BIOLOGY OF REPRODUCTION 36, 47–58 (1987)

# Interactions between Prolactin and Dopaminergic Neurons[1]

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## ABSTRACT

*The secretion of prolactin from the adenohypophysis is tonically inhibited by dopamine that is released into the hypophysial portal blood from terminals of tuberoinfundibular neurons located in the external layer of the median eminence. These tuberoinfundibular neurons are unique among other dopaminergic neurons in the brain (including the well-characterized nigrostriatal neurons) in that they are not directly regulated by dopaminergic receptor-mediated mechanisms, but instead are selectively responsive to changes in prolactin concentrations in blood and cerebrospinal fluid. In the rat, the activity of the tuberoinfundibular dopaminergic neurons 1) is higher in the female than in the male, 2) exhibits a characteristic cyclical pattern during the first half of pregnancy and is constantly high as a result of stimulation by placental lactogen during the last 9 days of pregnancy, and 3) is reduced in lactating animals and acutely inhibited during suckling.*

## INTRODUCTION

The secretion of adenohypophysial hormones is regulated by chemicals (releasing and inhibiting factors or hormones) that are released from neurosecretory neurons in the mediobasal hypothalamus and transported to the adenohypophysis via the hypophysial portal circulation. Release of these hypothalamic regulatory factors is under control of both hormonal and neuronal influences, that is, by the feedback actions of the adenohypophysial hormones or their target cell hormones, and by stimuli carried via afferent neuronal circuits that project to the hypothalamic neurosecretory cells. This review will consider the regulation of one adenohypophysial hormone, prolactin, by one inhibitory factor, dopamine (DA). The DA that inhibits prolactin secretion from the adenohypophysis is released from tuberoinfundibular neurosecretory neurons located in the mediobasal hypothalamus. Attention will focus on the regulation of these hypothalamic DA neurons by prolactin and on the changes in the activity of these neurons during different endocrine states.

## ANATOMICAL DISTRIBUTION OF DOPAMINERGIC NEURONS

The mammalian brain contains a number of anatomically distinct catecholaminergic neurons (Moore and Bloom, 1978, 1979). Their perikarya have been identified in the rat brain by a numbering system suggested by Dahlström and Fuxe (1964). As illustrated in Figure 1 (top), the caudal catecholaminergic nerve cells ($A_{1-7}$) in the pons-medulla contain norepinephrine, while the more rostral DA perikarya extend from the mesencephalon ($A_{8-10}$) to the hypothalamus ($A_{11-14}$) and olfactory bulb ($A_{16}$). DA perikarya located in pars compacta of substantia nigra ($A_{8-9}$) and in the ventral mesencephalon ($A_{10}$) give rise to the major ascending DA neuronal systems that terminate in the caudate/putamen (striatum), nucleus accumbens, olfactory tubercle, septum and several regions of the cerebral cortex. Collectively, these neurons are referred to as the mesotelencephalic system; one division of these neurons comprises the nigrostriatal DA system. These DA neurons have cell bodies in substantia nigra ($A_{8-9}$) and terminals in the striatum (Fig. 1, bottom); as part of the extrapyramidal system, they are involved with the regulation of motor functions. Nigrostriatal neurons constitute the largest group of DA neurons in the brain; consequently, they are the easiest to study. Therefore, most of what is known

[1] These studies were supported by USPHS grant NS09174.
[2] Reprint requests.

about DA neurons has been learned from studies on this system. It is, however, misleading to consider nigrostriatal DA neurons as models for other DA neurons; but in the present review, they will serve as a frame of reference for comparison with hypothalamic DA neurons.

There are 4 separate groups of DA perikarya located within the hypothalamus ($A_{11-14}$; Fig. 1, top); but, with exception of the $A_{12}$ group, the axonal projections from these neurons are not well defined. Axons from cell bodies of the $A_{12}$ group located in the rostral arcuate nucleus project through the median eminence, down the infundibular stalk, and terminate in the neural and intermediate lobes of the hypophysis; these neurons constitute the tuberohypophysial DA system (Fig. 2). Short axons of DA perikarya located in the periventricular and more caudal regions of the arcuate nucleus project to the external layer of the median eminence, where they terminate near perivascular spaces of the primary capillary loops of the hypophysial portal system; these are the tuberoinfundibular DA neurons. DA released from terminals of these neurons is transported to the adenohypophysis where it activates receptors on lactotrophs and thereby inhibits the release of prolactin. In turn, it is these tuberoinfundibular DA neurons that are selectively activated by prolactin. In the following sections, the properties of these tuberoinfundibular DA neurons will be compared with the "classical" DA neurons that constitute the nigrostriatal system. To study the mechanisms by which different DA neurons are regulated, one must be able to measure their activities. Methods for doing this are described in the next section.

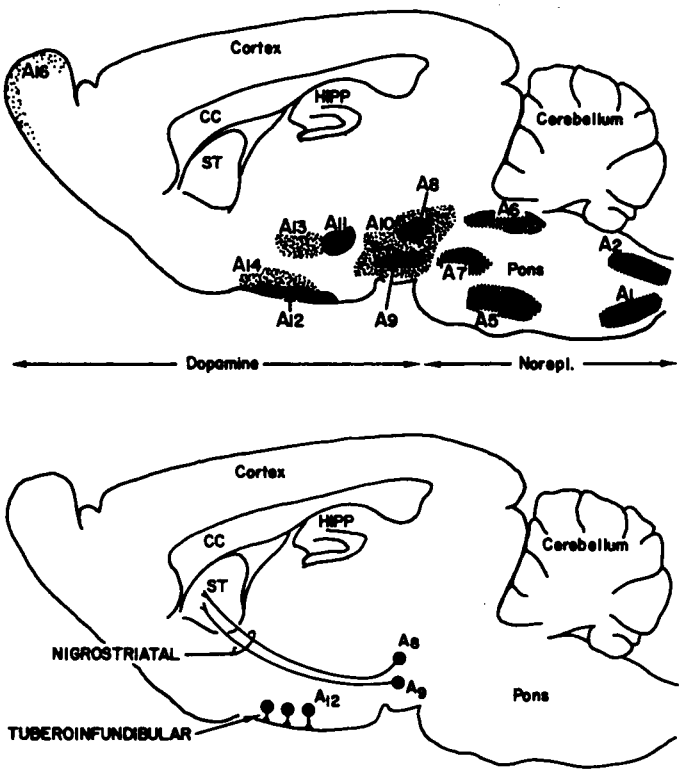


FIG. 1. Schematic depiction of sagittal sections of the rat brain. Shown are the location of noradrenergic ($A_{1-7}$) and dopaminergic ($A_{8-16}$) perikarya (*top*) (modified from Hökfelt et al., 1984), and the locations of nigrostriatal and tuberoinfundibular dopaminergic neurons (*bottom*). *CC*, corpus callosum; *ST*, striatum; *HIPP*, hippocampus.

