Brain Neuropeptides That Control Prolactin Secretion in Cattle

A.S. Leaflet R1786

Lloyd L. Anderson, distinguished professor of animal science

Summary

The objective was to test the hypothesis that dopamine regulates prolactin (PRL) secretion by determining acute changes in catecholamine concentrations in hypophyseal portal blood of cattle and their relation to peripheral blood concentration of PRL in hypophyseal stalk-transected (HST) and sham-operated control (SOC). Holstein heifers were subjected to neurosurgery to collect hypophyseal portal blood with a stainless steel cannula designed with a cuff placed under the pituitary stalk and peripheral blood via a jugular vein catheter. PRL plasma concentration was measured by radioimmunoassay, and dopamine and norepinephrine in portal plasma by radioenzymatic assay. During anesthesia before HST or SOC, PRL plasma concentration ranged from 20–40 ng/ml throughout 255 minutes. PRL abruptly increased and remained above 90 ng/ml after HST, compared with a steady decrease to <20 ng/ml in SOC heifers throughout 440 minutes. Within 5 minutes after severing of the hypophyseal stalk, dopamine in portal blood (>8 ng/ml) was significantly increased ($P<0.05$) compared with peripheral blood (<2 ng/ml). Norepinephrine concentration in portal blood was significantly greater ($P<0.05$) than in peripheral blood during the first 60 minutes. The sustained high PRL level in peripheral plasma after severing the hypophyseal stalk stimulated hypothalamic dopamine secretion from hypophyseal portal vessels during the prolonged period of blood collection. Norepinephrine concentration in these cattle was greater in hypophyseal portal blood than in peripheral blood, implicating both an important hypothalamic source of the catecholamine as well as an adrenal gland contribution during anesthesia.

Introduction

Prolactin (PRL) secretion in cattle is markedly affected by seasonal changes, with activity peaking in summer and reaching a nadir in winter. A hypophyseal stalk connects the pituitary gland to the hypothalamus of the brain at the median eminence by a region joining the tuber cinerum and infundibulum. A portal vascular system between the median eminence and anterior pituitary is the pathway for the hypothalamic regulation of pituitary function. Both prolactin-releasing factor (PRF) and prolactin-inhibiting factor (PIF) activities have been demonstrated in hypothalami of mammalian species, but available evidence indicates that PIF has a predominant role in monogastric species. PRL secretion in ruminants such as sheep and cattle is elevated immediately after HST but gradually drifts to lower basal blood concentration. PRF activity in hypothalamic extracts results partly from thyrotropin-releasing hormone (TRH); opiate peptides, β-endorphin and methionine-enkephalin, and vasoactive intestinal peptide (VIP) also can release PRL *in vivo*.

Catecholamines such as dopamine account for at least a part of the inhibition of PRL release, but it remains to be determined whether catecholamines represent physiological PIF; dopamine is released into portal blood in quantities sufficient to inhibit PRL secretion in rats and in rhesus monkeys. Haloperidol is a neuroleptic drug that blocks dopamine receptors on lactotrophs in the anterior pituitary. Haloperidol injected intravenously in cattle causes an immediate peak PRL release that is dose dependent and seasonally regulated, being greater in summer than winter. α-Methyl-*p*-tyrosine (αMT) inhibits catechoalmine synthesis in the hypothalamus by blocking activity of tyrosine hydroxylase. Intraperitoneal injection of αMT (250 mg/kg body weight) reduces dopamine concentration by half in rat median eminence, and intracarotid injection of αMT at 3–12 mg/kg body weight in lactating rats resulted in a dose-dependent increase in PRL plasma concentration. In cattle, intravenous injection of αMT at 0.1, 1.0, 10, and 30 mg/kg body weight causes an immediate dose-dependent increase in plasma PRL concentration that is seasonally affected, being greater in summer than winter. Additionally, TRH intravenously injected at 100 μg causes an immediate peak PRL release within 20 minutes in intact as well as HST heifers. Thus in cattle, the evidence suggests tonic hypothalamic inhibition of PRL secretion; but season has a marked influence on basal PRL blood concentration throughout the year. Although circulating PRL concentration remains elevated soon after HST compared with SOC cattle, PRL levels drift lower over time in HST calves but still remain seasonally responsive, with peak activity in summer and low basal circulating levels of the hormone in winter.

The objective was to test the hypothesis that hypothalamic dopamine regulates prolactin secretion in cattle. This was accomplished by determining acute changes in catecholamine concentrations in peripheral and hypophyseal portal blood, and their relation to peripheral blood concentration of prolactin in hypophyseal stalk-transected (HST) and sham-operated controls (SOC) cattle.

