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The Dipsogenic Effects of Rat Relaxin: The Effect of Photoperiod and the Potential Role of Relaxin on Drinking in Pregnancy*

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ABSTRACT

Experiments were done to examine whether rat relaxin is dipsogenic and whether such dipsogenic effects of rat relaxin are related to time of injection during the light-dark cycle. Female rats were fitted with a chronic intra-cerebro-ventricular (icv) cannula. Rat relaxin (2.5, 5, 10, 25, 50, or 100 ng/2 μl in 0.9% saline) was injected into the right lateral ventricle at either morning (0800–1000 h), afternoon (1400–1600 h), or night (2200–2400 h), and water consumption was measured. Relaxin caused a dose-dependent dipsogenesis at doses $\geq$ 5 ng, but the sensitivity and magnitude of the response varied with the photoperiod. Water consumption was smallest (3.5 $\pm$ 0.7 ml at 50 ng) and least sensitive (minimal effective dose at 25 ng) in the afternoon and maximal (17.7 $\pm$ 2.3 ml at 50 ng) and most sensitive (minimal effective dose 5 ng) at night. The latency from injection to drinking was 55.8 $\pm$ 10.4 sec (mean $\pm$ SEM) and did not vary significantly with either the dose or time of day.

A second set of experiments was done to examine the effects of neutralizing the central actions of relaxin on drinking behavior in pregnancy. Pregnant rats were injected daily, through a chronically implanted icv cannula, with either a specific monoclonal antibody raised against rat relaxin from day 12 to day 22 of gestation or with saline as a control. Drinking and eating behavior and weight gain were monitored every 12 h during pregnancy. There was a significant decrease in water consumed at night, but no effect on drinking during the day in relaxin-neutralized rats. These animals also showed a decrease in weight gain during pregnancy compared with controls and gave birth to lighter-weight litters.

These data provide evidence that the dipsogenic response to exogenous rat relaxin in female rats varies with time of injection during the light-dark cycle and suggest that relaxin in the brain may have a role in nighttime drinking behavior during the second half of pregnancy. (*Endocrinology* **139:** 2322–2328, 1998)

CIRCULATING relaxin is essential for successful birth and lactation in rats (1). Endogenous relaxin promotes growth and softening of the cervix (2) and growth of the vagina (3) to allow for delivery of the young. Uterine contractions during the second half of pregnancy until shortly before birth are reduced by the actions of relaxin (4), protecting fetuses against premature delivery. Finally, relaxin stimulates development of mammary nipples (5, 6) so the mother rat can suckle her young successfully.

Relaxin also has actions on the brain (7). Treatment of rats with exogenous relaxin suppresses reflex milk ejection (8, 9) and causes a profound pressor response (10–13). Relaxin affects the release of a number of hypothalamic and pituitary peptides including oxytocin and vasopressin (9, 12, 14, 15), LH (16), and PRL (17, 18). More recently there have been reports that centrally administered relaxin is dipsogenic (19, 20), although the physiological significance of these findings has not been proven.

During the second half of pregnancy in rats there are several changes in cardiovascular control (21, 22): plasma volume expands, blood pressure falls, plasma osmolality decreases, and glomerular filtration rate increases. These changes imply that there is a major shift in the central thresholds for cardiovascular control in pregnancy. To accommodate these changes, there is a substantial increase in drinking (22). Using monoclonal antibodies to neutralize the effects of endogenous relaxin in the periphery, Zhao and colleagues (23) demonstrated that endogenous circulating relaxin had marginal effects on drinking during pregnancy: relaxin-neutralized rats consumed less water during the day compared with intact controls. Recently Omi and colleagues (24) expanded these observations by showing that peripheral injection of exogenous relaxin promotes only moderate increases in water intake during late pregnancy in rats and does not affect drinking during the night. These authors suggested that the more likely explanation was that circulating relaxin might enter the cerebrospinal fluid and have a central action on drinking. However, they suggested that it was possible that relaxin produced within the brain could have a local action on drinking behavior. There is evidence that relaxin is synthesized within the brain (25), and recent data from our laboratory suggest that passive neutralization of relaxin in the brain may reveal roles for relaxin that are different from those observed for relaxin in the systemic circulation (26).

