Prolactin Regulation of Dopaminergic Neurons in the Infundibulum Pituitary Stalk of Bull Calves[1] (43008)

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Abstract. **The effects of elevated circulating concentrations of prolactin were examined on neurochemical estimates of activity of dopaminergic neurons in the infundibulum/pituitary stalk of Holstein bull calves (8–10 weeks of age). Activity of these neurons was estimated by measuring the accumulation of dihydroxyphenylalanine, the immediate precursor of dopamine, 15 min after an intravenous injection of the aromatic L-amino acid decarboxylase inhibitor, 3-hydroxybenzylhydrazine. Subcutaneous injections of the dopamine antagonist haloperidol every 6 hr for 1 day increased serum concentrations of prolactin and accumulation of dihydroxyphenylalanine in the infundibulum/stalk. Intravenous infusions of prolactin for 1 or 9 days increased accumulation of dihydroxyphenylalanine in the infundibulum/stalk, indicating that these neurons remain responsive to elevated prolactin for at least 9 days. It is concluded that elevated concentrations of prolactin in blood stimulate dopaminergic neurons in the infundibulum/pituitary stalk of bull calves. We speculate that these neurons may be analogous to the tuberoinfundibular dopaminergic neurons that regulate prolactin in rats.** [P.S.E.B.M. 1990, Vol 193]

Dopamine (DA) and (or) DA agonists (e.g., bromocriptine) inhibit secretion of prolactin from the bovine anterior pituitary gland *in vitro* (1, 2) and *in vivo* (2). Conversely, drugs that inhibit the synthesis of DA (α-methyltyrosine) or block DA receptors in the anterior pituitary gland (haloperidol) increase serum concentrations of prolactin in cattle (3). In rats, the DA which is responsible for tonic inhibition of prolactin release *in vivo* is released from terminals of the tuberoinfundibular dopaminergic (TIDA) neurons located in the median eminence (4, 5). This DA is transported in the blood of the hypothalamic-hypophysial portal system to the anterior pituitary gland where it activates receptors on lactotrophs and thereby inhibits release of prolactin (6).

Characteristics of TIDA neurons have been determined primarily from studies conducted in the rat (4, 5). Pharmacologic manipulations which increase (e.g., DA antagonists) or decrease (e.g., DA agonists) circulating concentrations of prolactin increase and decrease, respectively, TIDA neuronal activity (7, 8). Furthermore, systemic (9, 10) and intracerebroventricular injections of prolactin (11, 12) increase activity of TIDA neurons and decrease secretion of prolactin (13), suggesting that prolactin regulates its own secretion by altering the level of inhibitory dopaminergic tone (5). The objective of the present study was to determine if some of the properties of dopaminergic neurons that have been characterized in the rat also apply to the bull calf.

Changes in impulse traffic (activity) in DA neurons in the rat brain are not accompanied by changes in concentrations of DA in terminals of these neurons. This implies that synthesis of DA keeps pace with release so that the rate of DA synthesis reflects the activity of the DA neurons. The rate of synthesis of DA can be estimated *in situ* by measuring accumulation of dihydroxyphenylalanine (DOPA) following administration of a decarboxylase inhibitor. Specifically, activity of TIDA neurons in the rat is reflected in the rate of accumulation of DOPA in the median eminence (14, 15). Results of the present study reveal that the rate of DOPA accumulation in the infundibulum/pituitary stalk of bull calves, which contains relatively high con-

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centrations of DA and possibly contains terminals of TIDA neurons (16), is increased following manipulations which elevate circulating concentrations of prolactin.

Materials and Methods

Animals. Animals used in these studies were prepubertal Holstein bull calves of approximately 8–10 weeks of age (80–100 kg body wt). Calves were housed in individual stalls in temperature-controlled chambers (20 ± 2°C) and exposed to continuous light. One kilogram of a commercial calf diet (Startena; Purina Mills, St. Louis, MO) was provided at 0800 and at 1700 hr and water was provided *ad libitum*. Calves were acclimated to this environment for at least 1 week before the start of experiments.

