obtained when estimated by three different methods, one of which involved synthesis inhibition with α-methyltyrosine (Iversen and Glowinski, 1966). Furthermore, the decline of dopamine in the median eminence after α-methyltyrosine follows first order kinetics (see Results).

Two experimental designs were employed in turnover studies using α-methyltyrosine. In some experiments dopamine concentrations were determined 0, 60 and 120 minutes after the administration of

α-methyltyrosine methyl ester HCl (250 mg/kg of free amino acid, i.p.). Treatment differences were determined from a comparison of the calculated rate constants for the decline of dopamine concentrations. Alternatively, the extent of dopamine depletion at one time point, 1 hour, after α-methyltyrosine was used as an index of dopamine turnover. Differences in turnover in these studies were determined by comparing the percent depletion in various treatment groups.

E. Radioimmunoassays

Serum LH and FSH were measured by the NIAMDD RIA Kits in the laboratory of Dr. J. Meites. Results are expressed in terms of the standards NIAMDD-LH-RP-1 and NIAMDD-FSH-RP-1.

Serum prolactin was measured by the method of Niswender et al. (1969) or by the NIAMDD RIA Kit. In both cases the values are expressed in terms of NIAMDD-rat prolactin-RP-1.

F. Drugs

The following drugs were dissolved in saline: D,L-α-methyltyrosine methylester HCl (purchased from Regis Chemical Co.); piribedil mesylate, obtained through the courtesy of Dr. Derome-Tremblay (Les Laboratories Servier, Neuilly, France); ovine prolactin, obtained from the Pituitary Hormone Distribution Program; and baclofen (Lioresal), obtained through the courtesy of Dr. R. D. Robson, Ciba-Geigy Corp., Summit, NJ.

Apomorphine (obtained from Lilly Research Laboratories) was dissolved in 0.1% sodium metabisulfite. Estradiol benzoate was purchased from ICN Pharmaceuticals, Inc., and dissolved in corn oil. Haloperidol (obtained through the courtesy of Dr. John Kleis, McNeill Laboratories) was dissolved in 0.3% tartaric acid. Clozapine and thioridazine

(obtained through the courtesy of Ms. K. D. Roskaz, Sandoz Pharmaceuticals) were dissolved in 1.5% tartaric acid. γ-Butyrolactone was purchased from Matheson, Coleman and Bell, Norwood, OH.

The routes of administration of these agents are described in the Results.

G. Statistics

Differences in the steady state dopamine concentrations were analyzed by Student's t-test or an analysis of variance. After an analysis of variance, treatment differences were examined by the Student-Newman-Keuls test (Sokal and Rohlf, 1969).

Student's t-test was used to test the significance of the differences in the first order rate constants obtained by a least squares regression analysis (Goldstein, 1971). The effects of drug treatments on the α-methyltyrosine-induced depletion of dopamine in various brain regions were also examined by Student's t-test.

Serum prolactin concentrations were initially evaluated by a one-way analysis of variance. Differences between groups were then determined using the Student-Newman-Keuls test.

Serum LH and FSH concentrations were analyzed using Student's t-test.

RESULTS

I. Steady State Dopamine Concentrations

A. Dopamine concentrations in striatum, olfactory tubercle and median eminence

Values for the wet weights and protein contents of the corpus striatum, olfactory tubercle, and median eminence are listed in Table 1. The wet weight of the median eminence was not determined directly, but based on the protein contents in this region and the relative weights and protein contents of the other two brain regions, the median eminence samples were calculated to weigh 0.15 to 0.18 mg. These values are somewhat less than those reported by Kavanagh and Weisz (1974), but the dopamine concentration in these samples is essentially the same as that obtained by these investigators (12.5 µg/g). Despite the small amount of dopamine in the median eminence, approximately 2.5 ng/median eminence, the dopamine concentration in this region is higher than the other two brain regions examined. The dopamine concentration in the striatum and olfactory tubercle expressed on the bases of wet weight or protein content are essentially the same as those reported by other workers (Horn et al., 1974; Brownstein et al., 1974).

Table 1. Dopamine contents of striatum, olfactory tubercle and median eminence in rat brain

	Striatum	Olfactory Tubercle	Median Eminence
Wet weight (mg)	39.04 ± 1.91	10.10 ± 1.19	0.15 - 0.18*
Protein (μg)	4546 ± 242	998 ± 39	17.6 ± 1.5
Dopamine (ng/mg protein)	88 ± 3	60 ± 3	120 ± 13
Dopamine (μg/g wet weight)	10.19 ± 0.43	5.50 ± 0.34	10.8 - 13.7*

Values represent means ± 1 S.E.M. of 9 separate determinations.

*These values were calculated assuming the relationship between wet weight and mg protein for median eminence is the same as it is for striatum and olfactory tubercle.

B. Effects of γ-butyrolactone and baclofen on dopamine concentrations in the corpus striatum, olfactory tubercle and median eminence

Previous studies have demonstrated that γ-butyrolactone inhibits impulse flow in nigrostriatal (Walters et al., 1973) and, presumably, mesolimbic neurons resulting in a rapid accumulation of dopamine in the striatum (Gessa et al., 1966; Walters and Roth, 1972) and nucleus accumbens and olfactory tubercle (Aghajanian and Roth, 1970).

The purpose of this experiment was to compare the responses of tuberoinfundibular dopamine neurons to those of nigrostriatal and mesolimbic neurons to drugs which have been shown to influence dopaminergic systems and alter the steady state dopamine concentrations in the terminal region of these pathways.

The effects of γ-butyrolactone on dopamine concentrations in the striatum, olfactory tubercle and median eminence are presented in Table 2. In confirmation of previous reports, γ-butyrolactone increased dopamine concentrations in the striatum and olfactory tubercle 75% and 50%, respectively. In contrast, γ-butyrolactone had no effect on the dopamine concentration in the median eminence.

Kelly and Moore (1976) have reported that the systemic administration of baclofen increases the rat forebrain concentration of dopamine but not of norepinephrine. The effects of systemic baclofen administration on the dopamine concentrations of the three brain regions were similar to those of γ-butyrolactone (Table 2). Baclofen (40 mg/kg, i.p.) produced a 40% increase in the dopamine concentrations in the striatum and olfactory tubercle. Baclofen, like γ-butyrolactone, did not alter the dopamine concentration in the median eminence.

Table 2. Effects of γ-butyrolactone (GBL) and baclofen on dopamine concentrations in various brain regions

Treatment	N	ng dopamine/mg protein		
		Striatum	Olfactory Tubercle	Median Eminence
Control	5	82 ± 1	54 ± 3	112 ± 10
GBL	8	141 ± 7*	81 ± 4*	111 ± 12
Control	8	103 ± 5	70 ± 2	138 ± 7
Baclofen	8	142 ± 7*	97 ± 5*	138 ± 6

γ-Butyrolactone (GBL) (750 mg/kg, i.p.) was administered 90 minutes before sacrifice and baclofen (40 mg/kg, i.p.) 60 minutes before sacrifice. Values represent means ± S.E.M.

*Indicates values that are significantly different from control.

Thus, regardless of the mechanism by which γ-butyrolactone and baclofen increase endogenous dopamine concentrations, it is apparent that not all dopamine systems are influenced by these drugs.

C. Median eminence dopamine concentrations in female rats

Although steady state median eminence dopamine concentrations are not altered by γ-butyrolactone and baclofen, changes can be observed when the hormonal state of the animal is altered. Table 3 summarizes the dopamine and norepinephrine concentrations in the median eminence and hypothalamus of female rats in various hormonal states: at different times of the estrous cycle, after ovariectomy, and after estrogen treatment of ovariectomized rats.

No differences were observed in the dopamine concentration in the median eminence or hypothalamus throughout the estrous cycle, although a slight increase was observed in the median eminence on the day of estrus. However, the dopamine concentration in the median eminence of ovariectomized female rats was markedly increased, the value being approximately 140% of the values observed during the estrous cycle. Of further interest is the fact that seven daily injections of estradiol benzoate completely reversed this increase. The dopamine concentration in the median eminence of ovariectomized rats which received estradiol was the same as those observed throughout the estrous cycle. The increased dopamine concentration in the median eminence of ovariectomized rats may be a very selective effect since no corresponding change was observed in the concentration of this amine in the remaining hypothalamus. Norepinephrine concentrations were likewise unaltered in the median eminence and hypothalamus during the estrous cycle. In contrast to the elevation of the median

Table 3. Median eminence and hypothalamic catecholamine concentrations in the female rat

	Median Eminence		Hypothalamus	
	Dopamine	Norepinephrine	Dopamine	Norepinephrine
Diestrus 1	150 ± 16 (10)	49 ± 7 (10)	5.4 ± 0.4 (10)	22.7 ± 1.3 (10)
Diestrus 2	140 ± 10 (10)	40 ± 5 (10)	5.4 ± 0.3 (10)	22.7 ± 1.5 (10)
Proestrus	144 ± 10 (10)	44 ± 2 (10)	5.7 ± 0.4 (9)	24.7 ± 1.7 (9)
Estrus	165 ± 10 (10)	48 ± 5 (10)	5.3 ± 0.5 (10)	24.2 ± 1.7 (10)
OVX	213 ± 13 (11)	60 ± 5 (10)	5.7 ± 0.4 (11)	26.3 ± 2.7 (10)
OVX + Estradiol	139 ± 28 (5)	57 ± 13 (5)	5.9 ± 0.2 (6)	28.7 ± 2.6 (6)

Values are expressed in ng catecholamine/mg protein. Ovariectomized animals (OVX) were used 28-38 days after surgery. Animals which received estradiol benzoate (25 µg/kg, s.c.) were sacrificed 24 hours after the last of 7 daily injections.

eminence dopamine concentration after ovariectomy, castration did not significantly alter median eminence or hypothalamic norepinephrine concentrations, although a slight increase was observed in the median eminence.

The ability of ovariectomy to markedly increase the median eminence dopamine concentration and the ability of estradiol administration to reverse this effect support the contention that tuberoinfundibular dopamine neurons may be responsive to hormonal influences.

II. Dopamine Turnover Studies

Although the median eminence responds differently to the actions of certain drugs and hormonal changes when compared to other brain regions, the examination of steady state dopamine concentrations provides very little information regarding the activity of a neuronal pathway. Therefore, in order to examine the hypothesis that differences may exist in the regulatory mechanisms governing the activity of nigrostriatal, mesolimbic and tuberoinfundibular dopamine neurons, studies involving drug-induced changes in dopamine turnover in the terminal regions of these pathways were performed.

A. Differential effects of acute agonist and antagonist administration on dopamine turnover in the striatum, olfactory tubercle and median eminence

Dopamine antagonists (neuroleptics) increase the turnover rate of dopamine in whole brain (Andén et al., 1964; Neff and Costa, 1966; Nybäck et al., 1967). More recently neuroleptics have been shown to increase dopamine turnover within specific neuronal pathways. In particular, these drug effects have been observed in dopaminergic terminals of the nigrostriatal (Besson et al., 1971; Andén et al.,

1970) and mesolimbic (Andén, 1972; Zivkovic et al., 1975a; Wilk et al., 1975; Wiesel et al., 1975) pathways located in the striatum and olfactory tubercle or nucleus accumbens, respectively. On the other hand, dopaminergic agonists (apomorphine, piribedil) decrease dopamine turnover in the striatum (Corrodi et al., 1972; Bariletto et al., 1975) and limbic cortex (Scatton et al., 1975). It is generally thought that these effects of dopamine antagonists and agonists are mediated by a neuronal feedback loop subsequent to receptor blockade or stimulation, respectively (Carlsson and Lindqvist, 1963).

The effects of haloperidol and piribedil on dopamine turnover in the median eminence were examined in order to compare the response of tuberoinfundibular neurons to drugs which are known to influence the activity of nigrostriatal and mesolimbic neurons.

Neither haloperidol (0.5 mg/kg, i.p.) nor piribedil (30 mg/kg, i.p.) significantly altered the dopamine concentrations in the terminal regions of these three pathways before the administration of α-methyltyrosine (i.e., zero time or steady state concentrations). Nevertheless, changes in the rate constants for the α-methyltyrosine-induced decline of dopamine in the striatum and olfactory tubercle were observed in haloperidol- and piribedil-treated animals (Figure 6, Table 4). In the corpus striatum the dopaminergic antagonist, haloperidol, accelerated the rate of decline of dopamine, increasing the synthesis rate to 190% of control. Although the rate constant for the decline of the dopamine concentration in the olfactory tubercle was similarly increased, the difference was not significant. Nevertheless, the dopamine concentrations at 60 and 120 minutes after α-methyltyrosine administration in the haloperidol-treated group were significantly less than control values at these times. On the other

Figure 6. The effects of a dopaminergic agonist (piribedil) and antagonist (haloperidol) on the rate of decline of the dopamine concentration in the corpus striatum, olfactory tubercle and median eminence after i.p. injection of α-methyltyrosine (250 mg/kg). Haloperidol (0.5 mg/kg) or piribedil (30 mg/kg) were injected i.p. 60 or 30 minutes, respectively, before the administration of α-methyltyrosine. Animals were sacrificed immediately before (0 time) or 1 and 2 hours after α-methyltyrosine administration. Control animals received an i.p. injection of saline 60 minutes prior to sacrifice. Symbols represent means and vertical lines indicate $\pm$ 1 S.E.M. as determined from 6 to 9 animals. Where no S.E.M. is indicated the value is less than the radius of the symbol. See Table 4 for statistical treatment of the data.

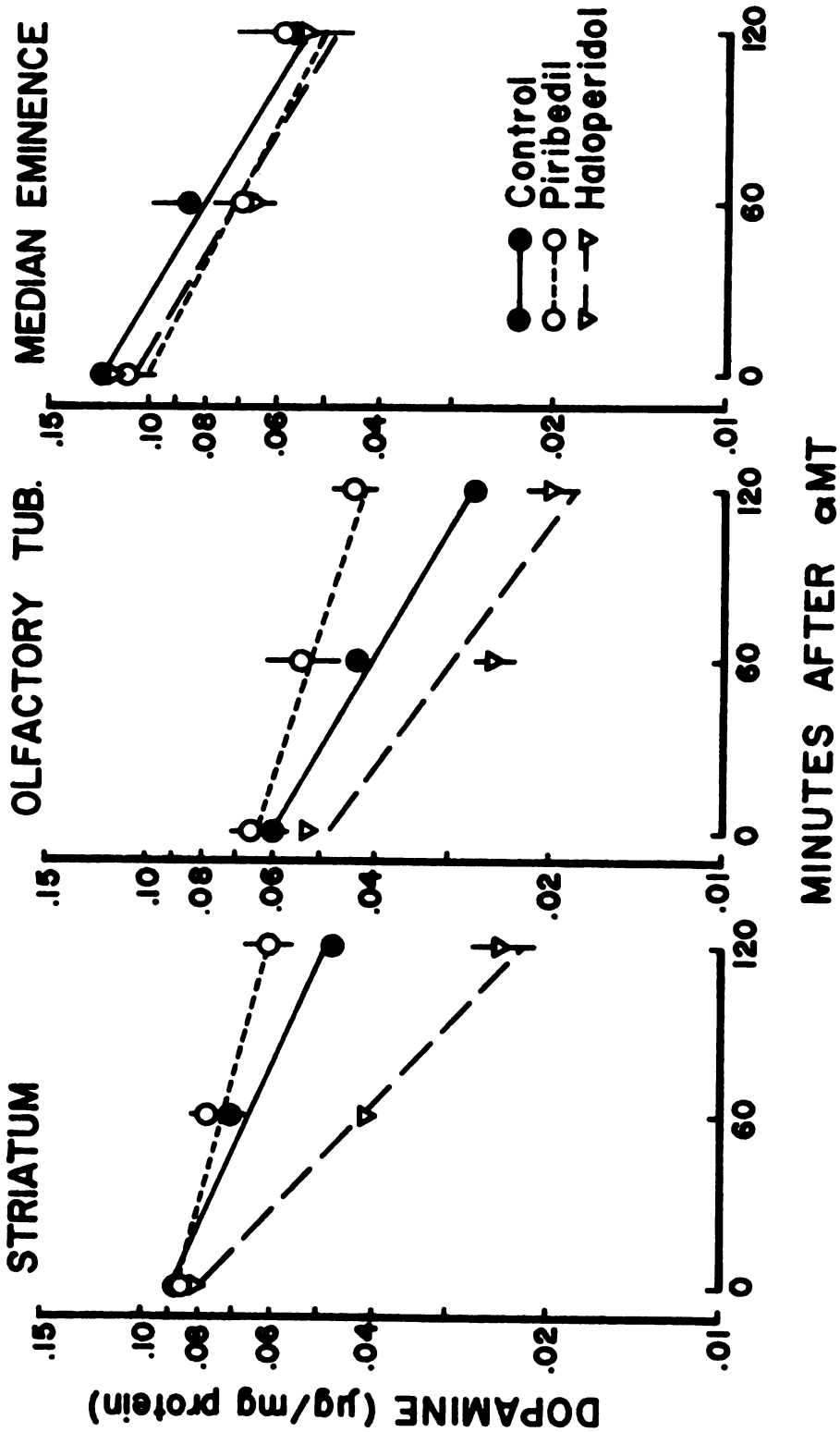

STRIATUM
OLFACTORY TUB.
MEDIAN EMINENCE
DOPAMINE (µg/mg protein)
MINUTES AFTER αMT
Control
Piribedil
Haloperidol
.15
.10
.08
.06
.04
.02
.01
0
60
120

Figure 6

Table 4. Effects of a dopaminergic agonist and antagonist on dopamine synthesis rates in various brain regions

	Rate Constant[†] k (h^{-1})	Synthesis Rate µg dopamine/mg protein/h
Median Eminence		
Control (19)	0.411 ± 0.170	0.049
Haloperidol (23)	0.399 ± 0.180	0.046
Piribedil (20)	0.353 ± 0.288	0.038
Olfactory Tubercle		
Control (21)	0.392 ± 0.073	0.023
Haloperidol (22)	0.487 ± 0.108	0.025
Piribedil (20)	0.230 ± 0.159*	0.015
Striatum		
Control (21)	0.311 ± 0.067	0.027
Haloperidol (23)	0.637 ± 0.096*	0.052
Piribedil (21)	0.178 ± 0.097*	0.015

See legend to Figure 6 for details of drug administration. Numbers in parentheses indicate number of observations in treatment groups.

[†]Values represent the mean ± 95% confidence interval calculated from the slopes of the plots of Figure 6. Synthesis rates were calculated by multiplying the rate constant times the zero-time endogenous content of dopamine.

*Indicates value different from control at $p<0.05$.

hand, the dopaminergic agonist, piribedil, reduced the rate of dopamine synthesis in the corpus striatum and olfactory tubercle to 55 and 65% of control, respectively. In contrast, neither haloperidol nor piribedil altered the rate constant for the α-methyltyrosine-induced decline of dopamine or the dopamine synthesis rate in the median eminence (Figure 6, Table 4).

These results suggest that a neuronal feedback mechanism may not regulate the activity of tuberoinfundibular dopamine neurons. Alternatively, dopamine receptors in the median eminence area may differ from those in the striatum and olfactory tubercle (e.g., the sensitivity of the receptors may be less). Thus, the lack of a drug-induced change in dopamine turnover in the median eminence may indicate a quantitative rather than a qualitative difference between tuberoinfundibular and nigrostriatal or mesolimbic neurons.

B. Time course for the effects of haloperidol on dopamine turnover in the striatum, olfactory tubercle and median eminence

In order to exclude the possibility that these differential drug effects reflected merely quantitative differences, the dose of haloperidol was increased and the time course for the effects of the neuroleptic on dopamine turnover in the striatum, olfactory tubercle and median eminence was examined.

Male rats were sacrificed 2, 8, 16, 24 and 72 hours after a single injection of haloperidol (2.5 mg/kg, s.c.). One hour before sacrifice and independent of the time of neuroleptic administration, half of the animals in each group received saline and the other half received α-methyltyrosine. At no time did haloperidol alter the steady state dopamine concentration in the striatum. That is, the

dopamine concentrations in this brain region of vehicle- and haloperidol-pretreated rats which received saline were the same. Consequently, these values were combined and the mean is represented by the horizontal line in Figure 7. One hour after α-methyltyrosine administration the striatal dopamine concentration in vehicle-pretreated animals was reduced to approximately 75% of the steady state value. Haloperidol enhanced the α-methyltyrosine-induced reduction of striatal dopamine concentrations to approximately 50% of the steady state value 2 and 8 hours after a single injection; no effect was observed at 16, 24 and 72 hours after haloperidol.

Similar results were obtained in the olfactory tubercle. As in the striatum, haloperidol did not alter the steady state dopamine concentration at any time. Steady state values for haloperidol- and vehicle-pretreated animals were thus combined and are depicted in Figure 8 as the horizontal line. The α-methyltyrosine-induced depletion of the dopamine concentration in the olfactory tubercle of vehicle-pretreated rats was similar to that seen in the striata (i.e., approximately 75% of the steady state concentration). Haloperidol again enhanced the α-methyltyrosine-induced reduction of the dopamine concentration to approximately 50% of the steady state value at the earlier times (2 and 8 hours), while no effect was observed at later times (16, 24 and 72 hours).