There were two objective for the current experiments: 1) to investigate the dipsogenic response to rat relaxin at different times of the light-dark cycle, and 2) to examine the effects of central administration of monoclonal antibodies specific to rat relaxin on drinking behavior of pregnant rats.

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Materials and Methods

All experiments were carried out in accordance with the guidelines established by the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Guelph.

Animals

Primiparous pregnant and nonpregnant retired-breeders Sprague-Dawley rats (230–320 g; Harlan Sprague-Dawley, Indianapolis, IN) were used in these studies. Pregnant animals were bred at approximately 90 days. The day on which sperm were found in the vagina was designated as day 1 of pregnancy, and 0300 h on day 1 was arbitrarily chosen to represent the estimated time of insemination. The rats were caged individually in the Central Animal Facility of the University of Guelph. Food and water were available *ad libitum*. All rats were maintained on a 12-h light, 12-h dark lighting regimen with lights on at 0600 h. The ambient room temperature was 18 C.

Surgical preparation of the animals

Rats were fitted with a chronic indwelling microcannula for injection into the ventricular system of the brain. The details of the technique for implantation of the microcannula are described elsewhere (19). Animals were anesthetized with diazepam (Valium, 10 0.8 mg ip; Hoffman La Roche Ltd., Etobicoke, Ontario, Canada), xylaxine (Rompun, 4 mg ip; Bayer Inc., Agriculture Division, Animal Health, Etobicoke, Ontario, Canada), and ketamine hydrochloride (Rogarsetic, 4 mg ip; Rogar STC Inc., London, Ontario, Canada) and a placed in a stereotaxic frame (Narishige SR6: Tokyo, Japan). Under strict aseptic conditions a sterilized microcannula (220A; Kopf Instruments, San Francisco, CA) was placed with its tip in the right lateral cerebral ventricle: coordinates = bregma, 2.5 mm lateral to the midline and 3.0 mm ventral to the cortical surface (27). The cannula was sealed in place using cold-curing acrylic, and the animals were allowed to recover. Postoperative analgesic [meperidine HCl (Demerol) 2.5 mg every 4 h im] was given for 24 h. As the operations took 2–3 h to complete, rats were given 15 ml lactated Ringer's solution (sc) to ensure adequate hydration but were not given postoperative antibiotic. The animals were allowed to recover for at least 2 days before treatment.

Rat relaxin and monoclonal antibodies to rat relaxin and fluorescein

Rat relaxin was a gift from D. G. Porter. Freeze-dried hormone was stored at 4 C until use when it was reconstituted in 0.9% sterile saline. Purified monoclonal antibody to rat relaxin and antibody to fluorescein were produced according to the methodology described by Lao Guico-Lamm *et al*. (28). Details of the purification and characterization of the monoclonals used in the current study are described elsewhere (26). Purified antibody was freeze-dried and stored at 4 C until use when it was reconstituted in PBS at a concentration of 1 mg protein/ml.

Exp 1: effect of exogenous rat relaxin on drinking behavior in rats

Eight nonpregnant rats were fitted with an icv microcannula as described above. Five days after implantation, a daily routine was established for testing the dipsogenic effects of rat relaxin. The dose-dependent effects of rat relaxin on drinking behavior were established at three time points in the circadian cycle: morning (0800–1000), afternoon (1400–1600), and evening (2200–2400). Rats were treated in a randomized, blinded fashion with one dose per day of either rat relaxin (2.5, 5, 10, 25, 50, 100 ng relaxin in 2 μl 0.9% saline), angiotensin II (Sigma Chemical Co., St. Louis, MO; 10 ng in 2 μl saline), monoclonal antibody (MCA-3) to rat relaxin (2 μg in 2 μl saline) plus 50 ng relaxin in 2 μl saline, or 2 μl saline alone. Four repeats of each dose were tested so each animal received a total of 36 injections (nine different treatments with four repeats of each treatment).