## ESTIMATIONS OF DOPAMINERGIC NEURONAL ACTIVITY

The activity (impulse traffic) of nigrostriatal DA neurons has been monitored by both electrophysiological and biochemical techniques. It has been relatively easy to record electrical unit activity from the densely packed nigrostriatal DA neurons in the substantia nigra (Bunney, 1979). Unfortunately, DA perikarya of tuberoinfundibular DA neurons are diffusely distributed within the periventricular and arcuate nuclei and, to date, it has not been possible to electrically record unit activity from confirmed DA cell bodies in these regions. Instead, investigators have had to rely on biochemical techniques to estimate impulse activity in tuberoinfundibular DA neurons. To understand the neurochemical techniques that are employed to estimate DA neuronal activity, it will be useful to review the dynamics of DA synthesis, storage, release and metabolism in the nerve terminals. The following sections describe what is known about these events as they occur at the terminals of nigrostriatal DA neurons; these are schematically depicted in Figure 3 (top).

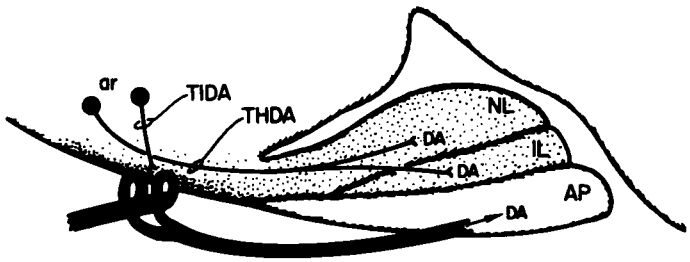


FIG. 2. Schematic depiction of projections of $A_{12}$ dopaminergic neurons in the rat brain. *TI*, tuberoinfundibular; *TH*, tuberohypophysial; *NL*, neural lobe; *IL*, intermediate lobe; *AP*, anterior lobe of the pituitary; *ar*, arcuate nucleus.

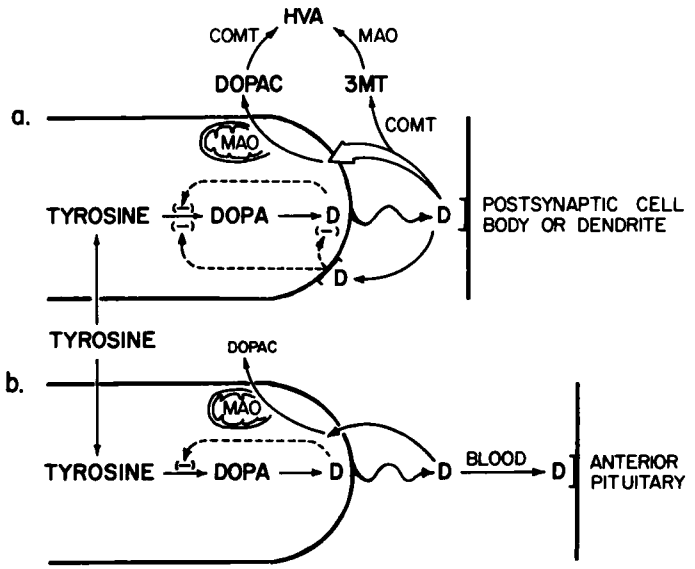


FIG. 3. Schematic diagram of a) nigrostriatal (*top*) and b) tuberoinfundibular (*bottom*) dopaminergic nerve terminals. Abbreviations: *COMT*, catechol-O-methyltransferase; *D*, dopamine; *DOPAC*, dihydroxyphenylacetic acid; *HVA*, homovanillic acid; *MAO*, monoamine oxidase; *3MT* 3-methoxytyramine.

Tyrosine is transported into the neuron where it is converted to L-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase, the enzyme that catalyzes this rate-limiting step in the synthesis of DA. DOPA, in turn, is rapidly decarboxylated to DA by aromatic L-amino acid decarboxylase. The newly synthesized DA can then be scavenged by intraneuronal monoamine oxidase (MAO), stored in synaptic vesicles, or released from the nerve terminal in response to the arrival of action potentials. Steady-state concentrations of DA in the nigrostriatal nerve terminal, which primarily reflect the amount of amine stored in synaptic vesicles, remain fairly constant despite alterations in the amount of DA released. This is due, in part, to end product regulation of tyrosine hydroxylase. That is, if the concentration of DA in some undefined pool within the neuron increases, the activity of tyrosine hydroxylase, and consequently the rate of DA synthesis, is reduced. On the other hand, when the concentration of DA in the same pool declines, for example, as a result of increased neuronal activity and transmitter release, tyrosine hydroxylase increases. Consequently, DA concentrations in the nerve terminal remain fairly constant despite marked alterations in the amount of transmitter released. Therefore, changes in DA neuronal activity are not reflected in changes in DA concentrations.

DA that is released from the nigrostriatal nerve terminal can diffuse across the synaptic cleft and activate receptors on membranes of dendrites or cell bodies of postsynaptic neurons. The released DA also can activate receptors located on the presynaptic nerve terminal. Activation of these presynaptic receptors (autoreceptors) is believed to inhibit the synthesis and release of DA. Thus, synaptic concentrations of DA appear to modulate DA synthesis and release via this autoreceptor-mediated "short loop" feedback mechanism.

DA is removed from the synaptic cleft, and thus from the regions of pre- and postsynaptic receptors, by a high-affinity uptake mechanism that transports the amine back into the nerve terminal where it can reenter the synaptic vesicle and subsequently be released, or it can be converted by mitochondrial MAO to dihydroxyphenylacetic acid (DOPAC). This metabolite then diffuses from the neuron, and some is converted to homovanilic acid (HVA) by catechol-O-methyltransferase (COMT). Both of these metabolites are removed from the brain by a probenecid-sensitive acid transport system. A small amount of DA that is not recaptured by the presynaptic nerve terminal can be converted to COMT to 3-methoxytyramine (3MT). Changes in the concentrations of all three metabolites in the striatum have been used as indices of nigrostriatal DA neuronal activity (Roth et al., 1976; Wood et al., 1982).