The steady state dopamine concentration in the median eminence was likewise unaltered by haloperidol. In contrast to the effects of haloperidol in the striatum and olfactory tubercle, this neuroleptic did not affect the α-methyltyrosine-induced depletion of dopamine 2 and 8 hours after a single injection (Figure 9). However, unlike the results obtained in the striatum and olfactory tubercle, haloperidol

Figure 7. The effect of haloperidol on the α-methyltyrosine-induced depletion of dopamine in the striatum. Rats were sacrificed at the indicated times after a single injection of haloperidol (2.5 mg/kg, s.c.) or vehicle. One hour before sacrifice half of the animals in each group received saline or α-methyltyrosine (250 mg/kg, i.p.), independent of the time of neuroleptic administration. No significant differences were observed in the endogenous striatal dopamine concentrations of haloperidol- and vehicle-pretreated animals (87.2 $\pm$ 2.7 and 89.8 $\pm$ 2.4 ng/mg protein, respectively). Accordingly, these values were combined; the horizontal line represents the mean and the shaded area $\pm$ 1 S.E.M. The means of the α-methyltyrosine-depleted values are depicted by the solid (haloperidol) and open (vehicle) columns; vertical lines represent $\pm$ 1 S.E.M. based upon 5 to 15 separate determinations. * indicates those values obtained from haloperidol-pretreated animals which differ significantly ($p<0.05$) from vehicle-pretreated animals.

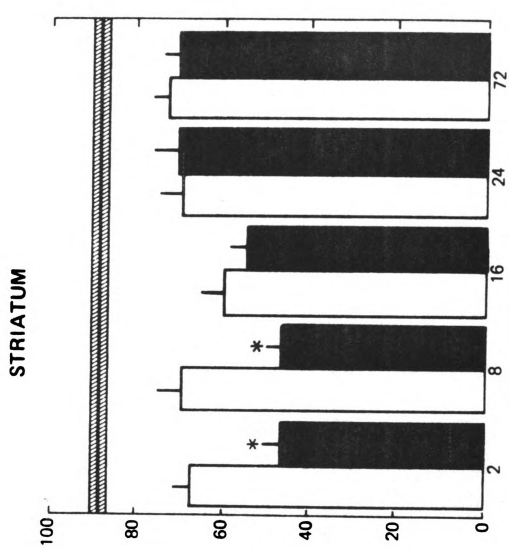

STRIATUM
DOPAMINE (ng/mg protein)
100
80
60
40
20
0
*
*
2
8
16
24
72
HOURS AFTER HALOPERIDOL

Figure 7

Figure 8. The effect of haloperidol on the α-methyltyrosine-induced depletion of dopamine in the olfactory tubercle. See legend to Figure 7 for details of drug administrations. No significant differences were observed in the endogenous dopamine concentrations of haloperidol- and vehicle-pretreated animals (63.4 $\pm$ 1.7 and 67.6 $\pm$ 2.0 ng/mg protein, respectively). Accordingly, these values were combined; the horizontal line represents the mean and the shaded area $\pm$ 1 S.E.M. The means of the α-methyltyrosine-depleted values are depicted by the solid (haloperidol) and open (vehicle) columns; vertical lines represent $\pm$ 1 S.E.M. based upon 5-15 separate determinations. * indicates those values of the haloperidol-pretreated animals which differ significantly ($p<0.05$) from the vehicle-pretreated animals.

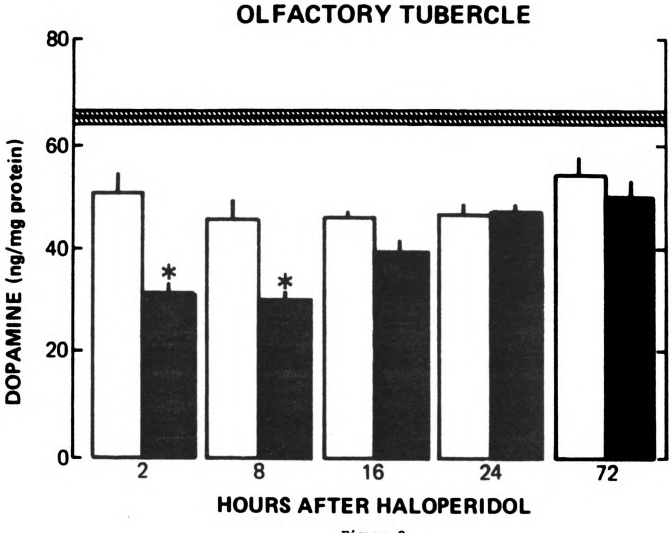

OLFACTORY TUBERCLE
DOPAMINE (ng/mg protein)
80
60
40
20
0
*
*
2
8
16
24
72
HOURS AFTER HALOPERIDOL

Figure 8

Figure 9. The effect of haloperidol on the α-methyltyrosine induced depletion of dopamine in the median eminence. See Figure 7 for details of drug administrations. No significant differences were observed in the endogenous dopamine concentrations of haloperidol- and vehicle-pretreated animals (132.2 ± 4.4 and 133.2 ± 3.7 ng/mg protein, respectively). Accordingly, these values were combined; the horizontal line represents the mean and the shaded area ± 1 S.E.M. The means of the α-methyltyrosine-depleted values are depicted by the solid (haloperidol) and open (vehicle) columns; vertical lines represent ± 1 S.E.M. based upon 6 to 18 separate determinations. * indicates those values of the haloperidol-pretreated animals which differ significantly ($p<0.05$) from the vehicle-pretreated animals.

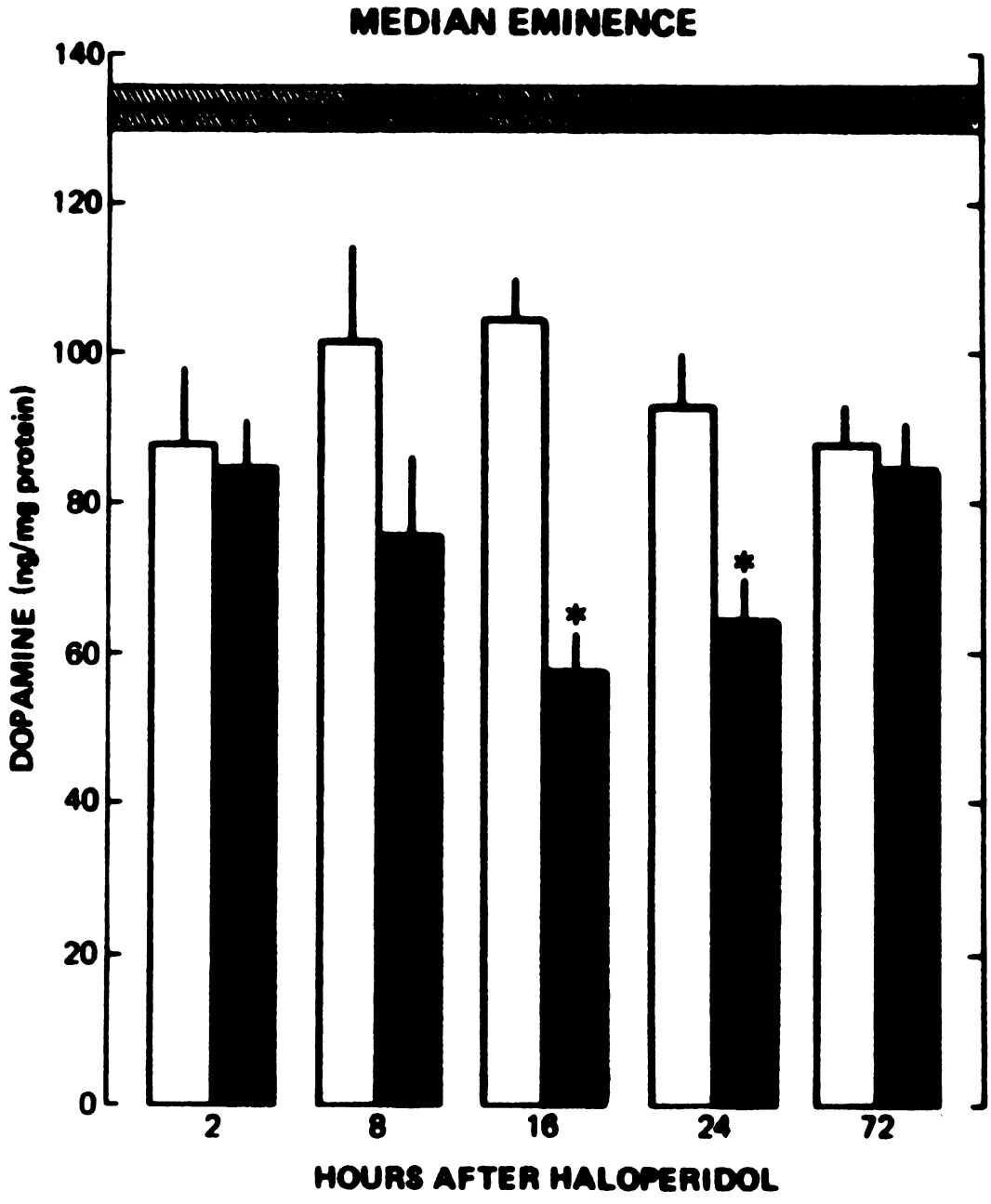

MEDIAN EMINENCE
140
120
100
80
60
40
20
0
DOPAMINE (ng/mg protein)
2
8
16
24
72
HOURS AFTER HALOPERIDOL
*
*

Figure 9

did enhance the α-methyltyrosine-induced reduction of median eminence dopamine concentrations at later times, 16 and 24 hours after administration.

Thus, haloperidol can increase dopamine turnover in all three brain regions, although apparently through different mechanisms. The rapid effect in the striatum and olfactory tubercle is probably mediated through a neuronal feedback loop, subsequent to the blockade of postsynaptic dopamine receptors. However, this rapid effect was not observed in the median eminence. Although haloperidol produced a delayed increase in dopamine turnover in the median eminence, it does not appear that this effect is mediated by a similar neuronal feedback mechanism.

C. Haloperidol in hypophysectomized rats

Since neuronal systems in the median eminence are involved in controlling the secretion of a number of anterior pituitary hormones, one possibility for the delayed increase of dopamine turnover in this brain region after haloperidol is that this action is mediated by hormones released from the pituitary. The possibility that the haloperidol-induced increase in dopamine turnover in the median eminence is hormonally mediated was investigated by examining the effects of haloperidol in hypophysectomized animals.

Sixteen hours after the subcutaneous injection of haloperidol (2.5 mg/kg), the rate constant for the decline of the dopamine concentration in the median eminence and, consequently, the calculated synthesis rates of dopamine were increased 100% (Figure 10A, Table 5).

Hypophysectomy resulted in a 30% reduction of the steady state (zero time) dopamine concentration in the median eminence (Figure 10).

Figure 10. The α-methyltyrosine-induced decline of the dopamine concentration in the median eminence of (A) unoperated control and (B) hypophysectomized rats 16 hours after the subcutaneous administration of vehicle (open circles) or haloperidol, 2.5 mg/kg (solid circles). Animals were sacrificed 1 hour after an i.p. injection of saline (zero time) or 1 or 2 hours after an i.p. injection of α-methyltyrosine (250 mg/kg). Symbols represent means and vertical lines ± 1 S.E.M. as determined for 7-9 animals. Where no S.E.M. is indicated the value is less than the radius of the symbol. See Table 5 for statistical treatment of the data.

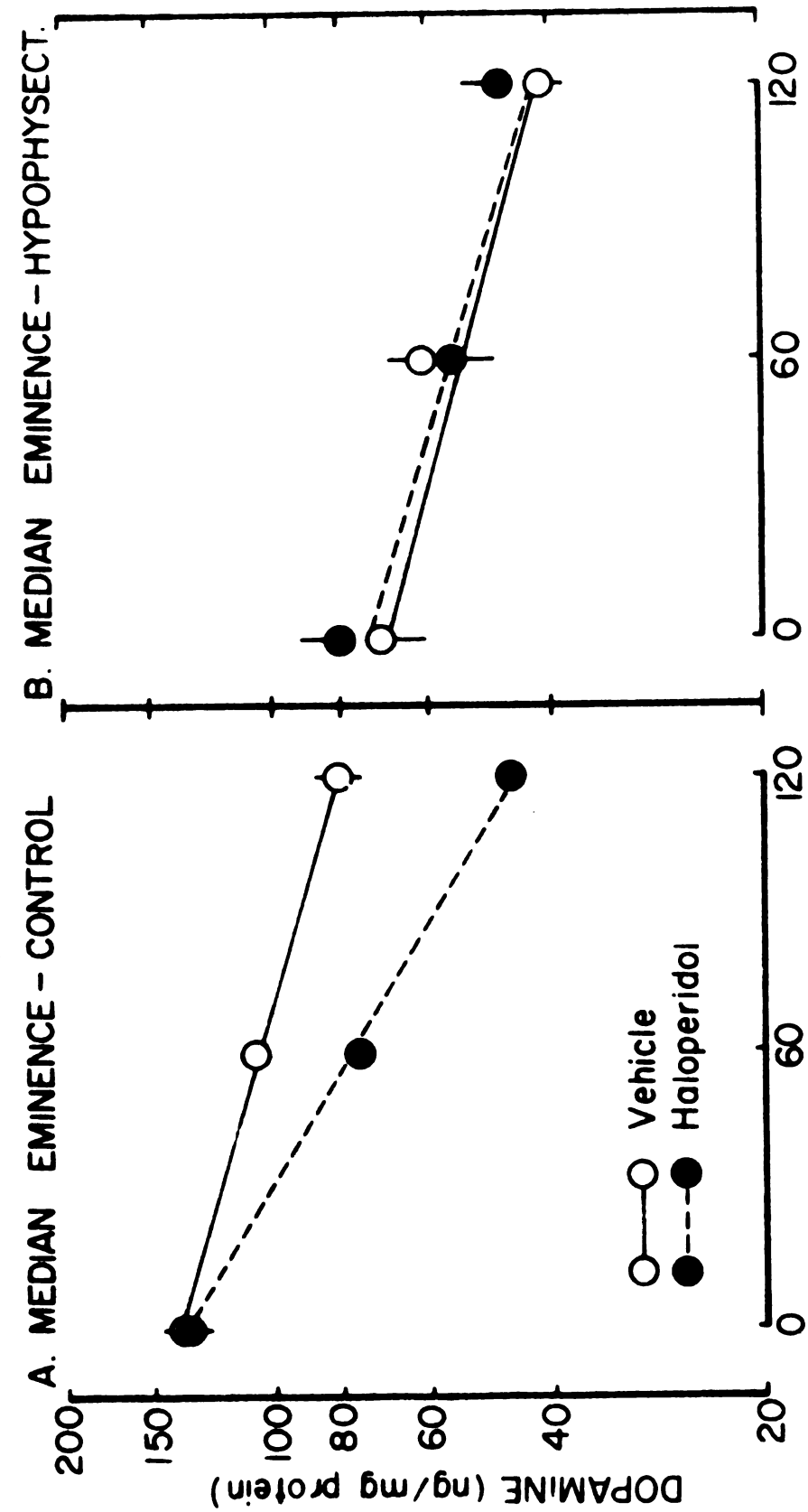

A. MEDIAN EMINENCE - CONTROL
B. MEDIAN EMINENCE - HYPOPHYSECT.
DOPAMINE (ng/mg protein)
200
150
100
80
60
40
20
0
60
120
MINUTES AFTER αMT
Vehicle
Haloperidol

Figure 10

Table 5. Effects of haloperidol on dopamine synthesis rates in the median eminence and striatum

	Rate Constant k (hr^{-1})	Synthesis Rate µg dopamine/mg protein/h
Median Eminence		
Controls		
Vehicle	0.26 ± 0.02	0.036
Haloperidol	0.53 ± 0.02*	0.072
Hypophysectomized		
Vehicle	0.24 ± 0.04	0.017
Haloperidol	0.26 ± 0.04	0.021
Striatum		
Controls		
Vehicle	0.28 ± 0.02	0.026
Haloperidol	0.29 ± 0.02	0.026
Hypophysectomized		
Vehicle	0.19 0.02	0.017
Haloperidol	0.40 0.02*	0.035

See legend to Figure 10 for details of drug administration.

Values for the rate constant represent the mean ± 95% confidence interval. Synthesis rates were calculated by multiplying the rate constant times the zero time endogenous concentration of dopamine.

*Indicates those values of haloperidol-pretreated animals which differ from those of the paired vehicle-pretreated group at $p<0.05$.

Despite this reduction, the rate constant for the decline of the dopamine concentration after α-methyltyrosine, 0.24 $hour^{-1}$, was the same as that observed in unoperated controls, 0.26 $hour^{-1}$ (Table 5). On the other hand, hypophysectomy did prevent the haloperidol-induced increase in dopamine turnover in the median eminence.

The effects of haloperidol on striatal dopamine turnover were also determined in the same unoperated and hypophysectomized animals. Consistent with the time course for the actions of haloperidol, this neuroleptic had no effect on dopamine turnover in the striata of unoperated controls 16 hours after a single administration (2.5 mg/kg, s.c.) (Figure 11A, Table 5). In contrast, haloperidol enhanced the rate of the α-methyltyrosine-induced decline of striatal dopamine concentrations of hypophysectomized animals by approximately 100% (Figure 11B, Table 5).

Thus, although haloperidol increased dopamine turnover in the median eminence, this action was dependent upon the presence of the pituitary gland. These results suggest, therefore, that the action of haloperidol on tuberoinfundibular neurons is indirect and most likely hormonally mediated.

It was of further interest to determine if other antipsychotic agents could increase dopamine turnover in the median eminence. Either thioridazine (16 mg/kg, s.c.) or clozapine (40 mg/kg, s.c.) was given 16 and 8 hours before sacrifice. Neither drug altered the steady state dopamine concentration in the median eminence (Table 6). However, similar to the actions of haloperidol, both thioridazine and clozapine pretreatment enhanced the α-methyltyrosine-induced depletion of dopamine in this brain region when compared to vehicle-pretreated controls.

Figure 11. The α-methyltyrosine-induced decline of the dopamine concentration in the striata of (A) unoperated controls and (B) hypophysectomized rats 16 hours after the subcutaneous administration of vehicle (open circles) or haloperidol, 2.5 mg/kg (solid circles). Animals were sacrificed 1 hour after an i.p. injection of saline (zero time) or 1 or 2 hours after an i.p. injection of α-methyltyrosine (250 mg/kg). Symbols represent means and vertical lines $\pm$ 1 S.E.M. as determined for 7-9 animals. Where no S.E.M. is indicated the value is less than the radius of the symbol. See Table 5 for statistical treatment of the data.

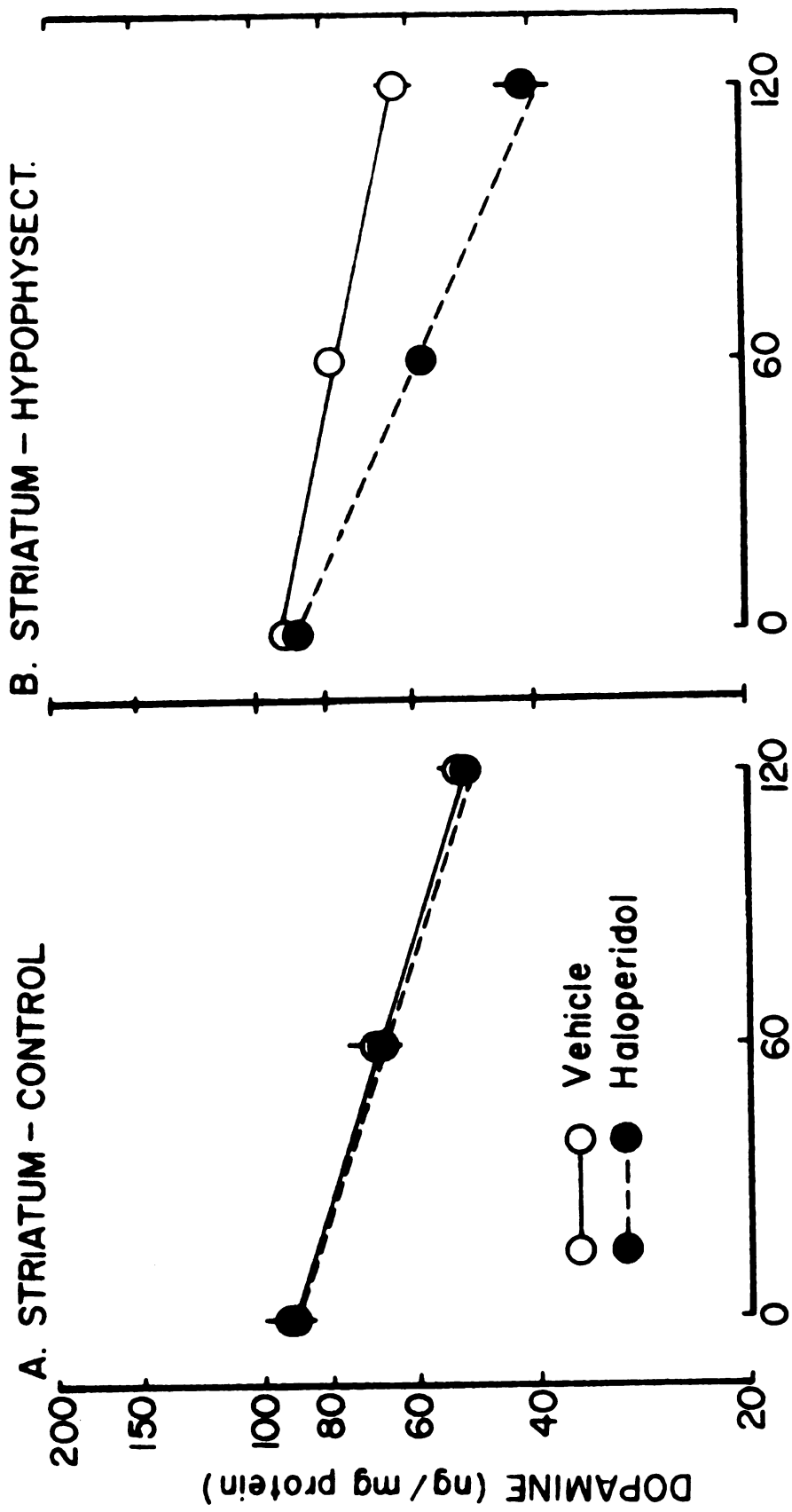

A. STRIATUM – CONTROL
B. STRIATUM – HYPOPHYSECT.
DOPAMINE (ng/mg protein)
200
150
100
80
60
40
20
0
60
120
MINUTES AFTER αMT
Vehicle
Haloperidol

Figure 11

Table 6. Effects of thioridazine and clozapine on the α-methyltyrosine induced depletion of dopamine in the median eminence

Pretreatment		Treatment: Saline (ng dopamine/mg protein)	Treatment: α-Methyltyrosine (ng dopamine/mg protein)	$\frac{\text{α-Methyltyrosine}}{\text{Saline}} \times 100$[a]
Vehicle	16 hr	120 ± 11 (7)	89 ± 8 (7)	75 ± 7
Thioridazine	16 hr	118 ± 8 (6)	49 ± 4 (7)	41 ± 4*
Vehicle	16 hr	122 ± 10 (9)	90 ± 8 (8)	70 ± 6
Clozapine	16 hr	137 ± 10 (8)	61 ± 7 (7)	47 ± 5*

Rats received thioridazine (16 mg/kg), clozapine (40 mg/kg) or vehicle subcutaneously 16 and 8 hours before sacrifice. One hour before sacrifice half of the animals in each group received saline and half received α-methyltyrosine (250 mg/kg) i.p. Neither thioridazine nor clozapine altered the steady state dopamine concentration in the median eminence. Numbers in parentheses represent the number of animals in each treatment group.