Drugs. Haloperidol (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.3% tartaric acid (2.5 mg/ml) and injected subcutaneously at a dose of 1 mg/kg, a dose that maintains elevated serum concentrations of prolactin for 6 to 8 hr (unpublished observations). Bovine prolactin (USDA b1) was dissolved in 0.85% saline (1 mg/ml) containing 0.1% bovine serum albumin and infused intravenously (0.5 mg prolactin/hr) using AS-2BH Autosyringe infusion pumps (Autosyringe, Inc., Hooksett, NH). Syringes were refilled daily at 1000 hr with fresh solutions of prolaction. 3-Hydroxybenzylhydrazine (NSD 1015; Sigma Chemical Co.) was dissolved in 0.85% saline (100 mg/ml) and injected intravenously at a dose of 25 mg/kg. Sodium pentobarbital (Sigma Chemical Co.) was dissolved in sterile water (388 mg/ml) and injected intravenously at a dose of 85 mg/kg, a dose that kills cattle within 15–20 sec.

Catheterizations. In experiments involving repeated collection of blood and intravenous drug injections, calves were fitted with a polyvinyl catheter in a jugular vein 1 day before the start of the experiment. In experiments involving intravenous infusion of prolactin, calves were fitted with an additional polyvinyl catheter in the contralateral jugular vein 1 day before the start of infusion.

Tissue Dissection. Following experimental treatments, calves were euthanized with an intravenous injection of sodium pentobarbital. Skin was then cut along the forehead, just above the eyes, and from the eyes to the poll on both sides of the head. The skull and brain were then cut with a reciprocating saw along the line of the skin cut. The optic nerves and pituitary stalk were cut at their distal junctions with the dura mater, and the ventral part of the brain was removed and placed on a petri dish over ice. A block of hypothalamic tissue extending from the optic chiasm to the mammillary bodies containing the mediobasal hypothalamus and pituitary stalk was dissected with a scalpel blade, placed with the ventral surface up on aluminum foil, and frozen on dry ice within 3–4 min of euthanasia. Frontal sections (1 mm) beginning at the optic chiasm were prepared in a cryostat (−9°C) and the mediobasal hypothalamus and infundibulum/stalk were dissected from these frozen sections with a scalpel blade (Fig. 1). Samples were placed in 60 μl of 0.1 *M* phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at −20°C until assayed.

Assays. On the day of assay, tissue samples were thawed, homogenized, and centrifuged for 1 min in a Beckman Microfuge. Concentrations of DA, DOPA, 5-hydroxytryptophan (5-HTP), and 5-hydroxytryptamine (5-HT) in supernatants of the mediobasal hypothalamus and infundibulum/stalk were determined by high-performance liquid chromatography with electrochemical detection as described previously (17). Tissue pellets were dissolved in 1.0 *N* NaOH and assayed for protein (18).

Serum was harvested from each blood sample and concentrations of prolactin were determined using a double antibody radioimmunoassay procedure described previously (19). The reference standard was NIH-bPRL-B4, the level of detection was 0.9 ng/ml, and intra- and interassay coefficients of variation were 3.9 and 8.8%, respectively.

Neurochemical Estimation of Neuronal Activity. Activities (or impulse traffic) in DA and 5-HT neurons were estimated by measuring concentrations of DOPA and 5-HTP 15 min after an intravenous injection of NSD 1015. NSD 1015 inhibits conversion of DOPA to dopamine and 5-HTP to 5-HT, and accumulation of