A similar series of biochemical events also occur at the terminals of tuberoinfundibular DA neurons in the external layer of the median eminence (Fig. 3, bottom). There are, however, some important differences in the events occurring at the terminals of nigrostriatal and tuberoinfundibular DA neurons (compare top and bottom of Fig. 3). First, tuberoinfundibular DA neurons do not form synapses but release DA directly into the perivascular spaces of the primary plexus of the hypothalamic-hypophysial portal system. Second, the amine-uptake system in terminals of tuberoinfundibular DA neurons has a lower affinity than nigrostriatal terminals for DA (Demarest and Moore, 1979a). Because of this and the fact that DA released from tuberoinfundibular DA terminals is quickly transported away by the blood, there is less likelihood that the released DA is recaptured by the nerve terminal and converted to DOPAC. This is probably why the median eminence contains relatively less DOPAC than does the striatum (Umezu and Moore, 1979). Third, tuberoinfun-

dibular DA neurons are unresponsive to the direct actions of DA agonists and antagonists (Gudelsky and Moore, 1976; Demarest and Moore, 1979b). Nigrostriatal and other mesotelencephalic DA neurons exhibit a characteristic compensatory response to activation or blockade of DA receptors; DA agonists reduce and DA antagonists increase nigrostriatal activity (see review, Moore and Wuerthele, 1979). This is believed to be due to DA receptor-mediated control of neuronal feedback loops (regulated by postsynaptic DA receptors) or short loop autoreceptor-mediated mechanisms. Tuberoinfundibular DA neurons are not regulated by DA receptor-mediated mechanisms; they do not make synaptic contact with other neurons so they cannot be influenced by neuronal feedback loops, and they lack DA autoreceptors (Demarest and Moore, 1979b).

Several different neurochemical procedures have been employed to estimate nigrostriatal and tuberoinfundibular DA neuronal activity in situ.

*Measurement of the Release of DA and/or Its Metabolites*

This is the most straightforward method, but it requires the employment of some rather specialized techniques. For estimating nigrostriatal DA neuronal activity, the concentrations of endogenous DA and its major metabolites, DOPAC and HVA, have been quantified in perfusates collected from push-pull cannulae or dialysis tubing implanted into the striatum (Bayõn and Drucker-Colin, 1985). The release of DA and DOPAC also have been estimated in vivo by using voltammetric electrodes inserted into the striatum (Knott et al., 1985). The release of DA from tuberoinfundibular DA neurons has been quantified by measuring the concentration of this amine in the hypophysial portal blood (Ben-Jonathan et al., 1977; Gibbs and Neill, 1978). This procedure provides a direct measure of tuberoinfundibular DA neuronal activity; but its disadvantage is that it requires extensive surgery and must be carried out in anesthetized animals, and some anesthetics alter the activity of these DA neurons (Pilotte et al., 1980).

*Measurements of the Concentrations of DA Metabolites*

The concentrations of DOPAC, HVA, and 3MT in the striatum have been used as estimates of nigrostriatal DA neuronal activity; increases and decreases in the concentrations of these metabolites have been associated with increases and decreases in impulse traffic in these neurons (Roth et al., 1976; Wood et al., 1982). The concentrations of DOPAC, relative to that of DA, are very low in the median eminence compared with that in the striatum, and it was originally believed that the concentrations did not reflect impulse traffic in tuberoinfundibular DA neurons (Umezu and Moore, 1979). The results of recent studies employing the more sensitive analytical techniques of high performance liquid chromatography (HPLC) suggest that changes in DOPAC concentrations in the median eminence do reflect tuberoinfundibular DA neuronal activity (Lookingland et al., 1986).

*Measurement of DA Synthesis (DOPA Accumulation)*

The release of DA is generally coupled to its synthesis. Since the rate of synthesis of this amine is regulated by tyrosine hydroxylase, estimates of DA neuronal activity have been made by measuring the activity of this enzyme. This can be done in vivo by measuring the rate of accumulation of DOPA after systemic administration of centrally active inhibitors of aromatic L-amino acid decarboxylase (e.g., NSD 1015; m-hydroxybenzylhydrazine, Sigma Chemical, St. Louis, MO). In the absence of a decarboxylase inhibitor, the concentration of DOPA in the brain is essentially zero, but after the administration of a drug such as NSD 1015, the concentration of this amino acid in the striatum increases linearly with time at a rate that is proportional to impulse activity in nigrostriatal neurons (Murrin and Roth, 1976). Similarly, the rate of accumulation of DOPA in the median eminence after the administration of a decarboxylase inhibitor reflects the activity of tuberoinfundibular DA neurons (Demarest and Moore, 1980).

*Measurement of the Rate of Decline of DA after Inhibition of Tyrosine Hydroxylase (DA Turnover)*

When the synthesis of DA is blocked, the amine continues to be released from the nerve terminal in response to the arrival of nerve action potentials. Since the released amine cannot be replaced by the synthetic process, the concentration of the amine declines at a rate proportional to the impulse traffic in the neuron. After the administration of α-methyltyrosine (αMT), an inhibitor of tyrosine hydroxylase, the rate at which the DA concentration declines in the striatum and median eminence reflects the

activity of nigrostriatal and tuberoinfundibular DA neurons, respectively. One advantage of this procedure is that the decline of DA after αMT administration can be followed by histofluorescent techniques. Fuxe and coworkers were the first to use this procedure to estimate the activity of tuberoinfundibular DA neurons by measuring the loss of DA fluorescence in the median eminence (Fuxe et al., 1969).

Each of these neurochemical methods has some limitations, so it has been advisable to confirm the results of experiments with more than one procedure. Nevertheless, results of experiments by different investigators, who have used these techniques to determine the effects of pharmacological and endocrinological manipulations on various DA neuronal systems, have been remarkably consistent. The following sections summarize the results of studies using these neurochemical procedures to estimate the activities of nigrostriatal and tuberoinfundibular DA neurons in a variety of situations.

