# THE RELATIONSHIP BETWEEN SERUM PROLACTIN CONCENTRATION AND TUBEROINFUNDIBULAR DOPAMINE ACTIVITY

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ABSTRACT

THE RELATIONSHIP BETWEEN SERUM PROLACTIN CONCENTRATION AND TUBEROINFUNDIBULAR DOPAMINE ACTIVITY

by

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Control of anterior pituitary hormone secretion involves the central nervous system, and more specifically, neural activity in the hypothalamus. The release of specific anterior pituitary hormones is determined by various releasing and/or inhibiting factors which are synthesized by neurosecretory cells in the hypothalamus. It is generally agreed upon that prolactin is under tonic inhibitory control.

Since the discovery and mapping of catecholaminergic nerve tracts in the medial basal hypothalamus near the primary capillary plexus of the hypophyseal portal system, efforts have been directed toward determining the relationship between medial basal hypothalamic catecholamine activity and the secretion of prolactin. It now appears that the release of dopamine by tuberoinfundibular dopamine neurons in the median eminence is the major factor in the tonic inhibitory control of serum prolactin concentrations. Thus, the determination of serum prolactin concentrations should prove to be an easily accessible biochemical measure of neuronal activity in a central dopamine system. Many of the pharmacologic responses and receptor characteristics of the tuberoinfundibular system remain to be quantified, however, before information

obtained by measuring prolactin responses can be extrapolated to other central dopamine systems. The experiments described in this thesis were performed with the objective of better defining the relationship between serum prolactin concentrations and tuberoinfundibular dopamine activity. This was done by studying the serum prolactin response to a number of pharmacological agents known to affect either dopaminergic activity or pituitary function.

Haloperidol and clozapine, two drugs with known antipsychotic activity, elevated serum prolactin concentrations in male rats in a dose-dependent fashion. Haloperidol was at least 160 times more potent than clozapine, minimum effective doses being 0.125 and 20 mg/kg, respectively, but both agents were capable of stimulating marked increases in the concentration of the hormone. It was thus concluded that both agents interfere with tuberoinfundibular dopamine transmission.

Baclofen is a new drug that has been shown to depress the firing of nigrostriatal and mesolimbic dopaminergic neurons. When baclofen was given to male rats, however, it failed to elevate serum prolactin except at the highest dose (20 mg/kg), and at this dose, the rats were anaesthetized and appeared to have difficulty breathing. Furthermore, when baclofen was administered to rats pretreated with a dose of haloperidol that is subthreshold for increasing prolactin, baclofen did not increase circulating hormone levels. These data suggest that the baclofen-induced rise in serum prolactin observed at 20 mg/kg is due to non-specific stress effects, and that baclofen does not inhibit tuberoinfundibular neuronal firing.

Alan Jay Ravitz

Treatment with exogenous estrogens increases serum prolactin levels and tuberoinfundibular dopamine turnover. By treating male rats with estrogen and then measuring the prolactin response to drugs which alter dopaminergic activity, the mechanism of action by which estrogens exert their effects on prolactin and tuberoinfundibular dopamine can be studied. It was found that chronic treatment with estradiol benzoate potentiates the prolactin-increasing effects of alpha-methyltyrosine, an agent which blocks dopamine synthesis. In addition, the prolactin-lowering effects of piribedil, a direct-acting dopamine agonist, were partially inhibited by prior treatment with estradiol benzoate. These findings provide evidence that estrogen-induced increases in tuberoinfundibular dopamine turnover are due to direct stimulation by estrogen of the anterior pituitary to increase prolactin release.

Supersensitivity of postsynaptic receptors to neurotransmitter stimulation commonly occurs in dopamine systems upon chronic blockade of impulse transmission. Whether or not supersensitivity develops in tuberoinfundibular dopamine receptors was tested by treating male rats with haloperidol for 2 weeks, and then challenging them with the dopamine agonist, piribedil, 72 hours after the last injection. If supersensitivity had developed, piribedil would have been more potent in blocking alpha-methyltyrosine-induced prolactin increases in rats which received chronic haloperidol than in chronic vehicle-treated controls. The results, however, showed that piribedil was equally potent in both chronic treatment groups. Therefore, it was concluded that chronic administration of dopamine blocking drugs does not alter the sensitivity of dopamine receptors associated with tuberoinfundibular system.

In the last series of experiments, the prolactin response to three indirect-acting dopamine agonists, d-amphetamine, methylphenidate, and cocaine, was examined both with and without alpha-methyltyrosine pretreatment. As all three of these agents have been shown to stimulate dopamine activity, it was expected that they would lower prolactin concentrations. This was found to be the case when the drugs were administered without any pretreatment. Alpha-methyltyrosine appeared to block the prolactin-lowering actions of d-amphetamine, but methylphenidate and cocaine did induce dose-dependent decreases of the hormone. Under both conditions, however, the decreases induced by the indirect-acting agonists were much smaller than those induced by direct-acting agonists in similar situations. To explain these differences in efficacy, it was hypothesized that less intraneuronal dopamine is available for release in tuberoinfundibular neurons than in neurons of other central dopamine systems.

In summary, the results presented indicate that while the tuberoinfundibular dopamine system does share certain characteristics with other more extensively studied dopamine systems, major differences appear to exist.

# THE RELATIONSHIP BETWEEN SERUM PROLACTIN CONCENTRATION AND TUBEROINFUNDIBULAR DOPAMINE ACTIVITY

By

Alan Jay Ravitz

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## DEDICATION

The time I spent working in Ken Moore's laboratory was not only valuable in terms of knowledge gained, it was also a positive, enjoyable experience. Therefore, I would like to dedicate this thesis to all of my co-workers: Gary, Geoff, Gerry, Ken, Mirdza, Nan, Pete, Sue, and Suzanne.

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I would like to thank the members of my committee for their time and suggestions: Ken Moore, Richard Rech, Theodore Brody, and Joseph Meites. I would also like to thank Dr. Meites for allowing me to use his laboratory facilities. Finally, I would like to thank the Pharmaceutical Manufacturer's Association Foundation and the Department of Pharmacology for providing me with financial support while I completed the work described in this thesis.

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## INTRODUCTION AND STATEMENT OF PURPOSE

Control of anterior pituitary hormone secretion involves the central nervous system and more specifically, neural activity in the hypothalamus (see Szentagothai, 1972). The release of specific anterior pituitary hormones is determined by various releasing and/or inhibiting factors which are synthesized by neurosecretory cells in the hypothalamus. These hypophysiotropic hormones enter the hypophyseal portal circulation where they are transported to the anterior pituitary. Here they act directly on the gland to influence the synthesis and release of the pituitary hormones. It is generally agreed upon that prolactin secretion is under tonic inhibitory control (see Meites, 1973).

Since the discovery and mapping of catecholaminergic nerve tracts which terminate in the medial basal hypothalamus near the primary capillary plexus of the hypophyseal portal system (see Ungerstedt, 1971a), a great deal of research has been done to determine the relationship between medial basal hypothalamic catecholamine activity and the secretion of prolactin. It now appears that the release of dopamine from tuberoinfundibular dopamine neurons into the median eminence is the major factor in the tonic inhibitory control of serum prolactin concentrations (see MacLeod, 1974). The tuberoinfundibular dopamine system has cell bodies in the arcuate nucleus (area A12 of rat brain)

with short axons projecting to the external layer of the median eminence (Ungerstedt, 1971a).

Tuberoinfundibular dopamine may itself be the physiologic prolactin inhibitory factor (PIF), or it may be that dopamine stimulates the release of a peptidergic PIF from neurosecretory cells in the median eminence. There is evidence to support both of these views, and it may also be possible that dopamine exerts both a direct and indirect effect on anterior pituitary prolactin release (see MacLeod, 1976). In any case, drugs that stimulate dopamine activity in the central nervous system have been found to depress serum prolactin. Conversely, drugs that block central dopamine activity increase prolactin concentrations.

Thus, the measurement of serum prolactin concentrations should prove to be an easily accessible, easily quantifiable, biochemical measure of activity in a central dopamine system. It is on this assumption that the following experimental studies are based, the ultimate object being the characterization of the tuberoinfundibular dopamine system and its utilization to clarify certain pharmacologic problems.

## REVIEW OF LITERATURE

### I. Biochemistry and Neuroanatomy of Central Dopaminergic Systems

#### A. Dopamine Biosynthesis

The first step in the synthesis of dopamine (Figure 1) is the active uptake from the circulation of the amino acid precursor tyrosine (Cooper et al., 1974). Tyrosine is then hydroxylated to form L-dihydroxyphenylalanine (L-DOPA), a catechol amino acid. This hydroxylation is catalyzed by L-tyrosine hydroxylase, an enzyme found only in catecholamine-containing neurons and chromaffin cells (Cooper et al., 1974). L-DOPA is then rapidly converted to dopamine via the action of aromatic L-amino acid decarboxylase.

Tyrosine hydroxylase is the rate-limiting enzyme for the synthesis of dopamine and other catecholamines, and its activity is regulated through end-product inhibition. Both dopamine and norepinephrine are strong inhibitors of this enzyme (Levitt et al., 1965, 1967). In addition, tyrosine hydroxylase is stereospecific for L-tyrosine, and it requires tetrahydrobiopterin as a cofactor, as well as molecular $O_2$ and $Fe^{++}$ for activity (Nagatsu et al., 1964; Levitt et al., 1967; Musacchio and D'Angelo, 1971). Aromatic L-amino acid decarboxylase is widely distributed throughout the brain and other tissues. It exhibits little substrate specificity and is involved in

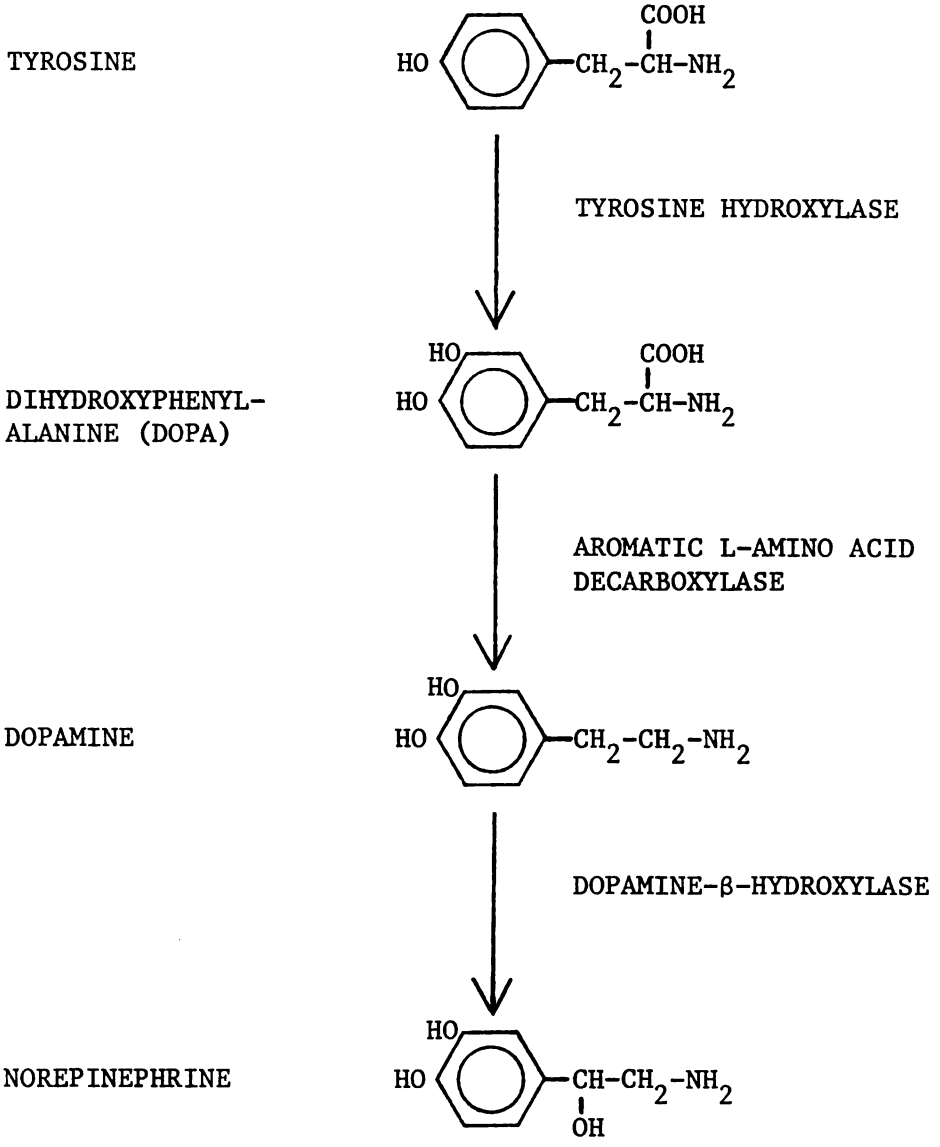


FIGURE 1. Biosynthesis of catecholamines.

the biosynthesis of both catecholamines and serotonin. The enzyme requires pyridoxal phosphate as a cofactor (Cooper et al., 1974).

In noradrenergic neurons, dopamine is subsequently hydroxylated at the beta carbon by dopamine-beta-hydroxylase to form norepinephrine. Dopamine-beta-hydroxylase is bound to the inner surface of norepinephrine-containing vesicles (Cooper et al., 1974).

When an impulse arrives at the terminals of a dopaminergic neuron, dopamine is released into the synaptic cleft where it exerts its action by combining with a dopamine receptor on the postsynaptic cell. The major route through which the action of the neurotransmitter is terminated is active reuptake by the presynaptic cell (Cooper et al., 1974).

B. Regulation of Dopamine Synthesis

Steady-state concentrations of catecholamines remain quite constant under a variety of physiological and pharmacological conditions (Cooper et al., 1974). Thus, there must be one or more homeostatic mechanisms responsible for the regulation of dopamine synthesis such that the steady-state is maintained.

As has already been mentioned, tyrosine hydroxylase is the usual rate limiting enzyme in catecholamine synthesis. Like other rate limiting enzymes in biosynthetic pathways, tyrosine hydroxylase is under end-product inhibition. Both dopamine and norepinephrine, presumably by competing with enzyme cofactors, can effectively inhibit enzyme activity (Nagatsu et al., 1964; Neff and Costa, 1966; Spector et al., 1967). Costa et al. (1974) have proposed that this end-product inhibition of tyrosine hydroxylase provides a mechanism

whereby dopamine levels could be regulated, but Carlsson et al. (1974) question its physiological importance. There is evidence, however, that treatments which acutely increase catecholaminergic activity also acutely increase enzymatic activity (Sedvall et al., 1968; Dairman et al., 1968), and that neuronal concentrations of tyrosine hydroxylase rise after chronic stimulation of neuronal activity (Mueller et al., 1969; Joh et al., 1973). These experiments all employed peripheral catecholamine systems, however, and there is no proof that similar phenomena would occur within central dopaminergic neurons.

