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Effect of Prolactin on Blood Pressure and Cardiovascular Responsiveness in the Rat (42217)

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Abstract. The effects of chronic ovine PRL (oPRL) infusion on resting systolic blood pressure (BP), heart rate, and pressor responsiveness to acute administration of norepinephrine and angiotensin were studied in adult male Sprague–Dawley rats. oPRL was administered over 7 days, via osmotic pump implanted ip on Day 1, at rates of 0, 0.15, 0.30, 0.60, 1.20, and 4.80 μg/hr. Resting BP and heart rate were indirectly determined in conscious rats by tail cuff technique on Days 1, 4, and 7 following pump implantation. In addition, acute pressor responses to ia norepinephrine (4.3 μg) and angiotensin (1.25 μg) were directly measured via arterial cannula in halothane-anesthetized rats on Day 7 of oPRL administration. oPRL infusion did not alter resting BP or heart rate over the 7 days. However, oPRL increased the BP response to norepinephrine at infusion rates of 0.60 and 4.80 μg/hr ($P < 0.01$ vs controls). Body weight increases during the study were also greater in groups receiving 0.15, 0.30, 0.60, and 4.80 μg oPRL/hr ($P < 0.05$) than those in control animals. oPRL decreased pressor responses to angiotensin at infusion rates of 0.30 and 1.20 μg/hr ($P < 0.01$). These data suggest that, although the vascular effects of oPRL may not be evident under resting conditions, oPRL enhances vascular reactivity to norepinephrine infusion and depresses vascular reactivity to angiotensin infusion. Furthermore, at oPRL infusion rates which affect pressor responses to norepinephrine, oPRL increases body weight gain. These findings support a role for PRL in cardiovascular regulation during conditions of altered sympathetic activity.

The role of prolactin (PRL) in mammalian blood pressure (BP) regulation is controversial. In animal studies, low doses of PRL have been reported to elicit systemic pressor responses as well as positive inotropic and chronotropic responses by the heart (1–6). High doses of PRL, in contrast, exhibited systemic depressor activity as well as negative inotropic and chronotropic actions on the heart (1–5, 7). However, other researchers have been unable to reproduce these findings, leading to speculation that a genetic predisposition to hypertension may be required for PRL to exert its cardiovascular effects (8, 9), or that seasonal variations in sensitivity to PRL may account for the discrepancy in results (10).

In the present study, the effects of increasing doses of chronically administered ovine PRL (oPRL) on resting systolic BP and heart rate were evaluated in genetically normotensive male rats. In addition, the effects of oPRL on cardiovascular responsiveness to norepinephrine (NOR) and angiotensin (ANG) were evaluated.

Methods. *Animals.* Fifty-six adult male Sprague–Dawley rats weighing 150–176 g were purchased from Charles River (St. Constant, Quebec). Animals were housed four per cage in a constant temperature (21 ± 1°C) and light cycle-controlled (12L:12D) room for the duration of the study. Rat chow and tap water were supplied *ad libitum.*

Agents administered. Physiological saline (controls, $n = 8$) and oPRL (NIADDK-oPRL-16, 30.5 IU/mg, in physiological saline adjusted to pH 9.0) were administered by constant flow (1 μl/hr), 7 day osmotic minipumps (Alza, No. 2001) implanted ip under halothane anesthesia on Day 1 of the study. Experimental animals received oPRL at calculated rates of infusion of 0.15, 0.30, 0.60, 1.20, 2.40, and 4.80 μg/hr ($n = 8$/group) over the 7 days of the study. The oPRL preparation used contained 0.1 μU AVP/0.6 μg oPRL, as determined by radioimmunoassay. Systemic infusion of 0.1 μU AVP/hr by osmotic pump has no effect on BP (unpublished observation).

In addition, on Day 7 of the study, femoral arterial cannulae were inserted under halothane anesthesia to the level of the abdominal aorta. Following cannulation, 4.3 μg norepinephrine and 1.25 μg angiotensin (Sigma) were

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injected ia. These doses of norepinephrine and angiotensin were previously determined to approximate the ED_{50} in similar animals.

Measurements. On Days 1 (prior to pump implantation), 4, and 7 of the study, resting BP and heart rate (HR) were determined in conscious animals using the tail cuff technique previously described (11). Body weights were also determined on these days.

At least 2 hr after indirect BP measurement on Day 7 the BP responses to bolus ia injection of NOR and ANG were measured directly via aortic cannula (Coulbourn Instruments) in anesthetized animals, as previously described.