## RESPONSES OF DA NEURONS TO PHARMACOLOGICAL MANIPULATIONS

### *Dopamine Agonists and Antagonists*

A well-recognized characteristic of mesotelencephalic DA neurons, including those that comprise the nigrostriatal system, is their responsiveness to the administration of DA agonists (e.g., piribedil and bromocriptine) and DA antagonists (e.g., haloperidol and thioridazine). Results of electrophysiological and biochemical studies reveal that DA agonists decrease and DA antagonists increase the activity of these neurons (see review, Moore and Wuerthele, 1979). The compensatory increases and decreases of the activity of these DA neurons appear to result from the ability of these drugs to activate or block DA autoreceptors and/or DA receptors on neurons that participate in neuronal feedback loops.

Tuberoinfundibular DA neurons, unlike the other DA neurons in the brain, are unresponsive to the direct actions of DA antagonists and agonists. For example, for up to 2 h after the administration of DA antagonists, such as haloperidol, the rates of DA synthesis, turnover, and metabolism are markedly increased in the striatum and other forebrain regions, but not in the median eminence. Conversely, DA agonists, such as piribedil, reduce the rate of synthesis, turnover, and metabolism of DA in forebrain regions, but are without effect in the median eminence. Figure 4 shows that acute administration of haloperidol increased and piribedil decreased the rate of turnover (αMT-induced decline) of DA in the striatum (left), but these drugs had no effect on the turnover of DA in the median eminence. These results indicate that unlike other DA neurons, the tuberoinfundibular DA neurons are not directly regulated by DA receptor-mediated mechanisms.

Although tuberoinfundibular DA neurons are not immediately responsive to DA antagonists, they do exhibit a delayed increase in activity if large doses of the drugs are administered or if the drugs are administered chronically. The time course of the effects of a large dose of haloperidol on the rates of DA synthesis in the striatum and median eminence are compared in Figure 5. Between 2 and 12 h after the injection of a large dose of haloperidol, the rate of DA synthesis (DOPA accumulation) increased in the striatum but not in the median eminence; this is consistent with the characteristic activation of nigrostriatal DA neurons by DA antagonists. At later times, however, the rate of DA synthesis increased in the median eminence, which suggests a delayed activation of the tuberoinfundibular DA neurons. A similar time course of the effects of DA antagonists is seen when

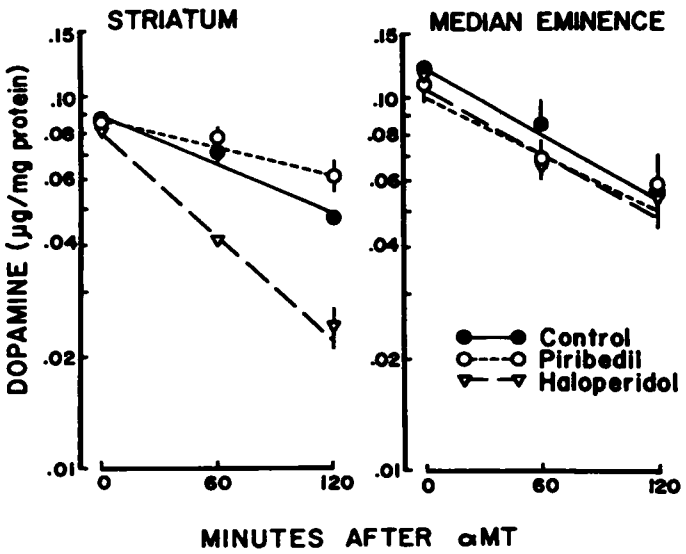


FIG. 4. Effects of a dopaminergic agonist (*piribedil*) and an antagonist (*haloperidol*) on the decline of dopamine concentrations in the striatum and median eminence of male rats after an injection of α-methyltyrosine (*αMT*; 250 mg/kg, i.p.). Haloperidol (0.5 mg/kg, i.p.) was injected 60 min and piribedil (30 mg/kg, i.p.) was injected 30 min before the administration of αMT. Animals were killed immediately before (0 time) or 1 and 2 h after αMT. *Symbols* represent means ± 1 SE as determined in 6–9 animals. (Modified from Gudelsky and Moore, 1976.)

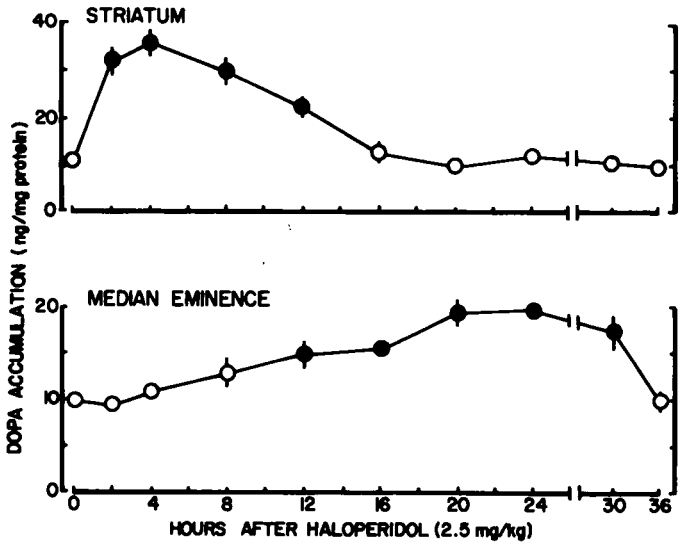


FIG. 5. Effects of *haloperidol* on the rate of dopamine synthesis (*DOPA accumulation* after administration of a decarboxylase inhibitor) in terminals of nigrostriatal neurons (*top*) and tuberoinfundibular neurons (*botton*). Male rats were killed at various times after a single injection of haloperidol (2.5 mg/kg, s.c.). Each animal was injected with a decarboxylase inhibitor (NSD 1015, 100 mg/kg, i.p.) 30 min before it was killed. *Symbols* represent means ± 1 SE as determined from 8 animals at each time point; *solid symbols* indicate those values that are significantly different ($p<0.05$) from zero time controls. (Demarest and Moore, unpublished.)

the rates of DA turnover ($\alpha$MT-induced decline of DA) are measured in the striatum and median eminence (Gudelsky and Moore, 1977). These results suggested that DA antagonists influence the activity of nigrostriatal and tuberoinfundibular DA neurons by different mechanisms. This difference becomes evident when DA antagonists are injected into hypophysectomized rats. The activation of nigrostriatal DA neurons by haloperidol is unchanged in hypophysectomized rats while the delayed activation of tuberoinfundibular DA neurons is completely blocked (Gudelsky and Moore, 1977; Demarest and Moore, 1980). These results imply that the delayed activation of tuberoinfundibular DA neurons by DA antagonists is not the result of a direct action on these neurons, but rather is the result of an indirect action of these drugs on the adenohypophysis. A most obvious action at this site is the ability of DA antagonists to increase the secretion of prolactin, and thereby increase the circulating levels of this hormone.

