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The Cardiovascular Effects of Porcine Relaxin in Brattleboro Rats

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The effects of porcine relaxin on arterial blood pressure, heart rate, and the release of vasopressin and oxytocin were investigated in homozygous diabetes insipidus (di/di) Brattleboro and Long-Evans rats. Acute iv injection of relaxin (5 μg) caused a significant increase in mean arterial, systolic and diastolic blood pressures in Long-Evans rats compared with control injections of saline, but had no pressor effect in Brattleboro rats. Circulating concentrations of vasopressin were also significantly elevated above baseline in the Long-Evans rats 1 min after relaxin treatment, but remained undetectable in the relaxin-treated Brattleboro rats. Relaxin increased heart rate in both groups of animals 4 min after injection. The chronotropic effect of relaxin was, however, attenuated in the Brattleboro rats. Intravenous relaxin injection also caused a significant increase in plasma oxytocin concentrations 5 min posttreatment in both the Long-Evans and Brattleboro rats. The change in plasma oxytocin above basal concentrations was significantly greater in Brattleboro rats compared with Long-Evans controls.

The data in this study demonstrate that iv relaxin increases heart rate, but not arterial blood pressure in Brattleboro rats. Furthermore, the relaxin-induced release of oxytocin in Brattleboro rats does not result in an acute pressor response.

Key Words: Blood pressure; Brattleboro; heart rate; oxytocin; relaxin; vasopressin.

Introduction

In 1926, Hisaw *(1)* discovered an ovarian hormone that caused relaxation of the pelvic ligament. The hormone was named relaxin. Since that time, there has been a great deal of work showing that circulating relaxin is essential for successful birth and lactation in rats *(2)*. Endogenous relaxin promotes growth and softening of the cervix at term *(3)* and growth of the vagina *(4)* to allow for delivery of the young. Contractions of the uterus in the second half of pregnancy, until immediately before birth, are reduced by the action of relaxin *(5)* preventing premature delivery. Relaxin also stimulates development of mammary nipples *(6,7)* so the mother can suckle her young successfully.

Relaxin also acts on the brain *(8)*. Treatment of rats with exogenous relaxin suppresses reflex milk ejection *(9,10)* and results in a prolonged pressor response *(11–14)*. Relaxin affects the release of a number of hypothalamic and pituitary peptides, including oxytocin and vasopressin *(10,13,15,16)*, luteinizing hormone *(17)*, and prolactin *(18,19)*. Recently it has been suggested that there is a separate relaxin "system" within the brain *(20,21)*, and that relaxin may be responsible for controlling the time of birth *(20)* and increased drinking seen in pregnancy *(21)*.

There are major changes in cardiovascular control during pregnancy *(22,23)*, and it has been suggested that endogenous relaxin may have a role in these changes *(21)*. It is therefore important to understand the effects of relaxin on the brain. The pressor response to exogenous relaxin appears to be mediated by the release of vasopressin *(11,15)*, whereas the tachycardic response may be owing to a direct action of relaxin on the heart *(24,25)*. There is some evidence that the tachycardia is modified by β-adrenoceptor blockade *(26)*.

Homozygous diabetes insipidus (di/di) Brattleboro rats have a single base deletion in the vasopressin gene *(27)*, which leads to the expression of an aberrant vasopressin precursor. This mutant vasopressin precursor appears to be retained in the endoplasmic reticulum and does not enter the secretory pathway *(28,29)*. Therefore, Brattleboro rats have the normal complement of hypothalamic vasopressin neurons, but neither the precursor nor the peptide is detected in secretory granules in the neural lobe.