With the exception of nighttime injections, water-replete rats were brought into the laboratory at least 30 min before testing and allowed free access to water. The animals were lifted from the cage, held lightly, and injected with the test solution through the neoprine seal of the microcannula using a sterilized Hamilton 10-μl microsyringe (701N; Hamilton Co., Reno, NV) and replaced in the observation cage. (The microsyringe needle was cut down to ~5 mm to make injection through the seal easier). Water consumption was measured from a graduated pipette attached to the side of a plexiglass observation cage. The tip of the pipette was modified in the laboratory to allow rats to drink without water loss from the tip of the pipette. The latency to the onset of drinking and the volume of water consumed were recorded every 5 min for 30 min. For the experiments at night (2200–2400), the animals were injected in the animal house under red light illumination.

Exp 2: effect of central injection of monoclonal antibodies to relaxin on drinking behavior in pregnancy

The effect of neutralizing endogenous rat relaxin on drinking behavior during pregnancy in 12 rats was examined. Rats were fitted with an icv cannula and left for at least 7 days before mating. The rats were divided, at random, into three experimental groups: group A rats were injected each morning (between 0900 h and 1000 h) from day 12 of pregnancy to day 22 with 1 μl monoclonal antibody to rat relaxin in PBS icv (concentration 1 mg/ml: n = 6); group B rats were injected with 1 μl PBS alone intra-cerebro-ventricular (icv) (n = 6); and group C rats were injected with 1 μl monoclonal antibody to fluorescein in PBS icv (concentration 1 mg/ml: n = 4). Rats were weighed daily, and water and food consumption were monitored twice each day at 0600 h and 2000 h from day 12 of gestation to delivery in rats. Water and food consumption were calculated by weighing the water bottle and food remaining at each sample period.

Animals were watched continuously from 0400 h on day 20 of pregnancy. Observations at night were carried out under red light illumination. The time to the onset of delivery of the first fetus, the number of young delivered, and the live birth rate were recorded. The length of time from putative insemination (0300 h on day 1) to the time of delivery of the first fetus was calculated.

Analysis of data

For Exp 1 (dose dependency), the mean values of each group were compared by one-way ANOVA and Neuman-Kreuls multiple comparison procedure to test for statistical difference between pairs of means. Two-way ANOVA was used for comparing the drinking response at different times of day. Students' *t* test was used for comparison of means in experiments in which independent groups of means were tested. Significance was determined at the 5% level.

For Exp 2, daily consumption of water and consumption during either the light or dark phase of the cycle were analyzed by a linear plot model for relaxin-neutralized and control-treated rats and the days of pregnancy. The effect of neutralizing relaxin was tested with an F statistic of the ratio of the variation between the relaxin-neutralized (group A), PBS-treated (group B), and monoclonal control group (group C).

Confirmation of the sterility of the relaxin and monoclonal antibody samples

Evidence indicates that cytokine and endotoxin induce the release of a variety of hypothalamic peptides (29, 30) and affects body temperature (31) when injected i.c.v; therefore, the sterility of the samples tested in the current study was examined for the presence of bacterial growth and endotoxin. Separate batches of the relaxin or monoclonal antibody to rat relaxin were made up in sterile solutions and frozen until use. Each batch was used for a maximum of 7 days. At the end of the experimental period, the samples were tested for bacterial or endotoxin contamination (Clinical Pathology Lab., Ontario Veterinary College, Guelph, Ontario, Canada).

Confirmation of the site of injection

At the end of the trials, rats were deeply anesthetized with an overdose of barbiturate ip and 5 μl Indian Ink injected through the microcannula before the animal stopped breathing. After death, the brain was removed, and the presence of ink in the fourth ventricle and the central canal of the spinal cord was taken as proof that the tip of the microcannula was in the ventricular system.