The mechanisms by which DA antagonists may increase the activity of nigrostriatal and tuberoinfundibular DA neurons are compared in Figure 6. In nigrostriatal DA neurons, pictured on the left, DA antagonists block DA autoreceptors located on cell bodies and terminals of these neurons; they also block DA receptors on striatonigral feedback loops. As a result, there is a prompt compensatory increase in the activity of the nigrostriatal neurons. Tuberoinfundibular DA neurons, on the other hand, are not directly regulated by DA receptor-mediated mechanisms. DA antagonists block DA receptors on lactotrophs in the adenohypophysis, thereby removing the tonic inhibitory effect of DA on prolactin secretion. As a result, circulating levels of prolactin increase and feedback to activate tuberoinfundibular DA neurons, either by acting directly on these neurons or on neurons that project to the tuberoinfundibular DA neurons. In this way, prolactin regulates its own release via a hormonal-neuronal feedback loop. If this scheme is correct, prolactin per se must be capable of activating tuberoinfundibular DA neurons.

### *Prolactin*

A number of investigators utilizing a variety of neurochemical techniques have demonstrated that systemic (Hökfelt and Fuxe, 1972; Gudelsky et al., 1976; Selmanoff, 1981) and intracerebroventricular (i.c.v.) (Annunziato and Moore, 1978; Johnston et al., 1980) injections of prolactin cause a delayed but selective activation of tuberoinfundibular DA neurons. An example of the effects of prolactin on a biochemical index of DA neuronal activity is depicted in Figure 7. Twelve h after a single i.c.v. injection of prolactin, the rate of DA synthesis is increased in the median eminence but not in the striatum. The mech-

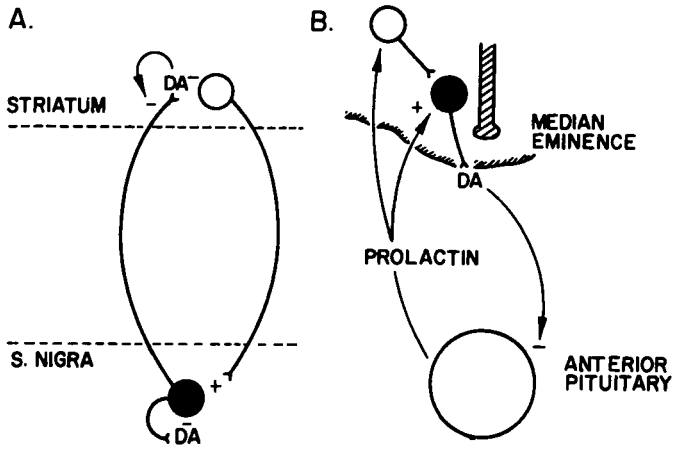


FIG. 6. Schematic representation of the regulation of *A*) nigrostrial and *B*) tuberoinfundibular dopaminergic neurons. (For details, see text.)

anism of the delayed activation of tuberoinfundibular DA neurons by prolactin is not understood; but, since it is blocked by cycloheximide, it appears to involve protein synthesis (Johnston et al., 1980). Subsequent studies have revealed that the activation of tuberoinfundibular DA neurons by prolactin actually has 2 components, a rapid "tonic" component that can be obtained by 2–4 h, and a delayed "induction" component that is seen by 12 h (Demarest et al., 1984, 1986). Only the latter component is blocked by cycloheximide. It is proposed, therefore, that the activity of tuberoinfundibular DA neurons at any point in time is dependent upon circulating levels of prolactin at the time of measurement ("tonic" component) and the past history of prolactin concentration ("induction" component). Further details on the actions of prolactin on tuberoinfundibular DA neurons can be found in several recent reports (Demarest et al., 1984, 1985a,b, 1986; Moore et al., 1985).

## TUBEROINFUNDIBULAR NEURONAL ACTIVITY IN DIFFERENT ENDOCRINE STATES

### *Male-Female Differences*

There is a marked sexual difference in the activity of tuberoinfundibular but not nigrostriatal DA neurons. The concentration of DA in the median eminence of male and female rats is the same, but the "basal" rates of synthesis and turnover of this amine in the median eminence, but not in the striatum, are 2–3 times greater in the female than in the male (Demarest et al., 1981). Consistent with these observations, the DA content in pituitary stalk blood is several times higher in the female (Ben-Jonathan et al., 1977). The sexual difference appears to be due to both androgen-induced changes in neuronal differentiation during development and to differences in hormonal environment in the adult. The rate of DA synthesis in the median eminence of the adult female rat administered testosterone on Day 5 after birth (androgenization) was reduced to values similar to those in the adult male. On the other hand, "female-like" higher values were obtained in adult male rats in which androgenization of the brain was prevented by castration on Day 1 after birth (Demarest et al., 1981). Thus, neonatal androgen exposure alters tuberoinfundibular DA neuronal activity, and this may contribute to the differences in the regulation of prolactin secretion in male and female rats.

DA concentrations in the median eminence of adult rats are not altered by castration, but the rates of synthesis and turnover of DA are increased in the male and decreased in the female after castration. Testosterone or estrogen replacement restores the castration-induced changes in tuberoinfundibular DA neuronal activity to values obtained in intact male and female rats, respectively (Fig. 8; also see Kizer et al., 1978; Demarest et al., 1985c). Sexual differences in the prolactin secretion (Neill, 1972) may be related, in part, to the male-female differences in tuberoinfundibular nerve activity.