*Indicates those values from thioridazine or clozapine-pretreated animals which differ significantly ($p<0.05$) from their vehicle-pretreated controls.

[a]These values represent the α-methyltyrosine values expressed as a percent of a combined saline and thioridazine or saline and clozapine zero time value.

D. Selective actions of prolactin administration on dopamine turnover

High circulating levels of prolactin have been shown to inhibit prolactin release by the *in situ* pituitary (Meites and Clemens, 1972; MacLeod, 1974) and may do so by increasing dopamine turnover in the median eminence (Fuxe and Hökfelt, 1969). Since haloperidol and other antipsychotic agents increase pituitary prolactin release (Dickerman et al., 1972; Clemens et al., 1974), drug-elevated serum prolactin concentrations may mediate the action of neuroleptics to increase dopamine turnover in the median eminence. The effect of exogenous prolactin administration on dopamine turnover in the median eminence was therefore examined.

Ovariectomized female rats received ovine prolactin (5.0 mg/kg, s.c.) or vehicle as follows. Two groups of rats were injected with prolactin or vehicle every 8 hours for 1 or 3 days and sacrificed 2 hours after the last injection, or 26 and 74 hours after the first injection, respectively. Another group was given prolactin 10 and 2 hours before decapitation, and a fourth group was given prolactin 2 hours before decapitation.

The steady state median eminence dopamine concentrations of prolactin- and vehicle-pretreated rats were similar (127.9 ± 5.7 and 129.7 ± 4.9 ng dopamine/mg protein, respectively). Therefore, these values were combined and the mean is represented as the horizontal line in Figure 12. α-Methyltyrosine reduced the dopamine concentration in the median eminence of vehicle-pretreated rats to 50% of the steady state concentration. After 2 and 10 hours of prolactin administration, no differences were observed in the α-methyltyrosine-induced decline of the dopamine concentration between the two groups.

Figure 12. Effects of prolactin (PRL) administration on the α-methyltyrosine-induced depletion of dopamine in the median eminence. Ovariectomized rats were treated with PRL or vehicle for the indicated times as described in Results. Animals in each group received saline or α-methyltyrosine 1 hour before sacrifice. PRL administration had no effect on the endogenous dopamine concentration in saline-pretreated animals (129.7 $\pm$ 4.9 and 127.9 $\pm$ 5.7 ng/mg protein in vehicle- and PRL-pretreated rats, respectively). Accordingly, these values were combined; the horizontal line represents the mean and the shaded area 1 S.E.M. Columns and vertical lines represent the means $\pm$ 1 S.E.M. of the α-methyltyrosine-depleted groups. * indicates those values of the PRL-pretreated animals which differ significantly ($p<0.05$) from the vehicle-pretreated animals.

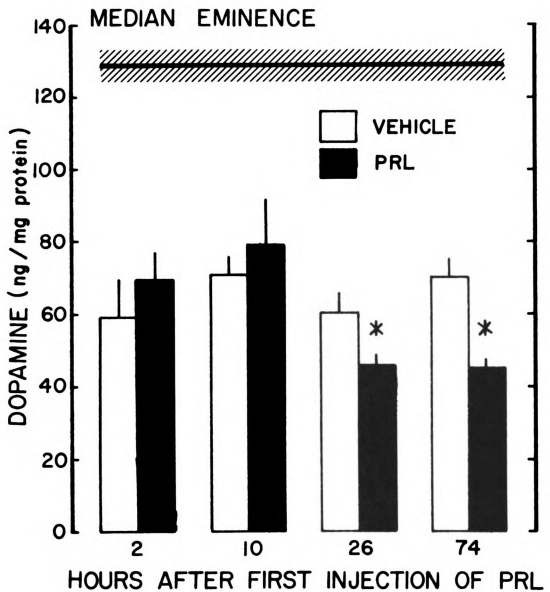

MEDIAN EMINENCE
VEHICLE
PRL
DOPAMINE (ng/mg protein)
140
120
100
80
60
40
20
0
2
10
26
74
HOURS AFTER FIRST INJECTION OF PRL

Figure 12

However, the α-methyltyrosine-induced depletion of dopamine in the prolactin-pretreated animals was significantly greater than in the vehicle-pretreated rats after 1 and 3 days of prolactin administration. This suggests that after a definite latent period, prolactin administration increases the turnover of dopamine in the median eminence. Indeed, this lag period is very similar to the delay seen before haloperidol increased dopamine turnover in the median eminence.

The effects of prolactin administration were also examined in the striatum (Figure 13). As in the median eminence, prolactin had no effect on the steady state dopamine concentration. However, in contrast to the results observed in the median eminence, prolactin administration did not influence the α-methyltyrosine-induced depletion of dopamine in the striatum at any of the times examined.

Serum LH and FSH concentrations in prolactin- and vehicle-pretreated rats were also determined (Table 7). Castration produced a marked elevation of serum LH and FSH levels when compared to the intact controls (diestrous). Prolactin administration partially prevented the post-castration rise of serum LH after 1 or 3 days of treatment. A similar effect of prolactin on serum FSH was observed. The post-castration rise of FSH was significantly reduced after 10 hours and 1 or 3 days of prolactin administration.

Thus, the exogenous administration of prolactin is capable of selectively increasing the activity of the tuberoinfundibular dopamine pathway. Since tuberoinfundibular neurons appear to be responsible for the tonic inhibition of prolactin secretion (Meites and Clemens, 1972), the prolactin-induced increase of dopamine turnover in the median eminence may be one mechanism by which prolactin regulates its own secretion.

Figure 13. Effects of prolactin (PRL) administration on the α-methyltyrosine-induced depletion of dopamine in the corpus striatum. Ovariectomized rats were treated with PRL or vehicle for the indicated times as described in Results. Animals in each group received saline or α-methyltyrosine 1 hour before sacrifice. PRL administration had no effect on the endogenous dopamine concentration (91.8 ± 3.7 and 89.6 ± 4.1 ng dopamine/mg protein in vehicle- and PRL-pretreated rats, respectively). Accordingly, these values were combined; the horizontal line represents the mean and the shaded area ± 1 S.E.M. Columns and vertical lines represent the means ± 1 S.E.M. for the α-methyltyrosine-depleted groups.

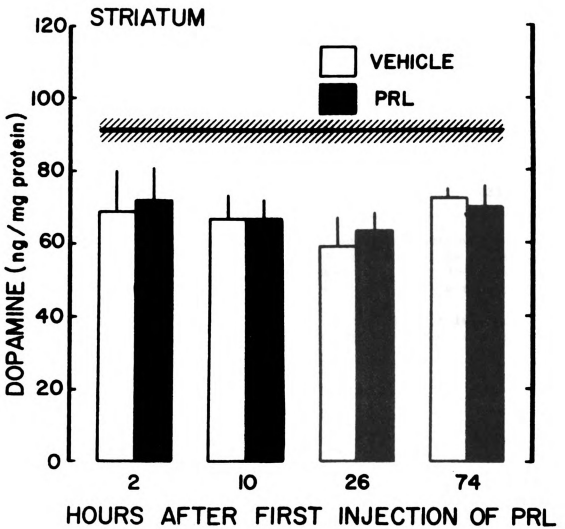

STRIATUM
VEHICLE
PRL
DOPAMINE (ng/mg protein)
120
100
80
60
40
20
0
2
10
26
74
HOURS AFTER FIRST INJECTION OF PRL

Figure 13

Table 7. Effects of prolactin (PRL) administration on serum LH and FSH concentrations of female ovariectomized rats

Duration of PRL Administration	LH (ng/ml)		FSH (ng/ml)	
	Vehicle	PRL	Vehicle	PRL
Intact Controls	22 ± 1		62 ± 20	
2 hours	192 ± 28	207 ± 41	886 ± 45	911 ± 23
10 hours	295 ± 44	195 ± 33	837 ± 17	765 ± 31*
26 hours	345 ± 40	226 ± 28*	888 ± 36	758 ± 17*
74 hours	481 ± 59	268 ± 41*	942 ± 56	633 ± 77*

All animals were sacrificed 4 days after ovariectomy and at the indicated times after the initiation of PRL administration as described in Results. Each value represents the mean ± 1 S.E.M. of 6-8 separate determinations at each of the times of PRL or vehicle pretreatment.

*Indicates those values of the PRL-pretreated animals which differ significantly ($p<0.05$) from the vehicle-pretreated animals.

The observation that the prolactin-induced increase of dopamine turnover in the median eminence was accompanied by decreases in the post-castration rise of serum LH and FSH suggests that tuberoinfundibular dopamine neurons may mediate these effects and supports the contention of Fuxe et al. (1976) that tuberoinfundibular neurons inhibit the release of LH and FSH.

The ability of elevated serum prolactin concentrations to increase dopamine turnover in the median eminence provides evidence for the hypothesis that dopaminergic neurons of the tuberoinfundibular pathway may be regulated by a hormonal-neuronal feedback mechanism in contrast to the neuronal feedback mechanism regulating nigrostriatal and mesolimbic neurons.

In addition, this hormonal-neuronal feedback mechanism appears to be a sluggish system, as evidenced by the lag time in the activation of tuberoinfundibular neurons after prolactin and haloperidol administration. This contrasts to the rapid response of neuronal feedback modulation of nigrostriatal and mesolimbic neurons.

E. Effects of estradiol benzoate on dopamine turnover and serum prolactin concentrations

Pituitary prolactin release is markedly enhanced by endogenous gonadal steroids. Since dopamine turnover in the median eminence was increased after the administration of exogenous prolactin, it was of interest to examine the effects of estradiol benzoate administration on serum prolactin concentrations and dopamine turnover in the median eminence. In order to examine the regional specificity of the actions of estradiol, its effects on dopamine turnover in the striatum were also determined.

Male rats were injected daily with estradiol benzoate, 25 µg/kg, or corn oil vehicle, subcutaneously, for 1, 3 or 5 days. Animals were sacrificed 24 hours after the last injection. Once again, half of the animals in each group received saline and the other half received α-methyltyrosine 1 hour before sacrifice. Since the dopamine concentrations in the median eminence of vehicle- and estradiol-pretreated rats which received saline were not significantly different, these values were combined and are represented by the horizontal line in Figure 14A. One hour treatment with α-methyltyrosine reduced the dopamine concentration in the median eminence of vehicle-pretreated rats to 55-70% of the values in saline-treated animals. The α-methyltyrosine-induced depletion of dopamine in the median eminence of animals treated for 1 day with estradiol or vehicle was the same. However, the α-methyltyrosine-induced decline of dopamine was significantly greater in the estrogen-pretreated group after 3 and 5 days of injections, the dopamine concentration being 43 and 30% of the control steady state value, respectively.

In the striatum, as in the median eminence, estrogen pretreatment did not alter the steady state concentration of dopamine. However, unlike the results observed in the median eminence, the α-methyltyrosine-induced depletion of dopamine in the striatum was not altered by estradiol treatment (Figure 14B). The dopamine concentrations in the α-methyltyrosine-treated groups ranged from 71-78% of the steady state value.

Serum prolactin concentrations were also determined in these same animals; the results are summarized in Table 8. When compared to the appropriate corn oil controls, estradiol increased serum prolactin concentrations, an increase which became progressively greater with

Figure 14. Effects of estrogen on the α-methyltyrosine-induced reduction of dopamine concentrations in the (A) median eminence and (B) striatum. Male rats were injected daily with estradiol benzoate (25 μg/kg, s.c.) or corn oil vehicle for 1, 3 or 5 days, the last injection being made 24 hours prior to sacrifice. One hour prior to sacrifice half of the animals at each time point were injected with saline (zero time controls) and the other half with α-methyltyrosine (250 mg/kg, i.p.). Since there were no significant differences in the dopamine concentrations of median eminence (114.8 ± 8.3 and 111.9 ± 7.4 ng/mg protein) and striatum (113.6 ± 3.4 and 107.2 ± 3.1 ng/mg protein) of vehicle- and estrogen-pretreated rats which received saline, these values were combined. The means of the zero time controls were set at 100% and represented by the horizontal lines and ± 1 S.E.M. by the shaded areas. The dopamine concentrations in the brain regions of animals sacrificed 60 minutes after the injection of α-methyltyrosine were calculated as a percentage of their respective zero time controls. Values from vehicle-pretreated animals which received α-methyltyrosine are represented by open bars and values from estrogen-pretreated animals which received α-methyltyrosine are represented by solid bars; vertical lines represent 1 S.E.M. based on 5-9 determinations. * indicates values in estrogen-pretreated rats which are significantly different from vehicle-pretreated rats ($p<0.05$).

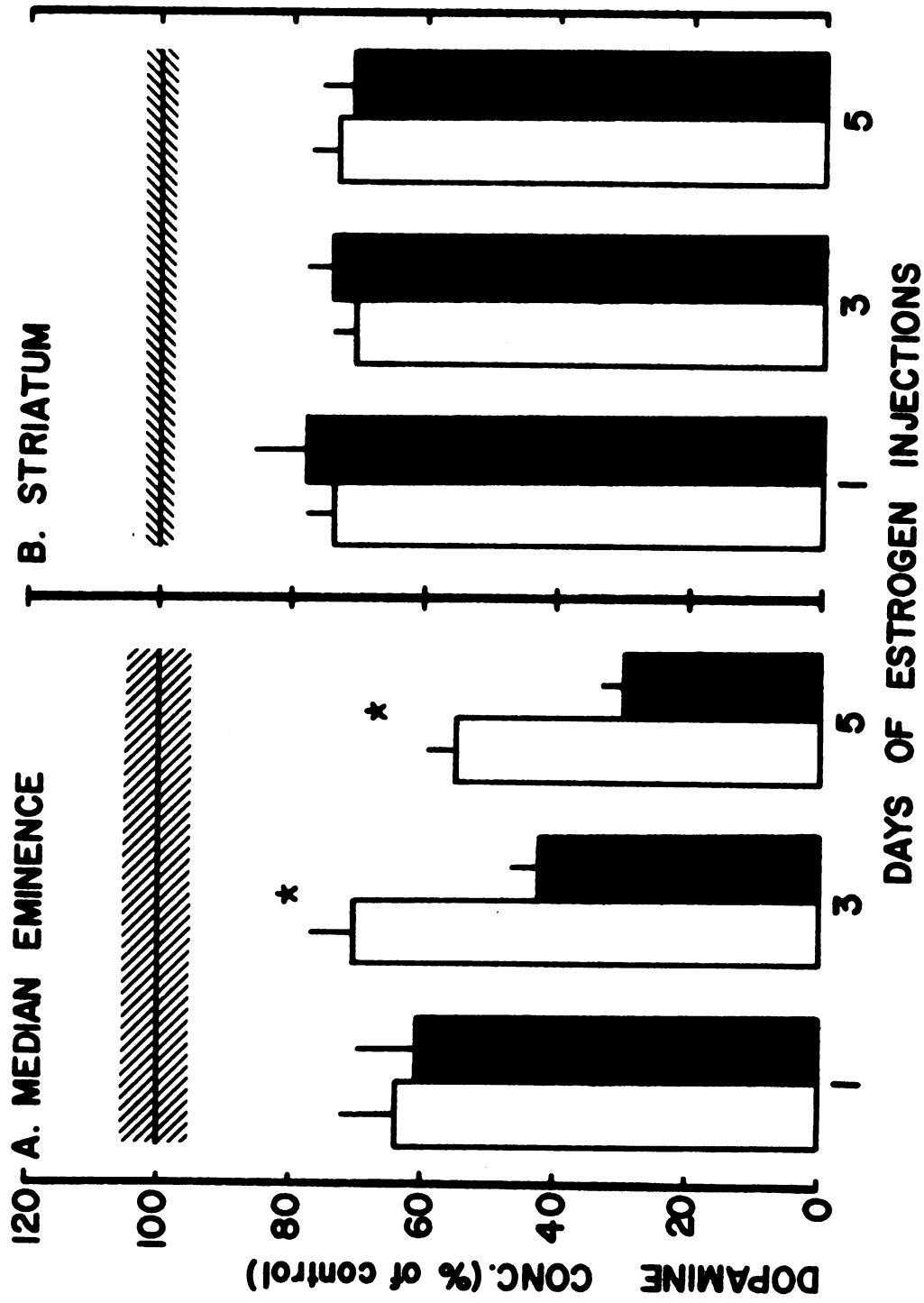

A. MEDIAN EMINENCE
B. STRIATUM
DOPAMINE CONC. (% of control)
120
100
80
60
40
20
0
1
3
5
*
*
DAYS OF ESTROGEN INJECTIONS

Figure 14

Table 8. Effects of estradiol benzoate and α-methyltyrosine on serum concentrations of prolactin

		Serum Prolactin (ng/ml)			
Pretreatment		Corn Oil		Estradiol Benzoate	
Treatment		Saline	α-Methyltyrosine	Saline	α-Methyltyrosine
Days of Pretreatment	N				
1	11	19 ± 3	113 ± 8*	40 ± 4*	190 ± 15†
3	6	33 ± 6	99 ± 25*	74 ± 7*	328 ± 23†
5	4	40 ± 23	101 ± 20*	141 ± 13*	288 ± 8†

Male rats were injected daily with estradiol benzoate (25 μg/kg, i.p.) or corn oil vehicle for 1, 3 or 5 days, the last injection being made 24 hours prior to sacrifice. One hour prior to sacrifice, half of the animals at each time point were injected with saline and the other half with α-methyltyrosine (250 mg/kg, i.p.).

*Indicates those values which differ significantly ($p<0.05$) from the appropriate values for corn oil-saline treated animals.

†Indicates a significantly greater ($p<0.05$) α-methyltyrosine-induced increase in estradiol-treated animals.

increasing duration of estradiol pretreatment. Prolactin concentrations rose from 40 ng/ml, after 1 day of estradiol, to 140 ng/ml after 5 days of injections. α-Methyltyrosine administration increased serum prolactin concentrations in vehicle-pretreated animals. This α-methyltyrosine-induced effect was enhanced in estradiol-pretreated animals. That is, serum prolactin concentrations in rats treated with both estradiol and α-methyltyrosine were significantly higher ($p<0.05$) than in rats treated with either agent alone. Thus, although serum prolactin concentrations are elevated after estradiol administration, they would be much greater without the inhibitory influence of tuberoinfundibular neurons.