Determination of plasma oPRL levels. Three groups of rats of similar body weight received ^{125}I-oPRL via ip osmotic pumps at calculated release rates of 0.60, 1.20, and 2.40 μg/hr. Blood samples were collected via jugular venipuncture under halothane anesthesia following 4 days of infusion. Plasma ^{125}I-oPRL levels were then calculated from ^{125}I activity in the plasma of these animals.

In order to verify that ^{125}I activity reflected plasma oPRL levels, decay curves of plasma ^{125}I activity were calculated following ^{125}I-oPRL injection, and the $T_{\frac{1}{2}}$ of ^{125}I activity was compared with that of oPRL. Blood samples (0.5 ml) were drawn for this purpose at T = 1, 2, 5, 10, 15, 30, 40, 50, 60 min post ^{125}I-oPRL injection. In addition, both ^{125}I-oPRL and plasma taken 50 min post ^{125}I-oPRL injection were passed through a Sephadex G-100 column (1.3 × 50 cm) in order to determine whether or not plasma ^{125}I activity reflected ^{125}I-oPRL activity or a combination of labeled hormone plus labeled metabolic products of hormone degradation.

Data analysis. Data from resting BP, HR, and body weight measures were analyzed using a two-way ANOVA on repeated measures. Data on ^{125}I-oPRL levels, as well as BP responses to NOR and ANG, were analyzed using a two-way ANOVA. When an ANOVA was significant at $P < 0.05$ a planned comparisons test was used to locate significant differences (12).

Results. *Resting BP and heart rate (Table I).* There were no significant changes in resting BP or heart rate at any dose of oPRL on Days 4 or 7 of the infusion period.

BP responses to NOR and ANG (Fig. 1). Acute systolic and diastolic BP responses to

TABLE I. CHANGES IN RESTING SYSTOLIC BP (mm Hg), HEART RATE (bpm), AND BODY WEIGHT FROM DAYS 1 TO 4 AND DAYS 1 TO 7 IN RATS RECEIVING CHRONIC oPRL BY OSMOTIC PUMPS IMPLANTED ip ON DAY 1 ($\bar{x} \pm$ SE)

Rate of oPRL infusion (μg/hr)	Day 1 BP	Δ BP from Day 1		Day 1 heart rate	Δ Heart rate from Day 1		Day 1 body weight	Δ Body weight control from Day 1	
		Day 4	Day 7		Day 4	Day 7		Day 4	Day 7
0.00 (n = 8)	124 ± 2	−4 ± 3	0 ± 3	385 ± 16	29 ± 15	33 ± 8	339.6 ± 8.7	−8.7 ± 2.5	5.3 ± 3.9
0.15 (n = 8)	127 ± 1	−6 ± 2	−6 ± 2	384 ± 8	26 ± 8	29 ± 8	373.8 ± 4.4	2.1 ± 2.2**	8.3 ± 4.2
0.30 (n = 8)	122 ± 1	−3 ± 1	−5 ± 1	390 ± 7	14 ± 7	6 ± 10	343.0 ± 5.8	0.0 ± 1.3*	12.3 ± 3.2
0.60 (n = 8)	124 ± 1	3 ± 1	−5 ± 2	401 ± 8	34 ± 6	20 ± 11	311.4 ± 4.4	3.0 ± 2.8**	14.1 ± 3.6
1.20 (n = 7)	125 ± 2	3 ± 4	2 ± 4	369 ± 12	20 ± 14	30 ± 14	352.5 ± 12.9	−6.3 ± 2.3	−4.4 ± 3.7
2.40 (n = 8)	125 ± 1	2 ± 1	2 ± 1	385 ± 23	28 ± 11	3 ± 10	349.0 ± 6.8	−2.7 ± 2.3	6.5 ± 3.0
4.80 (n = 8)	124 ± 1	−4 ± 1	−7 ± 1	401 ± 7	13 ± 8	12 ± 4	329.3 ± 5.8	2.3 ± 2.5**	18.8 ± 1.8**

* $P < 0.05$ vs controls.
** $P < 0.02$ vs controls.