Brattleboro rats therefore provide an ideal model to confirm the importance of the role of vasopressin in the car-

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Table 1
Effect of iv Injection of 5 µg Porcine Relaxin on Systolic and Diastolic Blood Pressures (Mean ± SEM mmHg) in Anesthetized Long-Evans and Brattleboro Rats

	0 Min	2.5 Min	5 Min	7.5 Min	10 Min	15 Min	20 Min	30 Min
Long-Evans rats after relaxin								
Systolic	114.4 ± 4.7	120.7 ± 3.4[a]	124.8 ± 4.3[a]	123.8 ± 4.2[a]	122.1 ± 5.0[a]	121.1 ± 5.2[a]	119.7 ± 4.1[a]	116.7 ± 3.6
Diastolic	86.0 ± 2.4	92.8 ± 2.5[a]	94.6 ± 2.3[a]	95.3 ± 2.8[a]	96.9 ± 3.1[a]	93.3 ± 1.5[a]	91.3 ± 2.3[a]	87.6 ± 2.5
Long-Evans rats after saline								
Systolic	111.4 ± 2.6	112.1 ± 2.8	114.8 ± 3.2	113.6 ± 3.3	112.9 ± 3.4	111.5 ± 2.1	112.9 ± 4.4	112.7 ± 3.5
Diastolic	86.5 ± 2.0	86.7 ± 1.9	84.6 ± 2.3	85.6 ± 1.8	84.9 ± 3.2	86.3 ± 1.5	85.3 ± 2.7	84.6 ± 3.0
Brattleboro rats after relaxin								
Systolic	110.2 ± 2.7	110.1 ± 1.0	112.3 ± 1.7	113.7 ± 1.5	114.0 ± 1.9	113.9 ± 1.8	110.0 ± 2.2	109.8 ± 2.6
Diastolic	77.1 ± 2.3	76.9 ± 2.1	79.0 ± 2.2	80.3 ± 2.7	79.4 ± 1.9	80.0 ± 2.1	77.6 ± 2.2	76.9 ± 2.7
Brattleboro rats after saline								
Systolic	112.3 ± 3.1	110.4 ± 4.4	112.8 ± 4.4	113.3 ± 3.9	112.0 ± 5.2	111.8 ± 3.7	110.8 ± 3.9	110.3 ± 3.9
Diastolic	76.0 ± 2.1	77.1 ± 1.1	76.8 ± 1.6	78.3 ± 2.5	74.5 ± 2.9	76.7 ± 2.0	79.0 ± 3.1	77.9 ± 3.5

[a]Significant increases in blood pressure compared with relaxin-treated Brattleboro rats and with saline controls.

diovascular responses to exogenous relaxin and to examine the relative importance of the direct chronotropic effect of relaxin without circulating vasopressin. Since exogenous relaxin also stimulates the release of oxytocin in intact rats, we also examined the effects on oxytocin secretion in these vasopressin-deficient animals.

Results

All data are expressed as mean ± SEM, and reference to statistical significance is at the 95% level ($p < 0.05$).

Effects of Exogenous Relaxin on Cardiovascular Parameters

Mean arterial pressure in anesthetized Brattleboro rats (88.1 ± 2.6 mmHg) was significantly lower than Long-Evans controls (95.5 ± 2.7 mmHg) during the 30 min pretreatment recording period. The data for the pretreatment period were averaged (Table 1). Control iv injections of saline produced no significant changes in blood pressure or heart rate in either group of rats.

Acute iv injection of 5 µg porcine relaxin caused a rise in systolic and diastolic blood pressure (Table 1) and mean arterial pressure (Fig. 1) in Long-Evans rats compared with both pretreatment values and control iv injection of saline. Increases in blood pressure were significant 2.5 min after relaxin treatment and remained elevated for 15 min (Fig. 1; Table 1). Relaxin also provoked a significant increase in heart rate compared with iv saline controls. Heart rate increased from basal (230.6 ± 3.6 beats/min) to a peak of 267.3 ± 3.3 beats/min 5 min after relaxin injection, and then remained significantly elevated above pretreatment values for 15 min (Fig. 2).