Estrogen clearly enhanced the α-methyltyrosine-induced reduction of the dopamine concentrations in the median eminence, an action which appears to be specific for this brain region since no effect was observed in the striatum. This action in the median eminence was accompanied by an estrogen-induced increase in serum prolactin concentrations. However, these two effects may or may not be causally related. For example, estrogen may increase dopamine turnover in the median eminence independent of its ability to increase serum prolactin concentrations. On the other hand, estrogen may increase dopamine turnover as a consequence of the elevated serum prolactin concentrations. In order to distinguish between these two possibilities, the effects of estrogen were determined on the turnover of dopamine in the median eminence of hypophysectomized rats. The results of this experiment are presented in Figure 15. The ability of 3 daily injections of estradiol to enhance the α-methyltyrosine-induced decline of median eminence dopamine concentrations in normal rats was completely abolished by hypophysectomy. Thus, estrogen does not appear to have

Figure 15. Effects of estrogen on the α-methyltyrosine-induced reduction of dopamine concentrations in the median eminence of control and hypophysectomized rats. Male rats were injected daily with estradiol benzoate (25 μg/kg, s.c.) or corn oil vehicle for 3 days. See legend to Figure 14 for details of drug administration. The means of zero time controls were set at 100% and represented by the horizontal lines and ± 1 S.E.M. by the shaded areas. Median eminence dopamine concentrations of animals sacrificed 60 minutes after the injection of α-methyltyrosine were calculated as a percentage of their respective zero time controls. Values from vehicle-pretreated animals which received α-methyltyrosine are represented by open bars and values from estrogen-pretreated animals which received α-methyltyrosine are represented by solid bars; vertical lines represent 1 S.E.M. based on 10-14 determinations. * indicates values in estrogen-pretreated rats which are significantly different from vehicle-pretreated rats ($p<0.05$).

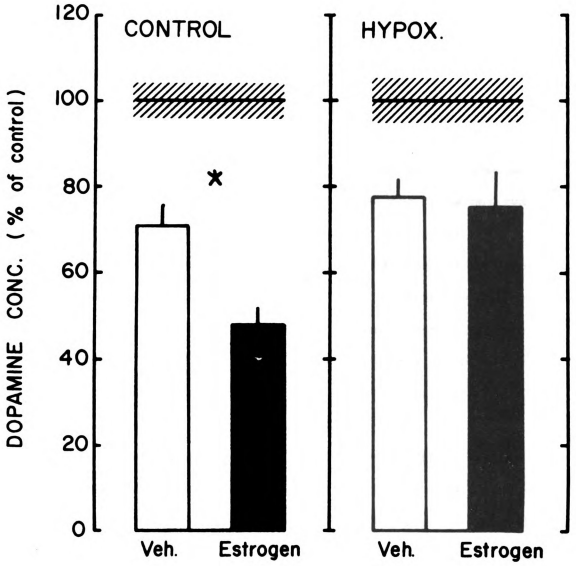

DOPAMINE CONC. (% of control)
120
100
80
60
40
20
0
CONTROL
HYPOX.
*
Veh.
Estrogen
Veh.
Estrogen

Figure 15

a direct influence on the activity of tuberoinfundibular neurons, but rather produces its effect by elevating serum prolactin concentrations.

F. Effects of hypothalamic deafferentation on drug-induced changes in serum prolactin concentrations and dopamine turnover in the median eminence

Although recent studies have attempted to identify components of the neuronal feedback mechanism governing the activity of nigrostriatal and mesolimbic neurons, very little work has been done on the pathways involved in the proposed hormonal-neuronal feedback regulation of tuberoinfundibular dopamine neurons. The observations that high circulating levels of prolactin or an implant of prolactin into the median eminence region blocks prolactin secretion associated with suckling, proestrus (Voogt and Meites, 1973) and pseudopregnancy (Dang and Voogt, 1977) and increases dopamine turnover in the hypothalamus (Fuxe and Hökfelt, 1969) suggest that the action of elevated serum prolactin concentrations to increase the activity of tuberoinfundibular neurons involves the hypothalamus.

The following experiment was designed to determine whether the proposed prolactin-mediated feedback mechanism involves extra- or intrahypothalamic neuronal pathways. A modified Halász knife (height 2.0 mm; radius 1.5 mm) was used to isolate a portion of the medial basal hypothalamus (Figure 16). The hypothalamic deafferentation extended from just caudal of the optic chiasma to the mamillary region (Figure 17). Although the lesion was intended to isolate the arcuate nucleus and median eminence (i.e., tuberoinfundibular neurons), the hypothalamic island also probably contained the rostral periventricular dopamine cell group (A14) and the caudal diencephalic

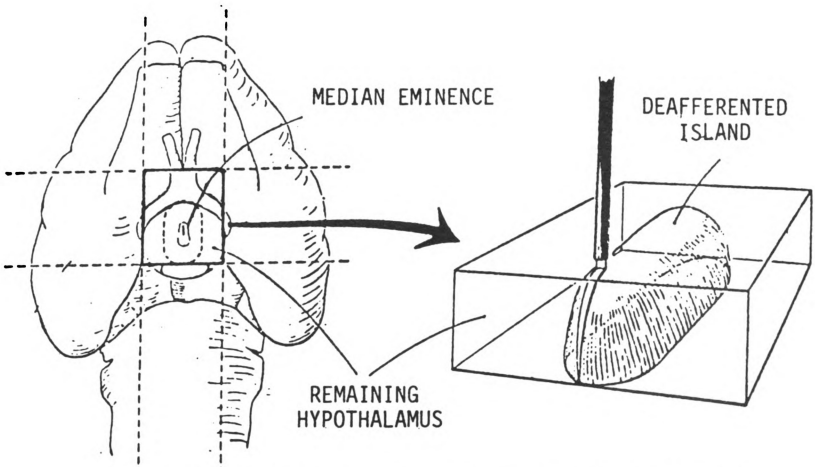


Figure 16. Relationship between median eminence, hypothalamic island and remaining hypothalamus. Broken lines represent planes of section through the brain. Solid lines outline hypothalamic fragment from which the island was taken. The insert at the right shows the location of the deafferented island within the hypothalamic fragment.

Figure 17. Sagittal view of the rat brain depicting the brain regions examined after hypothalamic deafferentation. Heavy dark lines indicate the borders used in dissecting the remaining hypothalamus. AHA, anterior hypothalamic area; CA, anterior commissure; CC, corpus callosum; CO, optic chiasm; CT, nucleus centralis tegmenti; DMH, dorsomedial nucleus; FX, fornix; IP, interpeduncular nucleus; NA, arcuate nucleus; PH, posterior hypothalamic nucleus; PVH, paraventricular nucleus; POA, preoptic area.

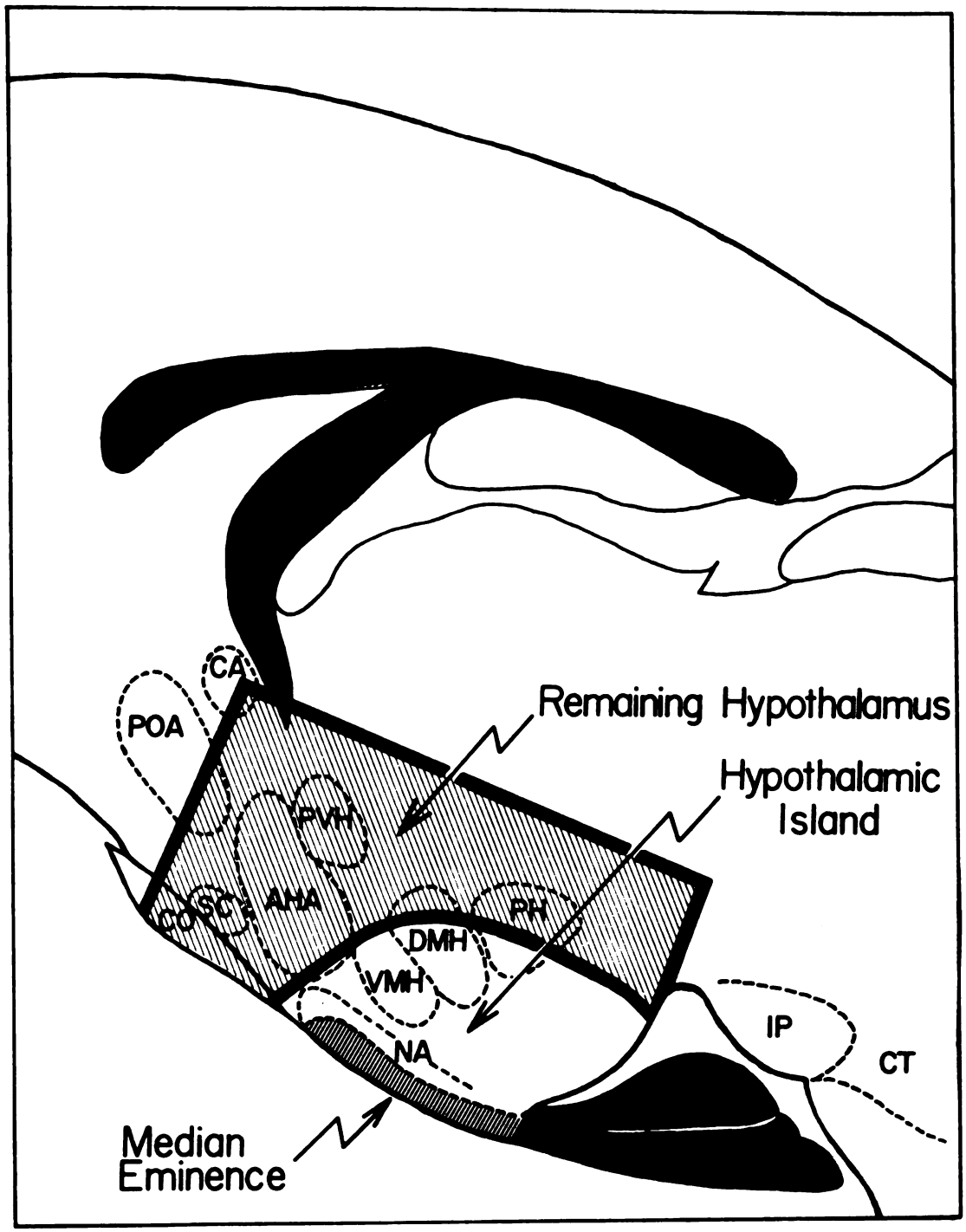

CA
POA
Remaining Hypothalamus
Hypothalamic Island
PVH
SCC
AHA
PH
DMH
VMH
IP
CT
NA
Median Eminence

Figure 17

dopamine cell group (A11), as described by Björklund and Nobin (1973) and Björklund et al. (1975).

The effects of hypothalamic deafferentation were first examined on the dopamine and norepinephrine concentrations in the three areas depicted in Figure 17--the median eminence, the hypothalamic island and the remaining hypothalamus. The results are presented in Figure 18. Sixteen days after hypothalamic deafferentation the dopamine concentration in the median eminence, hypothalamic island and remaining hypothalamus was not significantly different from the dopamine concentration in these same brain regions of control animals, which were sacrificed immediately after the lesion was made. In contrast, the norepinephrine concentration in the median eminence and hypothalamic island was reduced 40-50% in animals deafferented 16 days prior to sacrifice. The norepinephrine concentration in the remaining hypothalamus was similar in the two groups of animals. These results are in agreement with earlier histofluorescent (Löfström et al., 1976a, fluorescent (Weiner et al., 1972; Cuello et al., 1973c) and radioenzymatic (Brownstein et al., 1976) studies and support the conclusion that dopamine in the median eminence and hypothalamic island is contained in neurons which lie totally within the isolated area. However, the norepinephrine in these two regions is contained in neurons which originate in the brain stem (Ungerstedt, 1971a; and many others). Thus, hypothalamic deafferentation results in the transection of these noradrenergic neurons, degeneration of the nerve terminals and a subsequent reduction in the norepinephrine concentration.

Since animals which were sacrificed immediately after deafferentation were still anesthetized, it was necessary to determine whether anesthesia alone had any effects on median eminence or hypothalamic

Figure 18. Effects of hypothalamic deafferentation on the concentration of dopamine and norepinephrine in the median eminence, hypothalamic island and remaining hypothalamus. Animals were sacrificed immediately (open columns) or 16 days (solid columns) following complete hypothalamic deafferentation. Column heights represent the means and vertical lines $\pm$ 1 S.E.M. for 7-9 separate determinations. * indicates those values of the 16 day deafferented group which differ significantly ($p<0.05$) from the control group.

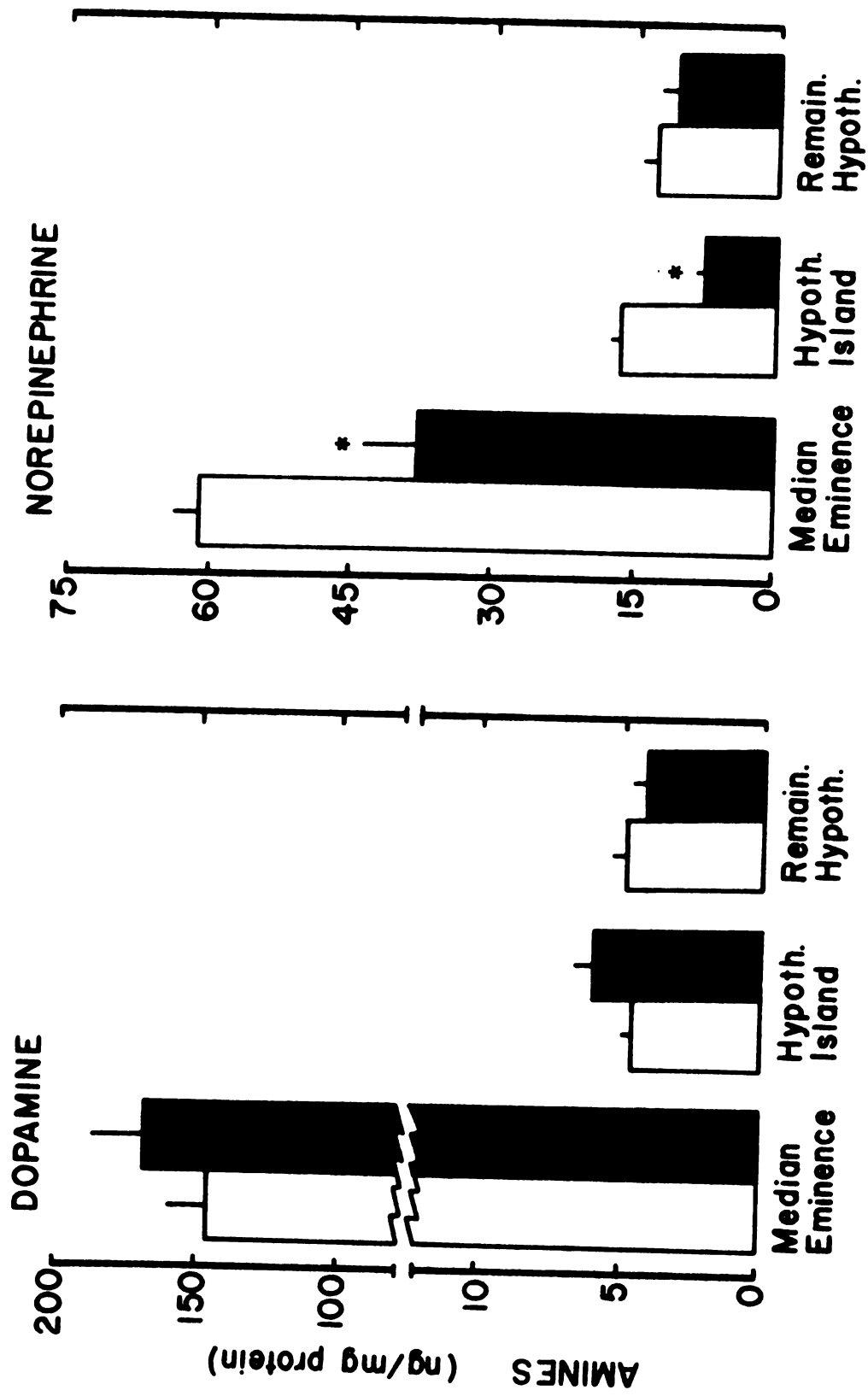

DOPAMINE
NOREPINEPHRINE
AMINES (ng/mg protein)
200
150
100
10
5
0
75
60
45
30
15
0
*
*
Median Eminence
Hypoth. Island
Remain. Hypoth.
Median Eminence
Hypoth. Island
Remain. Hypoth.

Figure 18

catecholamine concentrations. No differences were observed in the dopamine and norepinephrine concentrations in the median eminence and hypothalamus of Equithesin-anesthetized animals when compared to their untreated controls (Table 9).

Isolation of the medial basal hypothalamus did not alter basal serum prolactin concentrations (Figure 19). The serum prolactin concentration of deafferented animals which received saline, 13.7 $\pm$ 2.5 ng/ml, did not differ significantly from the serum prolactin concentration of normal intact male rats in our laboratory (11.4 $\pm$ 1.6 ng/ml). Furthermore, hypothalamic deafferentation did not appear to alter the ability of certain drugs to increase serum prolactin concentrations (Figure 19). One hour after the administration of α-methyltyrosine (250 mg/kg, i.p.), serum prolactin concentrations were increased fivefold to 61 ng/ml. Similar results have been reported by Weiner (1975). In addition, prolactin concentrations were still elevated (104 ng/ml) 16 hours after a single injection of haloperidol (2.5 mg/kg, s.c.). Thus, hypothalamic deafferentation does not appear to alter the ability of the pituitary to respond to drugs which normally increase prolactin secretion.

The central question, however, was: what effect would hypothalamic deafferentation have on the prolactin-induced increase in the activity of tuberoinfundibular dopamine neurons? The effects of haloperidol on dopamine turnover in the median eminence, hypothalamic island and remaining hypothalamus were, therefore, examined. The dose of haloperidol and time of administration were the same as those employed in a previous study (Figures 9 and 10) in which an increase in dopamine turnover in the median eminence of normal unoperated animals was

Table 9. Effects of anesthesia on median eminence and hypothalamic concentrations of dopamine and norepinephrine

	Dopamine*		Norepinephrine*	
	Control	Anesthetized	Control	Anesthetized
Median Eminence	141 ± 16 (6)	140 ± 9 (7)	66 ± 7 (6)	54 ± 4 (7)
Hypothalamus	3.9 ± 0.3 (7)	4.9 0.5 (7)	14.9 ± 1.4 (7)	14.7 ± 1.8 (7)

Numbers in parentheses indicate the number of animals in each group.

*ng/mg protein.

Figure 19. Serum prolactin concentrations in deafferented male rats. Deafferented male rats were pretreated with haloperidol (2.5 mg/kg, s.c.) or vehicle 16 hours before sacrifice. One hour before sacrifice half of the animals in each group were treated with saline and the other half with α-methyltyrosine (αMT) (250 mg/kg, i.p.). The means of 11-18 values are represented by the columns; the vertical lines represent ± 1 S.E.M. The horizontal line represents the serum prolactin concentration of normal unoperated male rats; the shaded area indicates ± 1 S.E.M.

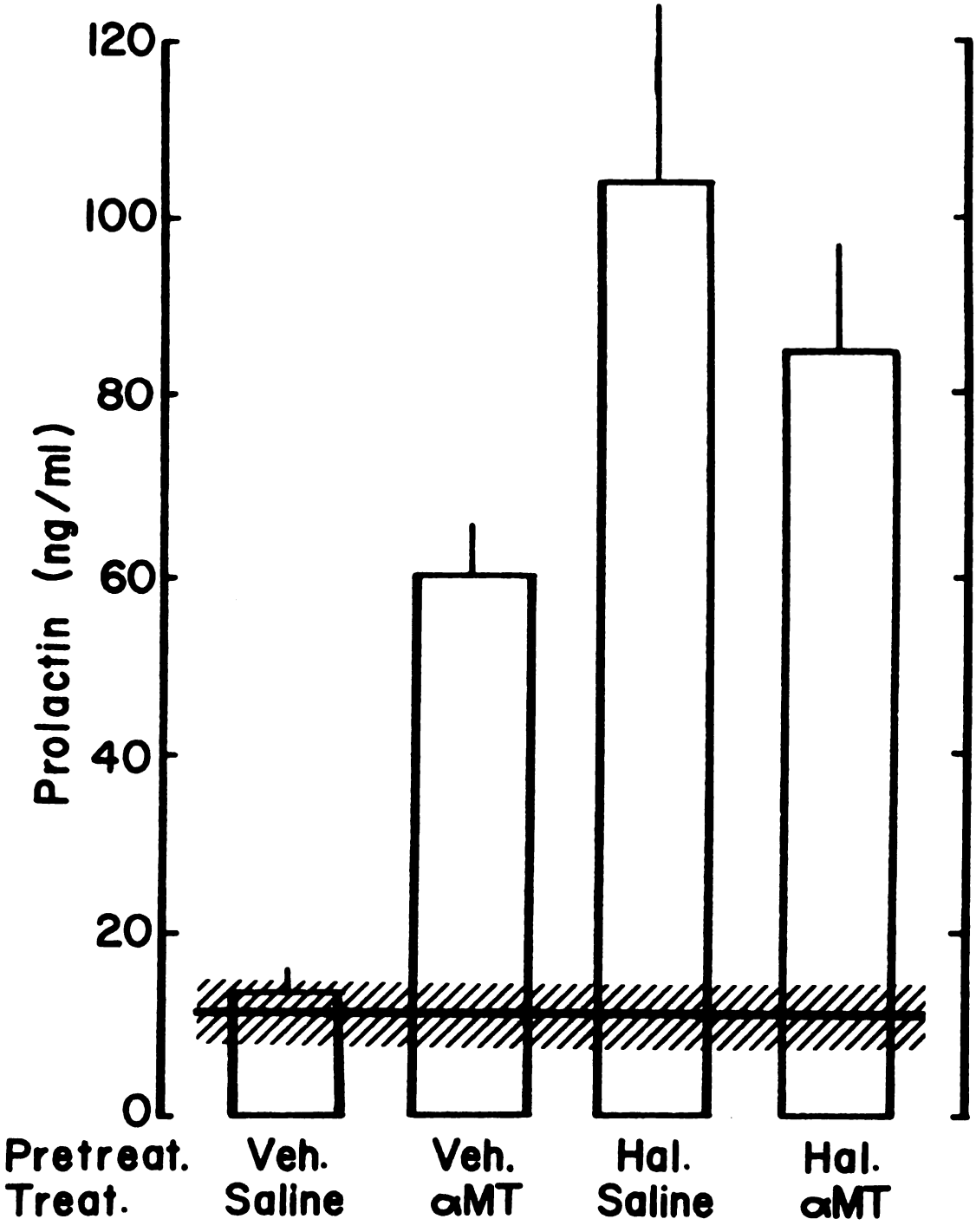

120
100
80
60
40
20
0
Prolactin (ng/ml)
Pretreat.
Treat.
Veh.
Saline
Veh.
αMT
Hal.
Saline
Hal.
αMT

Figure 19

observed. Steady state dopamine concentrations in the median eminence, hypothalamic island and remaining hypothalamus were unaltered 16 hours after a single administration of haloperidol (2.5 mg/kg, s.c.). Consequently, the steady state values of vehicle- and haloperidol-pretreated animals were combined and are represented by the horizontal lines in Figure 20. In vehicle-pretreated animals, the median eminence dopamine concentration was reduced to 65% of the steady state concentration 1 hour after the administration of α-methyltyrosine. Haloperidol enhanced the α-methyltyrosine-induced reduction of the dopamine concentration in the median eminence of deafferented rats to approximately 40% of the steady state concentration. The steady state dopamine concentration in the hypothalamic island and remaining hypothalamus were likewise unaffected by haloperidol. However, the ability of haloperidol to enhance the α-methyltyrosine-induced depletion appeared to be specific for the median eminence since the decline of the dopamine concentration in vehicle- and haloperidol-pretreated rats was similar in both the hypothalamic island and the remaining hypothalamus.