There are also marked sexual differences in the responses of tuberoinfundibular DA neurons to prolactin. Tuberoinfundibular DA neuronal activity is reduced if serum levels of prolactin are reduced by hypophysectomy or by treatment with a long-acting DA agonist, such as bromocriptine; this effect is much more pronounced in the female (Demarest and Moore, 1981; Demarest et al., 1984). Indeed, injections of bromocriptine reduce the rate of DOPA accumulation in the median eminence of the female rat to values equivalent to those in the male. This effect can be prevented by concurrent injections of prolactin, which indicates that the reduced tuberoinfundibular DA neuronal activity produced by DA agonists is due to the reduced stimulation of these neurons by prolactin, and not to a direct action of

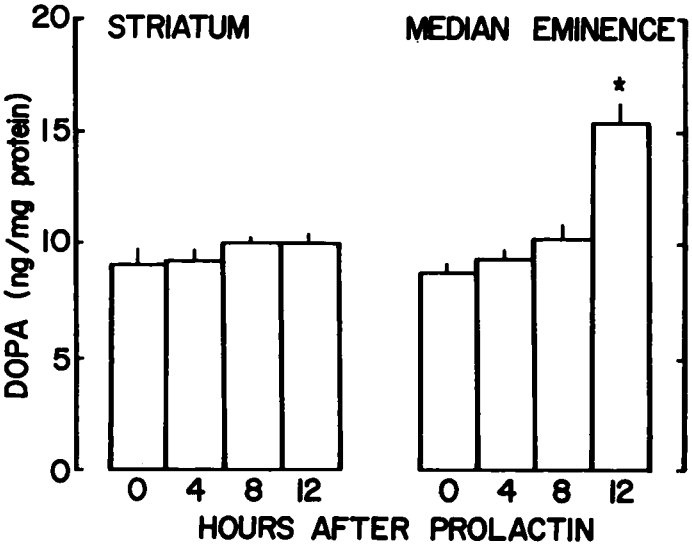


FIG. 7. Rate of *DOPA* accumulation in terminals of nigrostriatal (*left*) and tuberoinfundibular (*right*) dopaminergic neurons at various times after a single i.c.v. injection of rat prolactin (1 μg in 10 μl). The *height* of each *column* represents the mean accumulation of DOPA, and *vertical lines* represent 1 SE as determined from 8 rats. *Significantly different ($p<0.01$) from zero time control.

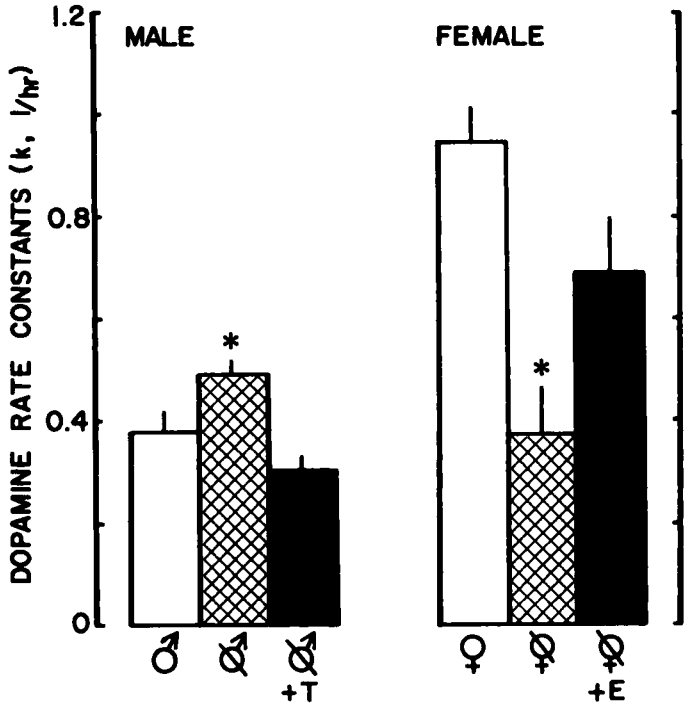


FIG. 8. Effects of castration and steroid replacement on the rates of dopamine turnover in male and female rats. Males were orchidectomized and received subcutaneous implants of empty or testosterone (*T*)-filled capsules 2 wk before they were killed; females were ovariectomized for 2 wk and implanted with capsules containing corn oil or estradiol benzoate (*E*) in corn oil 2 d before they were killed. *Columns* and *vertical lines* represent mean rate constants of decline (α-methyltyrosine technique, see Lookingland and Moore, 1984) ± SE of dopamine in the median eminence of intact rats, castrated rats, and castrated rats treated with steroid replacement. (Modified from Gunnet et al., 1986).

the drug (Demarest et al., 1984). Dose-response curves to i.c.v.-administered prolactin reveal that the magnitude of the stimulation of tuberoinfundibular DA neurons is greater and the dose of prolactin needed to produce this stimulation is lower in the female (Demarest and Moore, 1981). Thus, the increased activity of tuberoinfundibular neurons in the female appears to result from a greater sensitivity of these neurons to the stimulatory actions of prolactin.

### *Pregnancy*

During the first half of pregnancy in the rat, the secretion of prolactin exhibits two daily surges, a nocturnal surge between 0300 and 0600 h, and a diurnal surge at 1800–2100 h (Butcher et al., 1972). These surges are essential for the initiation and maintenance of luteal function (Smith et al., 1975). Tuberoinfundibular DA neurons (but not other DA neurons) also exhibit a semicircadian pattern of activity (as evidenced by changes in the rate of DA synthesis in the median eminence), with reduced activity occurring around 0600 and 2100 h (McKay et al., 1982). Thus, during early pregnancy, there appears to be a relationship between the surges of prolactin and the activity of tuberoinfundibular DA neurons, with neuronal activity decreasing during the surges of prolactin (see Fig. 9, Days 2 and 6).

There is also a temporal relationship between the cessation of these two effects. The surges of prolactin and the cyclical activity of tuberoinfundibular DA neurons disappear by Day 13 of pregnancy (Smith and Neill, 1976; McKay et al., 1982). By this time, and continuing until term, the serum concentrations of prolactin remain low while the activity of tuberoinfundibular DA neurons is constantly high throughout the 24-h period (Fig. 9, Day 13). During efforts to determine why cyclical patterns of serum prolactin levels and tuberoinfundibular DA neuronal activity cease at midpregnancy, it was found that lowering circulating levels of prolactin by injecting bromocriptine reduced DA synthesis in the median eminence on Day 6 but not on Day 13 of pregnancy (Fig. 10). This suggested that the high rate of DA synthesis in