Two other homeostatic mechanisms have been proposed for the regulation of steady-state dopamine concentrations in addition to end-product inhibition. Many investigators (Kehr, et al., 1972; Aghajanian and Bunney, 1974; Carlsson et al., 1974; Roth et al., 1974) have proposed that presynaptic dopamine receptors exist with which neuronally released dopamine can combine, thus providing the neuron with information concerning the amount of transmitter actually in the synaptic cleft. It has been shown that apomorphine, a direct dopamine agonist, when microiontophoretically applied to dopamine neurons can inhibit their firing, and that haloperidol and chlorpromazine cannot block this effect (Aghajanian and Bunney, 1973; Bunney et al., 1973). The conclusion is that apomorphine exerts a presynaptic action via receptors with which the two neuroleptics, both effective postsynaptic dopamine receptor blockers, cannot combine.

The third postulated feedback mechanism involves a multisynaptic neuronal feedback loop which is activated by postsynaptic dopamine receptors (Carlsson et al., 1974). Gamma-aminobutyric acid and acetylcholine have both been implicated as neurotransmitters in this feedback loop (Olivier et al., 1970; Kim et al., 1971; Precht and Yoshida, 1971; McGeer et al., 1973; Javoy et al., 1974). In this type of system, postsynaptic blockade of dopamine receptors by neuroleptics would feed back to the presynaptic neuron, causing an increased turnover of the neurotransmitter, while stimulation of postsynaptic receptors with direct-acting dopamine agonists would inhibit presynaptic dopamine release. This system does not appear to function in all central dopaminergic tracts (Gudelsky and Moore, 1976).

Figures 2 and 3 illustrate the various feedback systems mentioned above.

C. Location and Functional Importance of the Tuberoinfundibular Dopamine System

In addition to its more classically defined roles as a neurotransmitter in the nigrostriatal and mesolimbic systems (Ungerstedt, 1971a), there has been a recent accumulation of evidence that dopamine also functions as a neurotransmitter in the medial basal hypothalamus (Carlsson et al., 1962; Fuxe, 1963; Johnsson et al., 1972; Björklund et al., 1973). The tuberoinfundibular dopamine system has its cell bodies in the A12 cell group of the arcuate nucleus on the ventral aspect of the third ventricle (Ungerstedt, 1971a)(Figure 4). The short tuberoinfundibular dopamine axons project to the external layer of the median eminence where they terminate in close

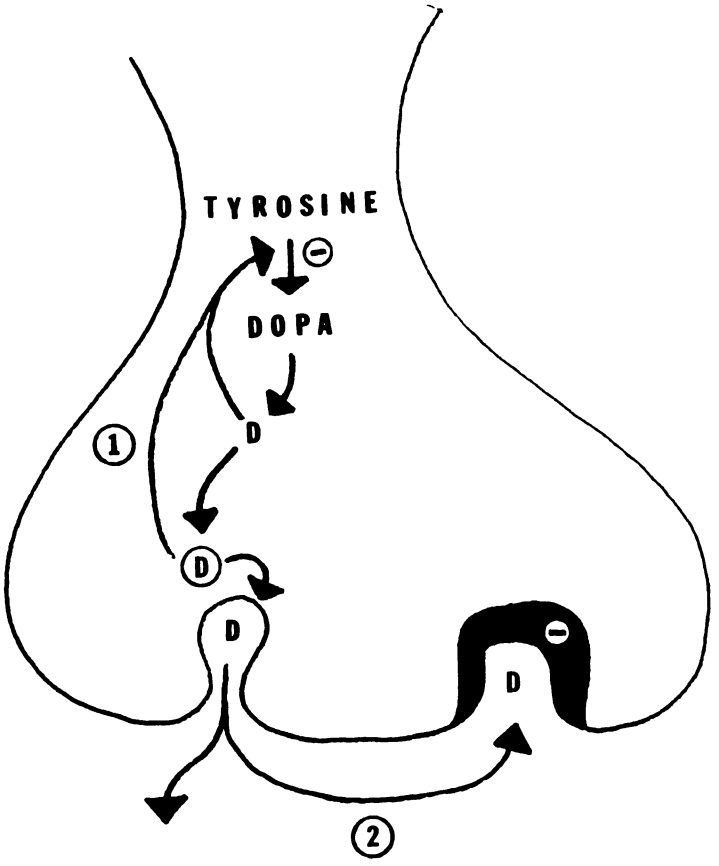


FIGURE 2. Presynaptic regulation of dopamine (D) activity. 1 Intraneuronal D, through feedback inhibition of tyrosine hydroxylase, blocks the conversion of tyrosine to DOPA, thus blocking D synthesis. 2 D in the synaptic cleft combines with presynaptic D receptors to inhibit the synthesis and release of dopamine.

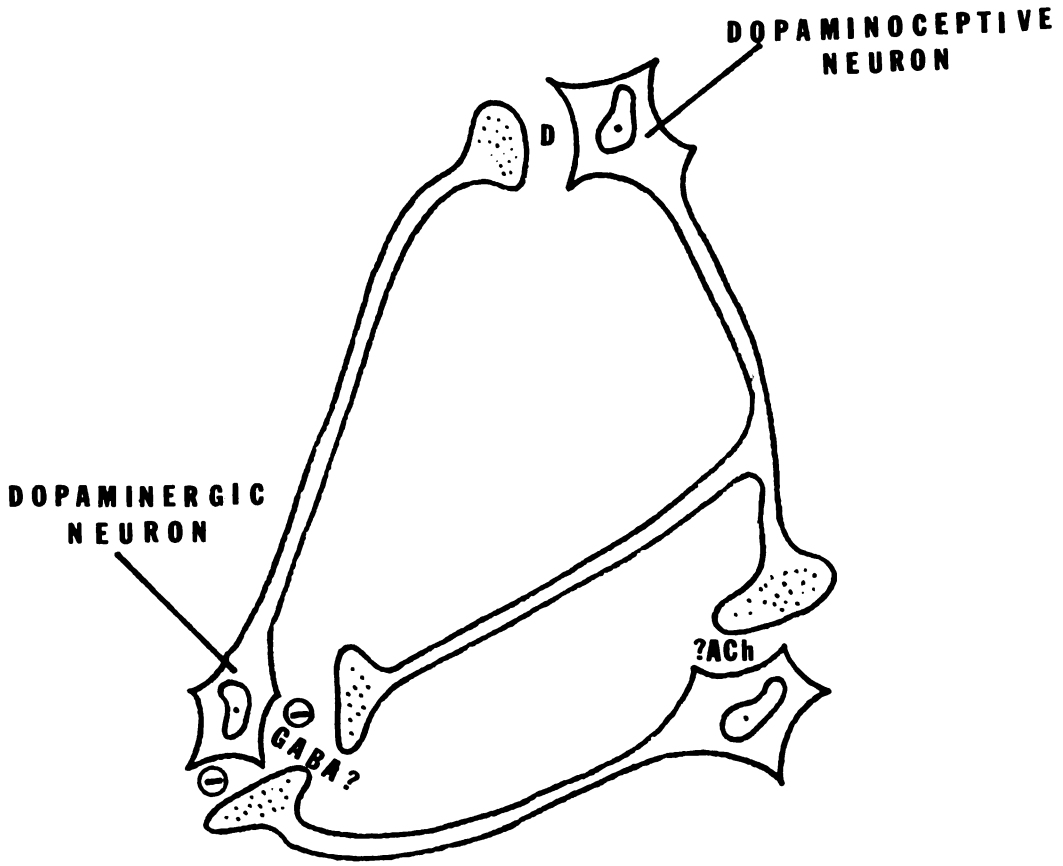


FIGURE 3. Multisynaptic feedback loop for the control of dopamine (D) activity. Abbreviations used: ACh, acetylcholine; GABA, gamma-aminobutyric acid (Adapted from Meltzer and Stahl, 1976).

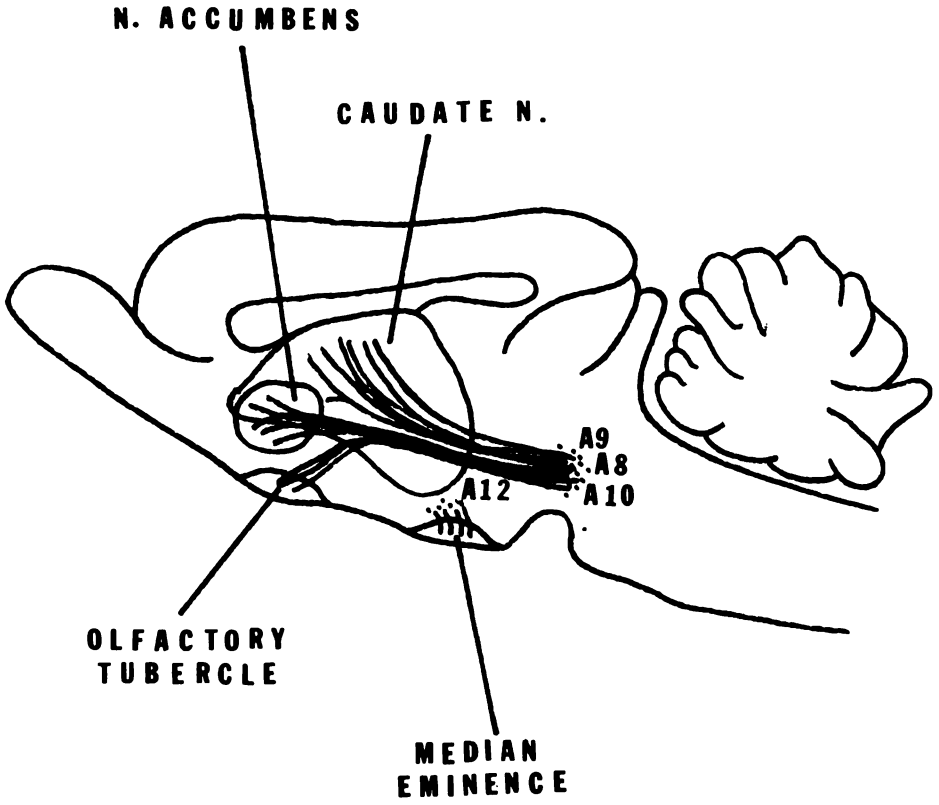


FIGURE 4. Dopaminergic tracts of the rat brain (Adapted from Ungerstedt, 1971a).

proximity to the primary capillary plexus of the hypophyseal portal system. This fact might lead one to suppose that the tuberoinfundibular dopamine system is involved with neuroendocrine function, and these suspicions have, indeed, been borne out experimentally. Not only do changes in the endocrine state of experimental animals affect the activity of tuberoinfundibular dopamine neurons (Fuxe et al., 1969a,b; Ahren et al., 1971; Hökfelt and Fuxe, 1972), but pharmacologic manipulations with various putative centrally acting dopamine agonists and antagonists alter serum levels of the anterior pituitary hormones (Donoso et al., 1971; Mueller et al., 1976). Although the tuberoinfundibular dopamine system has effects on many pituitary hormones, at present the most direct correlations can be made between dopamine activity and serum prolactin concentrations (MacLeod, 1974). This relationship will be discussed in detail in a later section.

It should be further noted that tuberoinfundibular dopamine neurons do not share all of the characterisitcs of the nigrostriatal or mesolimbic neurons. Recent work by Gudelsky and Moore (1976) indicates that the multisynaptic neuronal feedback loop which was mentioned in the previous section may not function in the tuberoinfundibular system. Haloperidol, a dopamine antagonist, did not increase, nor did piribedil, a dopamine agonist, decrease dopamine turnover in the median eminence upon acute administration. Both of these effects were observed in the mesolimbic and nigrostriatal systems. In addition, the inhibitory effects of gamma-butyrolactone on impulse flow which result in the accumulation of dopamine in

nigrostriatal and mesolimbic neurons were not demonstrated in the median eminence (Gudelsky and Moore, 1976).

## II. Selected Pharmacology of Various Drugs Which Affect Dopamine Transmission

### A. Drugs Which Increase Dopamine Activity

Dopamine agonists can be divided into two major groups depending upon their mechanism of action. Direct-acting agonists combine directly with postsynaptic dopamine receptors to activate them. Indirect-acting agonists exert their actions by stimulating the release and/or blocking the reuptake of endogenous dopamine. A number of biochemical and behavioral parameters can be measured to determine whether or not a drug influences dopamine transmission, and if so, the mechanism by which the drug exerts its effect. One of the most useful biochemical indicators is the effect a drug has on dopamine turnover in the telencephalon (Andén et al., 1967). Behavioral parameters include the stimulation of locomotor and stereotyped activities (Ernst, 1967; Moore and Thornburg, 1973; Randrup and Munkvad, 1974), which appear to be mediated by mesolimbic and nigrostriatal dopamine neurons, respectively (Kelly et al., 1975). Another useful behavioral test is the effect a drug has on circling behavior in animals with unilateral 6-hydroxydopamine-induced lesions of the corpus striatum (Ungerstedt, 1971b; Moore, 1974). This appears to be a specific measure of dopaminergic activity in the nigrostriatal pathway.

Apomorphine (Andén et al., 1967) and piribedil (ET 495) (Corrodi et al., 1971) are direct-acting dopamine receptor stimulants.

Both agents decrease dopamine turnover, probably through postsynaptic stimulation of the previously mentioned multisynaptic feedback loop. Both drugs elicit increased locomotor activity, and at higher doses, stereotypy. And lastly, both drugs cause contralateral circling in rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal pathway (Ungerstedt, 1971b; Thornburg and Moore, 1974). Piribedil appears to be less potent and have a longer duration of action than apomorphine (Corrodi et al., 1972).

Methylphenidate, cocaine, and d-amphetamine are three drugs which exhibit, among many other effects, indirect dopaminergic agonist properties. Scheel-Krüger (1972) demonstrated that all three agents stimulate locomotor activity and stereotypy. Chiueh and Moore (1975a,b) reported that d-amphetamine and methylphenidate were both capable of stimulating the release of endogenous dopamine, and Von Voigtlander and Moore (1972) observed that both agents stimulated ipsilateral turning in mice with unilateral 6-hydroxydopamine-induced lesions. Cocaine differs from the other two drugs in its mechanism of action. It has been shown in vitro (Heikkila et al., 1975) that cocaine is an example of a pure inhibitor of dopamine uptake.