Materials and Methods

Animals and Surgery

Holstein heifers (606 ± 21 kg body weight; mean ± SE) were subjected to two surgical interventions, 3 days apart. Anesthesia was induced by intravenous injection of sodium thiamlyal for endotracheal intubation and maintained on a

closed-circuit system of halothane and O_2. Hypophyseal stalk blood was collected by a supraorbital approach, with the animal suspended in ventral recumbency by canvas belts. An animal head restrainer, attached to the front of a cattle squeeze chute, permitted the head to be raised, lowered, tilted, and turned to the desired position for neurosurgical intervention. In the first intervention, the left frontal bone was removed from the nuchal eminence anterior to the lacrimal sinus, as previously described; the calvarium over the left cerebral hemisphere was removed to expose the dura mater. In the second intervention, 3 days later, an indwelling catheter was inserted into a jugular vein before surgery and maintained for sequential bleeding. The dura mater was cut, an adjustable brain retractor was mounted on the frontal bone, and the ventral surface of the left cerebral hemisphere was lifted to expose the hypothalamic area. The left internal carotid artery was clamped with two silver hemostatic clips and severed to allow an unobstructed view of the hypophyseal stalk. After severing of the hypophyseal stalk, a stainless steel cannula with a specially designed cuff was placed under the pituitary stalk and portal blood was collected for 275 minutes. After insertion of the portal blood cannula, the animal was intravenously injected with heparin (200,000 U).

Peripheral blood (8 ml) was collected at 5-minute intervals beginning 260 minutes before severing of the hypophyseal stalk (HST) or the sham operation (SOC) and continuing for 440 minutes. SOC included all surgical procedures, including placing two silver hemostatic clips on the left internal carotid artery, but without severing of the hypophyseal stalk. The plasma was separated by centrifugation ($1{,}500 \times g$) and frozen at –20°C for radioimmunoassay of prolactin plasma concentration. Portal blood from the severed hypophyseal stalk was collected at 5-minute intervals for assay of dopamine and norepinephrine concentration. There was no incidence of contamination of peripheral blood from the clamped and severed internal carotid artery with hypophyseal portal blood. The hypophyseal stalk blood was aspirated by attaching the soft tubing of a steel-cuff cannula to a syringe mounted on a Harvard infusion/withdrawal pump and maintained in a cold syringe with crushed ice packs in plastic below and above the syringe. A hematocrit indicated no incidence of contamination of hypophyseal portal blood with cerebrospinal fluid. Immediately after collection of a hypophyseal stalk blood sample, the blood was centrifuged. Plasma was collected from cuvettes and 1 part diluent (1 N perchloric acid, 50 mM $MgCl_2$, 10 mg/ml EGTA [ethylene-glycol-bis(β-amino-ethylether)N,N'-tetra-acetic acid]) was added in a ratio of 9:1, and the diluted plasma was frozen and stored in a mechanical ultra-low freezer (-80°C) for determination of catecholamines by radioenzymatic assay.

Hormone Assays

Prolactin was measured by radioimmunoassay of plasma by an iodination procedure using a solid-phase oxidation reaction (Iodobeads) for labeling highly purified bovine prolactin (bPRL-I-1, NIH) with ^{125}INa (IMS-30). A 25- to 100-µl aliquot of sample was added in duplicate tubes along with 150–225 µl of 0.05 M phosphate buffer (PB) containing 1% bovine serum albumin. A standard curve was prepared from bPRL-B-1 (NIH) and ranged from 0.2–80 ng/ml. Sensitivity of the assay was 0.35 ng/ml. Intrassay variations of medium and high pools were 4.6 and 4.9%, respectively. Interassay variations of these pools were 4.6% and 9.4%, respectively.

Dopamine and norepinephrine were measured by radioenzymatic assay. Briefly, bovine plasma blanks (25–100 µl) were dialyzed for 24 hours against 0.15 M NaCl to remove endogenous catecholamines. The unknown plasma samples consisted of 25–100 µl of heparinized bovine plasma. Equivalent volumes of nondialyzed, heparinized bovine plasma containing 0.05–1 ng each of dopamine and L-norepinephrine served as internal standards. The assay was linear from 0–5 ng for each catecholamine, and sensitivity was 10–30 pg.