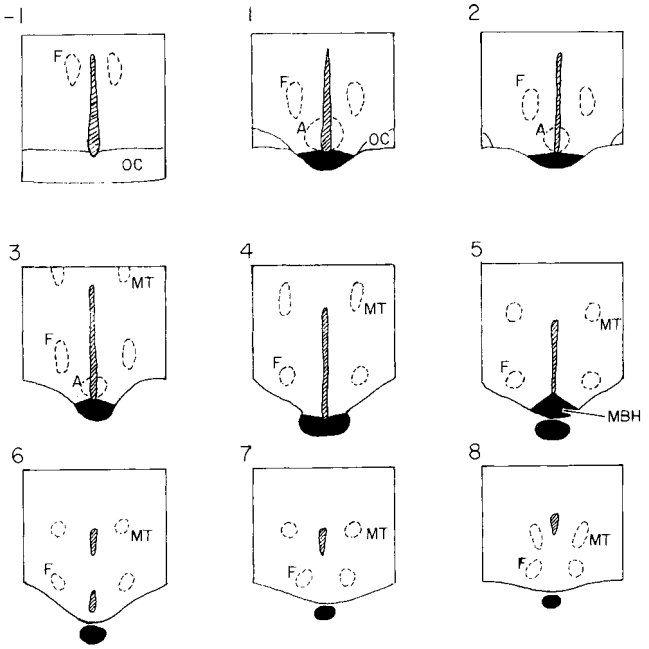

Figure 1. Schematic diagram of frontal sections of the hypothalamus from bull calves depicting the regions of dissection of the infundibulum/pituitary stalk and the mediobasal hypothalamus (MBH). Beginning at the optic chiasm (OC), consecutive 1-mm sections were prepared from a dissected block of hypothalamus and the attached pituitary stalk. Solid regions depict the infundibulum/pituitary stalk that was dissected from Sections 1–8 and the mediobasal hypothalamus that was dissected from Section 5. The central cross-hatched area represents the third cerebral ventricle. A, arcuate nucleus; F, fornix; MT, mammillothalamic tract.

these precursors over time reflects the *in vivo* rate of synthesis of DA and 5-HT, respectively (20). Since synthesis and release of neurotransmitters in these neurons are coupled, the rates of DA and 5-HT synthesis are used as indices of DA (14) and 5-HT neuronal activity, respectively (21, 22).

Statistical Analysis. Neurochemical data were analyzed by analysis of variance (23) and prolactin data were analyzed by split-plot analysis of variance with repeated measurements (24). Differences among means of the three treatment groups in Experiment 3 were determined by the Bonferroni-*t* procedure (23).

Experiment 1. Five bull calves were euthanized with sodium pentobarbital. Concentrations of DA were determined in the mediobasal hypothalamus and in each section of the infundibulum/pituitary stalk.

Experiment 2. To increase serum concentrations of prolactin, eight bull calves received subcutaneous injections of haloperidol at 6-hr intervals (total of five injections). Eight uninjected calves served as baseline controls.

The day after catheterization of a jugular vein, blood samples (5 ml) were collected and discarded every 10 min for 1.5 hr to accustom the animals to sampling procedures. Blood (10 ml) was sampled every 15 min for 1 hr before the start of haloperidol injections and then every 2 hr for the next 24 hr. One sample was collected 45 min after the last haloperidol injection and one sample was taken just before euthanasia.

Fifteen minutes before euthanasia (directly following collection of the penultimate blood sample) bulls were injected with NSD 1015. Bulls were then euthanized with sodium pentobarbital (60 min after the last haloperidol injection), and concentrations of DOPA, DA, 5-HTP and 5-HT were determined in each section of the pituitary stalk.

Experiment 3. Eighteen bull calves were randomly assigned to one of three groups. Six bulls were infused intravenously with prolactin for 9 days, six bulls were uninfused for 8 days and then infused intravenously with prolactin for 1 day, and six uninfused bulls were used as baseline controls.

Blood samples were collected over the 9 days. On Day 9 at 15 min before euthanasia, bulls were injected with NSD 1015. Subsequently, all bulls were euthanized with sodium pentobarbital, and concentrations of DOPA and DA were determined in each section of the infundibulum/pituitary stalk.

Results

Experiment 1. This initial experiment was performed to determine the distribution of DA in regions of the bovine brain that may contain terminals of dopaminergic neurons that control secretion of prolactin. The distribution of DA in the infundibulum/pituitary stalk and mediobasal hypothalamus of five bull calves is summarized in Table I. Because length of the infundibulum/stalk varied among individual animals (i.e., 6–9 mm), the number of sections analyzed per bull calf varied. Concentrations of DA were greater in the middle regions compared with those measured in either the proximal or distal ends of the infundibulum/stalk. Average concentrations of DA in the infundibulum/stalk for all five calves was greater ($P < 0.05$) than those determined in the mediobasal hypothalamus (6.80 ± 0.37 vs 1.85 ± 0.39 pg/μg protein, respectively). These results suggested that dopaminergic neurons in the bovine hypothalamus may terminate in the infundibulum/stalk, and changes in DA synthesis in this latter region became the focus of Experiments 2 and 3.