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Results

Exp 1: effect of exogenous rat relaxin on drinking behavior in rats

Rat relaxin icv caused an almost immediate and significant drinking response in rats at doses greater than 5 ng compared with saline-treated controls (Fig. 1). Most of the drinking (up to 80% of the volume consumed after treatment) occurred in the first 15 min after treatment (Fig. 2). There was no dose-dependent effect on the latency to the observed drinking response in any of the animals tested. During injections in the morning, the maximal response to relaxin was similar to drinking observed after 5 ng angiotensin II icv. Treatment of rats with a mixture of monoclonal antibody to rat relaxin and 50 ng rat relaxin did not show a drinking response (Fig. 1).

There were no significant differences detected between the drinking responses of individual rats at the same dose; therefore, the data were pooled for each treatment. Exogenous rat relaxin caused a dose-dependent dipsogenesis in rats; however, the magnitude and sensitivity of the response varied with the phase of the light-dark cycle (Fig. 3). The dipsogenic response was smallest and least sensitive during the afternoon (1400–1600 h) and maximal and most sensitive at night (2200–2400 h). The latency from the time of injection to the onset of drinking did not vary significantly with either the dose of relaxin or time of injection (Table 1).

Exp 2: effect of central injection of monoclonal antibodies to relaxin on drinking behavior in pregnancy

Water consumption in control rats (PBS-treated: group B) significantly increased during the second half of pregnancy: daily intake increased from about 40 ml on day 12 of pregnancy to approximately 55 ml on days 18–21 of pregnancy. There was no statistically significant difference in water consumption of the PBS-treated rats and rats treated with monoclonal antibody to fluorescein (group C). The increase in daily consumption in both control groups (groups B and C) was due to a change in the amount of water consumed at night (Fig. 4). In contrast, daytime drinking remained reasonably stable throughout pregnancy (Fig. 4). Injection of

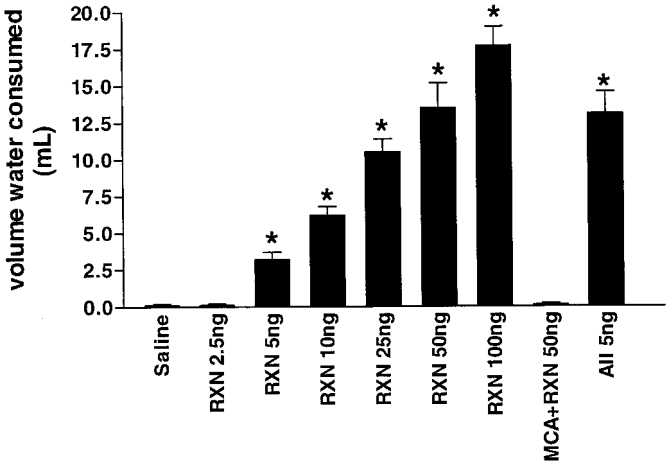

FIG. 1. The dose-dependent dipsogenic effects of rat relaxin icv Mean (± SEM) volume consumed by female rats in the 30 min after icv injection of either rat relaxin (dose range 2.5–100ng), saline alone, monoclonal antibody to rat relaxin (MCA-3) plus 50 ng rat relaxin, or 5 ng angiotensin. Experiments were done between 0800 and 1000 h. Each datum point represents the average of four trials at each dose in eight animals. Significant ($P < 0.05$) increases above baseline (saline alone) are shown by the *asterisks*.

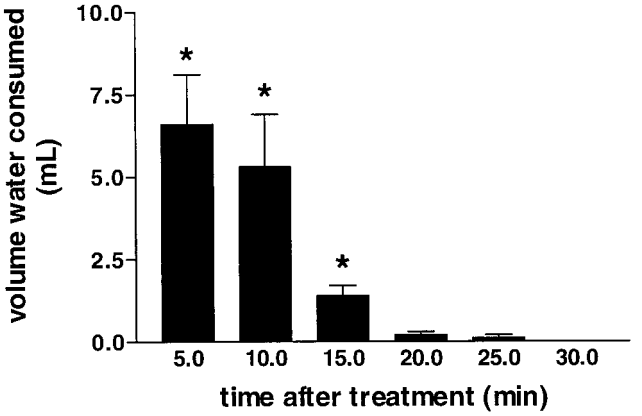

FIG. 2. Mean (± SEM) volume of water consumed by female rats in 5 min time-bins after icv injection of 50 ng rat relaxin. Experiments were done between 0800 and 1000 h. Each datum point represents the average of four trials in eight animals. Data for this figure were taken from experiments shown in Fig. 1 for treatment with 50 ng relaxin icv

