**Oxytocin-induced renin secretion in conscious rats**

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IN ADDITION to the well-known actions of oxytocin (OT) during lactation and parturition, OT is a natriuretic hormone (25). Indeed, neurohypophysial OT secreted in response to osmotic stimulation in rats has been documented to contribute importantly to the natriuresis observed under these conditions (5, 7). OT is also secreted in large amounts in response to hypotension or hypovolemia (20), although its actions under these conditions remain obscure.

Binding sites for OT exist in the macula densa (19). The macula densa is known to stimulate renin secretion (18), which contributes importantly to cardiovascular homeostasis during hypotension or hypovolemia. In recent studies we noted that intravenous infusion of OT in physiological doses stimulates renin secretion in anesthetized rats and that the action of OT on renin release is not secondary to its natriuretic effects (17). The present studies sought to determine whether infusion of OT increases plasma renin levels in conscious rats. Because the results indicated that infusion of OT did increase plasma renin levels, additional studies were conducted to determine whether this response required β-adrenoreceptor-dependent mechanisms, which would suggest an action independent of the macula densa.

**METHODS**

Animals. Adult male Sprague-Dawley rats (Zivic Laboratories, Zelienople, PA), weighing 350–400 g, were housed individually in wire-mesh cages in a colony room with ambient temperature maintained at 22–24°C and with lights on from 8 AM to 8 PM. Rats had ad libitum access to Purina Laboratory Chow pellets and tap water.

Experimental protocols. One day before the experiments, all rats were anesthetized with Equithesin (3.0 ml/kg body wt ip), a solution containing pentobarbital sodium (0.98 g/dl), chloral hydrate (4.25 g/dl), and MgSO4 (2.12 g/dl). Catheters were placed into the right femoral artery and the right femoral vein. The free ends of the two catheters were guided subcutaneously along the back to exit between the scapulae. On exiting, the catheters were encased in a steel spring to prevent them from being damaged. Rats were returned to their home cages, with the catheters leaving the cages to make them accessible without disturbing the rats.

On the following morning, water and food were removed from each cage, and the free end of the venous catheter was connected to an infusion pump (Harvard Apparatus, S. Natick, MA). The arterial catheter was connected via a pressure transducer to a physiograph (model 7, Grass Instruments, Quincy, MA) for the recording of mean arterial pressure (MAP) and heart rate (HR). Rats were used in one of the following three experiments.

Experiment 1 determined the effect of OT infusion on renin secretion. Rats received an infusion of isotonic saline (5 ml·kg⁻¹·h⁻¹) for 30 min, and then a baseline blood sample (~0.8 ml) was collected from the arterial catheter. The volume of this blood sample and subsequent samples was replaced with an equal volume of isotonic saline. Then, in one group (n = 7), the infusion was switched to saline containing OT, so that OT was given at a rate of 25 ng·kg⁻¹·h⁻¹ (~150 pg·rat⁻¹·min⁻¹) for 1 h and 125 ng·kg⁻¹·h⁻¹ (~750 pg·rat⁻¹·min⁻¹) for another hour. These infusion rates were selected to increase plasma OT levels to ~20 and ~80 pg/ml, respectively (17), which correspond to the levels attained in response to 24 h of water deprivation (5) or hypotension (14). Control rats (n = 7) continued to receive an infusion of isotonic saline throughout the 2-h period. The volume infused was 5 ml·kg⁻¹·h⁻¹ in all cases. At the beginning of each infusion, 0.4 ml of the solution was injected through the
venous catheter to fill the catheter with the new solution. Blood samples were collected after 20 and 60 min of infusion of each dose. MAP and HR were monitored for -10 min before each blood sample was collected.

Experiment 2 determined whether OT-induced renin secretion was mediated by an action on OT receptors and required β-adrenergic receptor-mediated mechanisms, by evaluating the effect of blocking OT receptors or β-adrenergic receptors on OT-induced renin secretion. In eight rats an OT receptor antagonist [1-(3-mercaptopropionic acid)-2-O-ethyl-D-Tyr, Thr⁶, Orn⁶]-OT; Ferring was administered before and during an infusion of OT. In these rats a baseline blood sample was taken after a 30-min period, during which the rats received an infusion of isotonic saline. Then the OT antagonist was infused at a rate of 40 µg·kg⁻¹·h⁻¹ in a volume of 5 ml·kg⁻¹·h⁻¹. This dose has been shown previously to block the natriuretic effects of OT but not to interfere with vasopressin receptors (6). After a 1-h pretreatment with the OT antagonist, a blood sample was taken and an infusion of OT was initiated (125 ng·kg⁻¹·h⁻¹). Additional blood samples were taken 30 and 60 min after the start of the OT infusion (time 0).