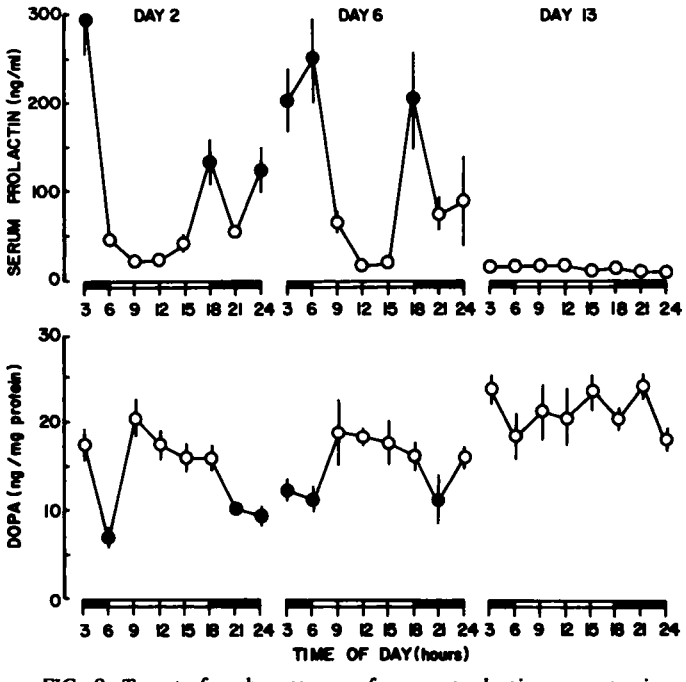


FIG. 9. Twenty-four-h patterns of *serum prolactin* concentrations (*top*) and the rate of *DOPA* accumulation in the median eminence on Days 2, 6, and 13 of pregnancy. Each *symbol* represents the mean ± 1 SE (N of 8–11); *solid symbols* represent values that are significantly different ($p<0.05$) from those obtained at 1200 h. (Modified from McKay et al., 1982).

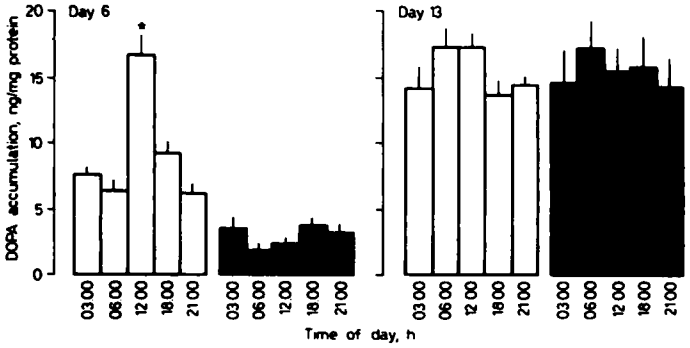


FIG. 10. Effect of bromocriptine on the pattern of *DOPA accumulation* in the median eminence on Days 6 and 13 of pregnancy. Pregnant rats were administered bromocriptine (3 mg/kg, s.c., *solid columns*) or 50% ethanol vehicle (*open columns*) 24 h before they were killed. Each *column* represents the mean, and *vertical lines* represent 1 SE of 8 determinations. *Value that is significantly different ($p<0.05$) from all others in the same group. (From Demarest et al., 1983b).

terminals of tuberoinfundibular neurons during the latter half of pregnancy was not a consequence of the activation by prolactin, but resulted from some endogenous factor that is not influenced by bromocriptine. Surgical removal of the fetal uterine-placental unit on Day 6 caused the cyclical pattern of prolactin secretion and tuberoinfundibular DA neuronal activity to be maintained on Day 13 (Voogt, 1980; Demarest et al., 1983b). This suggested that a factor secreted from the uterine-placental unit takes over from prolactin in stimulating tuberoinfundibular DA neurons shortly after midpregnancy, and this factor appears to be responsible for terminating the semicircadian pattern of prolactin secretion. A logical candidate for such a factor would be placental lactogen, which is secreted from the pregnant uterine placenta beginning on Days 10–12 of pregnancy and continuing until parturition; placental lactogen replaces prolactin as the major luteotrophic factor in late pregnancy (Shiu et al., 1973). Subsequent experiments (Demarest et al., 1983c) revealed that i.c.v. administration of human placental lactogen selectively increased the rate of DA synthesis in the median eminence in a manner similar to that of prolactin (see Table 1).

As a result of these experiments, a scheme illustrated in Figure 11 is proposed. During early pregnancy, tuberoinfundibular DA neurons and serum prolactin levels exhibit characteristic cyclical patterns with surges of prolactin occurring at times when neuronal activity is low. These patterns cease by Day 13 of pregnancy. At this time, serum prolactin levels remain low and tuberoinfundibular DA neuronal activity is high. These latter effects appear to be caused by the release from the uterine-placental unit of placental lactogen, which stimulates tuberoinfundibular DA neurons to release more DA into the hypophysial portal blood and thereby inhibit the release of prolactin from the adenohypophysis.

### *Lactation and Suckling*

In lactating rats, suckling markedly increases serum concentrations of prolactin, due in part to the reduced inhibitory control exerted at the adenohypophysis by DA. This is evidenced by a reduced rate of synthesis

TABLE 1. Effects of rat prolactin and human placental lactogen on the rate of DOPA accumulation in the median eminence and striatum of the rat.*

| Treatment† | Median Eminence | Striatum |
|---|---|---|
| Vehicle | 7.9 ± 0.8 | 7.2 ± 0.6 |
| Prolactin | 18.3 ± 1.9[a] | 8.1 ± 1.0 |
| Placental lactogen | 14.8 ± 1.0[a] | 7.7 ± 0.8 |

*Values represent ng DOPA/mg protein/30 min (mean ± 1 SE, N=8).

†Ovariectomized rats received i.c.v. injections of 10 μl saline alone (Vehicle) or saline containing 10 μg rat prolactin or 100 μg human placental lactogen 12 h before the rate of DOPA accumulation was determined in the median eminence or striatum.

[a]Values that are significantly different ($p<0.05$) from Vehicle controls. (Modified from Demarest et al., 1983c).