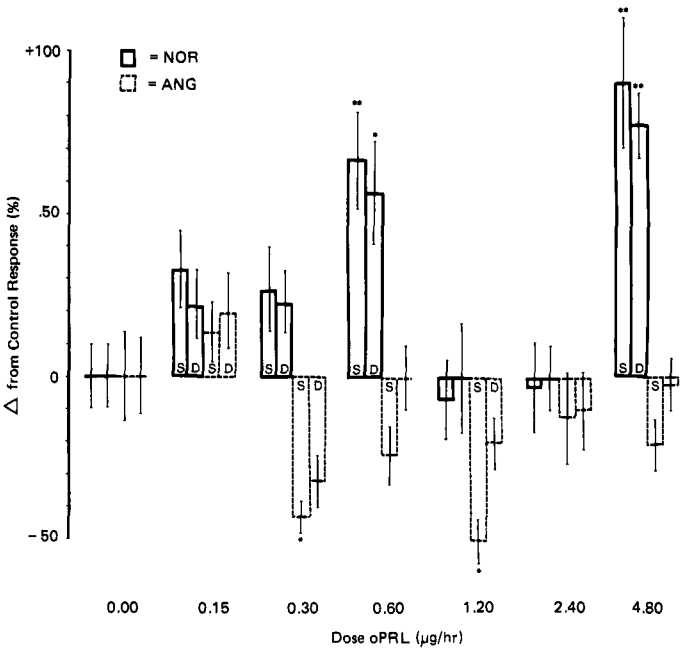

FIG. 1. Effects of chronic infusion of oPRL by osmotic pump on systolic(s) and diastolic (d) BP response to acute ia NOR (4.3 μg) and ANG (1.25 μg) in halothane-anesthetized rats (n = 8/group). Data are expressed as percentage change in response vs animals receiving NOR and ANG in the absence of oPRL ($\bar{x} \pm$ SE). * $P < 0.05$ vs controls; ** $P < 0.02$ vs controls.

ia NOR were significantly greater than controls in animals receiving 0.60 ($P < 0.02$ systolic, $P < 0.05$ diastolic) and 4.80 ($P < 0.02$ systolic and diastolic) μg oPRL/hr, and tended to be greater than controls in animals receiving 0.15 and 0.30 μg oPRL/hr. oPRL administration at rates of 1.20 and 2.40 μg/hr had no effect on the cardiovascular response to NOR.

There was a negative correlation between the systolic pressor response of anesthetized rats to NOR and the change in resting BP of conscious animals from Days 1 to 7 (r = -0.33, $P < 0.02$).

Acute systolic BP responses to ANG were significantly depressed, vs controls, in animals receiving 0.30 and 1.20 μg oPRL/hr, and tended to be lower than controls in groups receiving 0.60, 2.40, and 4.80 μg oPRL/hr. In contrast, 0.15 μg oPRL/hr tended to enhance the pressor response to ANG, although this effect was not statistically significant.

Body weight changes (Table I). Changes in body weight from Day 1 to Day 4 of the study were significantly greater in animals receiving 0.15 ($P < 0.02$), 0.30 ($P < 0.05$), 0.60 ($P < 0.01$), and 4.80 ($P < 0.02$) μg oPRL/hr than in controls. Body weight changes from Day 1 to Day 7 were significantly greater than controls in animals receiving 4.80 μg oPRL/hr ($P < 0.05$).

There was a significant correlation between the systolic BP response to NOR and body weight gain from Day 1 to Day 4 (r = 0.51, $P < 0.01$).

Circulating oPRL levels (Table II). Plasma ^{125}I-oPRL levels, calculated on Day 4 from plasma ^{125}I activity demonstrated a linear, dose-dependent increase with increasing infusion rate of the hormone (r = 0.99).

Plasma decay and size distribution of ^{125}I-oPRL. Serial lasma samples following iv ^{125}I-oPRL administration demonstrated a two-phase decay curve. There was a fast phase decay, with a $T_{\frac{1}{2}}$ of 4.3 ± 0.8 min which was evident over the first 20 min postinjection. A second slower phase was observed between 20 and 60 min postinjection. The $T_{\frac{1}{2}}$ of the latter phase was 19.9 ± 2.0 min.

Preinjection passage of a sample of ^{125}I-oPRL through Sephadex produced a peak at

TABLE II. DAY 4 PLASMA ^{125}I-oPRL CONCENTRATIONS DETERMINED BY PLASMA ^{125}I ACTIVITY

Infusion rate of hormone (μg/hr)	^{125}I oPRL determined by ^{125}I activity (ng/ml)
0.15	18*
0.30	21*
0.60	26
1.20	37
2.40	58
4.80	103*

* Calculated from extrapolation of regression line $Y = 18.3x + 15.4$, $r = 0.99$.

fraction 20 (60 ml). In contrast, ^{125}I peaked at fraction 35 (105 ml). In comparison, ^{125}I activity in plasma drawn 50 min postinjection of ^{125}I-oPRL also produced one peak corresponding to ^{125}I-oPRL. There were no other peaks detected, suggesting that plasma ^{125}I activity was a measure of ^{125}I-oPRL levels, and not smaller metabolic products.