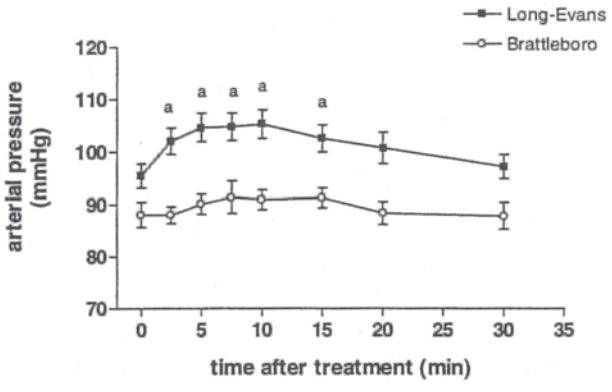

Fig. 1. Effects of iv injection of porcine relaxin (5 µg in 0.1 mL saline) on mean arterial blood pressure in Brattleboro rats (o: $n = 8$) and Long-Evans controls (■: $n = 8$). Data are expressed as mean ± SEM blood pressure (mmHg). [a]Significant elevations above pretreatment values.

In contrast, iv injection of relaxin did not induce a pressor effect in Brattleboro rats during the 20-min recording period (Fig. 1). The increase in both systolic and diastolic blood pressure observed in these animals between 5 and 10 min after relaxin administration (Table 1) was not statistically significant compared with iv saline injection in the same animal. However, there was a significant increase in heart rate above pretreatment values (242.9 ± 3.4 beats/min), 7.5 min after relaxin injection (Fig. 2A). Peak increases (254.5 ± 3.9 beats/min) were measured 10 min after relaxin injections, and heart rate remained elevated for a further 5 min posttreatment (Fig. 2A). In comparison with Long-Evans rats, the onset

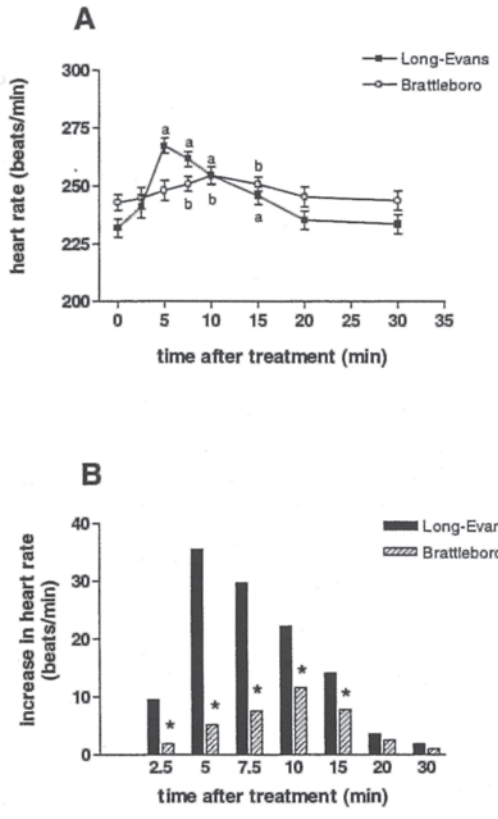

Fig. 2. Effects of iv injection of porcine relaxin (5 µg in 0.1 mL saline) on heart rate in Brattleboro rats (o: $n = 8$) and Long-Evans controls (■: $n = 8$). Data are expressed as mean ± SEM heart rate (beats/min). [a]Significantly ($p < 0.05$) elevated above baseline (pretreatment values) in Long-Evans and [b]Significantly ($p < 0.05$) elevated above baseline in Brattleboro rats. The upper graph represents heart rate data, whereas the lower histogram shows the increase in heart rates above pretreatment values in the two groups of rats. *Note that the increase in heart rate was significantly ($p < 0.05$) lower in Brattleboro rats compared with Long-Evans controls.