Statistical Analyses

The experimental units in this study were the individual cattle. Prolactin and catecholamine data were analyzed by a split-plot analysis using a one-way ANOVA, and Student's *t*-tests for continuous variables were used for comparisons between groups.

Results

Before HST or SOC surgery, PRL blood concentration ranged from 20–40 ng/ml throughout a period of 255 minutes (Fig. 1). After the dura mater was opened and reflected the left cerebral hemisphere lifted and the clamped left internal carotid artery severed and either HST or SOC performed, circulating PRL concentration increased and remained consistently elevated above 90 ng/ml in HST heifers compared with a steady decrease to less than 20 ng/ml in SOC animals throughout a period of 440 minutes. The experimental protocols were carried out during June and July, a time of maximal PRL secretion in cattle in Iowa (42°00' latitude).

Within 5 minutes after severing of the hypophyseal stalk and collection of portal blood, dopamine plasma concentration was significantly increased ($P < 0.05$) compared with peripheral blood concentration of the monoamine (Fig. 2). Dopamine concentration remained consistently elevated in portal versus peripheral blood throughout 240 minutes in Holstein heifers (Fig. 2; Table 1).

Norepinephrine concentration in portal blood was significantly greater than in peripheral blood during the first 60 min (Fig. 2; Table 1). Thereafter, there was considerable variation in portal versus peripheral blood concentration of norepinephrine (Fig. 2; Table 1).

Discussion

The sustained increase in PRL secretion after HST coincided with a similar sustained increase in portal blood concentration of dopamine compared with basal levels of the catecholamine in peripheral blood of cattle. In contrast, circulating PRL decreased to basal levels in SOC throughout a period of 8 hours. The increased PRL secretion immediately after HST in these heifers may stimulate an increase in dopamine secretion by two neuronal tracts: the tuberinfundibular, projecting to the median eminence, and the tuberhypophyseal, terminating in the neural and intermediate lobes of the pituitary.

Experimental evidence in rats, monkeys, and cattle suggests a role of dopamine in the regulation of PRL secretion. Dopamine binds to a D_2-type receptor subtype that is coupled to G_i proteins and the PRL gene. Intact and stalk-transected monkeys show a difference in the time course of PRL response to haloperidol that may indicate brain-mediated effects in intact monkeys and primarily a direct pituitary-mediated response in HST animals. In intact cattle, peak release of PRL to intravenous injection of haloperidol or αMT was rapid and dose dependent. In contrast, in HST heifers the same doses of intravenously administered HAL and αMT elicited a marked increase in PRL secretion in only one of five animals. Therefore, it seems that HST in cattle in some way interferes with the blockage of dopamine synthesis or decreased binding of dopamine to its receptors to allow increased PRL secretion by the pituitary gland. The pituitary gland of these HST heifers remains capable of secreting large quantities of PRL, however, as demonstrated by an acute elevation in peripheral PRL levels after intravenous administration of TRH. Rapid PRL release in response to TRH treatment was maintained 10 months later in these HST heifers.

Further evidence for a role of PRL in stimulating dopamine synthesis is indicated by the sustained high peripheral blood concentration of PRL immediately after HST in these heifers, compared with a decrease to basal levels of the hormone in SOC animals during the prolonged period of anesthesia. This suggests that PRL acts within the central nervous system to increase dopamine synthesis and secretion into the hypophyseal portal blood. For example, PRL binding occurs in some regions of the rat brain. The choroid plexus contains PRL receptors that function in part to transport PRL from blood into the cerebrospinal fluid (CSF). The results from *in vivo* autoradiography after the injection of ^{125}I-oPRL are consistent with up-regulation of PRL receptors in the choroid plexus by circulating PRL and the augmentation of transport of PRL from blood to CSF.

Although norepinephrine was in significantly greater concentration in hypophyseal portal blood of cattle than in the peripheral blood in this study, the peripheral plasma concentration may be mediated by peripheral conversion of dopamine to norepinephrine. The prolonged anesthesia in these cattle likely stimulated peripheral plasma concentrations of norepinephrine and epinephrine primarily of adrenal gland origin. The distribution of and physiological functions of dopamine outside the central nervous system are poorly understood. The presence of dopamine in blood circulation has been considered coincidental to the release of norepinephrine and epinephrine from the sympatho-adrenal system. Thus, the greater concentration of norepinephrine in hypophyseal portal blood compared with peripheral blood of cattle implicates the central nervous system as an important source of the catecholamine. However, the elevated peripheral blood concentrations of norepinephrine implicate a stimulated adrenal gland as an important source of catecholamine during this prolonged period of blood collection.