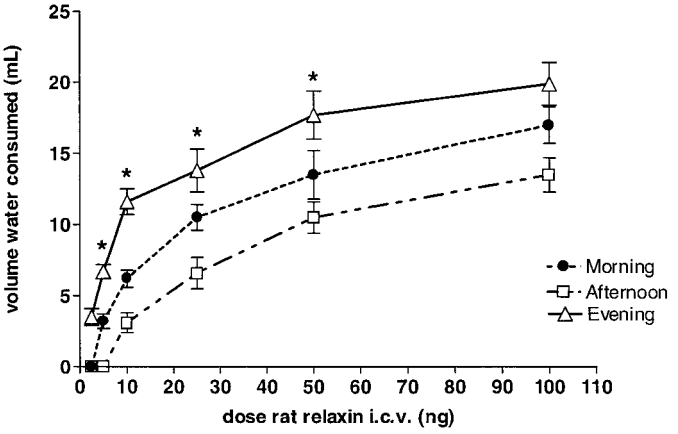

FIG. 3. The effects of rat relaxin icv on drinking behavior in rats. Mean (± SEM) water consumed is shown at different doses of rat relaxin (range 2.5–100 ng) given either during the morning between 0800 and 1000 h(●), in the afternoon between 1400 and 1600 h (□), or at night between 2200 and 2400h (△). Each datum point represents four trials at each dose in eight animals. *Asterisks* indicate where there were significant ($P < 0.05$) differences between the responses at the three times of day. Note that data for the morning injections are the same information shown in Fig. 1.

TABLE 1. The latency from injection of rat relaxin (icv) on the latency to an induced drinking response in conscious rats

Treatment	Latency to the onset of the drinking response(s)		
	0800–1000 h	1400–1600 h	2200–2400 h
No response	No response	No response	70.3 ± 23.7
Rat relaxin, 10 ng	75.5 ± 20.2	No response	52.1 ± 11.3
Rat relaxin, 25 ng	55.4 ± 9.7	49.3 ± 12.5	50.2 ± 10.9
Rat relaxin, 50 ng	57.7 ± 8.3	59.0 ± 11.6	50.8 ± 6.4
Rat relaxin, 100 ng	52.6 ± 10.4	57.1 ± 8.4	47.2 ± 11.1
No response	No response	No response	No response
Angiotensin II, 20 ng	55.7 ± 7.4	59.9 ± 11.8	50.4 ± 12.6

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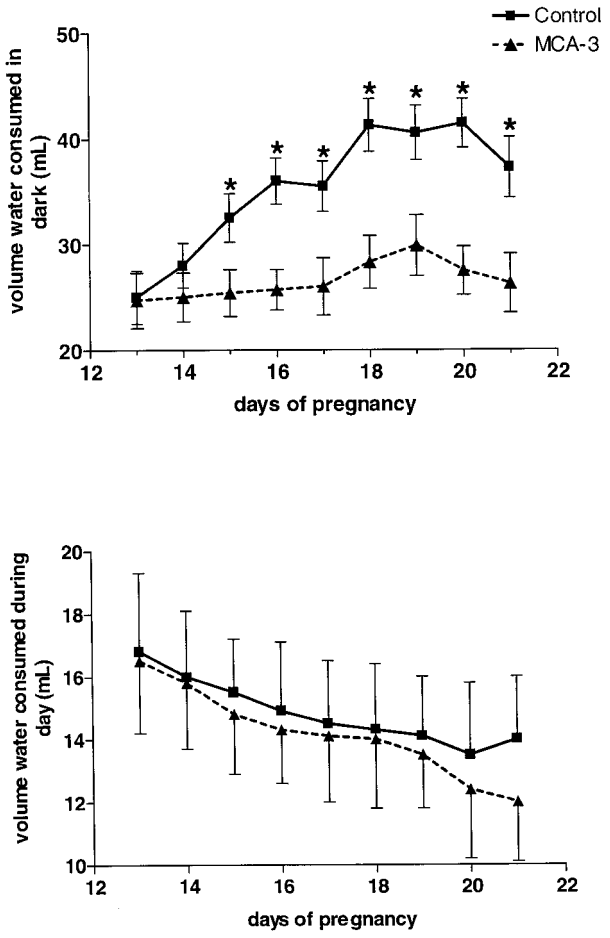