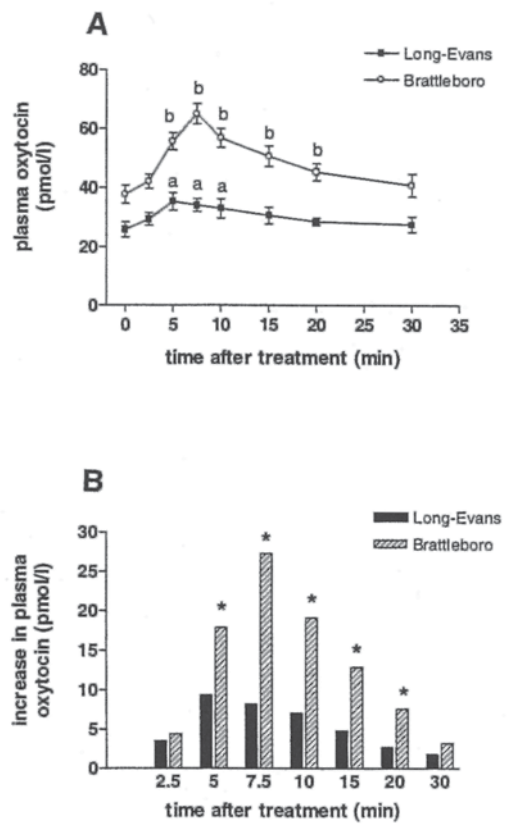

Fig. 3. Effects of iv porcine relaxin (5 µg in 0.1 mL saline) on mean ± SEM plasma oxytocin concentrations (pmol/L) in Brattleboro rats (o: $n = 9$) and Long-Evans controls (■: $n = 8$). [a]Significantly ($p < 0.05$) elevated above baseline (pretreatment values) in Long-Evans and [b]significantly ($p < 0.05$) elevated above baseline in Brattleboro rats. The upper graph shows plasma oxytocin concentrations, whereas the lower histogram shows the increase in oxytocin concentrations above pretreatment values. *Note that the increase in plasma oxytocin was significantly ($p < 0.05$) higher in Brattleboro rats compared with Long-Evans controls.

of the tachycardia in response to relaxin in the Brattleboro rats was delayed, and the increases in heart rate in Brattleboro rats were significantly lower compared with Long-Evans controls (Fig. 2B).

Effects of Exogenous Relaxin on Plasma Vasopressin and Oxytocin Concentrations

Mean basal plasma vasopressin and oxytocin concentrations in Long-Evans rats were 30.6 ± 2.2 and 26.3 ± 1.3 pmol/L, respectively. In Brattleboro rats, baseline vasopressin concentrations were below the sensitivity of the assay (<8.5 pmol/L), whereas oxytocin concentrations (36.4 ± 3.2 pmol/L) were significantly greater than Long-Evans controls (Fig. 3). Saline injection did not affect plasma oxytocin or vasopressin concentrations compared with pretreatment values in either group of rats.

Intravenous relaxin in the Long-Evans rats caused a significant increase in both plasma vasopressin and oxytocin compared with pretreatment levels. These increases were also significantly greater than those observed after control injections of saline (data not shown). Data for oxytocin levels are shown in Fig. 3. Peak increases (vasopressin: 81.3 ± 2.4 pmol/L; oxytocin: 35.3 ± 2.9 pmol/L) were measured between 5 and 10 min after relaxin injection. Thereafter only vasopressin concentrations remained significantly elevated above baseline for the 30-min sampling period. In contrast, iv injection of relaxin had no effect on plasma vasopressin concentrations in Brattleboro rats. However, there was a significant increase in plasma oxytocin 5 min after relaxin injection. Peak increases (65.0 ± 3.4 pmol/L) were measured 7.5 min after relaxin injection, and plasma oxytocin remained elevated for 20 min postinjection (Fig. 3A). In comparison with the Long-Evans rats, the relaxin-induced increases in plasma oxytocin in the Brattleboro rats were significantly higher between 5 and 20 min after treatment (Fig. 3B).

Discussion

Previous work has shown that the pressor effect of relaxin is mediated through the action of relaxin in the brain and the release of vasopressin from the neurohypophysis *(11,13)*. Since Brattleboro rats are incapable of synthesizing bioactive vasopressin *(27)*, experiments were carried out to investigate whether or not exogenous relaxin induced cardiovascular effects in Brattleboro rats.