Implications

This is the first study to show consistently elevated dopamine concentration in hypophyseal portal blood compared with peripheral blood of cattle. The immediate increase and sustained level of prolactin concentration in peripheral plasma after severing of the hypophyseal stalk likely stimulated hypothalamic dopamine secretion from hypophyseal portal vessels during the prolonged period of blood collection. Norepinephrine concentration in these cattle was greater in hypophyseal portal blood than in peripheral blood, implicating both an important hypothalamic source of the catecholamine as well as an adrenal gland contribution during the period of anesthesia.

Reference

Hard, D. L., R. K. Bhatnagar, J. R. Molina and L. L. Anderson. 2001. Secretion of dopamine and norepinephrine in hypophyseal portal blood and prolactin in peripheral blood of Holstein cattle. *Domest Anim Endocrinol* **20**:89-100.

Table 1. Dopamine and norepinephrine in sequential collections at 5-minute intervals of peripheral plasma and hypophyseal stalk plasma of cattle.

Period of blood collection (min)	No. of animals	Dopamine (ng/ml)		Norepinephrine (ng/ml)	
		Peripheral plasma	Hypophyseal stalk plasma	Peripheral plasma	Hypophyseal stalk plasma
0-30	5	1.5 ± 0.16	6.1 ± 0.88[a]	1.8 ± 0.22	3.8 ± 0.33[a]
31-60	5	1.3 ± 0.11	3.6 ± 0.42[a]	1.9 ± 0.15	3.6 ± 0.29[a]
61-120	5	1.4 ± 0.05	3.6 ± 0.25[a]	1.6 ± 0.11	3.1 ± 0.17

Values are the mean ± SE.

[a]$P < 0.05$ compared with peripheral plasma concentration.

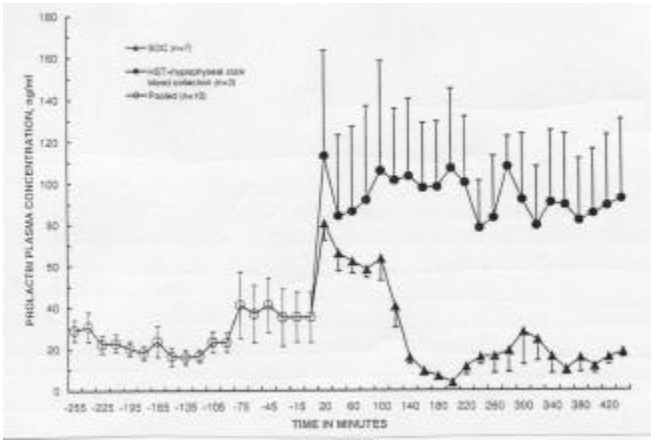

Figure 1. Plasma prolactin concentration in peripheral blood collected at 20-minute intervals from Holstein cattle beginning 255 minutes before either hypophyseal stalk transection (HST) or sham operation control (SOC) and continuing for 440 minutes. Values are mean ± SE.

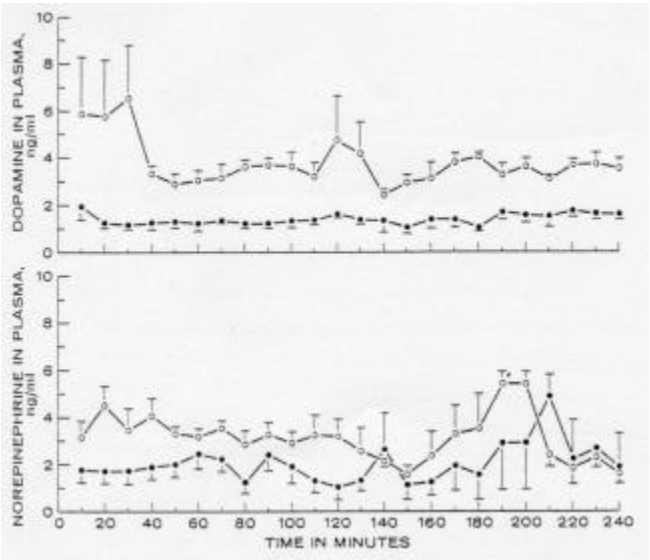

Figure 2. Dopamine and norepinephrine plasma concentration in hypophyseal portal blood (○) and peripheral blood (●) throughout 240 minutes in hypophyseal stalk-transected Holstein cattle (*n* = 5). Values are mean ± SE.