In other rats (n = 8), vehicle was infused for another hour after the basal period, within which rats received an intravenous injection of the β-adrenergic receptor antagonist nadolol (2.5 mg/kg in 1 ml/kg saline; Sigma Chemical, St. Louis, MO) 15 min before initiation of OT infusion (125 ng·kg⁻¹·h⁻¹). Preliminary studies indicated that this dose of nadolol blocked sympathetically mediated increases in HR evoked by intravenous injection of sodium nitroprusside for at least 2 h. Blood samples were collected during the baseline period, just before the start of OT infusion (time 0), and 30 and 60 min thereafter. In control rats (n = 7), a 1-h infusion of isotonic saline intervened between the baseline period and the start of OT infusion. Blood samples were collected after the 30-min baseline period (baseline), after the additional 1-h saline infusion (time 0), and 30 and 60 min after the start of OT infusion. In addition, after the 60-min blood sample, nadolol was injected (2.5 mg/kg iv), and an additional blood sample was collected 15 min later. Before each blood sample, MAP and HR were monitored for -15 min.

Experiment 3 evaluated the specificity of the OT antagonist for blocking OT-evoked renin secretion by determining the effect of OT receptor blockade on renin secretion evoked by β-adrenergic receptor stimulation. After a 30-min baseline period, rats received an intravenous infusion of OT antagonist (40 µg·kg⁻¹·h⁻¹, as described above; n = 7) or isotonic saline (n = 6). One hour later, each rat received an intravenous injection of isoproterenol (10 µg/kg in 1 ml/kg saline). Blood samples (0.4 ml) were collected just before and 5, 15, and 30 min after injection of isoproterenol. In preliminary experiments the dose of isoproterenol was determined to increase plasma renin activity (PRA) to approximately the same extent as did infusion of OT. MAP and HR were monitored throughout the experiment.

Analysis of plasma renin. All blood samples were withdrawn from the arterial catheters into tubes coated with 3.7 mg of potassium EDTA and bathed in ice. Blood samples were centrifuged (1,100 g for 10 min), and the plasma was removed and stored at -80°C.

In experiment 1, plasma renin concentration (PRC) was measured using the protocol of Lykkegaard and Poulsen (10). Aliquots of plasma were diluted 20- to 80-fold with Tris buffer containing human albumin, and 5-µl portions of these samples were incubated for 24 h at 37°C with 20 µl of a reaction mixture that contained purified rat renin substrate (~1,200 ng ANG I equivalents/ml) (10). This incubation was followed by RIA of generated ANG I. PRC was measured in reference to renin standards obtained from the Institute for Medical Research (Holly Hill, London, UK; 1 µGoldblatt unit = 160 pg ANG I·ml⁻¹·h⁻¹).

In experiments 2 and 3, PRA was measured by RIA of ANG I generated during a 1-h incubation at 37°C of the plasma samples diluted 1:1 with maleate buffer, as previously described (16). This assay differed from the measurement of PRC, in that exogenous renin substrate was not added to the incubation.

Statistics. Values are means ± SE. Data were analyzed by two-way (group × time) ANOVA (Systat, Evanston, IL) with repeated measures in the time parameter. The error terms and degrees of freedom from the ANOVA were used in t-tests to compare treatment values with baseline values within groups. Comparisons between groups at specific time points were done using Tukey's honestly significant difference test. P < 0.05 was considered to be statistically significant.

RESULTS

OT infused at 25 ng·kg⁻¹·h⁻¹ iv had no effect on PRC in conscious rats (Fig. 1). However, PRC was increased by twofold by infusions of OT at 125 ng·kg⁻¹·h⁻¹ (P < 0.05; Fig. 1). Infusion of OT at these rates altered neither MAP nor HR at any time during the infusion (data not shown).

In a separate group of rats, infusion of OT at 125 ng·kg⁻¹·h⁻¹ again increased PRA by twofold (P < 0.01; Fig. 2). Pretreatment with an OT receptor antagonist did not alter baseline PRA but completely prevented the OT-induced increase in PRA (Fig. 2). The β-adrenergic receptor antagonist nadolol (2.5 mg/kg iv) injected 15 min before infusion of OT slightly reduced baseline PRA and appeared to completely prevent the effects of OT on renin secretion (Fig. 2). Similarly, nadolol injected after 60 min of OT infusion abruptly reduced PRA (P < 0.05; Fig. 2). Neither OT nor the OT antagonist caused significant changes in MAP or HR compared with baseline values or control rats (both P < 0.05).
Effect of OT antagonist (OT-ant) or nadolol on OT-stimulated renin secretion. After collection of baseline blood samples for measurement of plasma renin activity (PRA), groups of rats (n = 7 or 8) were pretreated with OT antagonist, nadolol, or isotonic saline, and a 2nd blood sample was collected. Then all rats were infused with OT at 125 ng·kg⁻¹·h⁻¹, and blood samples were collected after 30 and 60 min of infusion. After 60 min of OT infusion, vehicle-pretreated rats were injected with nadolol (arrow), and an additional blood sample was taken 15 min later. OT significantly increased PRA from baseline only in the group pretreated with vehicle (P < 0.01). Nadolol also significantly reduced PRA when administered during OT infusion (P < 0.01).