Discussion. It has been suggested that PRL has no direct, independent actions on the heart or peripheral vasculature, and that it elicits its cardiovascular effects by altering the response of the system to other circulating pressor agents and/or by altering their release (1–7). This would suggest that the response of an animal to an oPRL infusion depends largely on the presence or absence of other circulating pressor substances.

In the present study, the administration of oPRL over a range comparable to the physiological range of endogenous PRL had no effect on resting BP or heart rate. This finding contradicts our earlier report (3), but is in agreement with the findings of McMurtry and Wexler (8). The discrepancy between the current observations and previous findings may be the result of differences in the activities of other circulating pressor substances, such as NOR and ANG, which are believed to mediate the cardiovascular effects of PRL (1, 7). It is possible that in the present study, factors such as blood volume, sympathetic activity, or salt balance were not the same as in the previous study, eliminating a pressor response in the resting animal.

Although oPRL did not visibly affect the resting, conscious animal, the cardiovascular response of anesthetized animals to bolus infusions of NOR and ANG were markedly affected by its presence. Previous studies conducted on the isolated superior mesenteric vascular bed have indicated that circulating concentrations of oPRL less than 100 ng/ml potentiate the effects of NOR and ANG, whereas concentrations of oPRL greater than 100 ng/ml inhibit the response to NOR and ANG (4, 12). Results of the present study support the observation that oPRL alters cardiovascular response to NOR and ANG, but reveal a different pattern. In the present study, oPRL potentiation of NOR occurred at calculated plasma levels of 26 ng/ml or less, and at 103 ng/ml, but not at 37 and 58 ng/ml. At no point was an inhibition of NOR effect observed. This pattern is more complex than that previously reported and may result from the use of a complex systemic model involving many, rather than one isolated, vascular beds. However, these findings do not preclude the possibility that higher oPRL levels may depress the cardiovascular response to NOR. It is also possible that the threshold for constrictor and/or depressor activity of PRL varies among vascular beds. If so, the net systemic effect of PRL might be negated at certain infusion rates, complicating the dose response curve. There is no possibility that nonfunctional osmotic pumps accounted for the absence of an alteration of reactivity to NOR at oPRL doses of 1.20 and 2.40 μg/hr, since an inhibition of the response to ANG could be demonstrated.

In contrast to its interaction with NOR, oPRL only depressed the systemic pressor response to ANG. This differs from earlier reports (1, 4) which describe a potentiation of ANG effects at oPRL levels less than 100 mg/ml, and an inhibition at oPRL levels above 100 ng/ml.

An interesting observation in the present study was that groups which tended to show an enhanced responsiveness to NOR also demonstrated a greater rate of weight gain than those groups not affected by oPRL. A number of studies have suggested that PRL acts on the mammalian kidney to promote Na^+, K^+, and water retention (13–15), suggesting that at the former doses, oPRL may also be altering water

and electrolyte balance and favoring fluid retention. However, whether this effect is primary or secondary to the altered vascular reactivity cannot be determined from the data.

Infusion rates of oPRL in the present study ranged from 2.5 to 80.0 ng/min. These are comparable with endogenous PRL production rates in the male rat which have been calculated to be in the range of 34–60 ng/min, and correspond to serum levels of 24–46 ng/ml (16, 17). Evidence that oPRL was, in fact, released in significant amounts was found by releasing ^{125}I-oPRL at rates similar to those used in the study and monitoring plasma ^{125}I activity on Day 4. Since the half-life of the ^{125}I activity was similar to that of PRL, and Sephadex chromatography demonstrated that all of the plasma ^{125}I activity eluted in the same fraction as oPRL, it can be concluded that the plasma ^{125}I activity was associated with ^{125}I-oPRL, rather than its metabolites. However, correlation of absolute plasma oPRL levels with various responses should be done with caution due to possible variations in biological activity of the oPRL in the rat system.

There are conflicting reports on the levels of PRL in hypertensive conditions (18–20), and the role of PRL in the development and maintenance of elevated blood pressure (21–23). The results of the present study support the idea that PRL may play a role in cardiovascular regulation. However, it appears that the effects of oPRL on the cardiovascular system may become evident only under circumstances in which they are accompanied by heightened sympathetic activity, such as during stress or disturbances in water and electrolyte regulation, and in certain forms of hypertension.

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