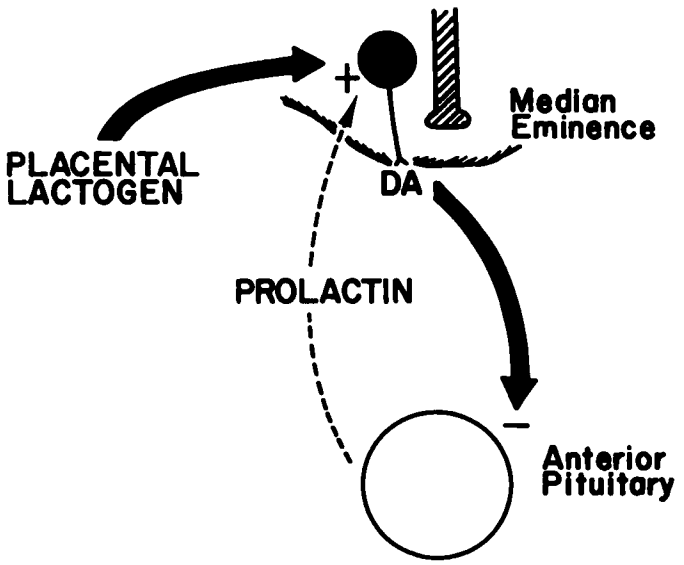


FIG. 11. Schematic representation of the role of *placental lactogen* in modulating the activity of tuberoinfundibular dopaminergic neurons during the latter half of pregnancy. (For details, see text.)

and turnover in DA in the terminals of the tuberoinfundibular neurons in the median eminence (Demarest et al., 1983a) and a reduced release of DA into hypophysial portal blood (Ben-Jonathan et al., 1980). In Figure 12, the serum concentrations of prolactin and the rate of DOPA accumulation in the median eminence are compared in "control" animals on Day 2 of diestrus and in lactating animals 12 days postpartum. In lactating rats deprived of their pups for 4 h, serum prolactin concentrations were significantly higher and the rate of DA synthesis in the median eminence significantly lower than they were in the diestrous control animals. These changes were enhanced if the lactating rats were permitted to suckle for 30 min. These results suggest that the activity of tuberoinfundibular DA neurons is reduced in the lactating rat and that it can be reduced further by suckling stimuli. It appears, therefore, that suckling activates neuronal afferent circuits that inhibit tuberoinfundibular neuronal activity and thereby removes the inhibitory control over prolactin secretion.

In view of the results of numerous experiments revealing that elevated blood levels of prolactin (e.g., following administration of the hormone, DA antagonists, estrogens, etc.) activate tuberoinfundibular DA neurons, it was surprising that in lactating rats, which have elevated circulating levels of prolactin, the activity of tuberoinfundibular DA neurons is actually reduced when compared with diestrous controls. This appears to be due to the fact that the properties of tuberoinfundibular DA neurons change in the lactating rat so that they become unresponsive to the stimulatory actions of prolactin. The data summarized in Figure 13 show that i.c.v. administration of prolactin and the systemic administration of haloperidol, both of which increase the rate of DA synthesis in the median eminence of diestrous rats, are without effect in the lactating rat. The mechanism by which the lactating rat becomes unresponsive to prolactin is currently unknown, but it permits these animals to maintain high serum levels of prolactin while they are suckling (i.e., the normal feedback control of tuberoinfundibular DA neurons by prolactin does not appear to operate in the lactating rat).

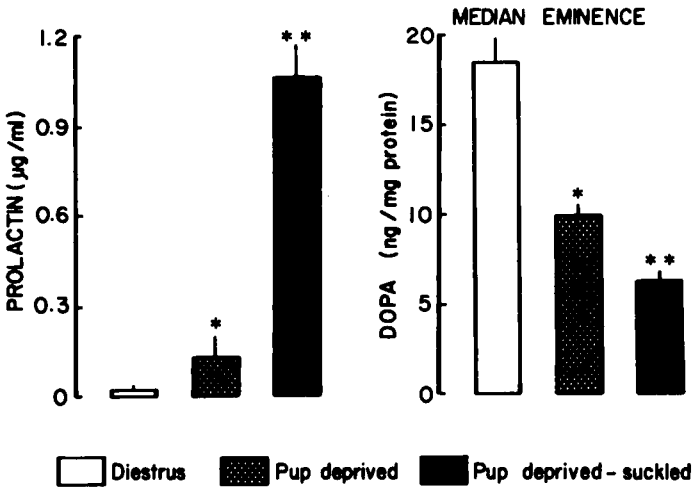


FIG. 12. Serum *prolactin* concentrations (*left*) and the rates of *DOPA* accumulation in terminals of tuberoinfundibular dopaminergic neurons (*right*) of diestrous and lactating rats. On Day 12 postpartum, lactating rats were separated from their pups for 4 h. Half of the animals were allowed to suckle for 30 min (pup-deprived suckled), while the other half remained separated from their pups for an additional 30 min before they were killed (pup-deprived). *Columns* represent means and *vertical lines* 1 SE of 8 determinations. *Values that are significantly different ($p<0.05$) from the diestrous group; **Values that are significantly different ($p<0.05$) from the pup-deprived group. (From Demarest et al., 1983a.)

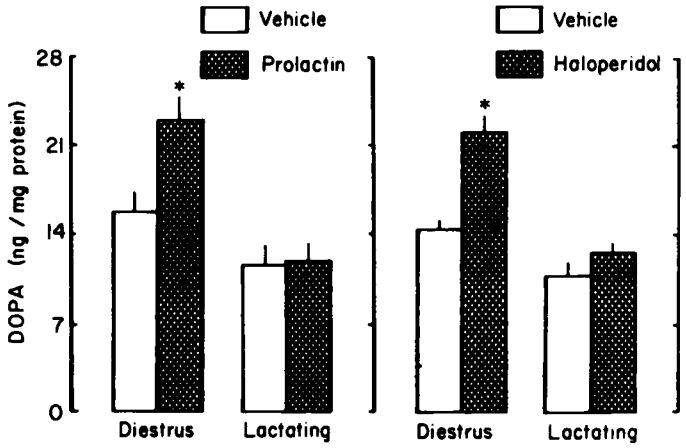


FIG. 13. *DOPA accumulation* in the median eminence of *diestrous* and *lactating* rats after i.c.v. administration of *prolactin* (10 μg/10 μl) or its saline *vehicle* 12 h before animals were killed (*left*), or s.c. administration of *haloperidol* (2.5 mg/kg) or its 0.3% tartaric acid *vehicle* 16 h before animals were killed (*right*). Lactating rats were removed from their pups 12 h before the adults were killed. *Columns* represent means and *vertical lines* 1 SE (N of 8–10). *Values that are significantly different ($p<0.05$) from appropriate vehicle-treated groups. (From Demarest et al., 1983a.)

## ACKNOWLEDGMENTS

The author gratefully acknowledges the contributions of students and coworkers (listed in the References) who worked on these studies, and the assistance provided by Diane Hummel in preparing this manuscript.

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