Temperature Effects on Serum Prolactin Concentrations and Activity of Dopaminergic Neurons in the Infundibulum/Pituitary Stalk of Calves (43227)

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Abstract. **The effects of ambient temperature on serum concentrations of prolactin and neurochemical estimates of activity of dopaminergic neurons projecting to the infundibulum/pituitary stalk were investigated in Holstein bull calves. Accumulation of 3,4-dihydroxyphenylalanine (DOPA) in the infundibulum/pituitary stalk after intravenous injection of a decarboxylase inhibitor was used to estimate activity of these dopaminergic neurons. Increasing ambient temperature from 21 to 33°C for 22 hr increased serum concentrations of prolactin and decreased activity of the dopaminergic neurons. Conversely, reducing ambient temperature from 22°C to 11°C decreased serum concentrations of prolactin and increased activity of these dopaminergic neruons. It is suggested that alterations in activity of dopaminergic neurons terminating in the infundibulum/pituitary stalk of bull calves may mediate acute temperature-induced changes in secretion of prolactin.** [P.S.E.B.M. 1991, Vol 197]

Increasing ambient temperature acutely increases the concentration of prolactin in serum of cattle, sheep, and rats (1–4). Conversely, reducing ambient temperature reduces the concentration of prolactin in serum (1, 2). Dopamine released from terminals of tuberoinfundibular neurons in the median eminence of rats tonically inhibits secretion of prolactin from the anterior pituitary gland (5, 6). The objective of the current study was to determine whether exposure to ambient temperatures that acutely alter serum concentrations of prolactin affect the activity of dopaminergic neurons that terminate in the infundibulum/pituitary stalk of bull calves.

Materials and Methods

Prepubertal Holstein bulls, 6–8 weeks after weaning, were housed in individual stalls in temperature-controlled chambers (21°C ± 1°C) and exposed to continuous light. Each calf was fed 1 kg of a commercial calf diet (Startena; Purina Mills, St. Louis, MO) at 0800 and 1700 hr, and water was provided *ad libitum*. Calves were acclimated to this environment for at least 1 week before initiation of experiments. On the last day of the acclimatization period, a jugular vein of each calf was catheterized as described previously (7).

In Experiment 1, nine calves were exposed to increasing ambient temperatures over a 2-hr adjustment period and then maintained at an average temperature of 33°C ± 0.4°C (warm) during a subsequent 22-hr "treatment" period. Nine control calves remained at moderate temperatures (21°C ± 0.2°C) during the 2-hr adjustment and the 22-hr treatment periods. In Experiment 2, eight calves were exposed to decreasing ambient temperatures over a 2-hr adjustment period and then maintained at 11°C ± 0.2°C (cool) for a subsequent 22-hr treatment period. Concurrently, eight control calves were maintained at 22°C ± 0.1°C.

In both experiments blood samples were collected at 2-hr intervals beginning 8 hr before the 2-hr adjustment period and continuing for 22 hr after the adjustment period. The blood was stored at 22°C for 2 to 4 hr and then stored at 4°C for 15 to 20 hr. The blood

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was then centrifuged at 1000*g* for 30 min, and serum was decanted and stored at −20°C until assayed for prolactin. Concentration of prolactin in serum was measured by a double antibody radioimmunoassay (8) using NIH-bPRL-B4 as the reference standard. The intraassay coefficient of variation averaged 7.7%, and the interassay coefficient of variation was 2.7%. The prolactin data used in the statistical analyses were from blood samples collected immediately after the adjustment period through 22 hr of treatment.

Fifteen minutes before euthanasia each calf was injected intravenously with 25 mg/kg of the decarboxylase inhibitor, *m*-hydroxybenzylhydrazine dihydrochloride (NSD 1015; Sigma Chemical Co., St. Louis, MO). NSD 1015, which inhibits conversion of DOPA to dopamine, causes DOPA to accumulate over the 15-min interval prior to euthanasia. The rate of DOPA accumulation, which reflects the *in vivo* rate of synthesis of dopamine (9), serves as an estimate of dopaminergic neuronal activity (10). Fifteen minutes after NSD 1015 treatment, the calves were sacrificed by an intravenous injection of sodium pentobarbital (85 mg/kg; Sigma); death occurred within 15–20 sec.

The brain was quickly removed from the skull and a block of hypothalamic tissue extending from the optic chiasm to the mammillary bodies and containing the mediobasal hypothalamus and infundibulum/pituitary stalk was dissected and frozen on dry ice within 3–4 min of death as described previously (11). Subsequently, 1-mm frontal sections beginning at the optic chiasm were prepared in a cryostat (−9°C). Infundibulum/pituitary stalk samples were placed in 10 μl of 0.1 *M* phosphate-citrate buffer (pH 2.5) containing 15% methanol and stored at −20°C until assayed for dopamine and DOPA (10). Samples of infundibulum/pituitary stalk were thawed, homogenized, and centrifuged for 1 min in a Beckman 152 microfuge. Concentrations of dopamine and DOPA in supernatants of the tissue homogenates were determined by high-performance liquid chromatography with electrochemical detection (12). Tissue pellets were dissolved in 1.0 *N* NaOH and assayed for protein (13). Accumulation of DOPA after injection of NSD 1015 was expressed relative to the protein content of the tissue and concentration of dopamine.