Table I. Concentrations of DA (pg/μg protein) in the Infundibulum/Pituitary Stalk and Mediobasal Hypothalamus of Bull Calves

	Calf				
	1	2	3	4	5
Infundibulum/stalk					
1[a] Proximal	3.06	1.90	1.71	5.06	1.72
2	10.00	5.42	6.99	7.86	5.52
3	12.05	7.19	9.00	8.47	12.43
4	10.40	8.81	10.23	8.36	10.36
5	9.65	5.31	10.68	7.45	10.11
6	8.45	5.62	5.04	4.51	6.58
7	6.83	6.84	1.83		3.23
8	8.83	8.90			2.47
9 Distal	3.78				
Average[b]	8.12	5.87	6.50	6.95	6.55
Mediobasal hypothalamus	1.87	1.33	3.37	1.38	1.30

[a] One-millimeter frontal sections were cut beginning at the junction of the pituitary stalk and infundibular recess (proximal) and ending at the junction of the pituitary stalk and anterior pituitary (distal) (see Fig. 1).
[b] Average for all sections of the infundibulum/pituitary stalk of individual calves.

Experiment 2. Activity of TIDA neurons in the rat is primarily regulated by positive feedback actions of prolactin; prolonged elevations in the circulating concentrations of this hormone increase synthesis, turnover, and metabolism of DA in the terminals of these neurons (5). To determine if dopaminergic neurons in cattle are also responsive to prolactin, the effects of procedures that increase circulating prolactin concentrations were examined on the rate of DA synthesis in the infundibulum/stalk of bull calves.

Concentrations of prolactin in serum of bull calves injected with haloperidol are shown in Figure 2. Within 2 hr after the first injection of haloperidol, serum prolactin concentrations were markedly increased compared with uninjected controls and remained elevated over the 25-hr experimental period. In addition, injection of NSD 1015 increased serum prolactin concentrations in uninjected controls, but not in haloperidol-treated bull calves.

In bulls not treated with NSD 1015, concentrations of DOPA in the infundibulum/stalk were undetectable (unpublished observations), but 15 min after intravenous injection of NSD 1015 DOPA accumulated in the

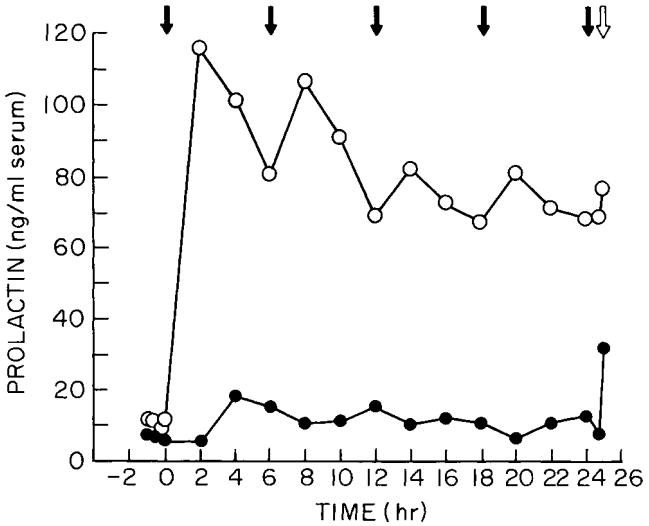

Figure 2. Concentrations of prolactin in serum of bull calves injected with haloperidol (1 mg/kg sc) every 6 hr (○) or uninjected controls (●). Time of haloperidol injections indicated by the solid arrows. All calves received NSD 1015 (25 mg/kg iv) 15 min before euthanasia as indicated by the open arrow. Each point represents the mean of eight samples. Pooled standard error = 5.2 ng/ml for haloperidol-injected bulls and 1.6 ng/ml for uninjected controls.

Table II. Effects of 25 hr of Exposure to Haloperidol on the Synthesis of DA and 5-HT in the Infundibulum/Pituitary Stalk of Bull Calves[a]

	Control	Haloperidol
DOPA	0.98 ± 0.09	1.57 ± 0.16[b]
DA	10.71 ± 0.54	6.80 ± 0.64[b]
DOPA/DA	0.09 ± 0.01	0.23 ± 0.03[b]
5-HTP	0.34 ± 0.03	0.29 ± 0.03
5-HT	5.20 ± 0.53	5.45 ± 0.97
5-HTP/5-HT	0.07 ± 0.01	0.06 ± 0.01

[a] Haloperidol-treated calves received five injections of haloperidol (1 mg/kg sc) at 6-hr intervals and were killed 1 hr after the last injection. Controls were not injected. Fifteen minutes before euthansia all animals were injected with NSD 1015 (25 mg/kg iv). Each amine was measured in each section of the infundibulum/pituitary stalk and then averaged for each bull calf. Values (pg/μg protein) represent mean ± SE of seven or eight bull calves.
[b] Means differ from controls ($P < 0.05$).

infundibulum/stalk (Table II). Relative to controls, haloperidol increased concentrations of DOPA and decreased concentrations of DA in the infundibulum/stalk. Accordingly, the ratio of DOPA to DA was increased in the infundibulum/stalk of haloperidol-treated calves. In contrast, haloperidol failed to alter concentrations of 5-HTP, 5-HT or the ratio of 5-HTP to 5-HT in the infundibulum/stalk.