FIG. 4. Mean (± SEM) water consumption of intact rats (■) or rats treated with monoclonal antibody to rat relaxin (MCA-3) icv (▲) during the 12-h dark period (*upper panel*) or the 12-h light period (*lower panel*). Significant ($P < 0.05$) differences are shown by the *asterisks*. Note: there was no statistically significant difference between the group treated with monoclonal to fluorescein and rats treated with PBS alone, although these data are not shown in the figure.

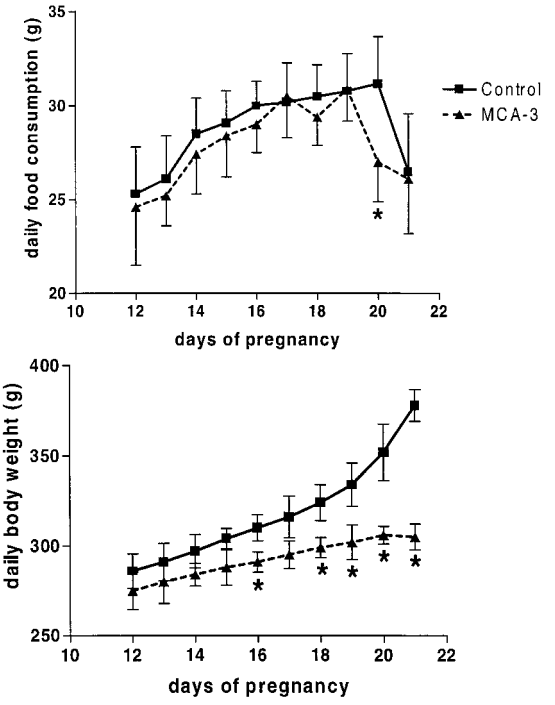

FIG. 5. Mean (± SEM) food consumption (*upper graph*) and body weight (*lower graph*) during pregnancy in intact rats (■) or relaxin-neutralized rats by injection of monoclonal antibody (MCA-3) to rat relaxin icv (▲). Significant ($P < 0.05$) differences are shown by the *asterisks*. Note the significantly reduced food intake on day 20 in relaxin-neutralized group may reflect the 24 h advance in birth seen in this group. Note: there was no statistically significant difference between the group treated with monoclonal to fluorescein and rats treated with PBS alone, although these data are not shown in the figure.

monoclonal antibody to relaxin icv affected drinking in pregnant rats. There was no significant change in the amount of water consumed per day throughout pregnancy in the antibody-treated rats, although there was a slight increase in the water intake at night. This increase was not statistically significant at any time between day 12 and day 21 of pregnancy. The amount of water consumed in the relaxin-neutralized rats (group A) is shown in Fig. 4.

There was no significant difference in the daily food consumption of the relaxin-intact groups (groups B and C) compared with the rats treated with monoclonal antibody to rat relaxin (group A). The exception was day 20 when there was a significant fall in food consumption in the relaxin-deficient rats. Relaxin-intact rats (groups B and C) showed significantly greater increases in body weight during pregnancy compared with the relaxin-neutralized group (group A). A comparison of food intake of PBS-treated (group B) and relaxin-neutralized animals (group A) and the effects on body weight are shown in Fig. 5.

Birth in the relaxin antibody-treated group occurred approximately 24 h in advance of the control groups (Table 2). There was no significant difference in the live birth rate and the number of young born between the three treatment groups, but pups in the relaxin-deficient group were significantly lighter in weight than pups from the control groups.