In general, the cardiovascular and hormonal responses of the Long-Evans rats were similar to those reported for Wistar *(10,14)* and Sprague-Dawley rats *(11,13)*. However, responses of the Brattleboro rats were different. In the Brattleboro rats, there was no pressor response to exogenous relaxin, and no vasopressin secretion was observed. There was a tachycardic response to exogenous relaxin, but this was attenuated in Brattleboro rats compared with the response in Long-Evans controls. In contrast, the oxytocinemic response to relaxin was exaggerated in Brattleboro rats compared with Long-Evans controls.

Evidence has accumulated to support the view that the pressor response to exogenous relaxin is primarily owing to the release of vasopressin. For example, central *(14,30)* and peripheral *(13,15)* injection of relaxin results in a rise in circulating plasma vasopressin concentrations, and blockade of vasopressin V1 receptors in the systemic circulation negates the pressor effect of relaxin *(11)*. This relaxin-induced release of vasopressin appears to be mediated by central angiotensin II, since blockade of angiotensin II receptors in the brain blocks the pressor response and the vasopressinemic response to relaxin *(12,15)*. Furthermore, Summerlee and Parry *(26)* reported that α- and β-adrenoceptor blockade and antagonism of the renin-angiotensin system in the periphery did not affect the pressor response to relaxin. In the current experiments, there was no pressor response to exogenous relaxin observed in the Brattleboro rats. Since these animals are incapable of releasing functional vasopressin *(27)*, the data are consistent with the view that vasopressin is the key component of the pressor response to exogenous relaxin in rats.

There was a difference in the tachycardia observed after relaxin treatment in the two groups of rats: in Long-Evans rats, there was a rapid and substantial increase in heart rate that was maintained for at least 15 min; in contrast, the onset of the relaxin-induced tachycardia was delayed in the Brattleboro rats and attenuated compared with the response in Long-Evans rats. The tachycardic response to exogenous relaxin in Long-Evans could be owing to both direct and indirect actions of relaxin. There are relaxin binding sites in heart muscle *(31)*, and experiments in vitro have confirmed that exogenous relaxin applied to cardiac tissue causes an increase in heart rate *(32,33)*. The second possible mechanism could be the indirect action of relaxin, releasing vasopressin, which causes a pressor response and a tachycardia. However, an increase in blood pressure is usually accompanied by a decrease in heart rate, which suggests that there is a direct action of relaxin on the heart. Both vasopressin and relaxin have chronotropic actions. Therefore, comparing the data in Fig. 2B, it is possible to determine the contribution of these two hormones to the rise in heart rate: Long-Evans rats (vasopressin-intact) show a greater increase in heart rate, which is presumably the combined effect of circulating vasopressin and the exogenous relaxin; in contrast, the smaller rise in heart rate seen in the Brattleboro rats (vasopressin-deplete) represents the tachycardic action of relaxin alone. The contribution from relaxin occurs later after treatment than the vasopressin effects. The third possible mechanism could be via relaxin-stimulated activation of the brainstem cardiovascular centers, although this is unlikely, since evidence suggests that central injection of relaxin is followed by a bradycardia in anesthetized rats *(14)*. Since a standard dose of relaxin was given in the current set of experiments to both the Brattleboro and Long-Evans rats, it is likely that the difference between the two groups of animals reflects the direct action of relaxin on the heart in the Brattleboro rats and this direct action plus the effects of increasing circulating vasopressin in the Long-Evans rats. It is also possible that relaxin could act through the circumventricular organs to activate the forebrain angiotensin system *(8)*, which, in turn, affects brainstem cardiovascular centres to increase heart rate. This second possibility needs to be studied further, especially in light of the evidence that function of the forebrain angiotensin system may be compromised in Brattleboro rats *(34)*.