Experiment 3. The increase in DOPA accumulation in the infundibulum/stalk following infusion of haloperidol in Experiment 2 could be interpreted in two ways: (i) a direct effect of the drug blocking either DA autoreceptors or neuronal feedback loops to cause a compensatory increase in the activity of DA neurons (7), or (ii) an indirect effect of haloperidol secondary to increased circulating levels of prolactin. To test the latter hypothesis, the effect of prolactin infusion on the synthesis of DA was examined in the infundibulum/stalk. Serum concentrations of prolactin were increased ($P < 0.05$) in bulls that were infused with bovine prolactin for 1 or 9 days (Fig. 3). A daily nadir in concentrations of prolactin in the serum of prolactin-infused calves was observed in the blood sample collected immediately before the prolactin solution was changed. This appears to be due to a reproducible loss of prolactin immunoreactivity 18 to 24 hr after the prolactin solution was changed. Injection of NSD 1015 increased serum prolactin concentrations within 15 min in controls, but not in calves infused with prolactin.

Compared with controls, infusion of prolactin for 1 or 9 days increased ($P < 0.05$) concentrations of DOPA in the infundibulum/stalk but had no effect on concentrations of DA (Table III). Thus, ratio of DOPA to DA was increased ($P < 0.05$) in bulls treated with prolactin.

Discussion

Although little is known regarding the location of terminals of TIDA neurons in the bovine hypothalamus, Cooper *et al.* (16) reported that in steers concentrations of DA and dihydroxyphenylacetic acid in the infundibulum and infundibular stalk were greater than in the anterior pituitary gland and zona tuberalis. In agreement, results from the present study indicate that average concentrations of DA in the infundibulum/stalk of bull calves were two to three times greater than concentrations in the mediobasal hypothalamus. Thus, in bull calves dopaminergic neurons may terminate in the infundibulum/stalk. This conclusion is consistent with previous reports that the pituitary stalk contains the greatest concentrations of 5-HT (25) and gonadotropin-releasing hormone (26, 27).

Haloperidol, a DA antagonist, blocks D2 receptors on lactotrophs in the anterior pituitary gland, thereby reducing the inhibitory influence of DA on secretion of prolactin (28). Similar to previous reports in rats (29), humans (30), and cattle (3), haloperidol increased serum concentrations of prolactin in bulls in this study. In addition, elevated serum concentrations of prolactin were maintained with an injection of haloperidol every 6 hr, indicating that bulls do not become refractory to repeated injections of haloperidol for at least 25 hr.

NSD 1015 blocks synthesis of DA (31) and reduces concentrations of DA in hypophysial portal blood (32) which results in increased serum concentrations of prolactin (33). Similarly in the present study, injection of NSD 1015 acutely increased serum prolactin in controls, indicating that newly synthesized DA inhibits secretion of prolactin in cattle. Since treatment with haloperidol blocks the action of DA at the pituitary gland and bulls infused with prolactin already had elevated circulating levels of prolactin, the acute effect of NSD 1015 on prolactin concentrations in serum of these animals was not observed. These results are con-