The action of haloperidol on dopamine neurons in the median eminence of deafferented animals was essentially the same as that seen on those in the median eminence of normal unoperated control animals (Figure 21). The steady state median eminence dopamine concentration and the α-methyltyrosine-induced depletion in vehicle-pretreated animals were similar in normal and deafferented male rats. More importantly, the haloperidol-induced increase in the α-methyltyrosine-induced depletion was almost identical in the two groups.

Figure 20. Effects of haloperidol on the α-methyltyrosine-induced decline of the dopamine concentration in the median eminence, hypothalamic island and remaining hypothalamus. Rats were sacrificed 16 hours after a single injection of haloperidol (2.5 mg/kg, s.c.) or vehicle. One hour before sacrifice half of the animals in each group received saline and the other half received α-methyltyrosine (250 mg/kg, i.p.). No significant differences were observed in the dopamine concentration in the median eminence, hypothalamic island and remaining hypothalamus of haloperidol- and vehicle-pretreated animals which received saline. The zero time values were combined and represented by the horizontal lines; the shaded areas indicate $\pm$ 1 S.E.M. The means of the α-methyltyrosine-depleted values are represented by the open (vehicle) and solid (haloperidol) columns and are based on 7-10 determinations. Vertical lines represent $\pm$ 1 S.E.M. * indicates a significantly ($p<0.05$) greater α-methyltyrosine-induced depletion.

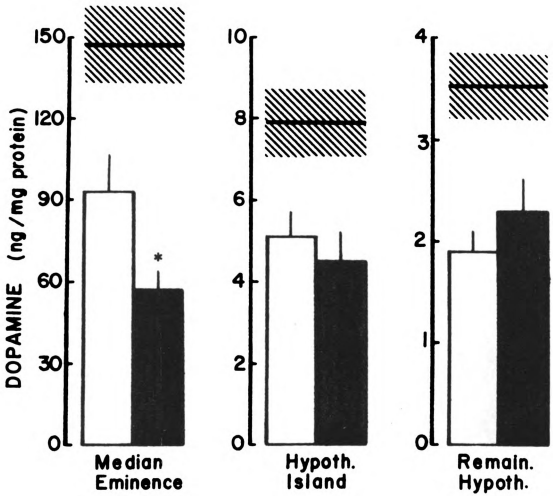

DOPAMINE (ng/mg protein)
150
120
90
60
30
0
*
Median Eminence
10
8
6
4
2
0
Hypoth. Island
4
3
2
1
0
Remain. Hypoth.

Figure 20

Figure 21. Effect of haloperidol on the α-methyltyrosine-induced decline of the dopamine concentration in the median eminence of normal unoperated control and deafferented male rats. See legend to Figure 20 for details of drug administration. No significant differences were observed in the median eminence dopamine concentration of vehicle- and haloperidol-pretreated normal unoperated or deafferented animals which received saline. These values were combined and are represented by the horizontal lines; the shaded areas represent $\pm$ 1 S.E.M. Columns represent the means of the α-methyltyrosine-depleted values from vehicle- (open) and haloperidol- (solid) pretreated animals; vertical lines indicate $\pm$ 1 S.E.M. for 8-10 determinations. * indicates a significantly ($p<0.05$) greater α-methyltyrosine-induced depletion.

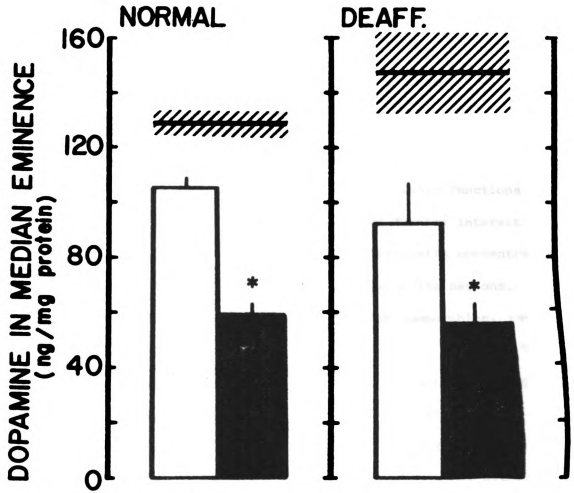

NORMAL
DEAFF.
160
120
80
40
0
DOPAMINE IN MEDIAN EMINENCE
(ng/mg protein)
*
*

Figure 21

Thus, the proposed hormonal-neuronal feedback mechanism regulating the activity of tuberoinfundibular dopamine neurons and prolactin secretion appears to involve an action of prolactin directly on neurons within the medial basal hypothalamus, possibly an action on the dopamine-containing cell bodies of tuberoinfundibular neurons in the arcuate nucleus.

G. Effects of subacute piribedil administration on dopamine turnover in the median eminence

The previous studies demonstrate that elevated serum prolactin concentrations may activate tuberoinfundibular dopamine neurons as part of a hormonal-neuronal feedback mechanism which functions to regulate pituitary prolactin release. It was of further interest to determine if a drug-induced reduction of serum prolactin concentrations would decrease the activity of tuberoinfundibular neurons.

Dopaminergic agonists, such as piribedil and apomorphine, reduce serum prolactin concentrations (Mueller et al., 1976). Despite the observation that acute piribedil administration did not alter dopamine turnover in the median eminence (Figure 6), the effects of subacute administration on dopamine turnover in the median eminence were examined in view of the time course for the actions of elevated prolactin concentrations to increase dopamine turnover in this region.

Male rats received piribedil (30 mg/kg, s.c.) or vehicle 26, 18, 10 and 2 hours before sacrifice. This dose of piribedil reduces serum prolactin concentrations for at least 8 hours (Table 10). As in the previous studies, steady state median eminence dopamine concentrations were unaltered by piribedil administration (Table 11). Somewhat surprisingly, however, this regimen of piribedil administration enhanced the α-methyltyrosine-induced depletion of dopamine in the

Table 10. Time course for the reduction of estradiol-elevated serum prolactin concentrations by piribedil

Treatment	Prolactin (ng/ml)
Vehicle	125 ± 14
Piribedil	
4 hours	55 ± 6
8 hours	88 ± 10
16 hours	103 ± 9

Male rats received estradiol benzoate (25 µg/kg, s.c.) daily for 5 days. Twenty-four hours after the last injection, animals received vehicle or piribedil (30 mg/kg, s.c.). Animals were sacrificed 4 hours after the vehicle injection and 4, 8 and 16 hours after administration of piribedil.

Table 11. Effect of piribedil on the α-methyltyrosine-induced depletion of dopamine in the median eminence

Pretreatment	Treatment: Saline (ng dopamine/mg protein)	Treatment: α-Methyltyrosine (ng dopamine/mg protein)	$\frac{\text{α-Methyltyrosine}}{\text{Saline}} \times 100$[a]
Vehicle	155 ± 9 (11)	97 ± 6 (11)	68 ± 4
Piribedil	131 ± 11 (11)	78 ± 4 (11)	55 ± 5*

Rats received piribedil (30 mg/kg) or vehicle subcutaneously 26, 18, 10 and 2 hours before sacrifice. One hour before sacrifice, half of the animals in each group received saline and half received α-methyltyrosine (250 mg/kg, i.p.). Piribedil did not alter the steady state dopamine concentration in the median eminence. Numbers in parentheses represent the number of animals in each group.

*Indicates the value for the piribedil-treated animals differs significantly ($p<0.05$) from the vehicle-pretreated control value.

[a]These values represent the α-methyltyrosine values expressed as a percent of a combined vehicle and piribedil zero time value.

median eminence, indicating that dopamine turnover was increased rather than decreased, as was expected.

These results differ from those observed 2 hours after the single injection of piribedil (Figure 6). The increase in turnover in the median eminence seen after multiple injections of piribedil every 8 hours may be due to the accumulation of the drug. Therefore, a dose response relationship was examined. The effects of piribedil (25, 50 and 100 mg/kg, i.p.) on the α-methyltyrosine-induced depletion of dopamine in the median eminence, olfactory tubercle and striatum are presented in Table 12. Piribedil (25 and 50 mg/kg) did not alter the steady state dopamine concentration in the striatum and olfactory tubercle. Consistent with the results observed in the acute study, piribedil reduced the α-methyltyrosine-induced decline of dopamine in the striatum and olfactory tubercle at 25 and 50 mg/kg. Although piribedil likewise did not alter the steady state dopamine concentrations in the median eminence, the effects of this dopamine agonist on the decline of dopamine in this brain region were completely opposite from the results observed in the striatum and olfactory tubercle. In a dose dependent manner, piribedil significantly enhanced the α-methyltyrosine-induced reduction of median eminence dopamine concentrations. The observation that the lowest dose of piribedil, 25 mg/kg, had no effect on dopamine turnover in the median eminence is consistent with the results observed in the acute study (Figure 6). Since larger doses of piribedil increased dopamine turnover in this brain region, the action of multiple injections of 30 mg/kg to enhance dopamine turnover probably reflects an accumulated drug effect. Nevertheless, the action of piribedil on the terminals of tuberoinfundibular neurons in the median eminence not only differs

Table 12. Effects of piribedil on the α-methyltyrosine-induced depletion of dopamine in various brain regions

Pretreatment	Treatment: Saline (ng dopamine/mg protein)	Treatment: α-Methyltyrosine (ng dopamine/mg protein)	$\frac{\text{α-Methyltyrosine}}{\text{Saline}} \times 100$[a]
Median Eminence			
Vehicle	128 ± 6	91 ± 4	68 ± 3
Piribedil			
25 mg/kg	152 ± 10	79 ± 7	60 ± 5
50 mg/kg	127 ± 9	66 ± 5	50 ± 3*
100 mg/kg	132 ± 9	64 ± 5	48 ± 4*
Olfactory Tubercle			
Vehicle	79 ± 5	60 ± 3	74 ± 3
Piribedil			
25 mg/kg	84 ± 5	73 ± 5	90 ± 6*
50 mg/kg	79 ± 3	73 ± 5	91 ± 6*
Striatum			
Vehicle	101 ± 5	74 ± 3	73 ± 3
Piribedil			
25 mg/kg	107 ± 5	94 ± 6	92 ± 6*
50 mg/kg	97 ± 5	93 ± 4	91 ± 4*

Rats received piribedil or vehicle subcutaneously 3 hours before sacrifice. One hour before sacrifice, half of the animals in each group received saline and the other half received α-methyltyrosine (250 mg/kg, i.p.). Piribedil did not alter the steady state dopamine concentration in any brain region at any time. Each value represents the mean of 7-17 determinations.

*Indicates those values from the piribedil-pretreated animals which differ significantly ($p<0.05$) from their vehicle-pretreated controls.

[a]These values represent the α-methyltyrosine values expressed as a percent of a combined vehicle and piribedil zero time value in each brain region.

but is completely opposite to the action of this agent on dopamine nerve terminals in the striatum and olfactory tubercle. Although the functional importance of this difference with respect to the median eminence is not certain, it is apparent that piribedil does not influence all dopaminergic systems in a similar manner.

DISCUSSION

I. Determination of Steady State Dopamine Concentrations

The median eminence dopamine concentration reported in the present studies, approximately 130 ng/mg protein, is much higher than that reported in earlier studies which examined catecholamines in this brain region. However, the prior use of fluorometric methods necessitated the use of large amounts of tissue (obtained by pooling samples or using large experimental subjects) which may have been contaminated by neighboring structures with lower dopamine concentrations (Laverty and Sharman, 1965; Rinne and Sonninen, 1968; Iwata and Ishii, 1969; Clementi et al., 1970). More recently, radioenzymatic procedures have been utilized to quantify catecholamine concentrations in small brain regions, including the median eminence (Cuello et al., 1973c; Palkovits et al., 1974; Versteeg et al., 1975; Chiocchio et al., 1976; Selmanoff et al., 1976). The median eminence dopamine concentration, as reported in these studies, appears to be much higher than previously believed and may, in fact, be the highest of any brain region. The median eminence dopamine concentrations reported in the present studies agree with these recently reported values.

One of the reasons for the disparity among literature values is that the dopamine concentration is dependent, to some extent, upon the size of the dissected median eminence fragment. Kavanagh and Weisz (1973) have shown that the dopamine concentration in the superficial

layer of the medial basal hypothalamus (i.e., median eminence) is reduced markedly when contaminated with surrounding tissue, in which the dopamine concentration is lower. Reported weights for the median eminence range from 65 µg (Chiocchio et al., 1976) to 440 µg (Kizer et al., 1975) and, accordingly, the dopamine concentrations range from 250 ng/mg protein to 72 ng/mg protein, respectively. Median eminence samples taken in the present studies were calculated to weigh approximately 200 µg. Thus, the weight and dopamine concentration of these samples represent intermediate values when compared to values reported in recent studies.

For the general purpose of characterizing the tuberoinfundibular dopamine system in relation to the nigrostriatal and mesolimbic systems, two types of studies were performed: drug effects on steady state dopamine concentrations and drug effects on dopamine turnover.

A. Effects of γ-butyrolactone and baclofen on steady state dopamine concentrations

γ-Hydroxybutyrate or its analog, γ-butyrolactone, has been shown to markedly increase the dopamine concentration in the striatum and olfactory tubercle without altering the concentration of norepinephrine or 5-hydroxytryptamine (Gessa et al., 1966; Aghajanian and Roth, 1970; Walters and Roth, 1972). This elevation of brain dopamine is associated with an increase in the synthesis of dopamine and an inhibition of nerve impulse flow (Spano et al., 1971; Walters et al., 1973).

Inhibition of nerve impulse flow by γ-butyrolactone excludes the possible participation of normal feedback mechanisms in the regulation of the synthesis and release of dopamine. Presynaptic or autoreceptors

are thought to regulate the synthesis and release of dopamine under these circumstances. Since nerve impulse flow is inhibited, less dopamine is released into the synaptic cleft. Consequently, presynaptic dopamine receptor activation is reduced. It is believed that the increased dopamine concentration in the brain results from a subsequent activation of tyrosine hydroxylase (Roth et al., 1975).

Baclofen may act in a manner similar to γ-butyrolactone. Dray and Straughn (1976) have demonstrated that baclofen inhibits the firing of nigral cells. Furthermore, baclofen, when administered systemically or applied directly to dopamine cell bodies, has been shown to increase the forebrain concentration of dopamine (Kelly and Moore, 1976).

In agreement with earlier studies, systemic administration of γ-butyrolactone and baclofen resulted in a marked elevation of dopamine concentrations in the striatum and olfactory tubercle (Table 2). However, neither drug altered the dopamine concentration in the median eminence.

One possible explanation for the inability of these two agents to alter the median eminence dopamine concentration is that the activity of tuberoinfundibular neurons is not reduced by γ-butyrolactone or baclofen. γ-Butyrolactone and baclofen have been considered γ-aminobutyric acid (GABA) receptor agonists (Andén and Stock, 1973; Fuxe et al., 1975c), although the GABA agonist properties of these agents has been challenged (Curtis et al., 1974; Davies and Watkins, 1974). If the effects of γ-butyrolactone and baclofen on nigrostriatal and mesolimbic neurons are mediated by an activation of GABA receptors, lack of a GABA input to tuberoinfundibular neurons

would account for the inability of these drugs to alter the dopamine concentration in the median eminence.

The intraventricular injection of GABA (Mioduszewski et al., 1976) and the systemic administration of γ-butyrolactone and baclofen (Meltzer and Fang, 1977, Ravitz and Moore, 1977) have been shown to increase serum prolactin concentrations. Although actions of these agents on prolactin secretion may be a nonspecific anesthetic or stress-related effect, the action of GABA, γ-butyrolactone and baclofen to elevate pituitary prolactin release may represent a GABA-mediated inhibitory influence on tuberoinfundibular dopamine neurons. If, indeed, nerve impulse flow is inhibited, the lack of a drug-induced elevation of dopamine may indicate that presynaptic receptors do not exist on the terminals of tuberoinfundibular neurons. In this case, a reduction of impulse flow and neurotransmitter release would be offset by a corresponding reduction of dopamine synthesis.

B. Hormonal influences on the dopamine concentration in the median eminence

Although the dopamine concentration in the median eminence was not altered by γ-butyrolactone and baclofen, the dopamine concentration was altered by endocrinological manipulations. Despite the fact that no changes in median eminence and hypothalamic concentrations of dopamine and norepinephrine were found during the estrous cycle, there was a marked elevation of the dopamine concentration in the median eminence of ovariectomized rats without a similar increase in the norepinephrine concentration. Furthermore, this increase in the median eminence dopamine concentration following ovariectomy was reversed by the administration of estradiol (Table 3).

Numerous studies correlating changes in the concentration and turnover of hypothalamic catecholamines with hormonal changes have been performed. The results of these studies support the general concept that hypothalamic catecholamines are involved in the regulation of anterior pituitary hormone secretion. Donoso and his coworkers have reported changes in the hypothalamic concentrations of norepinephrine during the estrous cycle and after castration (Stefano et al., 1965; Donoso et al., 1967; Stefano and Donoso, 1967; Donoso and De Gutierrez, 1970). Castration has also been shown to increase tyrosine hydroxylase activity in the hypothalamus (Beattie et al., 1972).

More recently, changes in the dynamics of catecholamine systems have been observed in individual hypothalamic nuclei. Kizer et al. (1974) have demonstrated that the castration-induced increase in hypothalamic tyrosine hydroxylase activity in male rats occurs in the median eminence exclusively. Furthermore, this increase was partially reversed by the administration of testosterone. These results are very similar to the observation that ovariectomy produced an increase in the dopamine concentration in the median eminence, an effect which was reversed by administration of estradiol. Thus, the increased median eminence concentration of dopamine in ovariectomized female rats apparently results from an increased synthesis of the neurotransmitter.

The interpretation of changes in catecholamine concentrations, such as that seen after ovariectomy, is difficult. However, the castration-induced increase in the median eminence dopamine concentration does reflect a movement away from the steady state and supports

the hypothesis that hormonal influences may alter the dynamics of tuberoinfundibular dopamine neurons.

II. Determination of Dopamine Turnover

A. Methodological considerations

One problem in the interpretation of concentration changes is that steady state concentrations of neurotransmitters are a poor index of the activity of a neuronal pathway. Therefore, dopamine turnover studies were performed to test the hypothesis that differences may exist in the regulatory mechanisms governing the activity of tuberoinfundibular, nigrostriatal and mesolimbic neurons.

Several steady state and nonsteady state methods have been utilized previously for the estimation of turnover rates and synthesis rates of central catecholamine neurotransmitters (Costa and Neff, 1966). Because of technical difficulties (e.g., determining the specific activity of ^{3}H-tyrosine), it is not currently possible to employ radiolabelled precursor methods to determine dopamine turnover in brain regions, such as the median eminence, which weigh less than 1 mg. Dopamine turnover in the present studies was estimated using the nonsteady state method of observing the decline of the endogenous dopamine concentration after synthesis inhibition with α-methyltyrosine. At the present time this is the only method which has been applied successfully to the determination of dopamine turnover in the median eminence.