B. Drugs Which Decrease Dopamine Activity

Two types of drugs which decrease dopamine activity will be discussed in this section. The first group of drugs, neuroleptics, are used clinically in the treatment of schizophrenia. They are thought to exert some of their clinical effects by the blockade of

central dopamine receptors (Hornykiewicz, 1966). The second type of drug decreases dopamine activity through the blockade of its synthesis.

Two different neuroleptics were used in the experiments to be reported on in this paper. Haloperidol is a member of the butyrophenone chemical class, and clozapine is one of the dibenzodiazepine class of drugs (Byck, 1975). Haloperidol appears to be a relatively specific blocker of dopamine receptors (Andén et al., 1970). It shows a typical activity spectrum in the behavioral tests widely used to detect neuroleptic activity, such as the induction of catalepsy or the antagonism of stereotyped behavior (Costall and Naylor, 1975). Clozapine, on the other hand, exhibits an atypical activity spectrum (Costall and Naylor, 1975), although it has been shown to be a clinically effective antipsychotic agent. In addition, it has been reported that clozapine stimulates dopamine turnover in the forebrain (Andén and Stock, 1973; Bürki et al., 1975).

Alpha-methyltyrosine is a tyrosine hydroxylase inhibitor (Spector et al., 1965), and as this is the rate limiting enzyme in catecholamine synthesis, treatment with alpha-methyltyrosine depletes brain contents of dopamine and norepinephrine.

Figure 5 is a diagram of a dopaminergic nerve terminal and synapse that indicates the location where the drugs mentioned in the last two sections are postulated to exert their effects.

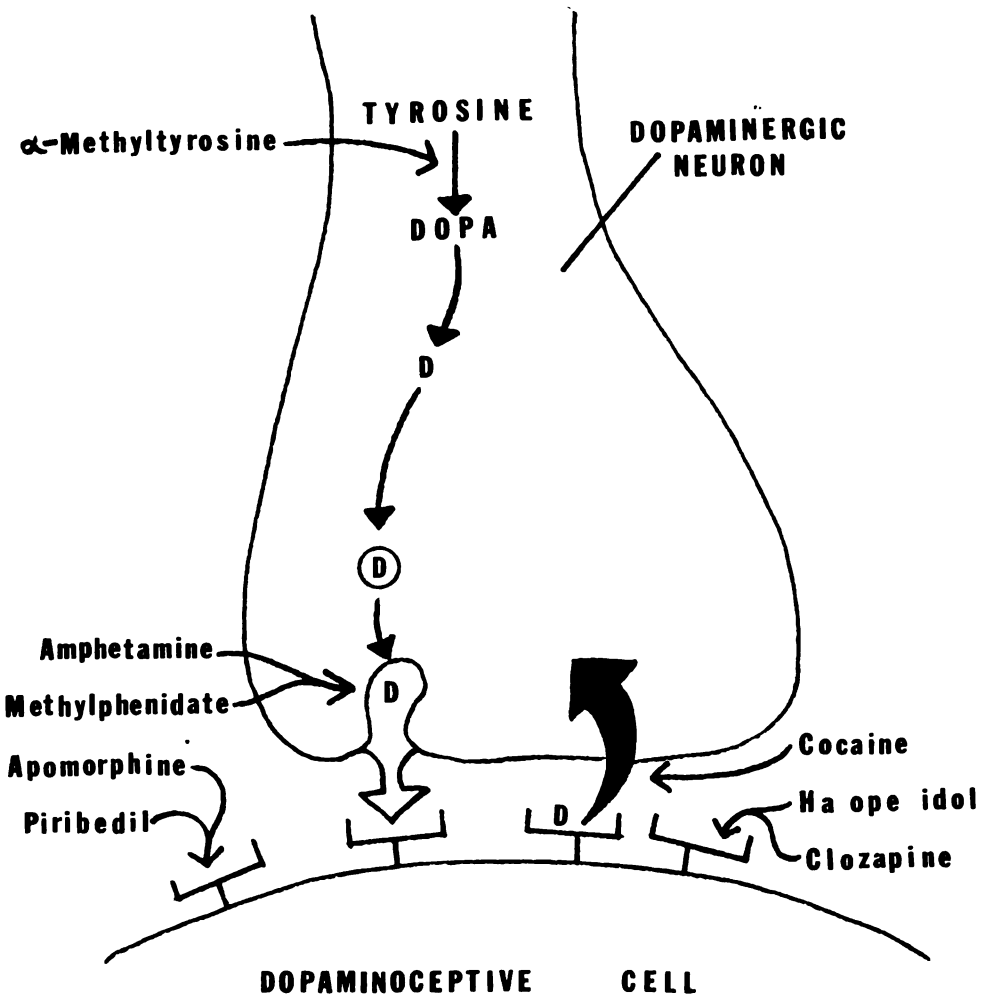


FIGURE 5. A dopaminergic synapse. Some of the drugs that alter dopaminergic transmission are depicted; details of the actions of these drugs are depicted in the text. Abbreviations used: D, dopamine; DOPA, dihydroxyphenylalanine.

C. Baclofen (beta-p-chlorophenyl gamma-aminobutyric acid)

Baclofen is a drug used to treat various forms of spasticity in humans (Burke et al., 1971). This structural analog of gamma-aminobutyric acid also has central effects. It has been reported that the clinically effective dosage of phenothiazine neuroleptics can be reduced when baclofen is administered concurrently (Robson, personal communication). It has also been observed to reverse certain d-amphetamine induced behavioral abnormalities (Ahlenius et al., 1975). Finally, baclofen has been shown to prevent the pimozide (a dopamine receptor blocker)-induced increase in mesolimbic dopamine turnover (Fuxe et al., 1975). It has been postulated that baclofen exerts its effects by somehow inhibiting the firing of dopamine neurons (Fuxe et al., 1975).

## III. Neuroanatomy of the Hypothalamo-pituitary System

### A. Neuroanatomy of the Hypothalamus

The functional neuroanatomy of the hypothalamus is quite adequately covered in many texts, e.g. Netter (1957), Szentagothai et al. (1972), and Jenkins (1972). This section, based on the above-mentioned works, will concern itself with those aspects of hypothalamic neuroanatomy most directly involved with anterior pituitary function.

The hypothalamus is a paired structure, divided into right and left halves by the vertically oriented third ventricle, in the center of the most ventral surface of the diencephalon. It comprises

the lateral walls and floor of the third ventricle and extends from the anterior border of the optic chiasm to the caudal border of the mammillary bodies. The thalamus and subthalamus make up the dorsal and lateral borders.

In the middle of the ventral surface of the hypothalamus is located the tuber cinereum, which gives rise to the infundibulum, or pituitary stalk. The tuber cinereum contains the arcuate nucleus, the primary capillary plexus of the hypophyseal portal system, and the median eminence. The arcuate nucleus, which contains the cell bodies of the tuberoinfundibular dopamine neurons, surrounds the most ventral aspect of the third ventricle. Just ventral to this is the median eminence, a small area of neural tissue composed of densely packed nerve terminals which surround the capillaries of the primary plexus (Figure 4). Within the region of the median eminence can be found high concentrations of hypothalamic hormones and dopamine (Szentagothai et al., 1972; Brownstein et al., 1976).

The hypothalamus receives afferent fibers from the fornix, medial forebrain bundle, thalamus, mammillary peduncle, stria terminalis, and perhaps other areas of the central nervous system. Major efferent tracts include the hypothalamo-hypophyseal, periventricular, and mammillary. Although it is known that other brain centers influence hypothalamic-pituitary function (Szentagothai et al., 1972), the nature of this relationship has not yet been elucidated.

B. Hypothalamo-hypophyseal Portal System and the "Chemotransmitter Hypothesis"

The pituitary gland, which lies just below the hypothalamus, is connected to it by the narrow infundibulum. This pituitary stalk is composed of: 1) nerve tracts along which are transported oxytocin and vasopressin from their origin in the hypothalamus to their site of release in the posterior pituitary, or neurohypophysis; 2) structural elements; and 3) blood vessels which extend from the hypothalamus to the anterior pituitary, or adenohypophysis. It has been found that removing the anterior pituitary from direct hypothalamic influence via either stalk section or transplantation to a peripheral site in the body causes gross changes in the anatomy and physiology of the pituitary and its target tissues (Harris, 1955; Everett and Nikitovitch-Winer, 1963). The vital importance of the hypothalamus in controlling anterior pituitary function has been deduced from these types of observations.

The two major influences on anterior pituitary secretion are circulating concentrations of target gland hormones and exteroceptive stimuli (Harris, 1955). In all probability, both influences are mediated through the central nervous system, yet there is no evidence of a direct nerve supply to the secretory cells of the adenohypophysis. This led Harris (1948) to propose the "chemotransmitter hypothesis", which states that neural stimuli can induce the secretion of various hypothalamic factors into the primary capillary plexus of the median eminence. These factors then traverse the hypothalamo-hypophyseal portal vessels to the adenohypophysis where they either stimulate or

inhibit the release of anterior pituitary hormones. Subsequently, hypothalamic extracts have been shown to be effective in altering pituitary secretion both *in vivo* and *in vitro*.

Green and Harris (1949) directly observed the hypothalamo-hypophyseal circulation in living rats. The portal vessels originate in capillary loops in the median eminence, and the blood flows caudally down the infundibulum to the pituitary. Another group of vessels arises from capillary loops located in the stalk itself. The portal vessels comprise the only blood supply to the anterior pituitary in rats. Upon entering the second capillary bed, the blood flows through the parenchyma of the anterior pituitary and then drains into venous sinuses which surround the gland. For a more thorough discussion of the anatomy of the hypophyseal portal system, see Daniel (1966) or Green (1966).

## IV. Control of Prolactin Secretion

### A. Prolactin Inhibitory Factor (PIF)

Prolactin differs from the other five hypophyseal hormones in that it is under tonic inhibitory hypothalamic control (Meites, 1973). There is a great deal of evidence to support this. Everett (1954) was the first to propose the concept. He found that hypophysectomy and autotransplantation of the pituitary under the kidney capsule leads to a marked hypersecretion of prolactin, as determined by the maintenance of luteal function for several months, while the other anterior pituitary hormones all decrease to basal levels. Section of the pituitary stalk, a somewhat less radical procedure

which nevertheless functionally severs the blood vessels connecting the median eminence and adenohypophysis, leads to the same results as does pituitary transplantation (Everett and Nikitovitch-Winer, 1963). More recently, it has been shown that transplantation of the pituitary under the kidney capsule results in elevated serum prolactin concentrations as measured by radioimmunoassay (Chen et al., 1970; Lu and Meites, 1972). Electrolytically-induced lesions in the hypophysiotropic area of the median eminence also lead to increases in serum prolactin and decreases in other pituitary hormones (Chen et al., 1970; Bishop et al., 1971). Finally, it has been shown that rat pituitaries when incubated in vitro will secrete large amounts of prolactin (Meites et al., 1961; Meites and Nicoll, 1966). All of the above indicate that the hypothalamus exerts an inhibitory influence on prolactin secretion. Subsequently, it has been reported both in vitro (Talwalker et al., 1963) and in vivo (Amenomori and Meites, 1970; Watson et al., 1971) that crude acid extracts of rat hypothalami will suppress the secretion of prolactin. It is generally agreed, then, that PIF does exist, but the nature of this substance is the subject of widespread disagreement.

B. Dopamine and PIF

As mentioned above, a controversy currently exists as to the exact mechanism through which the hypothalamus exerts its inhibitory effects on prolactin secretion (Figure 6). One school of thought supports the hypothesis that dopamine, released from tuberoinfundibular neurons into the hypophyseal portal vessels, exerts its effect

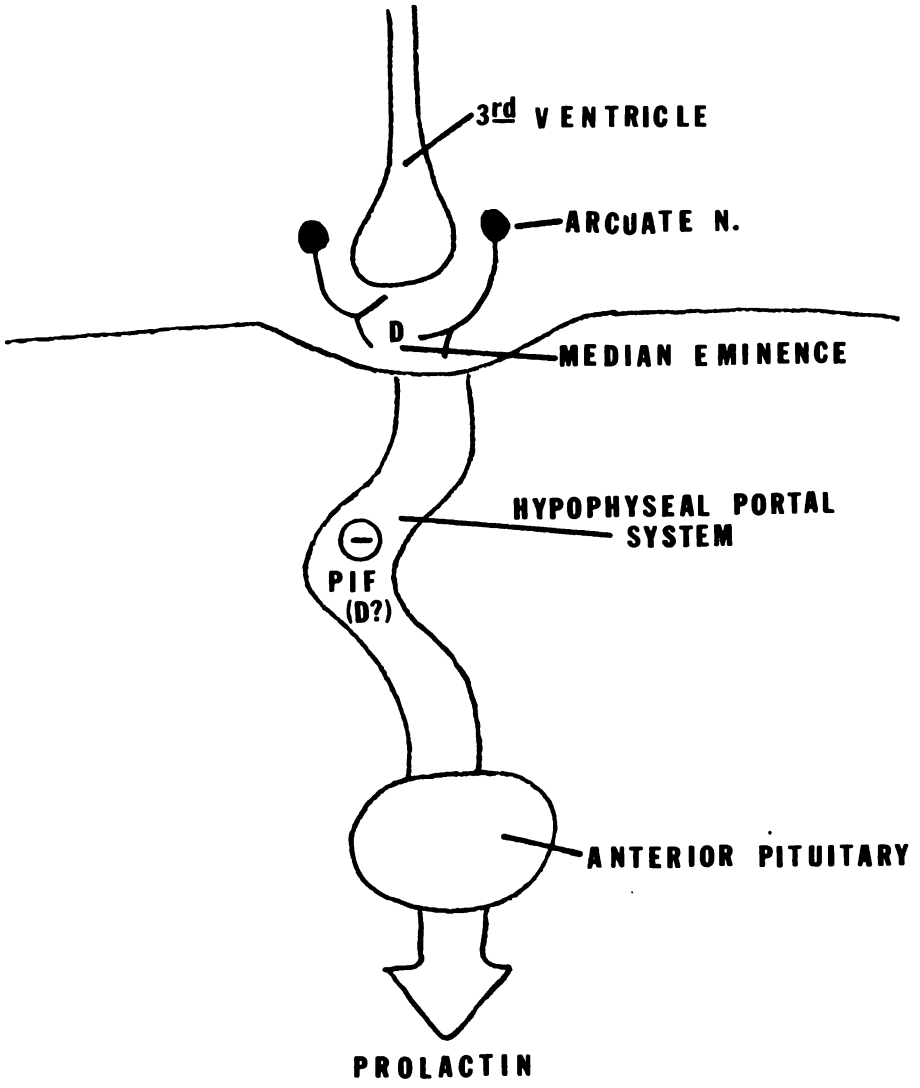


FIGURE 6. Hypothalamic control of prolactin secretion. Abbreviations used: D, dopamine; PIF, prolactin inhibitory factor.

directly on the anterior pituitary, and therefore is the physiologic PIF. The other group postulates the existence of a non-catecholamine PIF that is elaborated upon dopaminergic stimulation by neurosecretory cells in the median eminence. This PIF then enters the hypophyseal portal circulation and is transported to the pituitary where it acts to inhibit the secretion and/or synthesis of prolactin. There are a number of reviews on this subject (Neill, 1974; MacLeod, 1974, 1976).