Table 1. Effect of OT, administered with OT antagonist or nadolol, on MAP and HR

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT</td>
<td>111 ± 3</td>
<td>110 ± 3</td>
<td>109 ± 4</td>
<td>109 ± 3</td>
<td>107 ± 3</td>
</tr>
<tr>
<td>OT + OT antagonist</td>
<td>119 ± 3</td>
<td>118 ± 3</td>
<td>119 ± 4</td>
<td>118 ± 3</td>
<td></td>
</tr>
<tr>
<td>OT + nadolol</td>
<td>122 ± 4</td>
<td>119 ± 3</td>
<td>118 ± 4</td>
<td>118 ± 4</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT</td>
<td>401 ± 9</td>
<td>397 ± 9</td>
<td>397 ± 9</td>
<td>397 ± 7</td>
<td>366 ± 9*</td>
</tr>
<tr>
<td>OT + OT antagonist</td>
<td>399 ± 8</td>
<td>403 ± 6</td>
<td>404 ± 8</td>
<td>405 ± 3</td>
<td></td>
</tr>
<tr>
<td>OT + nadolol</td>
<td>383 ± 10</td>
<td>362 ± 8*</td>
<td>358 ± 9*</td>
<td>354 ± 7*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. OT, oxytocin; MAP, mean arterial pressure; HR, heart rate. After a 30-min baseline period during which MAP and HR were monitored, groups of rats (n = 7 or 8) were pretreated for 60 min with infusion of isotonic saline (OT group), OT antagonist (OT + OT antagonist group), or isotonic saline with an injection of nadolol (2.5 mg·kg⁻¹·h⁻¹) 45 min into infusion (OT + nadolol group). MAP and HR were measured at the end of this pretreatment period (time 0). Then all rats received an OT infusion at 125 ng·kg⁻¹·h⁻¹, and MAP and HR were measured after 30 and 60 min of OT infusion. After 60 min of OT infusion in the vehicle-pretreated group, rats were injected with nadolol, and MAP and HR were measured again 75 min after the start of the OT infusion. Data are from same experiment shown in Fig. 2. *Significantly different from baseline in that group (P < 0.05).

DISCUSSION

The major finding of the present study is that plasma renin levels were increased by intravenous infusion of OT in a physiological dose in conscious, freely moving rats. Furthermore, this substantial increase in plasma renin levels produced by OT was prevented by pretreatment with an OT receptor antagonist or with a β-adrenergic receptor antagonist.

OT was infused at 125 ng·kg⁻¹·h⁻¹ to simulate the increased plasma levels of OT measured during hypotension or hypovolemia (14, 20). Infusion of OT at this rate resulted in a doubling of PRC and PRA. Similar results were observed previously using thiobutabarbital (Inactin)-anesthetized rats (17). In contrast, infusion of OT at 25 ng·kg⁻¹·h⁻¹, a dose selected to mimic

Table 2. Effect of OT antagonist on cardiovascular effects of isoproterenol

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>0 min</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle + Iso</td>
<td>118 ± 4</td>
<td>118 ± 4</td>
<td>70 ± 5*</td>
<td>118 ± 5</td>
<td>116 ± 4</td>
</tr>
<tr>
<td>OT antagonist + Iso</td>
<td>116 ± 3</td>
<td>116 ± 3</td>
<td>71 ± 3*</td>
<td>119 ± 2</td>
<td>118 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle + Iso</td>
<td>398 ± 2</td>
<td>398 ± 2</td>
<td>564 ± 7*</td>
<td>418 ± 9</td>
<td>400 ± 3</td>
</tr>
<tr>
<td>OT antagonist + Iso</td>
<td>390 ± 10</td>
<td>380 ± 9</td>
<td>559 ± 11*</td>
<td>405 ± 10</td>
<td>385 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE. Groups of rats (n = 7 or 8) were infused with OT antagonist or vehicle for 1 h and then received an injection of isoproterenol (Iso, 10 µg·kg⁻¹·iv). MAP and HR were measured at the beginning of the experiment (baseline), just before injection of Iso (time 0), and 5, 15, and 30 min after injection of Iso. Iso produced a rapid and transient increase in HR and decrease in MAP, which were similar whether or not rats were pretreated with OT antagonist. Maximal Iso-evoked decrease in MAP was -47 ± 3 mmHg in rats pretreated with OT antagonist, which was not significantly different from response to Iso in control rats (-48 ± 4 mmHg). Similarly, maximal Iso-evoked tachycardia was similar in rats pretreated with OT antagonist (+169 ± 8 beats/min) or vehicle (+166 ± 7 beats/min). Data are from same experiment presented in Fig. 3. *Significantly different from baseline value in that group (P < 0.05).
the smaller increase in plasma OT levels caused by 24 h of water deprivation (5), did not significantly alter PRC in conscious rats. These observations allow the possibility that the high plasma OT levels observed during hypotension and hypovolemia may promote renin secretion and thereby make a useful contribution to the support of blood pressure, as does neurohypophysial vasopressin [which also is secreted under these conditions (14, 20)].