The use of α-methyltyrosine involves several assumptions, although there are shortcomings with all of the commonly employed methods for estimating turnover (see Weiner, 1974). One of these assumptions is that the inhibition of synthesis is complete in all of the brain

regions examined and that the degree of inhibition is not altered by drug administration. One disadvantage of this method is that it involves the perturbation of the system, and one must presume that the inhibition of synthesis per se does not affect the activity of the neuronal pathway. Finally, inhibition of dopamine synthesis eliminates the contribution of newly synthesized amine to the determination of dopamine turnover. Thus, this method of estimating catecholamine turnover assumes that the transmitter content of the nerve terminal behaves as a single pool of amine. Despite these disadvantages and assumptions, the same rate of catecholamine turnover in the hypothalamus was obtained when estimated by three different methods, one of which involved the inhibition of synthesis with α-methyltyrosine (Iversen and Glowinski, 1966).

That the dynamics of tuberoinfundibular neurons does not differ from nigrostriatal and mesolimbic neurons after synthesis inhibition is evidenced by the fact that the decline of dopamine in the median eminence after α-methyltyrosine followed first order kinetics, as did the decline in the striatum and olfactory tubercle (Figure 6).

B. Comparison of the synthesis rates for dopamine in the striatum, olfactory tubercle and median eminence

The rate constants for the decline of the endogenous dopamine concentrations after the administration of α-methyltyrosine and the calculated synthesis rates for dopamine in the striatum and olfactory tubercle of control rats were similar (Table 2), and the values for the striatum compare favorably with the results of Bariletto et al. (1975). The synthesis rate for dopamine in the median eminence was approximately twice the rates in the other two brain regions. The

rapid and marked decline of the dopamine concentration in the median eminence after α-methyltyrosine (Figure 6) is in agreement with the rate constants reported by Versteeg et al. (1975) and Löfström et al. (1976b) and contrasts with the recent work of Bacopoulos et al. (1975), who found the catecholamine stores in the median eminence to be resistant to depletion by α-methyltyrosine. Since the rate of decline of dopamine after α-methyltyrosine is believed to reflect the relative activity of a neuronal pathway (Andén et al., 1969), it appears that the activity of tuberoinfundibular neurons is at least as great as, if not greater than, that of nigrostriatal and mesolimbic dopamine neurons. Another indication of the tonic activity of tuberoinfundibular neurons is the rapid increase in serum prolactin concentrations after the administration of α-methyltyrosine (Donoso et al., 1971).

C. Acute effects of haloperidol and piribedil administration

The haloperidol-induced increase in dopamine turnover in the corpus striatum and olfactory tubercle is in agreement with many earlier studies (Andén et al., 1970; Andén, 1972; Wilk et al., 1975), as is the piribedil-induced decrease of dopamine turnover (Corrodi et al., 1972; Jori et al., 1974).

Numerous studies indicate that the effects of haloperidol and piribedil on dopamine turnover are mediated by a neuronal feedback mechanism subsequent to dopamine receptor blockade or stimulation, respectively (Carlsson and Lindqvist, 1963). Essential to the concept of neuronal feedback modulation of nigrostriatal and mesolimbic neurons is the assumption that piribedil and haloperidol are a postsynaptic dopamine receptor agonist and antagonist, respectively. Evidence for

the validity of such an assumption is several-fold. The dopamine receptor agonist properties of piribedil include its ability to induce contralateral rotation in rats with a unilateral 6-hydroxydopamine-induced lesion of the striatum (Corrodi et al., 1972). In addition, a metabolite of piribedil has been shown to stimulate the activity of a dopamine sensitive adenylate cyclase in the rat striatum (Miller and Iversen, 1974). Another indication of the dopamine receptor agonist activity of piribedil is its ability to reduce prolactin secretion *in vitro* and *in vivo* (MacLeod and Lehmeyer, 1974; Mueller et al., 1976). Haloperidol is believed to block postsynaptic dopamine receptors since this agent blocks drug-induced circling of animals with 6-hydroxydopamine-induced striatal lesions (Ungerstedt, 1971b), antagonizes the binding of dopamine to receptors (Burt et al., 1975), and increases serum prolactin concentrations (Dickerman et al., 1972).

Despite the evidence for the actions of haloperidol and piribedil on postsynaptic dopamine receptors, the possibility remains that the effects of these agents on cerebral dopamine metabolism *in vivo* may be mediated by a presynaptic action (e.g., on dopamine autoreceptors). One argument against this possibility is that dopamine receptor antagonists increase and dopamine receptor agonists decrease the firing rate of cells in the substantia nigra (Bunney et al., 1973; Walters et al., 1975). The concept of the regulation of the activity of nigrostriatal and mesolimbic neurons by a neuronal feedback loop is further supported by the fact that the haloperidol-induced increase in striatal tyrosine hydroxylase activity is blocked by cerebral hemisection, indicating that the integrity of the feedback loop is

required for the actions of this neuroleptic on dopamine metabolism (Zivkovic et al., 1975b).

Recent work suggests that the neuronal feedback mechanism regulating the activity of nigrostriatal neurons may be more complex than depicted in Figure 4. Destruction of GABA and acetylcholine neurons by kainic acid does not alter the ability of dopaminergic agonists and antagonists to influence the activity of nigrostriatal neurons (DiChiara et al., 1977; Wuerthele et al., 1977). Therefore, regulation of nigrostriatal neurons may involve yet another feedback mechanism. One possibility is that dopamine released in the substantia nigra from axon collaterals of nigrostriatal neurons may inhibit the release onto dopaminergic cell bodies of an excitatory neurotransmitter. Thus, the effects of dopamine agonists and antagonists may result from an action on dopamine receptors in the substantia nigra, rather than on receptors in the striatum.

Although haloperidol and piribedil altered dopamine turnover in the striatum and olfactory tubercle, neither drug had an effect on dopamine turnover in the median eminence. Fuxe and his co-workers (1975a) have likewise observed that acute administration of various neuroleptics has no effect on dopamine turnover in this brain region. These results suggest that a neuronal feedback loop may not regulate the activity of tuberoinfundibular dopamine neurons. In support of this conclusion is the fact that dopamine receptors have not been localized in the median eminence (Brown et al., 1976).

However, haloperidol and piribedil may alter the turnover of a small, newly synthesized pool of dopamine in the terminals of tuberoinfundibular neurons. These drug actions cannot be determined in turnover studies utilizing α-methyltyrosine.

Results from the examination of the time course for the effects of haloperidol on dopamine turnover in the striatum, olfactory tubercle and median eminence suggest that a qualitative difference exists in the regulatory mechanisms governing the activity of nigrostriatal, mesolimbic and tuberoinfundibular dopamine neurons (Figures 7-9). The rapid increase in the α-methyltyrosine-induced depletion of dopamine in the striatum and olfactory tubercle after haloperidol is consistent with the concept of neuronal feedback regulation. Haloperidol produced a delayed increase in dopamine turnover in the median eminence, an action not unlike the increase seen after multiple injections of pimozide (Fuxe et al., 1975b). Similarly, dopamine turnover in the median eminence was increased after two injections of clozapine and thioridazine (Table 6). However, this action of haloperidol appears to be hormonally mediated since it was eliminated by hypophysectomy (Figure 10, Table 5).

Hypophysectomy reduced the steady state dopamine concentration in the median eminence by 30%. Similar reductions have been reported by Fuxe et al. (1973) and Shoemaker and Schlumpf (1976). This reduction of the endogenous dopamine concentration might indicate that the surgery itself altered the dynamics of these dopamine neurons such that it was not possible to increase the turnover of dopamine in the median eminence in hypophysectomized animals. However, the rate constant for the decline of the dopamine concentration was almost identical to the value obtained from unoperated controls (Table 5). Furthermore, it has been previously demonstrated that prolactin administration is capable of increasing the activity of tuberoinfundibular neurons in hypophysectomized rats (Hökfelt and Fuxe, 1972).

Hypophysectomy unexpectedly enhanced the ability of haloperidol to increase dopamine turnover in the striatum. Haloperidol enhanced the α-methyltyrosine-induced decline of dopamine and the synthesis rate of dopamine in the striata of hypophysectomized rats at a time when no effect was observed in the striata of unoperated controls. From this observation alone it is not possible to determine whether this effect reflects an increased half-life of the drug or whether it represents a hormonal influence on the sensitivity of nigrostriatal neurons.

More importantly, however, the results of these experiments indicate that although neuroleptic-induced increases in dopamine turnover occur in all three brain regions, the action of haloperidol on tuberoinfundibular dopamine neurons is dependent upon the presence of the pituitary and, therefore, most likely hormonally mediated.

The delayed action of haloperidol on tuberoinfundibular neurons prompted an examination of the effects of piribedil at later times. Instead of observing a delayed decrease in dopamine turnover in the median eminence after piribedil administration, multiple injections of this drug resulted in an increased disappearance of dopamine after α-methyltyrosine in this brain region (Table 11). Indeed, piribedil increased dopamine turnover in the median eminence in a dose dependent manner and at doses which decreased turnover in the striatum and olfactory tubercle (Table 12). One explanation for this observation is that high doses of piribedil may enhance the release of dopamine from the terminals of tuberoinfundibular neurons in the median eminence. Presynaptic actions of piribedil have been reported (Fuxe, 1973). Despite the uncertain physiological significance of this action of piribedil in the median eminence, it appears that differential effects

of this dopaminergic agonist exist with regard to its action on central dopaminergic neurons. These results further support the hypothesis that the mechanisms governing the activity of tuberoinfundibular dopamine neurons differ from those governing the activity of nigrostriatal and mesolimbic neurons.

D. Selective actions of prolactin and estrogen on tuberoinfundibular dopamine neurons

The administration of exogenous prolactin selectively increased dopamine turnover in the median eminence (Figure 12) since no effect was observed in the striatum (Figure 13). These results confirm the histofluorescent study of Hökfelt and Fuxe (1972). Similarly, a pharmacologically-induced elevation of endogenous serum prolactin concentrations by estradiol also increased dopamine turnover in the median eminence (Figure 14). These results provide direct evidence that the activity of tuberoinfundibular neurons may be influenced by hormones. Specifically, high serum prolactin concentrations appear capable of activating these intrahypothalamic dopamine neurons.

A large number of studies indicate that dopamine released from the terminals of tuberoinfundibular neurons functions to inhibit the release of prolactin from the anterior pituitary. Thus, the prolactin-induced increase in the turnover of dopamine in tuberoinfundibular neurons not only represents a hormonal-neuronal feedback modulation of these neurons but also provides a possible mechanism by which prolactin may regulate its own secretion. Indeed, suppression of prolactin secretion from the *in situ* pituitary gland in situations of elevated serum prolactin concentrations has led to the suggestion

that prolactin inhibits its own secretion. This "short loop" feedback has been demonstrated in rats bearing pituitary tumor transplants which secrete large amounts of prolactin. The *in situ* pituitary prolactin concentration is markedly decreased in these animals (MacLeod et al., 1966; Chen et al., 1967). Transplants of pituitaries under the kidney capsule or implants of prolactin into the median eminence region have also been shown to reduce the pituitary and serum concentration of prolactin (Mena et al., 1968; Welsch et al., 1968; Clemens and Meites, 1968; Voogt and Dang, 1977). Of further interest in this regard are the effects of α-methyltyrosine on the serum prolactin concentration of estradiol-pretreated animals (Table 8). α-Methyltyrosine produced a much greater increase in the serum prolactin concentration of estradiol-pretreated rats when compared to the increase observed in vehicle-pretreated animals. Thus, despite elevations of serum prolactin concentrations in estrogen-treated rats, prolactin concentrations would be much greater without the inhibitory influence of tuberoinfundibular neurons. Furthermore, the enhanced response to α-methyltyrosine in these animals indicates an increased inhibitory influence of these neurons. An estrogen-induced, prolactin-mediated increase in the activity of tuberoinfundibular neurons may function to limit the estrogen-stimulated rise in serum prolactin concentrations. This provides further support for the concept of autoregulation of serum prolactin concentrations.

Tuberoinfundibular dopamine neurons may not be involved exclusively in the regulation of prolactin secretion. Fuxe and his coworkers have suggested that tuberoinfundibular dopamine neurons exert an inhibitory action on the pituitary secretion of gonadotropins (Fuxe et al., 1971; Fuxe et al., 1976). Indeed, dopaminergic agonists

have been shown to reduce serum LH concentrations (Mueller et al., 1976) and block ovulation in immature rats treated with pregnant mare serum (Fuxe and Hökfelt, 1973). The increase in dopamine turnover in the median eminence after the administration of exogenous prolactin was accompanied by reductions in the post-castration rise of serum LH and FSH concentrations (Table 7). This observation has been recently confirmed by Muralidhar et al. (1977), who reported that prolactin administration similarly reduced the post-castration rise of serum LH in suckling female rats. There are many indications that high serum concentrations of prolactin are associated with low secretion of gonadotropins. This is particularly noteworthy during postpartum lactation in rats where serum prolactin is high and serum LH and FSH are depressed (Lu et al., 1976). It is tempting to speculate that the prolactin-induced suppression of serum LH and FSH concentrations is mediated by the increased activity of tuberoinfundibular dopamine neurons. However, a direct action of prolactin on the pituitary cannot be excluded. Muralidhar et al. (1977) concluded that prolactin acts by altering the responsiveness of the pituitary to LHRH. Their conclusion does not exclude the possibility that a prolactin-induced increased release of dopamine into portal vessels acts on the pituitary to alter its responsiveness to LHRH.

The negative feedback mechanism by which estrogen inhibits pituitary gonadotropin secretion has been suggested to involve a direct action of estrogen on tuberoinfundibular dopamine neurons (Fuxe et al., 1969; Fuxe and Hökfelt, 1973). Results of the present studies do not support this contention (Figure 15). The ability of estradiol to increase dopamine turnover in the median eminence was completely blocked by hypophysectomy, indicating that estradiol

apparently has no direct action on tuberoinfundibular neurons. However, one cannot exclude the possibility that estrogen exerts a direct action on a small population of dopamine neurons or a small pool of newly synthesized dopamine.

E. Lag time in response of tuberoinfundibular dopamine neurons

One similarity in the actions of haloperidol, prolactin and estrogen on tuberoinfundibular neurons was the lag time observed between the elevation of serum prolactin concentrations and the increase of dopamine turnover in the median eminence. This lag time, 10-26 hours, was similar in all three cases. Although it may be argued that a continuous elevation of serum prolactin concentrations may not have been attained in the prolactin study, this does not appear to be a reasonable explanation for the delay observed in the haloperidol and estrogen experiments. Furthermore, a lag time has been observed after the administration of several other antipsychotic agents (Fuxe et al., 1975a). At the present time one can only speculate as to the reasons for the delayed response of tuberoinfundibular neurons. One explanation might be that prolactin is slowly accumulated in prolactin-sensitive neurons in the central nervous system. Indeed, Fuxe et al. (1977) have recently reported the existence of a protein immunoreactively related to prolactin stored in a network of nerve terminals in several hypothalamic nuclei. Alternatively, prolactin may cross permeable blood vessels in the median eminence and undergo retrograde axonal transport to prolactin-sensitive perikarya within the hypothalamus. Such a transport system has been described (Broadwell and Brightman, 1976). Regardless of what transpires between the elevation of serum prolactin concentrations and the

subsequent increase of dopamine turnover in the median eminence, it appears that the hormonal-neuronal regulation of tuberoinfundibular dopamine neurons is a sluggish system in contrast to the rapid neuronal feedback modulation of nigrostriatal and mesolimbic neurons.

F. Prolactin-induced changes in dopamine turnover in the median eminence of rats with an isolated hypothalamic island

The ability of prolactin implants into the median eminence region to suppress pituitary prolactin release indicates that the autoregulation of prolactin secretion may involve hypothalamic neuronal mechanisms (Voogt and Meites, 1973; Dang and Voogt, 1977). Results from the present studies indicate that prolactin-induced increases in the activity of tuberoinfundibular neurons may be one mechanism by which prolactin regulates its own secretion. However, many hypothalamic areas have been implicated in the control of prolactin secretion (see Neill, 1974), and connections between these areas and the median eminence have been described (Moss, 1976). There are no previous studies on the neuronal pathways which may be involved in the hormonal-neuronal feedback regulation of tuberoinfundibular dopamine neurons and prolactin secretion.

Hypothalamic deafferentations were performed so as to separate possible extra- and intrahypothalamic components of the proposed feedback mechanism. Within the hypothalamus the lesion was intended to separate the arcuate-median eminence region from other hypothalamic areas, such as the medial preoptic region (Figure 17). Completeness of the deafferntation was verified by gross inspection at the time of dissection, histological identification of the lesion and the depletion of norepinephrine observed within the median eminence and

hypothalamic island (Figure 18). The fact that only a 50% reduction of norepinephrine in these brain regions was observed might be taken as an indication that the lesion was not complete. However, Brownstein et al. (1976) have also found that isolation of the medial basal hypothalamus resulted in only a 60% reduction of median eminence norepinephrine, despite a 95% decline of the activity of dopamine-β-hydroxylase in this region. Some of this remaining norepinephrine may be present in nerve endings of peripheral sympathetic nerve fibers terminating in blood vessels in the median eminence. Another possibility is that some norepinephrine in the median eminence and hypothalamic island is contained within the terminals of a small number of intrahypothalamic noradrenergic neurons.

It is clear that hypothalamic deafferentation does not alter the ability of haloperidol to increase dopamine turnover in the median eminence (Figure 21). Since this action of haloperidol is most likely mediated by elevated serum prolactin concentrations, it appears that prolactin acts directly on neurons within the medial basal hypothalamus to increase the activity of tuberoinfundibular neurons. These results may indicate that prolactin acts directly on cell bodies of these dopamine neurons. However, one cannot exclude the possible involvement of other monoaminergic systems in the medial basal hypothalamus. If the ability of prolactin to increase dopamine turnover in the median eminence results from a direct action of this hormone on tuberoinfundibular neurons, it remains to be determined whether this action is mediated on the cell bodies or terminals of these neurons.

SUMMARY AND CONCLUSION

Sensitive radioenzymatic procedures were utilized in order to compare the responses of tuberoinfundibular dopamine neurons with those of nigrostriatal and mesolimbic neurons to various pharmacological agents. The abilities of drugs and endocrinological manipulations to alter steady state dopamine concentrations and dopamine turnover rates were examined in the median eminence, striatum and olfactory tubercle, regions containing the terminals of tuberoinfundibular, nigrostriatal and mesolimbic neurons, respectively. The significant observations and conclusive remarks of these studies are summarized below.

A. The systemic administration of γ-butyrolactone and baclofen markedly increased the steady state dopamine concentrations in the striatum and olfactory tubercle. However, neither drug altered the dopamine concentration in the median eminence. Regardless of the mechanism by which γ-butyrolactone and baclofen increase dopamine concentrations in the brain, these results indicate that these drugs do not influence all dopaminergic systems in a similar manner.

B. Although median eminence dopamine concentrations did not change during the estrous cycle, a marked increase in this brain region was observed four weeks after ovariectomy. Similar increases were not observed in the median eminence norepinephrine concentration nor in the concentration of dopamine in the hypothalamus, demonstrating

the selective nature of this increase. Thus, it appears that the dynamics of tuberoinfundibular dopamine neurons may be influenced by hormonal factors.

C. Dopamine turnover studies were performed to better characterize and compare the regulatory mechanisms governing the activity of tuberoinfundibular neurons with the neuronal feedback mechanism generally believed to regulate the activity of nigrostriatal and mesolimbic dopamine neurons. Dopamine turnover rates were estimated by observing the decline of the endogenous dopamine concentrations after synthesis inhibition with α-methyltyrosine. The dopaminergic antagonist, haloperidol, and the dopaminergic agonist, piribedil, produced a rapid increase and decrease, respectively, in the synthesis rates of dopamine in the striatum and olfactory tubercle. These agents did not produce similar effects in the median eminence. The actions of dopaminergic antagonists and agonists on cerebral dopamine metabolism have been used, previously, to support the concept of neuronal feedback modulation of nigrostriatal and mesolimbic neurons. The inability of these agents to alter dopamine turnover in the median eminence suggests that such a neuronal feedback loop does not influence the activity of tuberoinfundibular neurons.

Nevertheless, haloperidol did produce a delayed increase in dopamine turnover in the median eminence. However, this action of haloperidol in the median eminence appears to be hormonally mediated, since the effect was blocked by hypophysectomy.

D. The actions of exogenous prolactin and estradiol administration to selectively enhance the α-methyltyrosine-induced depletion of dopamine in the median eminence provides direct evidence for the

ability of hormones to influence the activity of tuberoinfundibular dopamine neurons. Thus, the activity of the tuberoinfundibular pathway may be regulated by a hormonal-neuronal feedback mechanism. In view of the inhibitory actions of dopamine on pituitary prolactin release, the action of prolactin to increase dopamine turnover in the median eminence may be one mechanism by which prolactin regulates its own secretion. Since the effects of estrogen on dopamine turnover in the median eminence were eliminated by hypophysectomy, the action of this steroid hormone in the median eminence is probably indirect and mediated by the estrogen-induced elevation of serum prolactin concentrations. The estrogen-induced, prolactin-mediated increased activity of tuberoinfundibular neurons may function to limit estrogen-stimulated prolactin secretion.

E. Isolation of a portion of the medial basal hypothalamus did not alter the ability of haloperidol to increase dopamine turnover in the median eminence. This observation indicates that the proposed hormonal-neuronal feedback regulation of tuberoinfundibular dopamine neurons involves an action of prolactin on intrahypothalamic neuronal pathways, possibly a direct action on tuberoinfundibular neurons (Figure 22).