There is much recent work which demonstrates that dopamine can act directly on rat pituitaries incubated in vitro to inhibit the release of prolactin. Birge et al. (1970) and MacLeod et al. (1970) reported that dopamine inhibits release of prolactin into the incubation medium while increasing pituitary prolactin content. More recently, Shaar and Clemens (1974) showed that dopamine, at concentrations stated to be less than those found in the hypothalamus, can significantly inhibit the in vitro release of prolactin by incubated rat pituitaries. It has also been shown that apomorphine, a direct-acting dopamine agonist (Andén et al., 1967), can inhibit in vitro prolactin release (MacLeod and Lehmeyer, 1974; Smalstig et al., 1974) and that this effect can be reversed by pimozide (Smalstig et al., 1974), a dopamine receptor blocker (Janssen et al., 1968).

Kamberi et al. (1971) observed that doses of dopamine which lowered serum prolactin concentrations when injected into the third ventricle of male rats had no effect when infused directly into the anterior pituitary via the hypophyseal portal vessels. These workers concluded that dopamine exerted its inhibitory effects by stimulating

the release of a non-catecholamine PIF. Kamberi, however, dissolved the dopamine in a saline vehicle. More recently, Takahara et al. (1974) and Schally et al. (1974) demonstrated that when dopamine is dissolved in a 5% glucose solution, it is effective in lowering serum prolactin when infused into the adenohypophysis via the hypophyseal portal system.

The work of Schaar and Clemens (1974) provides further evidence that the PIF activity of hypothalamic extracts can be accounted for by endogenous dopamine. As already mentioned, they demonstrated that very low concentrations of dopamine ($5x10^{-9}M$) can inhibit the in vitro release of prolactin from rat pituitaries. In addition they showed that: 1) if hypothalamic extracts are preincubated with monoamine oxidase they lose their ability to inhibit prolactin release, but when preincubated with both monoamine oxidase and a monoamine oxidase inhibitor simultaneously, the extracts retain their PIF activity; 2) if catecholamines from hypothalamic extracts are adsorbed onto alumina oxide columns, they lose their PIF activity, but the acid eluates from the columns are as active as the untreated hypothalamic extracts in inhibiting prolactin release; 3) preincubation with pepsin has no effect on the ability of the extracts to inhibit prolactin release. The authors note, however, that their in vitro system may not be sensitive enough to detect a peptidergic or other non-catecholamine PIF. Other criticisms of their work include: 1) the fact that monoamine oxidase preincubation may have effects on substances other than catecholamines; 2) that pH conditions necessary for alumina

adsorption of catecholamines are such that non-specific effects, including denaturation of proteins, may occur; and 3) small peptides are not always cleaved by proteolytic enzymes. The authors' data, nevertheless, do provide support for the hypothesis that dopamine itself is PIF. In addition, Donoso et al. (1973) have shown that L-DOPA, a dopamine precursor, is effective in lowering serum prolactin in rats with median eminence lesions, and in rats (Donoso et al., 1974) with anterior pituitary grafts under the kidney capsule.

One major obstacle to acceptance of the dopamine as PIF hypothesis is the inability, so far, of any group to consistently demonstrate the presence of dopamine in portal blood (Ben-Jonathan et al., 1975a,b, 1976). Quijada et al. (1973) have also reported that incubation of pituitaries with either dopamine or hypothalami will inhibit secretion of prolactin by pituitaries into the medium, but that pharmacologic blockade of dopamine receptors by haloperidol leads to a complete reversal of dopamine's inhibitory activity and only a partial antagonism of the inhibitory activity of the hypothalami. The authors conclude that PIF activity is being exerted by one or more hypothalamic factors other than dopamine. Finally, systemic administration in vivo of hypothalamic extracts (Amenomori and Meites, 1970; Watson et al., 1971) is very effective in lowering serum prolactin, while systemic administration of amounts of dopamine much greater than those found in the hypothalamic extracts has no effect at all on prolactin concentrations (Lu et al., 1970). The data cited in this paragraph support the hypothesis of a non-dopamine PIF. At the

present time, then, with so much contradictory evidence, the chemical nature of PIF(s) remains to be elucidated.

C. Other Factors in the Control of Prolactin Secretion

It should be noted here that the control of prolactin secretion is a very complex problem. Many substances other than dopamine have been implicated in this phenomenon. Included are norepinephrine, serotonin, acetylcholine, and gamma-aminobutyric acid. It is generally agreed upon that serotonin stimulates prolactin release, but the effects of the other substances are unclear. Stimulation, inhibition, and no change of serum prolactin concentrations have all been reported. Some of these neurotransmitters are postulated to work through modification of the dopamine-PIF system while others are postulated to exert effects independently of the dopamine-PIF system.

In addition, the existence of a prolactin surge center, located rostral to the medial basal hypothalamus, has been suggested by Neill (1974). Nicoll et al. (1970) observed that hypothalamic extracts had the ability to stimulate in vitro release of prolactin after the initial inhibitory phase. Based on this evidence, he postulated a prolactin releasing factor (PRF) in hypothalamic extracts. Subsequently, Valverde-R et al. (1972) reported the chromatographic separation of a fraction with PRF activity from porcine hypothalami. Lastly, there is little doubt that thyrotropin releasing hormone (TRH) can stimulate prolactin release (Tashjian et al., 1971; Mueller et al., 1973; Chen and Meites, 1975). However, under most physiological conditions thyrotropin and prolactin are not released

together, so it is doubtful if TRH plays a role in the physiologic control of prolactin secretion.

### D. Prolactin-sensitive Negative Feedback Control of Prolactin Secretion

Meites and Clemens (1972) proposed that, as prolactin target tissues do not seem to exert hormonal feedback control on the secretion of prolactin, it is possible that serum concentrations of prolactin itself may serve as the signal in a negative feedback loop. There is much evidence to support this hypothesis. It has been shown that serum and pituitary prolactin concentrations are decreased when prolactin is implanted in the median eminence of rats (Clemens and Meites, 1968; Voogt and Meites, 1971), and that such implants increase hypothalamic PIF activity (Clemens and Meites, 1968). Voogt and Meites (1973) also observed that such implants blocked the proestrous and suckling-induced increase in serum prolactin concentrations. Nicoll (1971) and Voogt and Ganong (1974), however, reported that prolactin has no direct effect on prolactin secretion by pituitaries incubated *in vitro*. This last observation, when combined with the data cited above, leads to the conclusion that the prolactin negative feedback loop is mediated through the central nervous system and thus is a hormonal-neuronal feedback loop.

In all probability, tuberoinfundibular dopamine neurons are involved in the feedback system. Fuxe and his group (Fuxe, *et al.*, 1969a,b; Ahren *et al.*, 1971; Hökfelt and Fuxe, 1972) have generated large amounts of data in support of this hypothesis. Utilizing

histofluorescence techniques, they have essentially demonstrated that dopamine turnover in the median eminence (i.e., tuberoinfundibular dopamine turnover) varies directly with concentrations of circulating prolactin. Thus, during pregnancy and lactation, or after anterior pituitary transplantation or administration of exogenous prolactin, dopamine turnover in tuberoinfundibular neurons is increased. This would have the effect of increasing hypothalamic PIF activity. Likewise, under conditions of low prolactin, turnover of dopamine is depressed. Administration of other pituitary hormones has no effect on the tuberoinfundibular system, and during administration of prolactin, only tuberoinfundibular neurons are stimulated, whereas nigrostriatal and mesolimbic dopamine neurons are not (Hökfelt and Fuxe, 1972; Gudelsky et al., 1977). The prolactin hormonal-neuronal negative feedback loop cannot explain the control of prolactin concentrations under all conditions (e.g., suckling-induced increases), but there is strong evidence that it does play a major role in determining rates of prolactin secretion.

### E. Effects of Dopamine Agonists and Antagonists on Serum Prolactin Concentrations

Whatever the intricacies involved in the physiological control of prolactin secretion, the effects of known dopamine agonists and antagonists on serum concentrations of the hormone are clear. Drugs which stimulate dopamine activity depress prolactin concentrations, and drugs which block dopamine activity cause increases in serum prolactin. Direct-acting agonists such as apomorphine (Andén et

al., 1967) and piribedil (Corrodi et al., 1971) depress serum prolactin in vivo (Smalstig et al., 1974; Lawson and Gala, 1975; Mueller et al., 1976), and apomorphine also decreases prolactin secretion into the medium by pituitary halves incubated in vitro (Smalstig et al., 1974). L-DOPA, which effectively increases the concentration of dopamine in the rat brain (Everett and Borcherding, 1970), is also effective in lowering serum prolactin (Donoso et al., 1971; Lu and Meites, 1971) when administered to rats.

Dopamine receptor blockers, such as pimozide, haloperidol, and the phenothiazines (Janssen et al., 1968) increase serum prolactin upon acute administration in vivo (Lu et al., 1970; Donoso et al., 1971; Clemens et al., 1974; Dickerman et al., 1974; Ojeda et al., 1974; Lawson and Gala, 1975). Pretreatment with these drugs (chlorpromazine and haloperidol) will block the in vivo inhibitory effects of apomorphine and piribedil on prolactin release (Smalstig et al., 1974; Mueller et al., 1976). In vitro, it has been demonstrated that haloperidol and perphenazine can block the inhibitory effects of dopamine and apomorphine on prolactin secretion in a dose-related fashion (Quijada et al., 1973; MacLeod and Lehmeyer, 1974).

Similarly, alpha-methyltyrosine, a tyrosine hydroxylase inhibitor which thus reduces brain dopamine content (Spector et al., 1965), produces a dramatic rise in serum prolactin upon administration to rats (Lu et al., 1970; Donoso et al., 1971). Carr et al. (1975) have reported that the increase in serum prolactin concentration

induced by alpha-methyltyrosine closely parallels the time course of catecholamine synthesis inhibition.

F. Effects of Estrogen on Prolactin Secretion

Nicoll and Meites (1962) demonstrated that estradiol can directly stimulate pituitary release of prolactin in a 3-day organ culture. Ratner et al. (1963) reported that in vivo treatment with estrogen increases in vitro release of prolactin into the incubation medium. More recently, Lu et al. (1971) confirmed the earlier work of Nicoll and Meites using a radioimmunoassay for prolactin. Also, it has been shown that the administration of exogenous estrogen to hypophysectomized rats with pituitary grafts is capable of increasing circulating prolactin concentrations (Chen et al., 1970; Lu et al., 1971). These observations all suggest a direct stimulation by estrogen of the adenohypophysis to secrete prolactin; however, Ratner and Meites (1964) reported that in vivo administration can depress the PIF activity of hypothalami in vitro. This would indicate that estrogen has a centrally mediated stimulatory effect as well.

Estrogens appear to be essential in the physiological control of prolactin concentrations. Ovariectomy leads to a decrease in basal prolactin concentrations which can be reversed by the administration of exogenous estradiol (Chen and Meites, 1970). In addition, the prolactin surge seen in rats on the afternoon of proestrous can be blocked by an injection of antiserum to estrogen on the day before (Neill et al., 1971). Neill postulates a centrally mediated mode of

action for estrogen; he has also presented evidence (Neill, 1970) that estrogens sensitize the prolactin release apparatus to exteroceptive stimuli such as stress.

V. Dopaminergic Supersensitivity

Chronic interruption of impulse traffic along a neuronal tract can lead to the development of postsynaptic supersensitivity to the neurotransmitter involved (see Fleming et al., 1973). Drug-induced supersensitivity in the central nervous system may have many important consequences. Thus, for example, as neuroleptics are thought to exert their antipsychotic effects via blockade of central dopamine receptors, supersensitivity of these receptors may develop. This hypothesis has been offered as an explanation for the phenomenon of tardive dyskinesia, a movement disorder which sometimes affects patients on long-term neuroleptic therapy (Gerlack et al., 1974; Klawans and Rubovits, 1974).

In the laboratory, a number of different treatments have been shown to be capable of inducing dopaminergic supersensitivity. Withdrawal of a chronic diet of alpha-methyltyrosine in mice leads to supersensitivity to the locomotor stimulant effects of d-amphetamine (Dominic and Moore, 1969). Ungerstedt (1971b) reported that 6-hydroxydopamine-induced destruction of nigrostriatal dopamine neurons in rats causes supersensitivity to the behavioral effects of apomorphine. Prolonged treatment of rats with a variety of neuroletpics has also been observed to induce supersensitivity to apomorphine (Gianutsos et al., 1974; Tarsy and Baldessarini, 1974).

Up to this time, the great preponderance of evidence for dopaminergic supersensitivity has been behavioral in nature. Recently, however, electrophysiological evidence for the development of supersensitivity in caudate neurons after chronic impulse interruption has been presented (Siggins et al., 1974; Yarbrough, 1975). It should also be mentioned that Gianutsos et al. (1975) have reported biochemical evidence for supersensitivity of dopamine receptors after chronic treatment with haloperidol.

## GENERAL MATERIALS AND METHODS

### I. Animals and Blood Collection

Mature male Sprague-Dawley rats (Spartan Research Animals, Haslett, Mich.) with weights between 175-250 grams were used in all experiments. They were housed, 4 rats to a cage, in a temperature (24°C) and light (lights on from 5:00 AM to 7:00 PM) controlled environment. Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) and tap water were provided *ad libitum*. Rats were maintained in this environment for at least 24 hours prior to each experiment.

All blood samples were collected from the body trunk following decapitation. The samples were centrifuged, and the serum was stored frozen at -20°C until assayed for prolactin content.

### II. Drugs

The following drugs were used in the experiments described in the *Experimental* section of this thesis: haloperidol (McNeil Laboratories, Fort Washington, Pa.), clozapine (Sandoz, Inc., E. Hanover, N.J.), baclofen (Ciba-Geigy, Inc., Summit, N.J.), estradiol benzoate (ICN Pharmaceuticals, Inc., Cleveland, Ohio), DL-alpha-methyltyrosine methylester HCL (Regis Chemical Co., Inc., Morton Grove, Ill.), piribedil monomethane sulfonate (ET 495, Les Laboratoires Servier, Orleans, France), *d*-amphetamine sulfate (Smith, Kline and French, Inc., Philadelphia, Pa.), methylphenidate HCl (Ciba-Geigy, Inc., Summit, N.J.), and cocaine HCl (Mallinckrodt Chemical Works, Inc., St. Louis, Mo.).