The current experiments revealed a difference in the oxytocinemic response to exogenous relaxin between Brattleboro and Long-Evans rats. Injection of exogenous relaxin in Long-Evans rats caused a small, but significant elevation in plasma oxytocin. This is similar to reports of the action of relaxin on oxytocin release in Wistar and Sprague-Dawley rats *(10,13,15)*. However, relaxin caused a substantial release of oxytocin in Brattleboro rats reaching peak levels twofold greater than the highest levels observed in Long-Evans rats. The mechanism of this enhanced response of the oxytocin system and its functional significance is not known. The finding that basal plasma oxytocin concentrations were significantly higher in Brattleboro rats compared with Long-Evans rats is similar to previous findings *(34)*, and implies that the oxytocin system is upregulated in Brattleboro rats and provides an exaggerated response to any stimulant for the neurohypophyseal hormone system.

In summary, these data provide confirmation that the pressor response to exogenous relaxin is owing to the release of vasopressin. They also show that the tachycardic response following an injection of relaxin is a combination of a direct action of vasopressin and relaxin on the heart in intact rats. The oxytocinemic response of the Brattleboro rats is exaggerated compared with Long-Evans controls, and this deserves further investigation.

Materials and Methods

Animals

Primiparous Long-Evans and homozygous diabetes insipidus Brattleboro rats (200–250 g body wt; Harlan Sprague Dawley, Indianapolis, IN) were used in these studies. Experiments and animal handling were carried out in accordance with the guidelines established by the Canadian Council for Animal Care and approved by the Animal Care Committee of the University of Guelph. The rats were caged singly in the Central Animal Facility and maintained under controlled environmental conditions (14-h light: 10-h dark lighting regime, ambient temperature 18°C) with food and water available ad libitum.

Relaxin

Highly purified porcine relaxin (cma' fraction: potency 3000 guinea pig U/mg) was used throughout this study. Although there are known differences in the amino acid structure of porcine vs rat relaxin, and there is a potential for different biopotency of the two peptides, porcine relaxin was used in the current study in order to make comparison with other studies where porcine relaxin was used. The hormone was prepared in the Department of Anatomy (University of Bristol, Bristol, Avon, UK) by extraction and purification of sow corpora lutea (ovaries supplied by the Meat and Livestock Commission, Bristol, Avon, UK), using the method of Sherwood and O'Byrne *(35)*. Purity of the relaxin preparation was established through SDS-PAGE and immunostaining *(36)*. Each animal was given only one injection of relaxin to avoid the possibility of tachyphylaxis.

Surgical Preparation of the Animals

Rats were anesthetized with a single injection of urethane (ethyl carbamate, 1.25 g/kg ip; Sigma Chemical Co., St. Louis, MO) and xylazine (Rompun, 2 mg/kg im; Bayvet, Chemagro Ltd., Etobicoke, Ontario). The left saphenous vein was cannulated in all rats (polythene tubing id 0.28 mm, od 0.61 mm Portex Tubing Ltd., Arnold and Horwell, London, UK) for iv administration of drugs.

Effects of Exogenous Relaxin on Cardiovascular Parameters

The right common carotid artery was cannulated (polyethylene tubing id 0.58 mm, od 0.96 mm) and connected to a Spectramed pressure transducer (Model P23XL; Grass Instrument Co., Quincy, MA) for the direct measurement of arterial pressure. To prevent clotting, the arterial cannula was filled with heparinized saline (heparin sodium injection USP, 100 U/mL: Allen and Hanburys, Toronto, Ontario). Heart rate was recorded using subdermal electrodes attached to a tachometer. After surgery, the animals were left undisturbed for 30 min. Baseline blood pressure and heart rate were recorded continuously for a further 30 min on a calibrated Grass polygraph (Model 7C) before any treatments were administered. Each animal then received an iv injection of 0.1 mL saline (control), followed 20 min later by an iv injection of a standard dose of 5 μg porcine relaxin (in 0.1 mL saline). The standard dose of relaxin was used to be able to compare findings with previously published work. Blood pressure and heart rate were recorded for a further 30 min after relaxin treatment.

Systolic and diastolic blood pressure, mean arterial pressure, and heart rate were measured 2.5, 5, 7.5, 10, 15, 20, and 30 min after each treatment. The effects of iv relaxin on systolic and diastolic blood pressures, mean arterial pressure, and heart rate in Brattleboro rats were then compared with pretreatment values, control injections of saline in the same animal, and the response to iv relaxin in Long-Evans rats.