F. In conclusion, dopaminergic systems within the central nervous system differ in their responsiveness to various pharmacological agents and endocrinological manipulations. Some of these differences may result from differences in the regulatory mechanisms governing the activity of these dopamine pathways. Whereas the activity of nigrostriatal and mesolimbic dopamine neurons may be modulated by a rapid neuronal feedback system, the activity of tuberoinfundibular dopamine

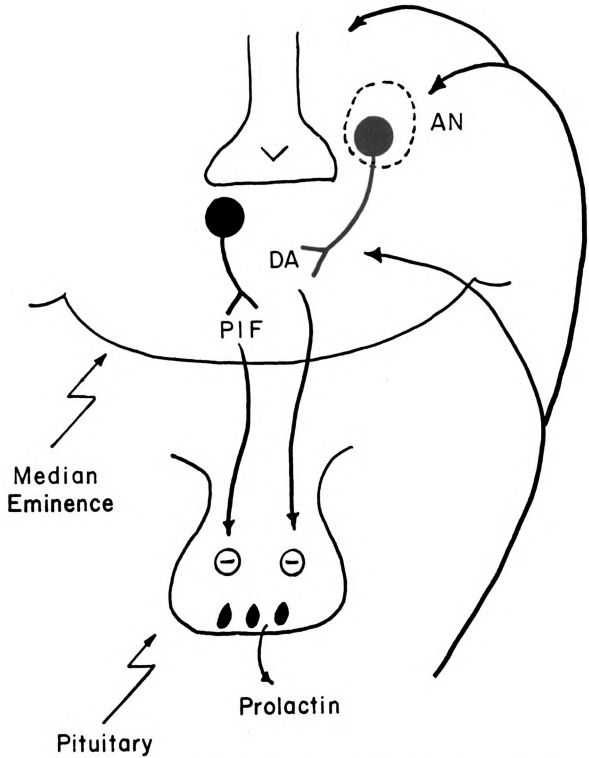


Figure 22. Prolactin-mediated feedback regulation of tuberoinfundibular dopamine neurons indicating possible sites of action of prolactin. AN, arcuate nucleus; DA, dopamine; PIF, prolactin inhibiting factor.

neurons may be influenced by a sluggish hormonal-neuronal feedback system.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aghajanian, G. K. and Roth, R. H.: γ-Hydroxybutyrate-induced increase in brain dopamine: Localization by fluorescence microscopy. J. Pharmacol. Exp. Ther. 175:131-138, 1970.

Amenomori, Y. and Meites, J.: Effects of a hypothalamic extract on serum prolactin levels during the estrous cycle and lactation. Proc. Soc. Exp. Biol. Med. 134:492-495, 1970.

Andén, N.-E.: Dopamine turnover in the corpus striatum and the limbic system after treatment with neuroleptic and antiacetylcholine drugs. J. Pharm. Pharmac. 24:905-906, 1972.

Andén, N.-E., Butcher, S. G., Corrodi, H., Fuxe, K. and Ungerstedt, U.: Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur. J. Pharmacol. 11:303-314, 1970.

Andén, N.-E., Corrodi, H. and Fuxe, K.: Turnover studies using synthesis inhibition. In *Metabolism of Amines in the Brain*, ed. by G. Hooper, pp. 38-47, Macmillan and Co., London, 1969.

Andén, N.-E., Roos, B.-E. and Werdinius, B.: Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci. 3:149-158, 1964.

Andén, N.-E. and Stock, G.: Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. J. Pharm. Pharmac. 25:346-348, 1973a.

Andén, N.-E. and Stock, G.: Inhibitory effect of gammahydroxybutyric acid and gammaaminobutyric acid on the dopamine cells in the substantia nigra. Naunyn-Schmiedeberg's Arch. Pharmacol. 297:89-92, 1973b.

Anlezark, G. M., Arbuthnott, G. W., Christie, J. E. and Crow, T. J.: Role of cerebral dopamine in the action of psychotropic drugs. Br. J. Pharmacol. 41:406-407, 1971.

Asher, J. M. and Aghajanian, G. K.: 6-Hydroxydopamine lesions of olfactory tubercles and caudate nuclei: Effect on amphetamine-induced stereotyped behavior in rats. Brain Res. 82:1-12, 1974.

Bacopoulos, W. G., Bhatnagar, R. K., Schnute, W. J. and VanOrden, L. S.: On the use of the fluorescence histochemical method to estimate catecholamine content in brain. Neuropharmacol. 14:291-299, 1975.

Bariletto, S., Dollar, E. and Leitz, F.: Effect of amantadine on the rate of dopamine synthesis in rat corpus striatum. J. Neurochem. 25:139-142, 1975.

Bartholini, G.: Differential effect of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. J. Pharm. Pharmac. 28:429-433, 1976.

Bartholini, G., Keller, H. H. and Pletscher, A.: Drug-induced changes of dopamine turnover in striatum and limbic system of the rat. J. Pharm. Pharmac. 27:439-442, 1975.

Beattie, C. W., Rodgers, C. H. and Soyka, L. F.: Influence of ovariectomy and ovarian steroids on hypothalamic tyrosine hydroxylase activity in the rat. Endocrinology 91:276-279, 1972.

Ben-Jonathan, N., Oliver, C., Weiner, H. J., Mical, R. S. and Porter, J. C.: Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. Endocrinology 100:452-458, 1977.

Ben-Jonathan, N. and Porter, J. C.: A sensitive radioenzymatic assay for dopamine, norepinephrine, and epinephrine in plasma and tissue. Endocrinology 98:1497-1507, 1976.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K. and Seiterberger, F.: Brain dopamine and the syndromes of Parkinson and Huntington. J. Neurol. Sci.: 415-455, 1973.

Bertler, A. and Rosengren, E.: Occurrence and distribution of catecholamines in the brain. Acta Physiol. Scand. 47:350-361, 1959.

Besson, M. J., Cheramy, A. and Glowinski, J.: Effects of some psychotropic drugs on dopamine synthesis in rat striatum. J. Pharmacol. Exp. Ther. 177:196-205, 1971.

Birge, C. A., Jacobs, L. S., Hammer, C. T. and Daughady, W. H.: Catecholamine inhibition of prolactin secretion by isolated rat adenohypophyses. Endocrinology 86:120-130, 1970.

Bishop, W., Krulich, L., Fawcett, C. P. and McCann, S. M.: The effect of median eminence (ME) lesions on plasma levels of LH, FSH and prolactin in the rat. Proc. Soc. Exp. Biol. Med. 136: 925-927, 1971.

Björklund, A., Lindvall, O. and Nobin, A.: Evidence of an incerto-hypothalamic dopamine neuron system in the rat. Brain Res. 89:29-42, 1975.

Björklund, A., Moore, R. Y., Nobin, A. and Stenevi, U.: The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. Brain Res. 51: 171-191, 1973.

Björklund, A. and Nobin, A.: Fluorescence histochemical and microspectrofluorometric mapping of dopamine and noradrenaline cell groups in the rat diencephalon. Brain Res. 51:193-205, 1973.

Broadwell, R. D. and Brightman, M. W.: Entry of peroxidase into neurons of the central and peripheral systems from extracerebral and cerebral blood flow. J. Comp. Neurol. 166:257-284, 1976.

Brown, G. M., Seeman, P. and Lee, T.: Dopamine/neuroleptic receptors in basal hypothalamus and pituitary. Endocrinology 99:1407-1410, 1976.

Brownstein, M. J., Palkovits, M., Tappaz, M., Saavedra, J. M. and Kizer, J. S.: Effect of surgical isolation of the hypothalamus on its neurotransmitter content. Brain Res. 117:287-295, 1976.

Brownstein, M., Saavedra, J. M. and Palkovits, M.: Norepinephrine and dopamine in the limbic system of the rat. Brain Res. 79:431-436, 1974.

Bunney, B. S., Walters, J. R., Roth, R. H. and Aghajanian, G. K.: Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity. J. Pharmacol. Exp. Ther. 185:560-571, 1973.

Burt, D. R., Enna, S. J., Creese, I. and Snyder, S. H.: Dopamine receptor binding in the corpus striatum of mammalian brain. Proc. Nat. Acad. Sci. 72:4655-4659, 1975.

Carlsson, A.: Receptor mediated control of dopamine metabolism. In *Pre- and Postsynaptic Receptors*, ed. by E. Usdin and W. E. Bunney, pp. 49-63, Marcel Dekker, New York, 1975.

Carlsson, A., Falck, B. and Hillarp, N.-A.: Cellular localization of brain monoamines. Acta Physiol. Scand. Suppl. 196:1-28, 1962a.

Carlsson, A., Falck, B., Hillarp, N.-A. and Torp, A.: Histochemical localization at the cellular level of hypothalamic noradrenaline. Acta Physiol. Scand. 54:385-386, 1962b.

Carlsson, A. and Lindqvist, M.: Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. et Toxicol. 20:140-144, 1963.

Carlsson, A., Lindqvist, M. and Magnusson, T.: On the biochemistry and possible functions of dopamine and noradrenaline in brain. In *Ciba Symposium on Adrenergic Mechanisms*, ed. by J. R. Vane, G. E. W. Wolstenholme, M. O. Connor and J. Churchill, pp. 432-439, London, 1960.

Carlsson, A., Lindqvist, M., Magnusson, T. and Waldeck, B.: On the presence of 3-hydroxytyramine in brain. Science 127:471, 1958.

Chen, C. L., Amenomori, Y., Lu, K. H., Voogt, J. L. and Meites, J.: Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendocrinology 6:220-227, 1970.

Chen, C. L., Minaguchi, H. and Meites, J.: Effects of transplanted pituitary tumors on host pituitary prolactin secretion. Proc. Soc. Exp. Biol. Med. 126:317-320, 1967.

Chiocchio, S. R., Cannata, M. A. and Tramezzani, J. H.: The size, weight and catecholamine content of the median eminence of the rat. Brain Res. 110:612-618, 1976.

Clemens, J. A. and Meites, J.: Inhibition by hypothalamic prolactin implants of prolactin secretion, mammary growth and luteal function. Endocrinology 82:876-881, 1968.

Clemens, J. A., Smalstig, E. G. and Sawyer, B. D.: Antipsychotic drugs stimulate prolactin release. Psychopharmacol. (Berl.) 40:123-127, 1974.

Clementi, F., Ceccarelli, B., Cerati, E., Delmonte, M. L., Felici, M. Motta, M. and Pecile, A.: Subcellular localization of neurotransmitters and releasing factors in the rat median eminence. J. Endocrinology 48:205-213, 1970.

Corrodi, H., Farnebo, L.-O., Fuxe, K., Hamberger, B. and Ungerstedt, U.: ET 495 and brain catecholamine mechanisms: Evidence for stimulation of dopamine receptors. Eur. J. Pharmacol. 20: 195-204, 1972.

Costa, E., Guidotti, A. and Zivkovic, B.: Short- and long-term regulation of tyrosine hydroxylase. In *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, ed. by E. Usdin, pp. 143-159, Raven Press, New York, 1974.

Costa, E. and Neff, N. H.: Isotopic and non-isotopic measurements of the rate of catecholamine biosynthesis. In *Biochemistry and Pharmacology of the Basal Ganglia*, ed. by E. Costa, L. Côté and M. D. Yahr, pp. 141-155, Raven Press, New York, 1966.

Costall, B. and Naylor, R. J.: The behavioral effects of dopamine applied intracerebrally to areas of the mesolimbic system. Eur. J. Pharmacol. 32:87-92, 1975.

Crane, G. E.: Pseudoparkinsonism and tardive dyskinesia. Arch. Neurol. 27:426-430, 1972.

Creese, I. and Iversen, S. D.: Amphetamine response in rat after dopamine neuron destruction. Nature New Biol. 238:247-248, 1972.

Creese, I. and Iversen, S. D.: The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. Psychopharmacol. (Berl.) 39:345-357, 1974.

Creese, I. and Iversen, S. D.: The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Res. 83:419-436, 1975.

Cuello, A. C., Hiley, R. and Iversen, L. L.: Use of catechol-O-methyltransferase for the enzyme radiochemical assay of dopamine. J. Neurochem. 21:1337-1340, 1973a.

Cuello, A. C., Horn, A. S., Mackay, A. V. P. and Iversen, L. L.: Catecholamines in the median eminence: New evidence for a major noradrenergic input. Nature 243:465-467, 1973b.

Cuello, A. C., Weiner, R. I. and Ganong, W. F.: Effect of lateral deafferentation on the morphology and catecholamine content of the medial basal hypothalamus. Brain Res. 59:191-200, 1973.

Curtis, D. R., Game, C. J. A., Johnston, G. A. R. and McCulloch, R. M.: Central effects of β-(p-chlophenyl)γ-aminobutyric acid. Brain Res. 70:493-499, 1974.

Dahlström, A. and Fuxe, K.: Evidence for the existence of monoamine containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. Suppl. 232:1-55, 1964.

Dang, B. T. and Voogt, J. L.: Termination of pseudopregnancy following implantation of prolactin. Endocrinology 100:873-880, 1977.

Davies, J. and Watkins, J. C.: The action of β-phenyl-GABA derivatives on neurons of the cat cerebral cortex. Brain Res. 70: 501-505, 1974.

DiChiara, G., Porceddu, M. L., Fratta, W. and Gessa, G. L.: Postsynaptic receptors are not essential for dopaminergic feedback regulation. Nature 267:270-272, 1977.

Dickerman, S., Clark, J., Dickerman, E. and Meites, J.: Effects of haloperidol on serum and pituitary prolactin and on hypothalamic PIF in rats. Neuroendocrinology 9:332-340, 1972.

Donoso, A. O., Banzan, A. M. and Barcaglioni, J. C.: Further evidence on the direct action of L-dopa on prolactin release. Neuroendocrinology 15:236-239, 1974.

Donoso, A. O., Bishop, W., Fawcett, C. P., Krulich, L. and McCann, S. M.: Effects of drugs that modify brain monoamine concentrations on plasma gonadotropin and prolactin levels in the rat. Endocrinology 89:774-784, 1971.

Donoso, A. O., Bishop, W. and McCann, S. M.: The effects of drugs which modify catecholamine synthesis on serum prolactin in rats with median eminence lesions. Proc. Soc. Exp. Biol. Med. 143:360-363, 1973.

Donoso, A. O. and DeGutierrez Moyano, M. B.: Adrenergic activity in hypothalamus and ovulation. Proc. Soc. Exp. Biol. Med. 135: 633-635, 1970.

Donoso, A. L., Stefano, F. J. E., Biscardi, A. M. and Cukier, J.: Effects of castration on hypothalamic catecholamines. Am. J. Physiol. 212:737-739, 1967.

Dray, A. and Straughan, D. W.: Synaptic mechanisms in the substantia nigra. J. Pharm. Pharmac. 28:400-405, 1976.

Enjalbert, A., Priam, M. and Kordon, C.: Evidence in favor of the existence of a dopamine-free prolactin-inhibiting factor (PIF) in rat hypothalamic extracts. Eur. J. Pharmacol. 41: 243-244, 1977.

Everett, J. W.: Luteotropic function of autografts of the rat hypophysis. Endocrinology 54:685-690, 1954.

Everett, J. W. and Nikitovitch-Winer, M.: Physiology of the rat pituitary gland as affected by transplantation or stalk section. In *Advances in Neuroendocrinology*, ed. by A. V. Nalbandov, pp. 289-313, University of Illinois Press, 1963.

Falck, B. and Hillarp, N.-A.: On the cellular localization of catecholamines in the brain. Acta Anat. 38:277-279, 1959.

Fibiger, H. C., Fibiger, H. P. and Zis, A. P.: Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. Br. J. Pharmacol. 47:683-692, 1973.

Fog, R. and Pakkenburg, H.: Behavioral effects of dopamine and p-hydroxyamphetamine injected into corpus striatum of rats. Exp. Neurol. 31:75-86, 1971.

Fog, R. L., Randrup, A. and Pakkenburg, H.: Aminergic mechanisms in corpus striatum and amphetamine-induced stereotyped behavior. Psychopharmacol. (Berl.) 11:179-183, 1967.

Fog, R., Randrup, A. and Pakkenburg, H.: Lesions in corpus striatum and cortex of rat brains and the effect on pharmacologically induced stereotyped, aggressive and cataleptic behavior. Psychopharmacol. (Berl.) 18:346-356, 1970.

Fuxe, K.: Cellular localization of monoamines in the median eminence and infundibular stem of some mammals. Acta Physiol. Scand. 58:383-384, 1963.

Fuxe, K.: Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. Supp. 247:39-85, 1965.

Fuxe, K.: Tools in the treatment of Parkinson's disease: Studies on new types of dopamine receptor stimulating agents. In *Advances in Neurology,* Vol. 3, ed. by D. B. Calne, pp. 273-279, Raven Press, New York, 1973.

Fuxe, K., Agnati, L., Tsuchiya, K., Hökfelt, T., Johansson, O., Jonsson, G., Lindbrink, P., Löfström, A. and Ungerstedt, U.: Effect of antipsychotic drugs on central catecholamine neurons of rat brain. In *Antipsychotic Drugs, Pharmacodynamics and Pharmacokinetics,* ed. by G. Sedvall, pp. 117-132, Pergamon Press, New York, 1975a.

Fuxe, K., Agnati, L., Hökfelt, T., Jonsson, G., Lindbrink, P., Ljungdahl, A., Löfström, A. and Ungerstedt, U.: The effect of dopamine receptor stimulating and blocking agents on the activity of supersensitive dopamine receptors and on the amine turnover in various dopamine nerve terminal systems in the rat brain. J. Pharmacol. (Paris) 6:117-129, 1975b.

Fuxe, K. and Hökfelt, T.: Further evidence for the existence of tuberoinfundibular dopamine neurons. Acta Physiol. Scand. 66:243-244, 1966.

Fuxe, K. and Hökfelt, T.: Catecholamines in the hypothalamus and the pituitary gland. In *Frontiers in Neuroendocrinology,* ed. by W. Ganong and L. Martini, pp. 47-96, Oxford University Press, New York, 1969.

Fuxe, K. and Hökfelt, T.: The effects of hormones and psychoactive drugs on the tubero-infundibular neurons. In *Some Aspects of Hypothalamic Regulation of Endocrine Function,* pp. 51-61, F. K. Schattauer Verlag, Stuttgart, 1973.

Fuxe, K., Hökfelt, T., Agnati, L., Löfström, A., Everitt, B. J., Johansson, O., Jonsson, G., Wuttke, W. and Goldstein, M.: Role of monoamines in the control of gonadotropin secretion. In *Neuroendocrine Regulation of Fertility,* ed. by T. C. Anand Kumar, pp. 123-140, Karger, Basle, 1976.

Fuxe, K., Hökfelt, T., Eneroth, P., Gustafsson, J.-A. and Skett, P.: Prolactin-like immunoreactivity: Localization in nerve terminals of rat hypothalamus. Science 196:899-900, 1977.

Fuxe, K., Hökfelt, T. and Jonsson, G.: The effect of gonadal steroids on the tuberoinfundibular dopamine neurons. In *Hormonal Steroids,* ed. by V. H. T. James and L. Martini, pp. 806-813, Excerpta Medica Foundation, Amsterdam, 1971.

Fuxe, K., Hökfelt, T., Jonsson, G. and Löfström, A.: Recent morphological and functional studies on hypothalamic dopaminergic and noradrenergic mechanisms. In *Frontiers in Catecholamine Research*, ed. by E. Usdin and S. Snyder, pp. 787-794, Pergamon Press, Oxford, 1973.

Fuxe, K., Hökfelt, T., Ljungdahl, A., Agnati, L., Johansson, O. and Perez de la Mora, M.: Evidence for an inhibitory gabergic control of the meso-limbic dopamine neurons: Possibility of improving treatment of schizophrenia by combined treatment with neuroleptics and gabergic drugs. Med. Biology 53:177-183, 1975c.

Fuxe, K., Hökfelt, T. and Nilsson, O.: Castration, sex hormones, and tuberoinfundibular dopamine neurons. Neuroendocrinology 5: 107-120, 1969.

Fuxe, K. and Ungerstedt, U.: Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In *Amphetamines and Related Compounds*, ed. by E. Costa and S. Garattini, pp. 257-288, Raven Press, New York, 1970.

Gessa, G. L., Vargai, L., Caralai, F., Bocro, C. C., Caboni, R. and Comba, R.: Selective increase of brain dopamine induced by gammahydroxybutyrate. Life Sci. 5:1921-1930, 1966.

Gianutsos, G., Thornburg, J. E. and Moore, K. E.: Differential actions of dopaminergic agonists and antagonists on the γ-butyrolactone-induced increase in mouse brain dopamine. Psychopharmacology 50:225-229, 1976.

Goldstein, A.: *Biostatistics*, p. 144, The Macmillan Co., New York, 1971.

Hökfelt, T.: The possible ultrastructural identification of tuberoinfundibular dopamine-containing nerve endings in the median eminence of the rat. Brain Res. 5:121-123, 1967.

Hökfelt, T. and Fuxe, K.: Effects of prolactin and ergot alkaloids on the tuberoinfundibular dopamine (DA) neurons. Neuroendocrinology 9:100-122, 1972.