Discussion

The work in this paper concerns the dipsogenic action of rat relaxin. There were two objectives to the study: 1) to establish whether or not exogenous rat relaxin has different effects on drinking behavior at different times in the light-dark cycle, and 2) to determine whether or not the action of relaxin in the brain affects drinking during pregnancy. The results show that the dipsogenic response exogenous rat relaxin varies during the light-dark cycle, the most powerful effect being seen at night. They also show that neutralizing the central actions of relaxin prevents the increase in drinking that occurs, predominantly at night, in the second half of pregnancy.

There are several lines of evidence to suggest that the circumventricular organs mediate the central actions of relaxin on drinking behavior: lesion of the subfornical organ (SFO) blocks the action of relaxin on blood pressure (32) and on birth (33); there are relaxin-binding sites in the SFO and organum vasculosum of the lamina terminalis (OVLT) (34–36); icv treatment with relaxin is followed by c-*fos* expression within the SFO (37), and finally the SFO and OVLT contain

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TABLE 2. The effects of neutralizing central relaxin on birth in rats

Parameter	Group A Monoclonal to relaxin	Group B PBS	Group C Monoclonal to fluorescein
Duration of pregnancy (h)	505.4 ± 3.1[a]	524.6 ± 0.5	525.9 ± 0.7
Number of pups born	10 ± 2.5	11 ± 2.1	10 ± 2.7
Live birth rate	98%	98%	98%
Birth weight (g) per pup	5.7 ± 1.3[b]	6.3 ± 1.5	6.2 ± 0.9

There was a significant ($P < 0.05$) advance in the time [a] to the onset of straining (duration of pregnancy) and the birth weight of the pups [b] compared with PBS-treated and antibody to fluorescein-treated controls.

osmoreceptors that regular thirst and vasopressin secretion (38–40). Pathways that emanate from the SFO and OVLT are involved in the forebrain angiotensin system and use angiotensin II as a neurotransmitter (41). It has been shown that the action of relaxin on drinking (19), oxytocin and vasopressin release (14), and on LH release (16) is negated by specific blockade of the action of angiotensin II, suggesting that the central actions of relaxin may be mediated through angiotensin. There are, however, no data suggesting that relaxin acts in the systemic circulation through the renin-angiotensin system.

Nocturnal animals tend to drink more at night during the active phase of the light-dark cycle. This was demonstrated by Zhao and colleagues (23) in rats. Data from our experiments indicate that rats are differentially sensitive to exogenous rat relaxin at different times of the light-dark cycle. The underlying cause of this varying response is not known. The nucleus medianus (another component of the forebrain angiotensin system) has been shown to be differentially sensitive to angiotensin-induced dipsogenesis at different times of the light-dark cycle (42), and recent evidence from our laboratory (43) shows that the vasopressinemic but not the oxytocinemic response to exogenous relaxin depends on the nucleus medianus. It is possible, therefore, that the nucleus medianus is involved in the effects of the light-dark cycle on the drinking response to exogenous relaxin. This remains to be determined.

Two laboratories have reported that exogenous porcine relaxin injected into brain is dipsogenic (19, 20), which could imply a role for relaxin in increased drinking during pregnancy. However, recent work indicates that neutralizing endogenous relaxin in the systemic circulation only moderately affects water intake during pregnancy in rats (23). Furthermore, this was supported by experiments in which water intake was monitored in ovariectomized rats given systemic replacement therapy with and without relaxin during pregnancy (24). In these experiments relaxin only moderately increased daytime drinking and did not appear to affect nighttime drinking. Recently, Summerlee and colleagues (26) demonstrated that neutralizing the action of relaxin within the brain had different effects on the physiology of birth compared with the actions of neutralizing relaxin in the systemic circulation (44). These data suggest that the actions of relaxin in the brain might be separate and different from its actions in the periphery. The current work shows that neutralizing the action of relaxin inside the ventricular system disrupts the normal increase in drinking that occurs in the second half of pregnancy in rats and implies that relaxin may have a physiological role in water balance in late pregnancy. During the second half of pregnancy in rats, there are several cardiovascular changes (21, 22): plasma volume expands; blood pressure falls; plasma osmolality decreases; and glomerular filtration rate increases. These changes imply a major shift in the central thresholds for cardiovascular control in pregnancy. It is possible that relaxin might be responsible for resetting these central thresholds (7). However, previous work reviewing the possibility of a role for relaxin in the blunted response to vasoconstrictors during gestation in both normotensive and hypertensive rats suggests that the changed response may be due to an action of relaxin on blood vessels in the periphery (45).