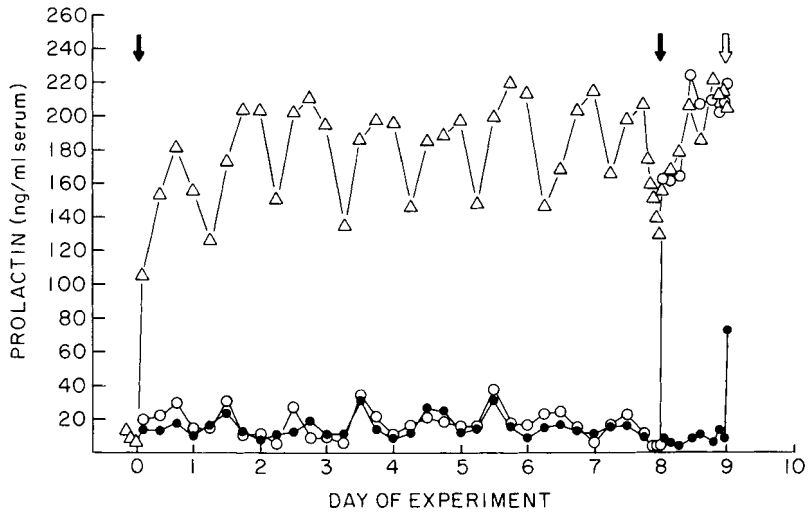

Figure 3. Concentrations of prolactin in serum of prepubertal bull calves infused intravenously with prolactin (USDA b1, 0.5 mg/hr) for 9 days (△), for 1 day (○) and noninfused controls (●). Beginning of infusion on Days 0 and 8 indicated by the solid arrows. All animals received NSD-1015 (25 mg/kg iv) 15 min before euthanasia as indicated by the open arrow. Each point represents the mean of five or six samples. Pooled standard errors = 4.3 ng/ml during periods of noninfusion and 11.2 ng/ml during periods of infusion.

Table III. Effects of Infusion of Prolactin for 1 or 9 Days on Synthesis of DA in the Infundibulum/Pituitary Stalk of Bull Calves[a]

	Control	1 Day	9 Days	SE[b]
DOPA	1.69	3.17[c]	3.83[c]	0.47
DA	11.56	9.70	10.10	1.80
DOPA/DA	0.15	0.33[c]	0.38[c]	0.04

[a] Prolactin-treated calves received intravenous infusions of bovine prolactin (USDA b1) at a dose of 0.5 mg/hr for 1 or 9 days. Controls were not infused. Fifteen minutes before euthansia all animals were injected with NSD 1015 (25 mg/kg iv). Each amine was measured in each section of the infundibulum/pituitary stalk and then averaged for each bull calf. Values (pg/μg protein) represent means of six bull calves.

[b] Pooled SEM.

[c] Means greater than controls ($P < 0.05$).

sistent with an inhibitory role of dopaminergic neurons in the regulation of secretion of prolactin in cattle.

Accumulation of DOPA following NSD 1015 administration is an index of the *in vivo* rate of DA synthesis in the rat brain (20). Alterations in impulse traffic flow in TIDA neurons induced by electrical stimulation of the perikarya of these neurons in the arcuate nucleus produce corresponding changes in the rate of DOPA accumulation in the median eminence of rats (15). Thus, in rats DOPA accumulation provides a good index of TIDA neuronal activity (31). In rats, haloperidol does not have a direct action on those DA neurons (i.e., TIDA neurons) that tonically inhibit the release of prolactin. For example, haloperidol does not alter the rate of DOPA accumulation in the median eminence of hypophysectomized rats (7) or in rats treated with a prolactin-antibody (34). On the other hand, TIDA neurons in the rat are activated by systemic and intracerebroventricular injections of prolactin (5, 34). Similarly, in the present study, procedures that increased serum concentrations of prolactin (i.e., haloperidol and infusion or prolactin) increased accumulation of DOPA in the infundibulum/stalk of bull calves within 1 day. These results in cattle are consistent with the stimulatory action of prolactin on TIDA neurons in the rat (5, 9–12). Therefore, the rate of accumulation of DOPA reflects activity of DA neurons in the infundibulum/stalk of cattle. Furthermore, increased rates of DOPA accumulation in the infundibulum/stalk were maintained after 9 days of infusion of prolactin, indicating that DA neurons in the infundibulum/stalk of bulls do not become refractory to prolactin-induced stimulation for at least 9 days. A similar observation was made in rats; increased activity of TIDA neurons was maintained for up to 11 days in response to elevated blood levels of prolactin induced by daily injections of haloperidol (35).

There was no effect of haloperidol on synthesis or storage of 5-HT in the infundibulum/stalk, indicating that haloperidol does not alter the activity of 5-HT neurons in this brain region.

High concentrations of DA were localized in the infundibulum/pituitary stalk of bull calves, and procedures that increase serum concentrations of prolactin increased accumulation of DOPA in the infundibulum/pituitary stalk. These results suggest that dopaminergic neurons in the bovine infundibulum/pituitary stalk are activated by prolactin.

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