The drug doses are reported as the salts if supplied in that form. All drugs were prepared immediately before administration. The specific drugs, doses, vehicles, and routes of administration used in each experiment are indicated under "Materials and Methods" for that experiment.

III. Radioimmunoassay for Rat Prolactin

Serum prolactin concentrations were measured by the double antibody radioimmunoassay (RIA) method of Niswender et al. (1969). Values are expressed in terms of NIAMDD-rat prolactin-RP-1. Each sample was assayed at three different dilutions and the values obtained were then averaged to determine serum prolactin concentrations.

IV. Methods of Statistical Analysis

All statistical analysis was carried out as described by Sokal and Rohlf (1969). The specific tests used are indicated under "Materials and Methods" for each experiment. In all cases, the level of significance was set at $p<0.05$.

# EXPERIMENTAL

## I. Acute Effects of Haloperidol and Clozapine on Serum Prolactin Concentrations

### A. Objectives

Haloperidol has been shown to be a very effective agent in the treatment of schizophrenia (Byck, 1975). Unfortunately, however, the drug quite commonly produces extrapyramidal signs as a side effect (see Snyder et al., 1974). The extrapyramidal signs are characterized by abnormal involuntary muscle movements, alterations in muscle tone, and disturbances in bodily posture. These side effects are also frequently seen during treatment with the great majority of other neuroleptics. Both antipsychotic activity and the development of extrapyramidal signs are postulated to be due to blockade of central dopaminergic receptors (Hornykiewicz, 1973; also see Snyder et al., 1974, and Meltzer, 1976), and classically, the antipsychotic potency of neuroleptics and their ability to produce extrapyramidal signs are thought to be directly related.

Recently, however, the drug clozapine has been reported to exert antipsychotic activity in man with the production of little or no extrapyramidal signs (Matz et al., 1974). A number of explanations have been proposed to explain this dissociation of antipsychotic activity and motor disturbances, but the problem remains controversial.

The present experiment, then, serves a dual purpose: 1) It has been shown that various antipsychotic agents such as chlorpromazine and haloperidol will increase serum prolactin (Ben-David et al., 1970; Meites and Clemens, 1972; Clemens et al., 1974; Dickerman et al., 1974; Ojeda et al., 1974; Lawson and Gala, 1975). It is generally accepted that these agents exert their effects on prolactin via blockade of tuberoinfundibular dopamine neurons (see MacLeod, 1974). Thus, if clozapine can stimulate increases in serum prolactin, it will provide evidence favoring an anti-dopaminergic mode of action for the drug. 2) Since clozapine has been shown to be a clinically effective antipsychotic drug that does not display the typical behavioral profile of neuroleptic drugs in laboratory animals (Stille et al., 1971), its ability or inability to increase prolactin will help to determine whether the measurement of prolactin concentrations after drug administration will be of value in predicting the clinical effectiveness of new antipsychotic agents.

B. Materials and Methods

Adult male Sprague-Dawley rats weighing 200-225 g received either haloperidol (0.125, 0.25, 0.5 mg/kg, i.p.), clozapine (10, 20, 40 mg/kg, i.p.), or an appropriate vehicle, and were killed by decapitation 1 hour later. Haloperidol was dissolved in 0.3% tartaric acid. Clozapine was dissolved in 1.5% tartaric acid. The mean serum concentration of prolactin from each drug-treatment group was compared to the appropriate vehicle-control using Student's t-test.

C. Results

As depicted in Figure 7, both haloperidol and clozapine produced dose-dependent increases in serum concentrations of prolactin. Haloperidol was at least 160 times more potent than clozapine, minimum effective doses being 0.125 and 20 mg/kg, respectively.

D. Discussion

As previously mentioned, clozapine does not display the same spectrum of behavioral actions as do most other antipsychotic agents (Stille et al., 1971). It is not, for example, cataleptogenic, nor does it antagonize apomorphine- or d-amphetamine-induced stereotypies. Coupled with the ability of clozapine to decrease psychotic symptoms without producing extrapyramidal side effects, the type of behavioral results cited above stimulated a great deal of biochemical investigation into the mechanism of action of the drug. As a result, a number of hypotheses have been offered to explain clozapine's unique pharmacological profile. Basically, two groups can be distinguished. One supports the view that clozapine does block central dopamine receptors, as do the other neuroleptics, but that the drug also exerts potent anticholinergic effects which serve to modify the classic behavioral and biochemical responses expected from a dopamine receptor blocking agent. Evidence for this position includes dopamine turnover studies in which the accumulation of dopamine metabolites was reported to be increased in both nigrostriatal and mesolimbic areas after administration of clozapine (Bartholini et al., 1972; Andén and Stock, 1973; Wilk et al., 1975), and binding studies in which clozapine was shown to have

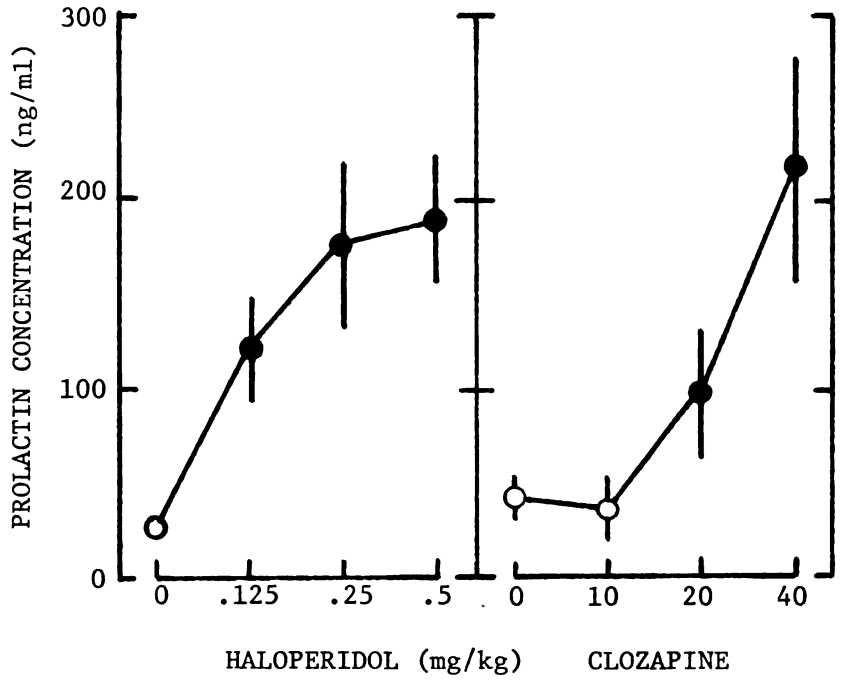


FIGURE 7. Effects of haloperidol and clozapine on serum prolactin concentrations. Rats received either haloperidol (.125, .25, .5 mg/kg, i.p.), clozapine (10, 20, 40 mg/kg, i.p.), or appropriate vehicle (0.3% or 1.5% tartaric acid) and were killed one hour later. Each symbol represents the mean ± S.E. of 8 determinations; where not shown, S.E. is less than radius of symbol. Solid symbols indicate values that are significantly different ($p<0.05$) from vehicle (zero-dose value).

a very high affinity for central cholinergic muscarinic receptors when compared to many other neuroleptic agents (Miller and Hiley, 1974; Snyder et al., 1974).

There is another group of investigators, however, who hold to the position that antipsychotic activity of neuroleptic drugs is not necessarily related to their ability to block dopamine receptors per se. Rather, these drugs may be inhibiting dopamine release (Seeman and Lee, 1975), or their activity may be due to effects on other neurotransmitter systems (e.g., norepinephrine, serotonin, acetylcholine, gamma-aminobutyric acid). Bürki et al. (1975a) observed that increases in dopamine metabolites after clozapine administration are accompanied by increases in dopamine itself. They further noted that in experiments with other known postsynaptic dopamine receptor blockers (e.g., haloperidol), increases in dopamine metabolites have been accompanied by decreased concentrations of the neurotransmitter. The authors therefore concluded that the clozapine-induced increases in brain homovanillic acid and dihydroxyphenylacetic acid concentrations are due to some mechanism other than increased dopamine turnover stimulated by postsynaptic receptor blockade. It is also reported in the study that clozapine stimulates norepinephrine turnover, and that the drug influences serotonin metabolism as well.

Two studies have been done to determine the effect of clozapine on serum prolactin concentrations. Bürki et al. (1975b) reported that clozapine (p.o.) in doses up to 80 mg/kg had no effect on prolactin while other neuroleptics (pimozide and clothiapine) induced

large increases. Meltzer et al. (1975), however, reported increases in prolactin after 5 mg/kg (i.p.) of clozapine that were maximal at 10 mg/kg. He also reported that the rise in serum prolactin induced by 10 mg/kg clozapine was between 4 and 5 times as great as that induced by 0.5 mg/kg (i.p.) haloperidol. The results of the present experiment, although differing from those of Meltzer et al. both in terms of the relative potency and efficacy of haloperidol and clozapine, nevertheless support the same type of conclusion: i.e., that clozapine-induced increases in serum prolactin are due to blockade of tuberoinfundibular dopamine activity, either by blocking postsynaptic dopamine receptors, or by inhibiting dopamine release.

In addition, the present results indicate that measurement of prolactin concentrations after the administration of a drug may indeed be of value in predicting the possible effectiveness of the drug as an antipsychotic agent in man. This is in agreement with the work of Clemens et al. (1974b).

## II. Effects of Baclofen (beta-p-chlorophenyl gamma-aminobutyric acid) on Serum Prolactin Concentrations

### A. Objectives

Gamma-aminobutyric acid has been implicated in the presynaptic inhibition of central dopamine neurons (see Meltzer, 1976). Because of the relative impermeability of gamma-aminobutyric acid through the blood-brain barrier, however, it has been difficult to do in vivo studies which examine the postulated presynaptic effects mentioned above. Baclofen is a structural analog of gamma-aminobutyric

acid which shows central effects (Ahlenius et al., 1975; Fuxe et al., 1975; Naik et al., 1976). There is, in addition, evidence that baclofen can act presynaptically to inhibit the firing of dopamine neurons in the mesolimbic system (Fuxe et al., 1975). The purpose of the present study is to determine what effects, if any, baclofen will exert on the dopamine neurons of the tuberoinfundibular tract. If baclofen does, indeed, inhibit the firing of these neurons, it should increase serum prolactin concentrations.

In addition, it has been reported that baclofen is capable of potentiating the antipsychotic effects of certain neuroleptics in man (Robson, personal communication). A second experiment was designed to determine if baclofen can significantly potentiate the stimulatory effect of haloperidol on prolactin concentrations. A dose of haloperidol which has been shown to be just subthreshold for increasing prolactin (Mueller et al., 1976) was therefore administered 2 hours prior to a range of doses of baclofen which also have been shown to have no effect on prolactin (see "Results") to determine whether the two drugs, acting in concert, can induce an increase in the concentration of the hormone.

B. Materials and Methods

Adult male Sprague-Dawley rats weighing about 200-250 g were used in both experiments. In the first experiment rats received baclofen (5, 10, 20 mg/kg, i.p.) or vehicle (saline) and were decapitated one hour later. In the second experiment, rats received either haloperidol (0.03 mg/kg, s.c.) or vehicle (0.3% tartaric acid) 2 hours

prior to treatment with baclofen (0.1, 0.3, 1.0, 3.0, 10.0 mg/kg, i.p.), or vehicle (saline), such that there was a haloperidol and vehicle pretreated group at each dose of baclofen. Animals were then killed by decapitation one hour after baclofen administration.

In the first experiment, means of each treatment group were compared with the control prolactin value using Student's t-test. In the second experiment, means of haloperidol and vehicle pretreatment groups were compared with appropriate controls using Student's t-test.

C. Results

As summarized in Figure 8, baclofen treatment caused an increase in serum prolactin only at a dose of 20 mg/kg. At 5 and 10 mg/kg, a slight but statistically significant decrease was observed.

When baclofen was administered after 0.03 mg/kg haloperidol pretreatment (Table 1), serum prolactin concentrations did not differ significantly from the haloperidol-pretreated control value. Likewise, in this experiment, baclofen alone, at the doses used, had no effect on circulating prolactin concentrations.

D. Discussion

Although baclofen did increase prolactin at 20 mg/kg, one must be careful in attributing this rise to a specific pharmacologic effect of the drug on tuberoinfundibular neurons; rather, a nonspecific stress-induced increase must be considered, as the rats which received 20 mg/kg are anesthetized and appear to have respiratory difficulties. As it is known that many types of stressful situations will induce rises in serum prolactin (see Neill, 1974), and as it has been demonstrated that baclofen exhibits many of its other postulated dopamine

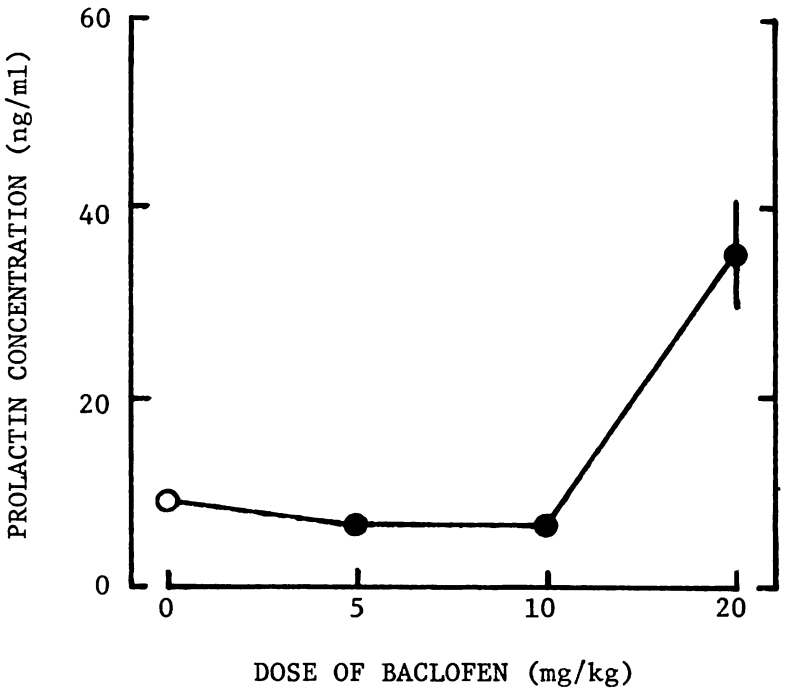


FIGURE 8. Effect of baclofen on serum prolactin concentrations. Rats received baclofen (5, 10, 20 mg/kg, i.p.) or vehicle (saline) and were killed one hour later. Each symbol represents the mean ± S.E. of 8 determinations; where not shown, S.E. is less than radius of symbol. Solid symbols indicate values that are significantly different ($p<0.05$) from vehicle treatment (zero-dose value).