Original work on the central actions of relaxin on the brain (46, 47) was based on the premise that high levels of relaxin in the plasma at the end of pregnancy might 'spill over' and affect the central nervous system. The circumventricular organs could mediate the actions of systemic relaxin. However, there is a growing body of evidence to support the concept that relaxin is synthesized and active within the brain (7), which could be independent from relaxin in the systemic circulation. For example, relaxin message is localized in several discrete regions of the rat brain (25, 34), and relaxin-binding sites have been demonstrated in a variety of sites (34–36). As the brain is separated from the systemic circulation by the blood-brain barrier that only allows passive access to molecules of less than 2 kDa (48), it is likely that relaxin in the systemic circulation may not reach the brain. While there are high concentrations of relaxin binding in some of the circumventricular organs (34, 36) there are other areas where the blood-brain barrier is intact that express high concentrations of receptors. However, to date, there have been no reports of successful relaxin extraction from neural tissue nor any evidence published that relaxin is secreted from the brain.

There was a difference in the experimental protocol between the daytime and nighttime injections. Experiments during the day were carried out in the laboratory while injections at night were done in the animal house under re-light illumination to avoid the possibility that moving the animals at night might disrupt the circadian rhythmicity of the rats. It is possible that this difference of protocol could explain the different drinking responses seen at night. This is unlikely, however, as preliminary experiments, in which rats were brought to the laboratory for nighttime injections (not reported in this paper), showed that relaxin caused an increased dipsogenesis at night compared with the responses during the day.

There is no adequate explanation for the results of water consumption, food intake, and body weight in relaxin-deficient rats on day 20 of pregnancy (Figs. 4 and 5). In these animals, food consumption significantly decreased on day 20 of gestation, yet body weight increased despite no change in water consumption. One possible explanation for this anom-

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aly could be the fact that the rats in the relaxin-deficient groups were observed to spend more time on day 20 (the day before birth occurred) nest building and chewing on bedding compared with rats in control groups. It is possible that relaxin-deficient rats ate bedding and feces during day 20, which resulted in no change in body weight, but this needs further investigation.

Two sets of experimental data support the contention that there is a separate central and systemic relaxin 'system': Hwang and colleagues (44) used monoclonal antibodies specific to rat relaxin to establish the role of relaxin in birth. They showed that neutralizing systemic relaxin disrupted the process but not the onset of birth in rats. In contrast, Summerlee *et al.* (26) injected antibody into the ventricular system of the brain and showed that the timing, but not the process, of birth was disrupted. The experiments reported in the current paper are the second line of evidence: injection of monoclonal antibodies into the ventricular system affects the drinking response in pregnant rats by blocking the increase in nighttime drinking. There is no evidence that relaxin has a physiological action(s) during the estrous cycle, but the possibility that relaxin may affect drinking behavior in nonpregnant rats deserves further study.

In conclusion, this paper provides evidence that the dipsogenic response to injection of exogenous rat relaxin into the ventricular system of the brain varies with the light-dark cycle. Furthermore, injection of antibodies specific to rat relaxin into the ventricles disrupts the increase in nighttime drinking that occurs in the second half of pregnancy in rats, implying a role for relaxin in the brain on drinking behavior in pregnancy.

Acknowledgments

This paper is dedicated to the memory of David G. Porter. Rat relaxin used in these experiments was inherited from David. The authors would like to thank O. David Sherwood for inspiring this work and Drs. Laura Parry and Brian Wilson for their patience, support, and encouragement.

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