Effects of Exogenous Relaxin on Vasopressin and Oxytocin Release

A second set of experiments was done to examine the effects of exogenous relaxin on vasopressin and oxytocin release in eight Brattleboro and eight Long-Evans rats. The rats were anesthetized as described above and the left femoral artery of each animal was cannulated (polyethylene tubing id 0.58 mm, od 0.96 mm). The cannula was connected to a Spectramed pressure transducer for the direct measurement of systemic blood pressure. The right common carotid artery (id 0.58 mm, od 0.96 mm), for taking blood samples, and the left external jugular vein was cannulated in the midneck region (id 0.63 mm, od 1.4 mm) and connected to a Sage infusion pump (Model 351; Sage Instruments, Orion Research Inc., Boston, MA) for simultaneous replacement of fluid during blood sampling. All cannulae were filled with heparinized saline (100 U/mL) to prevent the blood from clotting. Animals were left for at least 30 min after surgery.

Blood samples (1 mL) were collected from the carotid cannula into chilled, heparinized (100 U) polypropylene 1.5 mL microcentrifuge tubes immediately before, and 2.5, 5, 7.5, 10, 15, 20, and 30 min after iv injection of either saline (0.1 mL: $n = 3$) or porcine relaxin (5 μg relaxin in 0.1 mL saline: $n = 5$). At the same time, an equal volume of heparinized whole blood from donor rats was infused through the jugular vein at a rate equivalent to the blood loss (approx 1 mL/min). Blood pressure was monitored throughout the sampling period. This technique has been shown previously to have no significant effect on resting blood pressure, heart rate, or basal vasopressin or oxytocin concentrations in anesthetized Sprague-Dawley rats *(6)*. The blood samples were stored on ice for a maximum of 15 min before centrifugation at 2000*g* for 15 min. Plasma was decanted and acidified (1 *M* HCl: 0.1 mL/mL plasma) and stored at –70°C until assay.

The effects of iv relaxin on mean plasma vasopressin concentrations in Brattleboro rats were compared with pretreatment basal values, plasma concentrations in

saline-treated controls, and the response to iv relaxin in Long-Evans rats.

Radioimmunoassays

Plasma arginine vasopressin and oxytocin concentrations were measured in triplicate by specific radioimmunoassay *(37)*. The First International Standard 77/501 (National Biological Standards Board, London, UK) was used as the reference peptide for the vasopressin assay with antiserum 84/1 at a final dilution of 1:140,000. The lower limit of detection was 8.5 pmol/L, and the inter- and intra-assay coefficients of variation were 8.1 and 2.6%, respectively. The oxytocin RIA used the Fourth International Standard and antiserum 85/2 at a final dilution of 1:16,000. The lower limit of detection was 4.8 pmol/L, and the inter- and intra-assay coefficients of variation were 6.8 and 2.1%, respectively. Both antisera were raised and characterized by S. D. Birkett in the Department of Anatomy, University of Bristol, UK. Crossreactivities of these antisera were <0.02% for a variety of hypothalamic peptides (oxytocin and vasopressin neurophysins, vasotocin, somatostatin, and GnRH).

Statistical Analysis

Differences in mean blood pressure, mean heart rate, and mean plasma hormone concentrations were analyzed by two-way analysis of variance (ANOVA), and significant differences between pairs of means were assessed using the Neuman-Kreuls means comparison test (SYSTAT). To compare the data between Long-Evans and Brattleboro rats, it was necessary to calculate the change in blood pressure, heart rate, and hormone concentration above basal because the two groups of animals had significantly different baseline values. Differences in the mean change above baseline (pretreatment) between the Brattleboro and Long-Evans rats were then analyzed by one-way ANOVA. In all cases, statistical significance was set at the 95% level ($p < 0.05$).

Acknowledgment

This article is dedicated to the memory of David G. Porter from whom the supplies of porcine relaxin were inherited.

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