TABLE 1

Effects of haloperidol pretreatment on prolactin response to baclofen

| | PROLACTIN CONCENTRATION (ng/ml) | | | |
|---|---|---|---|---|
| PRETREATMENT | VEHICLE | | HALOPERIDOL | |
| DOSE BACLOFEN (mg/kg) | | N | | N |
| VEHICLE | 11 ± 2 | 8 | 17 ± 3 | 7 |
| 0.1 | 10 ± 1 | 8 | 22 ± 3 | 7 |
| 0.3 | 12 ± 1 | 8 | 14 ± 1 | 8 |
| 1.0 | 11 ± 1 | 8 | 13 ± 1 | 8 |
| 3.0 | 7 ± 1 | 8 | 11 ± 1 | 8 |
| 10.0 | 7 ± 1 | 8 | 13 ± 2 | 8 |

Rats were pretreated with haloperidol (0.03 mg/kg, s.c.) or vehicle (0.3% tartaric acid) two hours prior to treatment with baclofen (0.1, 0.3, 1, 3, 10 mg/kg, i.p.) or vehicle (saline). Rats were killed one hour after baclofen. Numbers represent mean ± S.E. Treatment values were not significantly different ($p<0.05$) from appropriate controls.

inhibitory actions at much lower doses (Ahlenius et al., 1975; Fuxe et al., 1975), stress-induced increases of prolactin are highly probable. This should be tested directly by determining if baclofen increases plasma concentrations of corticosterone.

The decreases in prolactin seen at 5 and 10 mg/kg of baclofen should also be interpreted with caution. Although statistically significant, differences between control and treatment values in both cases were only 3 ng/ml. A difference of this small magnitude approaches the limits of sensitivity of the prolactin radioimmunoassay; and the fact that similar doses of baclofen in the second experiment did not have any significant effect on prolactin values when compared to controls casts doubt on the small decreases observed in the first experiment.

Since a stress-induced increase of prolactin cannot be ruled out in the first experiment, and since baclofen did not appear to potentiate the effects of haloperidol on prolactin concentrations in the second experiment, the evidence at the present time does not support an inhibitory action by baclofen on the firing of tuberoinfundibular neurons. There are two possible explanations for this: 1) If the effects of baclofen are mediated by gamma-aminobutyric acid receptors on dopaminergic neurons, it may well be that there is no gamma-aminobutyric acid-mediated input to the tuberoinfundibular neurons. There is at least some evidence for this (Gudelsky and Moore, 1976), especially as the multisynaptic negative feedback loop for the regulation of dopamine, in which gamma-aminobutyric acid is implicated as a neurotransmitter (see Meltzer, 1976), does not appear to function

in the tuberoinfundibular dopamine system (Gudelsky and Moore, 1976). 2) It is also possible, however, that baclofen simply does not function as a gamma-aminobutyric acid agonist. Curtis et al. (1974), Davies and Watkins (1974), and Naik et al. (1976) have all published evidence to support this position.

## III. Effects of Estrogen Pretreatment on: 1) Serum Prolactin Concentrations, and 2) Serum Prolactin Response to Agents Which Modify Dopamine Activity

### A. Objectives

It has previously been shown that estrogens increase pituitary prolactin secretion both by a direct effect on the adenohypophysis (Nicoll and Meites, 1962) and by a centrally mediated mechanism (Ratner and Meites, 1964). It has also been shown that estrogens are necessary for the maintenance of normal physiologic levels of circulating prolactin in females. Thus, ovariectomy leads to decreases in circulating prolactin, and the administration of exogenous estrogens to ovariectomized females results in increased serum prolactin concentrations (Chen and Meites, 1970).

Another study has demonstrated that estrogens may exert effects on tuberoinfundibular dopamine activity. Fuxe et al. (1969) reported that ovariectomy results in decreased tuberoinfundibular dopamine turnover, and that this effect can be reversed by administering exogenous estrogens. These effects on tuberoinfundibular dopamine activity, however, may be indirectly mediated through the estrogen-induced increase of circulating prolactin, as it has been demonstrated that prolactin itself is capable of stimulating dopamine

turnover in the median eminence (Hökfelt and Fuxe, 1972; Gudelsky et al., 1977).

This series of experiments is designed to examine the estrogen-prolactin relationship, and to determine how estrogen pretreatment modifies the prolactin response to an agent that disrupts tuberoinfundibular neuronal activity by blocking dopamine synthesis (alpha-methyltyrosine) and another that stimulates tuberoinfundibular dopamine receptors (piribedil).

B. Materials and Methods

1) Adult male Sprague-Dawley rats weighing 175-200 g were treated with estradiol benzoate (5, 25 µg/kg, s.c.) daily for 1, 3, or 5 days, the last treatment being 24 hours before sacrifice. Control animals received the corn oil vehicle only for one day 24 hours before sacrifice.

2) Adult male Sprague-Dawley rats weighing 200-325 g were treated with estradiol benzoate (25 µg/kg, s.c.) daily for 1, 3 or 5 days, the last treatment being 24 hours before sacrifice. Control animals received the corn oil vehicle for 1, 3, or 5 days. In each treatment group, half of the rats were given DL-alpha-methyltyrosine methylester HCl (250 mg/kg, i.p.) and the other half saline, one hour before sacrifice.

3) Adult male Sprague-Dawley rats received estradiol benzoate (25 µg/kg, s.c.) daily for 5 days. Control animals received the corn oil vehicle only. Twenty-four hours after the last injection, the

rats were challenged with piribedil monomethane sulfonate (0.3, 1.0, 3.0, 10.0, 30.0, 100.0 mg/kg, s.c.) or saline vehicle one hour before sacrifice.

The effects of varying estradiol benzoate treatment regimens on serum prolactin concentrations, and the effects of estradiol benzoate pretreatment on prolactin response to alpha-methyltyrosine were evaluated as follows. Data were first analyzed by one way analysis of variance. Treatment means were then compared with each other using the Student-Newman-Keuls test. The effects of estradiol pretreatment on the prolactin response to piribedil were analyzed by comparing treatment group means to control values using Student's *t*-test.

C. Results

The effects of various estradiol benzoate treatment regimens on serum prolactin concentrations in male rats are summarized in Table 2. Estradiol benzoate treatment caused increased prolactin concentrations, and a dose of 25 μg/kg for either 3 or 5 days caused a significantly greater prolactin rise than does a dose of 5 μg/kg given for 5 days. Furthermore, it can be seen in Table 3 that prolactin concentrations increased with increasing duration of estradiol benzoate pretreatment, and that although acute treatment with alpha-methyltyrosine also increased prolactin in vehicle-pretreated animals, this alpha-methyltyrosine-induced effect was enhanced in estrogen-pretreated animals. That is, serum prolactin concentrations in rats treated with both estradiol benzoate and alpha-methyltyrosine were significantly greater than in rats treated with either agent alone. Finally, it can

TABLE 2

Effects of estradiol benzoate treatment regimens on serum prolactin concentrations

| | PROLACTIN CONCENTRATION (ng/ml) | | |
|---|---|---|---|
| DOSE OF ESTRADIOL (µg/kg) | VEHICLE | 5 | 25 |
| DAYS OF TREATMENT | | | |
| 1 | 5 ± 1 | 10 ± 2 | 13 ± 2 |
| 3 | | 14 ± 3 | 31 ± 3* |
| 5 | | 1 ± 3 | 35 ± 3* |

Male rats received estradiol benzoate (5, 25 µg/kg, s.c.) daily for one, three, or five days; the last injection was twenty-four hours prior to sacrifice. Control animals received corn oil vehicle for one day. Numbers represent mean ± S.E. of 8 determinations. * indicates that values are greater than all others ($p<0.05$), but are not significantly different from each other.

TABLE 3

Effects of estradiol benzoate and alpha-methyltyrosine (αMT) on serum concentrations of prolactin

| | | PROLACTIN CONCENTRATION (ng/ml) | | | |
|---|---|---|---|---|---|
| PRETREATMENT | | CORN OIL | | ESTRADIOL | |
| TREATMENT | | SALINE | αMT | SALINE | αMT |
| DAYS OF PRETREATMENT | N | | | | |
| 1 | 11 | 9 ± 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, s.c.) or corn oil vehicle for one, three, or five days, the last injection being made twenty-four 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 alpha-methyltyrosine methylester HCl (250 mg/kg, i.p.). Numbers represent mean ± S.E.

be seen in Figure 9 that the smallest dose of piribedil that significantly lowered serum prolactin concentrations was 1.0 mg/kg, and that even a dose of 100 mg/kg did not decrease prolactin concentrations to normal (vehicle pretreated) values.

D. Discussion

Chen and Meites (1970) reported that the maximally effective dose of estradiol benzoate for increasing serum prolactin in ovariectomized females is approximately 25 μ g/kg administered for 5 days. The results obtained in the present experiments (Tables 2, 3) are in general agreement with the earlier observation. Thus, it appears that the estrogen-induced release of prolactin in male rats is similar to that of the female.

The results obtained upon dopamine receptor stimulation and dopamine synthesis blockade after pretreatment with estradiol benzoate may have some important implications concerning the mechanism through which estrogen exerts its effects on both prolactin secretion and dopamine turnover in the median eminence. The present studies show that estrogen priming increases the sensitivity of the prolactin releasing apparatus to dopamine synthesis blockade (Table 3). They also show that estrogen priming renders the animals resistant to the prolactin lowering actions of piribedil (Figure 9) in that: 1) it took a higher dose of drug to depress prolactin than has previously been reported in normal males (Mueller *et al.*, 1976), and 2) even when very high doses of piribedil were administered, prolactin values in estrogen-primed animals did not return to normal male basal levels.

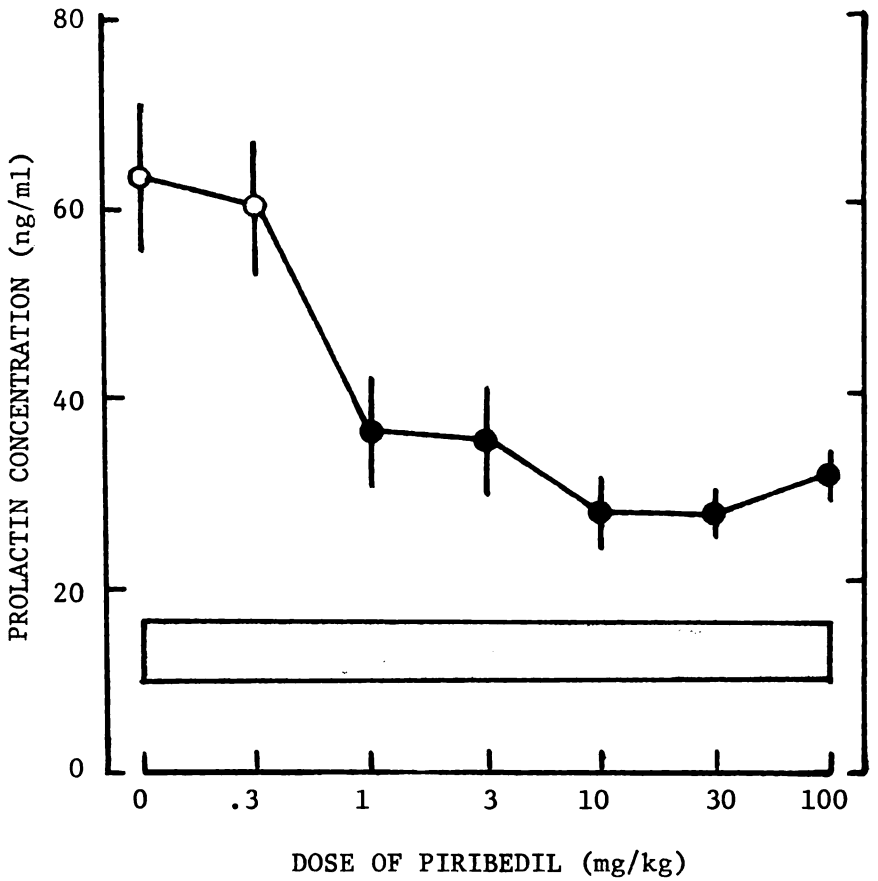


FIGURE 9. Effects of piribedil on serum prolactin after chronic treatment with estradiol benzoate. Male rats received estradiol benzoate (25 μg/kg, s.c.) or corn oil vehicle for 5 days. Twenty-four hours after the last injection, rats received piribedil monomethane sulfonate (0.3, 1, 3, 10, 30, 100 mg/kg, s.c.) or vehicle (water), and were killed one hour later. Symbols represent mean ± S.E. of 8 determinations. Solid symbols indicate values that are significantly different ($p<0.05$) from estradiol benzoate-treated vehicle (zero-dose value). Shaded area represents mean ± S.E. of corn oil-water control value.

It has been shown by Fuxe et al. (1969) that pretreatment of male rats with estrogens increases dopamine turnover in the median eminence as measured by a semiquantitative histochemical technique. This has been recently confirmed using a sensitive quantitative radioenzymatic assay procedure for dopamine (Eikenburg et al., 1977). Furthermore, the administration of estrogen to hypophysectomized rats has been demonstrated to have no effect on tuberoinfundibular dopamine turnover (Eikenburg et al., 1977). Although it has been shown that estradiol is concentrated in certain nerve cells of the arcuate nucleus and anterior pituitary (Stumpf, 1968), Ahrén et al. (1971) have suggested that estrogen itself does not exert any direct action on the uptake or release of amines in dopaminergic nerve terminals. Therefore, as previously mentioned, the ability of estrogen to increase dopamine turnover in the median eminence may be mediated through stimulation of the anterior pituitary to secrete prolactin (Nicoll and Meites, 1962; Lu et al., 1971).

The present results support this hypothesis. Thus, under conditions of estrogen priming alone, the tuberoinfundibular dopamine neurons are stimulated to increase their activity in an effort to reduce the estrogen-induced rise in prolactin concentrations. Despite increased activity, however, the dopamine-PIF system is not completely successful in inhibiting the increased prolactin secretion. The enhanced prolactin secretory response to acute administration of alpha-methyltyrosine, then, probably results from the removal of a damping influence on a system which is primed to secrete its product. Likewise, the administration of even high doses of dopamine agonists (e.g.,

piribedil) would not be expected to alter prolactin concentrations very much in estrogen-pretreated animals, as increased quantities of endogenously released dopamine are already occupying most of the prolactin-inhibitory receptor sites.