Neurochemical data were analyzed by Student's *t* test (14) and prolactin data were analyzed by split-plot analysis of variance with repeated measurements (15). Mean prolactin concentrations in serum were compared by Student's *t* test (14). $P < 0.05$ was considered significant.

Results

The results of Experiments 1 and 2 are summarized in Table I. During 8 hr before the beginning of the adjustment period serum concentrations of prolactin among calves in Experiment 1 averaged 12.2 ± 1.8 ng/ml and 17.0 ± 1.3 ng/ml among calves in Experiment 2. The changes in serum concentration of prolactin in response to ambient temperature occurred within the 2 hr adjustment period. Relative to values in control calves maintained at a moderate (21°C) temperature, increasing the ambient temperature to 33°C during the 22-hr treatment period increased the average serum concentration of prolactin to 161% of control. There was no difference in the dopamine concentration, but DOPA accumulation and the DOPA to dopamine ratio were significantly lower in the infundibulum/pituitary stalk of the animals exposed to the warm ambient temperatures. Conversely, the concentration of prolactin in serum of calves exposed to the cool ambient temperature (11°C) during the 22-hr treatment period was reduced to 50% of that in the controls maintained at a moderate ambient temperature. The dopamine concentration did not change, but accumulation of DOPA and the DOPA to dopamine ratio in the infundibulum/pituitary stalk of the animals exposed to the cool ambient temperature were significantly higher than values in animals maintained at the moderate temperature.

Discussion

Serum prolactin concentrations in cattle are greater during warmer, long day seasons than during cooler, short day seasons (16). Two of the variables associated with season, ambient temperature and photoperiod, each affect concentrations of prolactin in serum. Thus, confirming previous reports (1–4), in the present study exposure to a warm ambient temperature acutely increases the serum concentration of prolactin whereas exposure to a cool ambient temperature rapidly reduces concentrations of prolactin in serum.

Release of dopamine from tuberoinfundibular neurons in rats inhibits secretion of prolactin (6). In the present study, it was shown for the first time that increases in ambient temperature for 22 hr decreases activity of dopaminergic neurons terminating in the infundibulum/pituitary stalk of calves. Conversely, reducing ambient temperature for 22 hr increased activity of these neurons. These responses are consistent with the hypothesis that dopaminergic neurons terminating in the infundibulum/pituitary stalk mediate acute temperature-induced changes in secretion of prolactin. Neurosecretory activity of tuberoinfundibular dopaminergic neurons in rats decreases in response to suckling or restraint stress (17, 18), and this decrease probably contributes to the increase in prolactin secretion associated with these physiologic states. This decreased activity in tuberoinfundibular dopaminergic neurons in response to suckling or restraint stress in rats, or warm temperatures in calves (present study) occurs despite high concentrations of prolactin in serum which nor-

Table I. Concentrations of Prolactin in Serum and Concentrations of Dopamine and DOPA in the Infundibulum/Pituitary Stalk of Bull Calves Exposed to Moderate, Warm, or Cool Ambient Temperatures

Ambient temperature	Experiment 1		Experiment 2	
	Moderate	Warm	Moderate	Cool
Prolactin (ng/ml serum)	15.3 ± 2.6	24.7 ± 2.5[a]	21.5 ± 1.9	10.8 ± 1.9[a]
Dopamine (ng/mg protein)	11.2 ± 0.8	12.3 ± 0.5	14.6 ± 0.7	14.4 ± 1.3
DOPA (ng/mg protein)	1.04 ± 0.06	0.44 ± 0.04[a]	1.9 ± 0.1	2.6 ± 0.2[a]
DOPA/dopamine	0.10 ± 0.01	0.04 ± 0.01[a]	0.13 ± 0.01	0.19 ± 0.02[a]

Note. Before the experiments calves were maintained at moderate ambient temperatures (21°C ± 1°C). In Experiment 1, nine calves were maintained at a moderate temperature (21°C ± 0.2°C) and nine were subjected to a warmer ambient temperature (33°C ± 0.4°C) for 22 hr prior to euthanasia. In Experiment 2, eight calves were maintained at moderate temperatures (22°C ± 0.1°C) and eight were subjected to a cooler ambient temperature (11°C ± 0.2°C) for 22 hr prior to euthanasia. During the experimental period, blood samples were collected every 2 hr and prolactin concentrations were determined in serum. The serum concentration of prolactin was averaged for each calf and the mean for each group ± 1 SE represents values from eight or nine calves. DOPA and dopamine concentrations represent mean ± 1 SE.

[a] Values in calves subjected to warm or cool ambient temperature that are different ($P < 0.05$) from calves maintained at moderate temperatures.

mally increase activity of tuberoinfundibular dopaminergic neurons in rats (19) and cattle (10). It is suggested that high temperatures suppress the feedback of prolactin on the tuberoinfundibular dopaminergic neurons.

Abruptly increasing daily exposure from 8 to 16 hr of light/day gradually increases serum concentrations of prolactin (20), but activity of the dopaminergic neurons in the infundibulum/pituitary stalk after 4 weeks of exposure to the long day photoperiod is not altered (7). Thus, ambient temperature and photoperiod each affect serum concentrations of prolactin, but the neuronal mechanisms that regulate prolactin secretion differs between these two environmental signals.

It is concluded that acute temperature-induced changes in secretion of prolactin in bull calves are mediated by alterations in activity of dopaminergic neurons terminating in the infundibulum/pituitary stalk.

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