OT likely evokes renin release via its action on OT receptors, because this effect was prevented by pretreatment with a selective OT receptor antagonist. The antagonist used in this study has been shown previously to block the natriuretic effects of OT but not to interfere with vasopressin receptors (6). The specificity of this antagonist for renin release induced by OT was suggested by the observation that it did not block renin secretion induced by isoproterenol.

Although these studies were originally prompted by the observation that OT binding sites are present in the macula densa, the macula densa is probably not the site at which OT acts to elicit renin secretion in the present experiments. This view is based on observations that renin secretion stimulated by the macula densa is independent of β-adrenergic receptor stimulation (9, 15), whereas in the present study OT-induced renin secretion was largely attenuated, if not prevented, by injection of nadolol. This effect of nadolol suggests that OT acts to increase renal sympathetic nerve activity or adrenal medullary catecholamine secretion (8). Further studies are needed to determine whether OT acts on renal sympathetic nerve terminals, on sympathetic ganglia, directly in the central nervous system to increase sympathoadrenal outflow, or on afferent nerves to reflexly elicit this response. Responses mediated by β-adrenergic receptors independent of the sympathoadrenal system are also a possibility (11, 23). In this regard, a previous study (2) in which renin secretion was stimulated by infusion of OT into the vertebral artery of anesthetized dogs points to the brain as a likely site of action. Although OT is unlikely to penetrate the blood-brain barrier, its action on a circumventricular organ is possible.

The general hypothesis that a circulating factor may influence renin secretion via β-adrenergic receptors is consistent with previous reports (8, 23, 24). Adrenomedullary secretions during stress are well known to stimulate renin secretion, but they may not be the only blood-borne factors to do so (1, 8). Morton et al. (11) and Van de Kar and Richardson-Morton (23) reported that serotonin agonists cause renin release in rats that is blocked by β-adrenergic receptor antagonists but does not require intact renal sympathetic outflow. Many of the features of their blood-borne renin-releasing factors are consistent with the active agent being OT; like OT, it is a 1,000- to 5,000-Da peptide present in the hypothalamus (12, 22, 24). Furthermore, serotonin agonists are known to cause pituitary OT release (13). Although Van de Kar et al. (24) failed to find renin-releasing activity in the pituitary gland, it is possible that such activity was obscured by the high concentra-

tion of vasopressin, which is known to inhibit renin secretion (4). De Vito et al. (3) also presented evidence for a blood-borne renin-releasing factor that appeared in the circulation in response to hypotension in dogs.

In summary, the present results indicate that renin secretion is stimulated by increases in plasma OT similar to those produced physiologically by hypotension or hypovolemia. This finding raises the possibility that renin secretion elicited in response to OT contributes to the homeostatic response to such cardiovascular challenges. Indeed, we recently noted that renin release in response to hydralazine-induced hypotension is markedly attenuated by systemic infusion of an OT receptor antagonist (21). Further work is needed to confirm these observations and to elucidate the mechanisms by which OT exerts this effect.

Perspectives

The regulation of OT release suggests that it has actions independent of its effects during lactation and parturition. OT is a natriuretic hormone (25) secreted in response to increases in plasma osmolality (20). OT is also secreted in response to hypotension and hemorrhage (14, 20), during which the natriuretic actions of OT are negated by the increased secretion of the antinatriuretic hormone aldosterone as well as by decreased urine excretion secondary to reduced renal perfusion pressure and blood flow. However, the present studies suggest another important role for OT secreted in response to decreased blood pressure or blood volume: stimulation of renin secretion. Increased renin secretion has long been appreciated to contribute to cardiovascular homeostasis, and the present data suggest that OT may promote this response. Additional studies are needed to further define the role of OT in these homeostatic responses and to determine how these observations in rats apply to other species.

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REFERENCES