## IV. Effect of Chronic Dopamine Receptor Blockade by Haloperidol on Prolactin-Lowering Actions of Piribedil

### A. Objectives

If the transmission of impulse traffic along a neuronal tract is in some way interrupted for a prolonged period of time, postsynaptic supersensitivity to the neurotransmitter involved can develop (see Fleming et al., 1973). This type of phenomenon has been demonstrated in dopamine systems after various types of treatments including: 1) chronic alpha-methyltyrosine, which blocks dopamine synthesis; 2) chronic neuroleptics, such as haloperidol, which block dopamine receptors; and 3) 6-hydroxydopamine, which selectively destroys catecholaminergic nerve terminals (see Moore and Thornburg, 1975). In most demonstrations of dopaminergic supersensitivity, however, behavioral parameters, such as d-amphetamine-induced locomotor activity or apomorphine-induced stereotypy, have been measured. There is very little biochemical evidence for supersensitivity of dopamine receptors after chronic interruption of impulse transmission.

This experiment was undertaken with the intention of determining whether or not supersensitivity to the prolactin-lowering effects of piribedil (Mueller et al., 1976), a direct-acting dopamine agonist (Corrodi et al., 1971), develops after chronic treatment with haloperidol, a dopamine receptor blocker (Janssen, 1967). If supersensitivity does develop, the dose-response curve for piribedil will

show a shift to the left in the neuroleptic-treated animals as compared to animals that receive chronic vehicle treatment (i.e., an identical dose of piribedil will depress prolactin concentrations to a greater extent in animals that have been chronically treated with haloperidol).

A problem encountered when measuring decreases in prolactin concentrations in male rats is that basal hormone levels are already so low that further decreases cannot accurately be determined using the radioimmunoassay. This problem was avoided, however, by pretreating all animals with alpha-methyltyrosine 30 minutes before injecting piribedil. Alpha-methyltyrosine inhibits tyrosine hydroxylase (Spector et al., 1965), and thus it effectively increases circulating prolactin concentrations by decreasing endogenous dopamine without blocking postsynaptic dopamine receptors. Therefore, this pretreatment would not be expected to interfere with the prolactin-lowering actions of piribedil, as these actions are exerted through direct stimulation of the postsynaptic tuberoinfundibular dopamine receptors.

B. Materials and Methods

Male Sprague-Dawley rats weighing 150-175 g received haloperidol (2.5 mg/kg, s.c.) every 12 hours for 7 consecutive days, then haloperidol (5.0 mg/kg, s.c.) every 12 hours for an additional 7 days. A second group of rats received vehicle (0.3% tartaric acid) twice daily for 14 consecutive days. Seventy-two hours after the last injection, rats received DL-alpha-methyltyrosine methylester HCl (250 mg/kg, i.p.) or vehicle (saline) 30 minutes before receiving piribedil monomethane sulfonate (0.1, 0.3, 1.0, 3.0 mg/kg, s.c.) or vehicle

(water). One hour after the last injection, rats were killed by decapitation. At each dose of piribedil, group means of animals which received chronic haloperidol and chronic vehicle were compared using Student's t-test.

C. Results

As depicted in Figure 10, piribedil caused a dose-related reversal of the alpha-methyltyrosine-induced elevation of serum prolactin concentrations. Prior chronic treatment with haloperidol, however, did not alter this response to piribedil in any way.

D. Discussion

As there was no shift to the left in the dose-response curve (Figure 10), it must be concluded that chronic treatment with haloperidol does not induce supersensitivity to the prolactin-lowering effects of piribedil. It appears, then, that in this respect (i.e., the development of supersensitivity after chronic neuroleptic blockade) the dopamine receptors of the tuberoinfundibular system which mediate prolactin secretion differ from the dopamine receptors of the mesolimbic and nigrostriatal systems which mediate locomotor and stereotyped activity.

There is some evidence for this type of difference in the clinical literature. It has been shown that the ability of neuroleptics to induce extrapyramidal side effects decreases after chronic treatment with these agents (Byck, 1975). As extrapyramidal signs are thought to be due to blockade of dopamine receptors in the neostriatum (Hornykiewicz, 1973), tolerance to these side effects can be postulated to be due to the development of supersensitivity in these receptors,

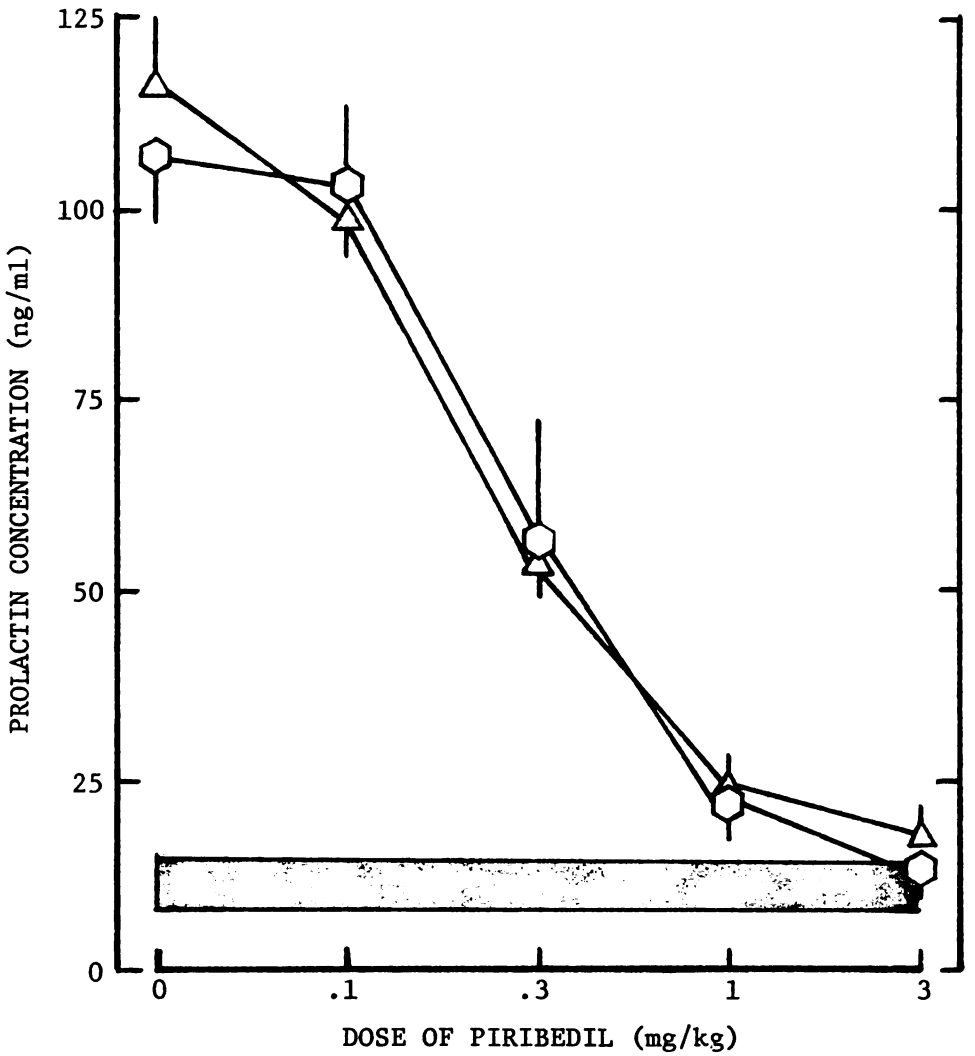


FIGURE 10. Antagonism of alpha-methyltyrosine (αMT)-induced rise in serum prolactin by piribedil in chronic haloperidol and vehicle treated rats. Rats received chronic haloperidol or vehicle (0.3% tartaric acid) as described in text. Seventy-two hours after last injection, rats received αMT methylester HCl (250 mg/kg, i.p.) or vehicle (saline) 30 minutes before receiving piribedil monomethane sulfonate (0.1, 0.3, 1, 3 mg/kg, s.c.) or vehicle (water). Rats were killed one hour after last injection. Symbols represent mean ± S.E. of 8 determinations. Hexagon represents values obtained from chronic vehicle treated rats; triangle represents values obtained from chronic haloperidol treated rats. Prolactin concentrations of animals treated chronically with vehicle (9±1) or haloperidol (12±2) and then with appropriate vehicles were not significantly different ($p<0.05$). Thus, these values were combined and mean ± S.E. represented by shaded area.

such that the few receptors that are not blocked by the drug are capable of transmitting an increased amount of impulse traffic. Meltzer and Fang (1976), however, have shown that serum prolactin concentrations are elevated in patients receiving neuroleptic therapy even after 3 months of continuous treatment. This would indicate that tolerance does not develop to the prolactin-increasing actions of neuroleptics, and by analogy, that tuberoinfundibular dopamine receptors are not capable of modifying their sensitivity to the prolactin-lowering effects of dopamine in order to normalize prolactin levels.

## V. Effects of Indirect-Acting Dopamine Agonists on Serum Prolactin Concentrations

### A. Objectives

Methylphenidate, d-amphetamine, and cocaine have all been shown to exert central psychomotor stimulant actions, such as the induction of locomotor activity and stereotypy in the rat (Scheel-Krüger, 1972; Von Voigtlander and Moore, 1973). Biochemical evidence indicates that these agents have effects on a number of neurotransmitters in the brain (see Costa and Garattini, 1970), but it appears that the behavioral actions mentioned above are due to stimulation of dopaminergic transmission (Moore and Thornburg, 1973). Furthermore, it is widely recognized that these drugs exert their effects by facilitating the release, and/or blocking the reuptake of neurotransmitter at catecholaminergic nerve terminals (Van Rossum et al., 1962; Ross and Renyi, 1967; Moore, 1971; Creese and Iversen, 1975). Thus, methylphenidate, d-amphetamine, and cocaine can all be characterized as indirect-acting dopamine agonists.

Many, although not all, of the central actions of d-amphetamine can be blocked by prior treatment with alpha-methyltyrosine (Weissman et al., 1966), but this pretreatment does not block the stimulant effects of methylphenidate or cocaine (Scheel-Krüger, 1971, 1972). On the other hand, d-amphetamine-induced psychomotor stimulation is not blocked by reserpine (Van Rossum, 1962), an agent that depletes the brain of catecholamines and serotonin by disrupting neuronal storage vesicles (Moore, 1971). Reserpine does, however, inhibit methylphenidate- and cocaine-induced locomotor activity and stereotypy (Van Rossum, 1962; Scheel-Krüger, 1971). The difference in sensitivity to alpha-methyltyrosine and reserpine has been explained by postulating two separate pools of intraneuronal dopamine (Glowinski, 1973), such that d-amphetamine induces the release of a newly synthesized, "functional" pool of dopamine while methylphenidate stimulates the release of a reserpine-sensitive, "storage" pool of the neurotransmitter (Scheel-Krüger, 1971; Chiueh and Moore, 1975a,b). Although cocaine, a reuptake inhibitor (Heikkila et al., 1975), does not really fit this model, it nevertheless appears to preferentially affect the reserpine-sensitive pool (Van Rossum, 1962).

The objectives of this series of experiments are to determine the effects of methylphenidate, d-amphetamine, and cocaine on serum prolactin concentrations, and to determine whether or not alpha-methyltyrosine pretreatment will alter the prolactin response to the drugs. Since these 3 agents all exhibit the properties of indirect-acting dopamine agonists, it is expected that they will depress prolactin

concentrations when administered alone; and according to the model presented in the preceding paragraph, it is further expected that alpha-methyltyrosine pretreatment will block the prolactin-lowering actions of d-amphetamine, but not those of methylphenidate and cocaine.

B. Materials and Methods

Adult male Sprague-Dawley rats weighing 175-250 g received d-amphetamine sulfate (0.5, 1, 2, 4, 8 mg/kg, s.c.), methylphenidate HCl (1, 3, 10, 30 mg/kg, s.c.), or the appropriate vehicle and were killed by decapitation one hour later. Another group of rats received cocaine HCl (5, 10, 20, 40 mg/ml, i.p.) or vehicle and were killed by decapitation 30 minutes later. In a second series of experiments, rats received DL-alpha-methyltyrosine methylester HCl (250 mg/kg, i.p.) or vehicle 30 minutes prior to receiving d-amphetamine sulfate (0.1, 0.3, 1, 3, 10 mg/kg, s.c.), methylphenidate HCl (0.3, 1, 3, 10, 30 mg/kg, s.c.), cocaine HCl (5, 10, 20, 40 mg/kg, i.p.), or appropriate vehicle. Rats were killed by decapitation one hour after receiving d-amphetamine and methylphenidate, and 30 minutes after receiving cocaine. d-Amphetamine, methylphenidate, and cocaine were dissolved in water. Alpha-methyltyrosine was dissolved in saline. The mean serum prolactin concentration from each drug-treatment group was compared to the appropriate vehicle-control using Student's t-test.