Hökfelt, T., Johansson, O., Fuxe, K., Löfström, A., Goldstein, M., Park, D., Ebstein, R., Fraser, H., Jeffcoate, S., Efendic, S., Luft, R. and Arimura, A.: Mapping and relationship of hypothalamic neurotransmitters and hypothalamic hormones. In *Proceedings of the Sixth International Congress of Pharmacology*, ed. by J. Tuomisto and M. K. Paasonen, pp. 93-104, Pergamon Press, New York, 1976.

Hökfelt, T., Ljungdahl, A., Fuxe, K. and Johansson, O.: Dopamine nerve terminals in the rat limbic cortex. Science 184:177-179, 1974.

Horn, A. S., Cuello, A. C. and Miller, A. J.: Dopamine in the mesolimbic system of the rat brain: Endogenous levels and the effects of drugs on the uptake mechanism and stimulation of adenylate cyclase activity. J. Neurochem. 22:265-270, 1974.

Hornykiewicz, O.: Dopamine and brain function. Pharmacol. Rev. 18: 925-962, 1966.

Iversen, L. L. and Glowinski, J.: Regional studies of catecholamines in the rat brain. II. Rate of turnover in various brain regions. J. Neurochem. 13:671-682, 1966.

Iversen, S. D. and Kelly, P. H.: The use of 6-hydroxydopamine (6OHDA) techniques for studying the pathways involved in drug-induced motor behaviors. In *Chemical Tools in Catecholamine Research,* ed. by G. Jonsson, T. Malmfors and G. Sachs, pp. 327-333, North Holland/American Elsevier, New York, 1975.

Iwata, T. and Ishii, S.: Chemical isolation and determination of catecholamines in the median eminence and pars nervosa of the rat and horse. Neuroendocrinology 5:140-148, 1969.

Jonsson, G., Fuxe, K. and Hökfelt, T.: On the catecholamine innervation of the hypothalamus, with special reference to the median eminence. Brain Res. 40:271-281, 1972.

Jori, A., Cecchetti, G., Dolfini, E., Monti, E. and Garattini, S.: Effect of piribedil and one of its metabolites on the concentration of homovanillic acid in the rat brain. Eur. J. Pharmacol. 27:245-248, 1974.

Kavanagh, A. and Weisz, J.: Localization of dopamine and norepinephrine in the medial basal hypothalamus of the rat. Neuroendocrinology 13:201-212, 1974.

Kelly, P. H. and Iversen, S. D.: Selective 6-hydroxydopamine induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant induced locomotor activity. Eur. J. Pharmacol. 40:45-56, 1976.

Kelly, P. H. and Moore, K. E.: Actions of GABA, γ-butyrolactone and baclofen (Lioresal) on mesolimbic and nigrostriatal dopaminergic neurons. Pharmacologist 18:130, 1976.

Kelly, P. H., Seviour, P. W. and Iversen, S. D.: Amphetamine and apomorphine responses in the rat following 6OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94:507-522, 1975.

Kizer, T. S., Arimura, A., Schally, A. V. and Brownstein, J. M.: Absence of luteinizing hormone-releasing hormone (LH-RH) from catecholamine neurons. Endocrinology 96:523-525, 1975.

Kizer, J. S., Palkovits, M., Zivin, J., Brownstein, M., Saavedra, J. M., and Kopin, I. J.: The effect of endocrinological manipulations on tyrosine hydroxylase and dopamine-β-hydroxylase activities in individual hypothalamic nuclei of the adult male rat. Endocrinology 95:799-812, 1974.

Laverty, R. and Sharman, D. F.: The estimation of small quantities of 3,4-dihydroxy-phenylethylamine in tissues. Br. J. Pharmacol. 24:538-548, 1965.

Lindvall, O., Björklund, A., Moore, R. Y. and Stenevi, U.: Mesencephalic dopamine neurons projecting to neocortex. Brain Res. 81:325-331, 1974.

Lloyd, K. G., Davidson, L. and Hornykiewicz, O.: Metabolism of levodopa in the human brain. In *Advances in Neurology*, Vol. 3, ed. by D. B. Caine, pp. 173-188, Raven Press, New York, 1973.

Lloyd, K. G. and Hornykiewicz, O.: Catecholamines in regulation of motor function. In *Catecholamines and Behavior*, Vol. 1, ed. by A. J. Friedhoff, pp. 41-57, Plenum Press, New York, 1975.

Löfström, A., Jonsson, G. and Fuxe, K.: Microfluorometric quantitation of catecholamine fluorescence in rat median eminence. I. Aspects on the distribution of dopamine and noradrenaline nerve terminals. J. Histochem. Cytochem. 24:415-429, 1976a.

Löfström, A., Jonsson, G., Wiesel, F.-A. and Fuxe, K.: Microfluorimetric quantitation of catecholamine fluorescence in rat median eminence. II. Turnover changes in hormonal states. J. Histochem. Cytochem. 24:430-442, 1976b.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

Lu, K. H., Amenomori, Y., Chen, C. L. and Meites, J.: Effects of central acting drugs on serum and pituitary prolactin levels in rats. Endocrinology 87:667-670, 1970.

Lu, K. H., Chen, H. T., Huang, H. H., Grandison, L., Marshall, S. and Meites, J.: Relationship between prolactin and gonadotropin secretion in post-partum lactating rats. J. Endocrinology 68: 241-250, 1976.

Lu, L. H. and Meites, J.: Inhibition of L-dopa and monoamine oxidase inhibitors of pituitary prolactin release: Stimulation by methyldopa and d-amphetamine. Proc. Soc. Exp. Biol. Med. 137: 480-483, 1971.

MacLeod, R. M.: Influence of norepinephrine and catecholamine-depleting agents on the synthesis and release of prolactin and growth hormone. Endocrinology 85:916-923, 1969.

MacLeod, R. M.: Regulation of pituitary function by catecholamines. In *Mammary Cancer and Neuroendocrine Therapy*, ed. by B. A. Stoll, pp. 139-159, Butterworths, London, 1974.

MacLeod, R. M. and Abad, A.: On the control of prolactin and growth hormone synthesis in rat pituitary glands. Endocrinology 83: 799-806, 1968.

MacLeod, R. M. and Lehmeyer, J. E.: Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinology 94:1077-1085, 1974.

MacLeod, R. M., Smith, M. C. and DeWitt, G. W.: Hormonal properties of transplanted pituitary tumors and their relation to the pituitary gland. Endocrinology 79:1149-1156, 1966.

Meites, J. and Clemens, J. A.: Hypothalamic control of prolactin secretion. Vitamins and Hormones 30:165-221, 1972.

Meites, J. and Nicoll, C. S.: Adenohypophysis: Prolactin. Ann. Rev. Physiol. 28:57-88, 1966.

Meites, J., Nicoll, C. S. and Talwalker, P. K.: The central nervous system and the secretion and release of prolactin. In *Advances in Neuroendocrinology*, ed. by A. V. Nalbandov, pp. 238-288, University of Illinois Press, Urbana, 1963.

Meltzer, H. Y. and Fang, V. S.: Effect of gamma-butyrolactone and baclofen on plasma prolactin in male rats. Biochem. Pharmacol. 26:645-646, 1977.

Mena, F., Maiweg, H. and Grosvenor, G. E.: Effect of ectopic pituitary glands upon prolactin concentration by the *in situ* pituitary of the lactating rat. Endocrinology 83:1359-1362, 1968.

Miller, R. J. and Iversen, L. L.: Stimulation of a dopamine sensitive adenylate cyclase in homogenates of rat striatum by a metabolite of piribedil (ET495). Naunyn Schmiedebergs Arch. Pharmacol. 282:213-216, 1974.

Mioduszewski, R., Grandison, L. and Meites, J.: Stimulation of prolactin release in rats by GABA. Proc. Soc. Exp. Biol. Med. 151:44-46, 1976.

Montagu, K. A.: Catechol compounds in rat tissues and in brains of different animals. Nature 180:244-245, 1957.

Moore, K. E. and Phillipson, O. T.: Effects of dexamethasone on phenylethanolamine N-methyltransferase and adrenaline in the brains and superior cervical ganglia of adult and neonatal rats. J. Neurochem. 25:289-294, 1975.

Moss, R. L.: Unit responses in preoptic and arcuate neurons related to anterior pituitary function. In *Frontiers in Neuroendocrinology*, ed. by W. F. Ganong, pp. 95-128, Raven Press, New York, 1976.

Mueller, G. P., Simpkins, J., Meites, J. and Moore, K. E.: Differential effects of dopamine agonists and haloperidol on release of prolactin, thyroid stimulating hormone, growth hormone and luteinizing hormone in rats. Neuroendocrinology 20:121-135, 1976.

Muralidhar, K., Maneckjee, R. and Moudgal, N. R.: Inhibition of *in vivo* pituitary release of luteinizing hormone in lactating rats by exogenous prolactin. Endocrinology 100:1137-1142, 1977.

Nagatsu, T., Levitt, M. and Udenfriend, S.: Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910-2917, 1964.

Naylor, R. J. and Olley, J. E.: Modification of the behavioral changes induced by amphetamines in the rat by lesions in the caudate nucleus, the caudate-putamen and globus pallidus. Neuropharmacol. 11:91-99, 1972.

Neff, N. H. and Costa, E.: Effect of tricyclic antidepressants and chlorpromazine on brain catecholamine synthesis. In *Proceedings of the First International Symposium on Antidepressant Drugs*, ed. by S. Garattini and M. N. C. Dukes, pp. 28-34, Excerpta Medica Foundation, New York, 1966.

Neill, J. D.: Prolactin: Its secretion and control. In *Handbook of Physiology-Endocrinology*, Vol. 4, ed. by E. Knobil and W. H. Sawyer, American Physiology Society, Washington, DC, 1974.

Nikodejevic, B., Senoh, S., Daly, J. W. and Creveling, C. R.: Catechol-O-methyltransferase. II. A new class of inhibitors of catechol-O-methyltransferase; 3,5-dihydroxy-4-methoxybenzoic acid and related compounds. J. Pharmacol. Exp. Ther. 174: 83-93, 1970.

Niswender, G. D., Chen, C. L., Midgley, A. R., Ellis, S. and Meites, J.: Radioimmunoassay for rat prolactin. Proc. Soc. Exp. Biol. Med. 130:793-797, 1969.

Nybäck, H., Sedvall, G. and Kopin, I. J.: Accelerated synthesis of dopamine-^{14}C from tyrosine-^{14}C in rat brain after chlorpromazine. Life Sci. 6:2307-2312, 1967.

Ojeda, S. R., Harms, P. G. and McCann, S. M.: Effect of blockade of dopaminergic receptors on prolactin and LH release: Median eminence and pituitary sites of action. Endocrinology 94: 1650-1657, 1974.

Palkovits, M., Brownstein, M., Saavedra, J. M. and Axelrod, J.: Norepinephrine and dopamine content of hypothalamic nuclei. Brain Res. 77:137-149, 1974.

Palkovits, M. and Jacobowitz, D. M.: Topographic atlas of catecholamine and acetylcholinesterase containing neurons in the rat brain. J. Comp. Neurol. 157:13-28, 1974.

Pijnenburg, A. J. J., Honig, W. M. M., Van der Heyden, J. A. M., and Van Rossum, J. M.: Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharmacol. 35:45-58, 1976.

Pijnenburg, A. J. J., Honig, W. M. M. and Van Rossum, J. M.: Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacol. (Berl.) 41:87-95, 1975a.

Pijnenburg, A. J. J., Honig, W. M. M. and Van Rossum, J. M.: Antagonism of apomorphine and d-amphetamine-induced stereotyped behavior by injection of low doses of haloperidol into the caudate nucleus and the nucleus accombens. Psychopharmacol. (Berl.) 45:65-71, 1975b.

Pijnenburg, A. J. J. and Van Rossum, J. M.: Stimulation of locomotor activity following injections of dopamine into the nucleus accombens. J. Pharm. Pharmac. 25:1003-1005, 1973.

Pijnenburg, A. J. J., Woodruff, G. N. and Van Rossum, J. M.: Ergometrine induced locomotor activity following intracerebral injection into the nucleus accumbens. Brain Res. 59:289-302, 1973.

Quijada, M., Illner, P., Krulich, L. and McCann, S. M.: The effect of catecholamines on hormone release from anterior pituitaries and ventral hypothalami incubated *in vitro*. Neuroendocrinology 13:151-163, 1973.

Randrup, A. and Scheel-Krüger, J.: Diethyldithiocarbamate and amphetamine sterotyped behavior. J. Pharm. Pharmac. 18:752, 1966.

Ravitz, A. J. and Moore, K. E.: Concentration of prolactin in serum of rats treated with baclofen. J. Pharm. Pharmac. (in press) 1977.

Rinne, U. K. and Sonninen, V.: The occurrence of dopamine and noradrenaline in the tubero-hypophyseal system. Experentia 24:177-178, 1968.

Roth, R. H., Walters, J. R., Murrin, L. C. and Morgenroth, V. H.: Dopamine neurons: Role of impulse flow and pre-synaptic receptors in the regulation of tyrosine hydroxylase. In *Pre- and Postsynaptic Receptors*, ed. by E. Usdin and W. E. Bunney, pp. 5-48, Marcel Dekker, New York, 1975.

Scatton, B., Thierry, A. M., Glowinski, J. and Julou, L.: Effects of thioproperazine and apomorphine on dopamine synthesis in the mesocortical dopaminergic system. Brain Res. 88: 389-393, 1974.

Scheel-Krüger, J. and Randrup, A.: Stereotyped hyperactive behavior produced by dopamine in the absence of noradrenaline. Life Sci. 6:1389-1398, 1967.

Selmanoff, M. K., Pramik-Holdaway, M. T. and Weiner, R. I.: Concentrations of dopamine and norepinephrine in discrete hypothalamic nuclei during the rat estrous cycle. Endocrinology 99:326-329, 1976.

Shaar, C. J. and Clemens, J. A.: The role of catecholamines in the release of anterior pituitary prolactin *in vitro*. Endocrinology 95:1202-1212, 1974.

Shoemaker, W. J. and Schlumpf, M.: Dopamine levels in rat diencephalon cell groups: Alteration by endocrine manipulation. Neuroscience Abstracts 2:684, 1976.

Sokal, R. R. and Rohlf, F. J.: *Biometry*, p. 410, W. H. Freeman and Co., San Francisco, 1969.

Spano, P. F., Tagliamonte, A., Tagliamonte, P. and Gessa, G. L.: Stimulation of brain dopamine synthesis by γ-hydroxybutyrate. J. Neurochem. 18:1831-1836, 1971.

Spector, S., Gordon, R., Sjoerdsma, A. and Udenfriend, S.: Endproduct inhibition of tyrosine hydroxylase as a possible mechanism for regulation of norepinephrine synthesis. Mol. Pharmacol. 3: 549-555, 1967.

Stawarz, R. J., Hill, H., Robinson, S. E., Setter, P., Dingell, J. V. and Sulser, F.: On the significance of the increase in homovanillic acid (HVA) caused by antipsychotic drugs in corpus striatum and limbic forebrain. Psychopharmacol. (Berl.) 43:125-130, 1975.

Stefano, F. J. E. and Donoso, A. O.: Norepinephrine levels in the rat hypothalamus during the estrous cycle. Endocrinology 81: 1405-1406, 1967.

Stefano, F. J. E., Donoso, A. O. and Cukier, J.: Hypothalamic noradrenaline changes in ovariectomized rats. Acta Physiol. Latinoam. 15:425-427, 1965.

Takahara, J., Arimura, A. and Schally, A. V.: Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophysial portal vessel. Endocrinology 95:462-465, 1974.

Talwalker, P. K., Ratner, A. and Meites, J.: *In vitro* inhibition of pituitary prolactin synthesis and release by hypothalamic extracts. Am. J. Physiol. 205:213-218, 1963.

Thierry, A. M., Blanc, G., Sobel, A., Stinus, L. and Glowinski, J.: Dopaminergic terminals in the rat cortex. Science 182:499-501, 1972.

Thierry, A. M., Stinus, L., Blanc, G. and Glowinski, J.: Some evidence for the existence of dopaminergic neurons in the rat cortex. Brain Res. 50:230-234, 1973.

Ungerstedt, U.: Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. 367:1-48, 1971a.

Ungerstedt, U.: Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367:69-93, 1971b.

Van Maanen, J. H. and Smelik, P. G.: Induction of pseudopregnancy in rats following local depletion of monoamines in the median eminence of the hypothalamus. Neuroendocrinology 3:177-186, 1968.

Versteeg, D. H. G., Van der Gugten, J. and Vanree, J. M.: Regional turnover and synthesis of catecholamines in rat hypothalamus. Nature 256:502-503, 1975.

Vogt, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. (London) 123:451-481, 1954.

Voogt, J. L. and Meites, J.: Effects of an implant of prolactin in median eminence of pseudopregnant rats on serum and pituitary LH, FSH and prolactin. Endocrinology 88:286-292, 1971.

Voogt, J. L. and Meites, J.: Suppression of proestrous and suckling-induced increase in serum prolactin by hypothalamic implant of prolactin. Proc. Soc. Exp. Biol. Med. 142:1056-1058, 1973.

Waldmeier, P. C. and Maître, L.: On the relevance of preferential increases of mesolimbic versus striatal dopamine turnover for the prediction of antipsychotic activity of psychotropic drugs. J. Neurochem. 27:589-597, 1976.

Walters, J. R. and Roth, R. H.: Effect of gamma-hydroxybutyrate on dopamine and dopamine metabolites in the rat striatum. Biochem. Pharmacol. 21:2111-2121, 1972.

Walters, J. R. and Roth, R. H.: Dopaminergic neurons: Drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by a cessation of impulse flow. J. Pharmacol. Exp. Ther. 191:82-91, 1974.

Walters, J. R., Bunney, B. S. and Roth, R. H.: Piribedil and apomorphine: Pre- and postsynaptic effects on dopamine synthesis and neuronal activity. In *Advances in Neurology*, Vol. 9, ed. by D. B. Calne, T. N. Chase and A. Barbeau, pp. 273-284, Raven Press, New York, 1975.

Walters, J. R., Roth, R. H. and Aghajanian, G. K.: Dopaminergic neurons: Similar biochemical and histochemical effects of γ-hydroxybutyrate and acute lesions of the nigro-neostriatal pathway. J. Pharmacol. Exp. Ther. 186:630-639, 1973.

Watson, J. T., Krulich, L. and McCann, S. M.: Effect of crude rat hypothalamic extract on serum gonadotropin and prolactin levels in normal and orchidectomized male rats. Endocrinology 89:1412-1418, 1971.

Weiner, N.: A critical assessment of methods on the determination of monoamine synthesis turnover rates *in vivo*. In *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, ed. by E. Usdin, pp. 143-157, Raven Rress, New York, 1974.

Weiner, R. I.: Role of brain catecholamines in the control of LH and prolactin secretion. In *Hypothalamic Hormones*, ed. by M. Motta, P. G. Crosignani and L. Martini, pp. 249-253, Academic Press, London, 1975.

Weiner, R. I., Shryne, J. E., Gorski, P. A. and Sawyer, C. H.: Changes in catecholamine content of the rat hypothalamus following deafferentation. Endocrinology 90:867-873, 1972.

Welsch, C. W., Negro-Vilar, A. and Meites, J.: Effect of pituitary homografts on host pituitary prolactin and hypothalamic PIF levels. Neuroendocrinology 3:238-245, 1968.

Westerink, B. H. C. and Korf, J.: Influence of drugs on striatal and limbic homovanillic acid concentration in the rat brain. Eur. J. Pharmacol. 33:31-40, 1975.

Wiesel, F.-A. and Sedvall, G.: Effect of antipsychotic drugs on homovanillic acid levels in striatum and olfactory tubercle of the rat. Eur. J. Pharmacol. 30:364-367, 1975.

Wilk, S. and Glick, S. D.: Dopamine metabolism in the nucleus accumbens: The effect of clozapine. Eur. J. Pharmacol. 37: 203-206, 1976.

Wilk, S., Watson, E. and Stanley, M. E.: Differential sensitivity of two dopaminergic structures in rat brain to haloperidol and to clozapine. J. Pharmacol. Exp. Ther. 195:265-270, 1975.

Woodruff, C. N., Kelly, P. H. and Elkhawad, A. O.: Effects of dopamine receptor stimulants on locomotor activity of rats with electrolytic or 6-hydroxydopamine-induced lesions of the nucleus accumbens. Psychopharmacology 47:195-198, 1976.

Wuerthele, S. M., Kelly, P. H. and Moore, K. E.: Biochemical properties of nigrostriatal dopaminergic neurons following intrastriatal administration of kainic acid. Pharmacologist (in press) 1977.

Zivkovic, B., Guidotti, A., Revuelta, A. and Costa, E.: Effect of thioridazine, clozapine and other antipsychotics on the kinetic state of tyrosine hydroxylase and on the turnover rate of dopamine in striatum and nucleus accumbens. J. Pharmacol. Exp. Ther. 194:37-46, 1975a.

Zivkovic, B., Guidotti, A. and Costa, E.: Role for post-synaptic dopamine receptors in the regulation of the kinetic state of striatal tyrosine hydroxylase. In *Chemical Tools in Catecholamine Research,* ed. by O. Almgren, A. Carlsson and J. Engel, pp. 45-52, North Holland, Amsterdam, 1975b.