C. Results

The results of these experiments are depicted in Figures 11 and 12. When administered alone, d-amphetamine (Figure 11) caused a dose related decrease in prolactin concentrations, with significant

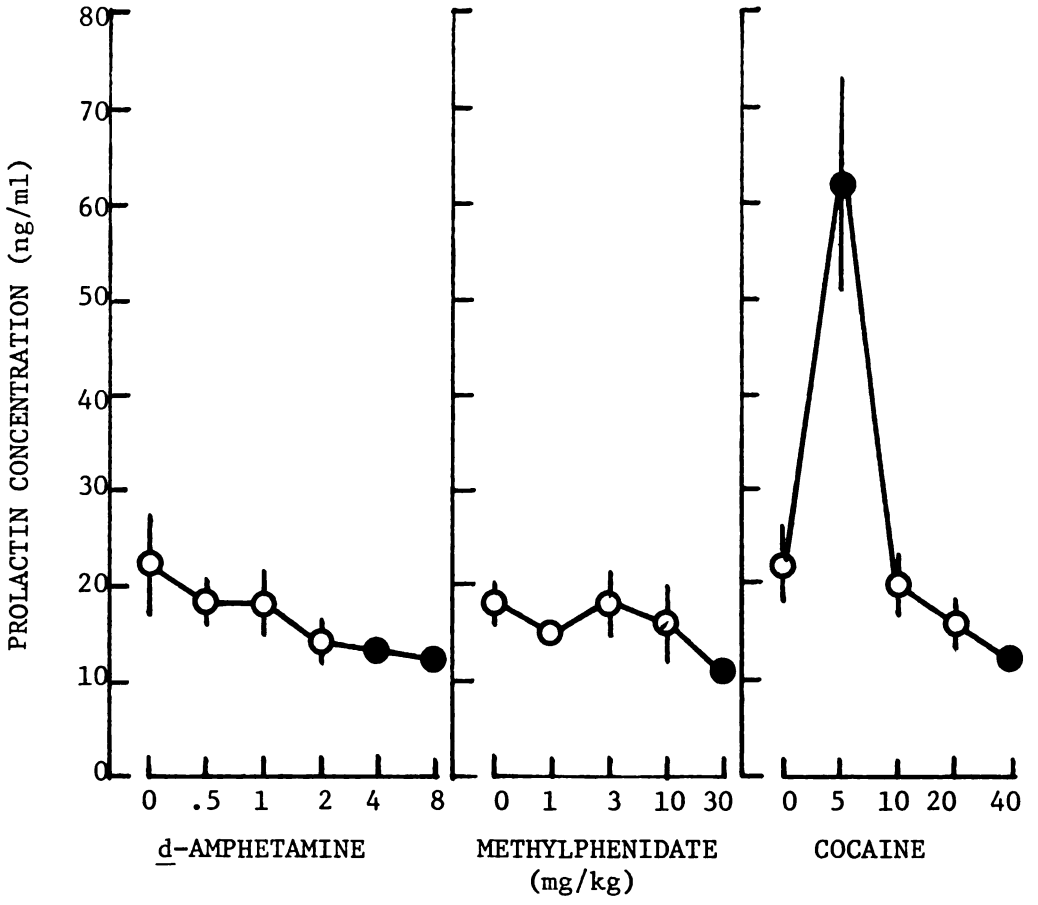


FIGURE 11. Effects of d-amphetamine, methylphenidate, and cocaine on serum prolactin concentrations. Male rats received d-amphetamine sulfate (.5, 1, 2, 4, 8 mg/kg, s.c.), methylphenidate HCl (1, 3, 10, 30 mg/kg, s.c.), or vehicle (water), and were sacrificed one hour later. Another group of rats received cocaine HCl (5, 10, 20, 40 mg/kg, i.p.) or vehicle (water) and were sacrificed 30 minutes later. Symbols represent mean ± S.E. of 8 determinations; where not shown, S.E. is less than radius of symbol. Solid symbols indicate values that are significantly different ($p<0.05$) from appropriate vehicle (zero-dose value).

effects occurring at 4 and 8 mg/kg; methylphenidate (Figure 11) had no effect except at the highest dose (30 mg/kg), which caused a decrease; and cocaine (Figure 11) exhibited a biphasic action, the lowest dose (5 mg/kg) increasing, and the highest dose (40 mg/kg) decreasing serum prolactin values. After alpha-methyltyrosine pretreatment, d-amphetamine (Figure 12) induced a slight, but significant, decrease in prolactin at all doses administered. The decrease did not, however, appear to be dose-dependent. Methylphenidate (Figure 12) did cause a dose-dependent decrease in serum prolactin after alpha-methyltyrosine pretreatment. The minimal effective dose was 3 mg/kg. Cocaine (Figure 12), when administered after alpha-methyltyrosine, significantly depressed prolactin values at 40 mg/kg but had no effect at lower doses. None of the drugs, when administered after alpha-methyltyrosine pretreatment, were able to lower prolactin to vehicle-pretreated control values.

D. Discussion

Although all three drugs, when administered in sufficiently high doses, were able to lower serum prolactin concentrations, the decreases observed were much smaller than those induced by direct-acting dopamine agonists such as apomorphine or piribedil (Mueller et al., 1976). The fact that tuberoinfundibular neurons tonically inhibit prolactin release (MacLeod, 1974), and the fact that this inhibition is dependent upon the continued synthesis and release of dopamine (Carr et al., 1975), may provide an explanation for the difference in efficacy between direct- and indirect-acting agonists. If one postulates a very large "functional" dopamine pool in tuberoinfundibular neurons that is essentially being released as fast as it is synthesized, then there

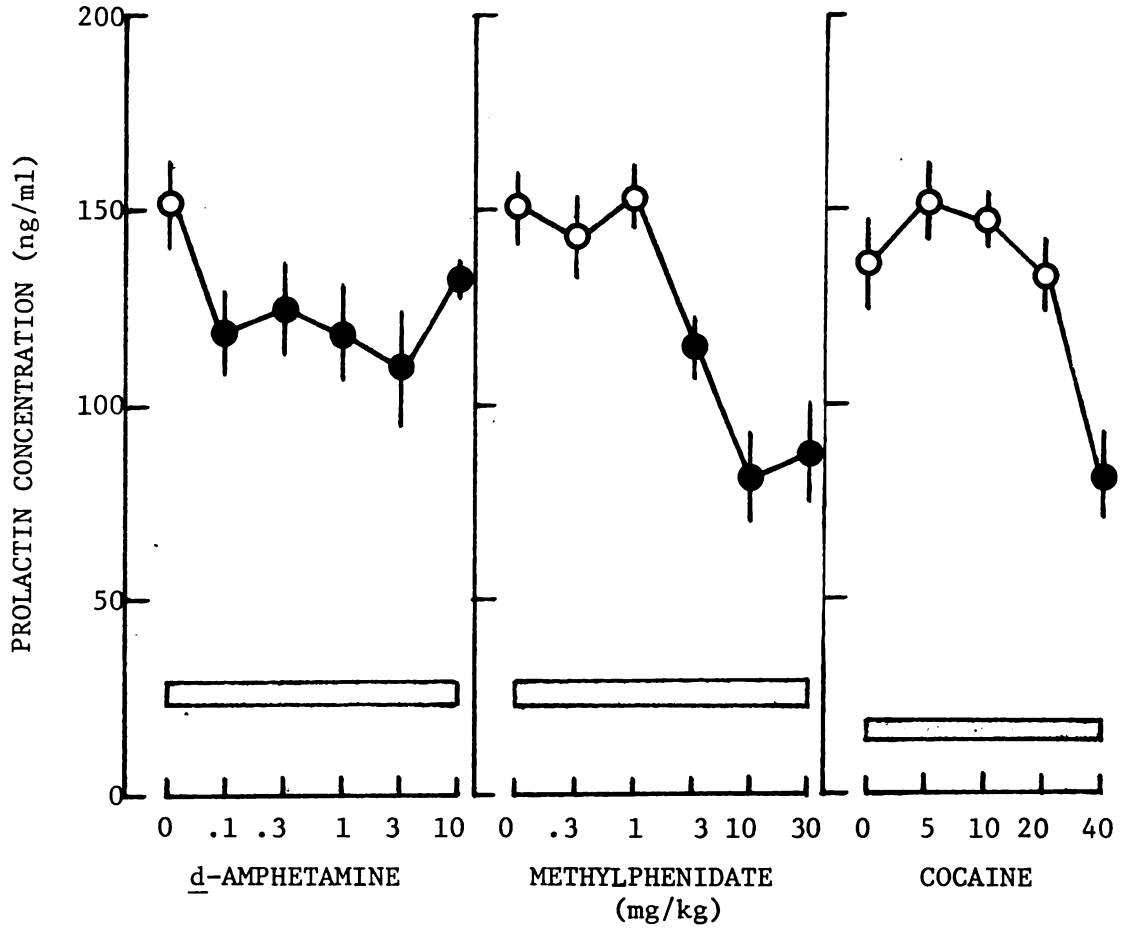


FIGURE 12. Effects of d-amphetamine, methylphenidate, and cocaine on serum prolactin concentrations after pretreatment with alpha-methyltyrosine (αMT). Rats received αMT methyl-ester HCl (250 mg/kg, i.p.) or vehicle (saline) 30 minutes prior to d-amphetamine sulfate (.1, .3, 1, 3, 10 mg/kg, s.c.), methylphenidate HCl (.3, 1, 3, 10, 30 mg/kg, s.c.), cocaine HCl (5, 10, 20, 40 mg/kg, i.p.), or appropriate vehicle (water). Rats were killed one hour after d-amphetamine and methylphenidate, and thirty minutes after cocaine. Symbols represent mean ± S.E. of 8 determinations. Solid symbols indicate values significantly different ($p<0.05$) than αMT-pretreated controls (zero-dose values). Shaded area indicates mean ± S.E. of values obtained from vehicle (saline)-pretreated controls.

would only be small amounts of intraneuronal "storage" dopamine susceptible to the actions of the indirect agonists. As such, the release of this dopamine would only slightly increase the concentration of neurotransmitter at postsynaptic receptor sites. And thus, there would only be a slight increase in receptor stimulation. Direct agonists, on the other hand, depending on the dose administered would act to dramatically increase postsynaptic receptor stimulation since these agents themselves can react with the receptors.

The same type of comparison can be made concerning the prolactin response to direct- and indirect-acting dopamine agonists after treatment with alpha-methyltyrosine. d-Amphetamine did not cause a dose-related decrease, and therefore, it can be hypothesized that its effects on prolactin under these conditions are not specifically mediated through stimulation of tuberoinfundibular neuronal activity. This is, of course, consistent with prior observations that the dopaminergic stimulation induced by d-amphetamine is sensitive to blockade by alpha-methyltyrosine (Weissman et al., 1966). Such pretreatment did not block the effects of methylphenidate or cocaine, and this, once again, is consistent with previous reports (Scheel-Krüger, 1971, 1972). These agents, at high doses, depressed prolactin (Figure 12), but the depression of hormone concentrations induced by piribedil after alpha-methyltyrosine was of much greater magnitude (Figure 10). Once again, the indirect agonists may have had only a minimal effect on postsynaptic dopamine concentrations due to the small amount of intraneuronal dopamine available for release, while piribedil, via direct interaction

with the receptors involved, would much more effectively increase the percentage of receptors stimulated.

The hypothesis presented above, however, does not account for all of the data obtained. The effects of cocaine, supposedly due to blockade of reuptake (Heikkila et al., 1975), do not fit the model, and even if a dopamine-releasing effect is postulated, the increase in prolactin at 5 mg/kg remains unexplained. Not only are neurotransmitters other than dopamine involved in the control of serum prolactin levels, but afferent input from brain centers other than the hypothalamus appear to play a role (Neill, 1974). It may well be that the drugs used in this series of experiments are exerting effects unrelated to actions on hypothalamic dopamine receptors. If this is the case, then unknown nonspecific factors may be modifying the prolactin response to specific tuberoinfundibular actions of d-amphetamine, methylphenidate, and cocaine.

The experiments described in this section are only preliminary studies. Much more work must be done to correlate changes in prolactin concentrations induced by various doses of these agents with changes in neurochemistry and neurotransmitter activity before any valid conclusions can be drawn concerning the relationship between these drugs, tuberoinfundibular dopamine, and the control of prolactin secretion.

# GENERAL DISCUSSION

Although control of prolactin secretion is a highly complex process, there is general agreement that dopamine plays a major inhibitory role (MacLeod, 1976). Furthermore, it has been shown that the dopamine involved in this inhibition is contained in tuberoinfundibular neurons (Fuxe et al., 1969a,b; Ungerstedt, 1971a; Carr et al., 1975). Therefore, examination of the prolactin response to various physiological and pharmacological stimuli may prove to be an easily accessible, quantitative measure of activity in a central dopaminergic system. This would, of course, have beneficial implications for the development of effective therapies for certain psychological and neurological disorders (e.g., schizophrenia and Parkinsonism), the etiologies of which appear to involve abnormalities of dopaminergic transmission in the central nervous system. At the present time, however, much work still needs to be done to define the pharmacological responses and receptor characteristics of the tuberoinfundibular dopamine system before direct extrapolations from this system to other central dopamine systems can be made. It was the purpose of the studies presented in this thesis to better define the nature of the tuberoinfundibular dopamine system and to better characterize the relationship between tuberoinfundibular activity and serum prolactin concentrations.

Clemens et al. (1974) and Meltzer and Fang (1976) have advocated the measurement of the effects of drugs on serum prolactin concentrations as a method of screening drugs for possible antipsychotic activity. The data in this thesis show that when two drugs with known antipsychotic activity, haloperidol and clozapine, were tested, they both increased serum prolactin as expected. Other, more classical behavioral tests (e.g. cataleptogenesis, blockade of circling, etc.) used to predict the antipsychotic activity of drugs have not predicted clozapine's clinical effectiveness (Costall and Naylor, 1975). Thus, the present data lends support to the contention of Clemens et al. (1974) that drugs with antipsychotic activity increase serum prolactin. Likewise, the studies with direct- and indirect-acting dopamine agonists show the prolactin response to be a relatively accurate reflection of central dopaminergic activity.

On the other hand, much of the data obtained shows the prolactin response to certain pharmacological treatments to differ from what might be expected on the basis of results obtained in studies of mesolimbic and nigrostriatal dopamine systems. Baclofen, an agent reported to depress dopamine neuronal activity in both mesolimbic and nigrostriatal neurons (Fuxe et al., 1975), had no effect on prolactin concentrations at doses reported to inhibit neuronal firing. In addition, estrogen treatment was shown to increase prolactin concentrations, to sensitize the prolactin release apparatus to alpha-methyltyrosine stimulation, and to desensitize the apparatus to piribedil inhibition. Hökfelt and Fuxe (1972) and Gudelsky et al. (1977) have shown that

estrogen preferentially affects tuberoinfundibular turnover without influencing mesolimbic or nigrostriatal systems. This type of work, plus the baclofen and estrogen data presented in this thesis, indicate that the feedback mechanism controlling tuberoinfundibular dopamine activity differs from that postulated for other dopaminergic systems.

Furthermore, evidence was presented that supersensitivity to receptor stimulation, a phenomenon that commonly develops in other dopamine systems after chronic treatment with neuroleptics (Moore and Thornburg, 1975), does not occur in the tuberoinfundibular system. Thus, animals that received chronic haloperidol did not exhibit an enhanced response to the prolactin-lowering effects of piribedil when compared to animals that did not receive the neuroleptic. This leads to the conclusion that tuberoinfundibular dopamine receptors differ from other dopamine receptors in the brain. Actually, this might be expected, as tuberoinfundibular dopamine receptors are postulated to be located on either neurosecretory cells in the median eminence or else on pituitary lactotrophs (MacLeod, 1976), while most other central dopamine receptors are probably located on other neurons (Moore, 1971).

The data presented in this thesis, then, show that while the tuberoinfundibular dopamine system does share certain characteristics with the more extensively studied nigrostriatal and mesolimbic systems, many differences appear to exist as well. All of the results were obtained, however, by measuring the prolactin response to various pharmacologic treatments. Whether the discrepancies between expected and observed results are due to actual differences in neuronal and

receptor characteristics between tuberoinfundibular and other dopaminergic systems, or whether the discrepancies are due to modulation of prolactin release by other non-dopaminergic influences, remains to be elucidated.

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