Salt Appetite and the Renin-Angiotensin System
Effect of Oxytocin Deficiency
Katya Rigatto, Robert Puryear, Iveta Bernatova, Mariana Morris

Abstract—To explore the role of oxytocin in the regulation of salt appetite and blood pressure, we conducted studies in oxytocin gene–knockout mice and determined (1) blood pressure and heart rate during day and night periods, (2) salt appetite after iso-osmotic volume depletion, and (3) salt appetite and blood pressure after central injection of angiotensin II. Long-term arterial catheters were inserted, and blood pressure and heart rate were recorded for 24 hours. There was a modest decrease in blood pressure and heart rate in knockout mice. Salt appetite was measured with a 2-bottle choice (water and 2% NaCl), with measurement of licking activity. Mice were injected subcutaneously with 30% polyethylene glycol (0.5 mL), and voluntary intakes were measured for 24 hours. Knockout mice consumed 3 times the amount of NaCl than did controls, 276±77 vs 90±38 licks/24 h (P<0.05). Water consumption was similar between groups. Angiotensin II (5, 50, and 200 ng/3 μL) injected intracerebroventricularly produced dose-related increases in intake, with no differences between the groups. The 50-ng dose of angiotensin II elicited salt and water intakes of 151±43 vs 160±33 licks and 250±53 vs 200±51 licks, respectively (control vs knockout). Thepressor response to angiotensin II was not different between the groups. Results suggest that oxytocin plays a role in the regulation of blood pressure and salt appetite, specifically as mediated by volume receptors, and that the renin-angiotensin system is not involved in these changes. (Hypertension. 2003;42[part 2]:793-797.)

Key Words: mice ■ blood pressure ■ angiotensin ■ renin-angiotensin system ■ water-electrolyte balance ■ sodium

Oxytocin (OT) is a posterior pituitary hormone that has a wide range of effects on target tissues from the brain to the vasculature. Although OT is well recognized for its role in reproduction, recent discoveries suggest that OT is also involved in regulation of fluid balance, blood pressure (BP), and cardiac function.1–4 Oxytocinergic neurons innervate brain regions important in cardiovascular control, such as the nucleus tractus solitarius, locus ceruleus, dorsal motor nucleus of the vagus, and intermediolateral cell column in the spinal cord.5–7 OT and OT receptors are present in the vasculature, heart, and kidney, and OT has effects on BP, renal function, and salt intake.1,3

Hypovolemia is a stimulus for cardiovascular and neuroendocrine reflexes, resulting in sympathetic, adrenal, and hypothalamic activation.8 Experimentally, volume depletion is often induced by the injection of large-molecular-weight colloids, such as polyethylene glycol (PEG), which acts to draw iso-osmotic fluid from the tissues. Time-course and pharmacologic antagonist studies suggest that PEG-induced OT release inhibits salt intake.9,10 There is also evidence for an OT-specific response to increased osmolality, rather than sodium.11 A role for central angiotensin II (Ang II) in mediating the OT responses was suggested, because OT antagonists potentiated the salt intake induced by Ang II.12,13 In addition, central administration of Ang II provoked systemic release of OT14,15 and vasopressin14–16 in rats. There are also studies that have explored the synaptic mechanisms involved in Ang II–induced secretion of vasopressin,17,18 whereas there is less information on the OT system.

A new method for the study of physiologic control is the use of genetically modified mouse strains. Animals are available that either lack or overexpress a wide range of receptors and peptides. To further explore the nature of OT’s role in volume and pressure regulation, we used the OT gene-disruption model.19 This strain lacks the ability to synthesize OT; however, they are able to procreate but not to nurse their young. Initial studies in our laboratory examined the voluntary intake of salt and water (choice test) in OT-knockout mice (OTKO). Results showed that OTKO mice consumed significantly more of an aversive NaCl solution under baseline conditions20 or after dehydration.21 These animals also showed imbalances in autonomic control, an enhanced sympathetic reserve, and alterations in baroreflex function.4

The present studies were designed to expand these findings in the OTKO model: (1) to determine the effect of volume
depletion on salt and water intake; (2) to determine the effect of central Ang II stimulation on salt and water intakes; and (3) to determine baseline BP and the response to central Ang II. Volume depletion was produced by PEG treatment, which activates neural and endocrine reflexes, resulting in enhanced intake of salt and water to compensate for fluid losses. If OT is involved in the volume restoration, we propose an enhancement of sodium intake in mice with an OT deficiency. To explore a role for the renin-angiotensin system (RAS) in volume-induced appetite changes, we proposed to test the effect of central Ang II injections. If Ang II/OT interactions are critical to the regulation of volume control, we predict an increase in salt intake in OT-deficient mice.

Methods

Animals

Male control (OT+/−) and OTKO (OT−/−) mice were produced from a cross of heterozygous parents. The colony founders were developed by Young et al.19 The gene was deleted by crossing a genetic construct with the wild-type mouse OT allele in a manner that replaced the last 2 exons. Animals were genotyped with a polymerase chain reaction method from DNA extracted from the tail.21 Mice (25 to 35 g) were given ad libitum access to standard chow (Harlan Teklad, 0.5% sodium by weight) and tap water. They were kept on a 12-hour:12-hour light (5 AM)/dark (5 PM) cycle at a temperature of 21°C. During the experiment, mice were housed in individual, clear, cylindrical cages (Instech) that had been modified to support an additional water bottle. Animal protocols were approved by the Institutional Animal Care and Use Committee.

Volume Depletion

To establish a protocol for volume depletion in mice, we determined the time course of changes in hematocrit, plasma osmolality, and body weight after subcutaneous injections of PEG (0.5 mL of 30% PEG in isotonic saline). Three groups of male C57BL/6 mice (n = 6) were injected with PEG and decapitated 0, 1, and 4 hours later, followed by collection of trunk blood.

Volume Depletion and Salt Appetite

Control (n = 6) and OTKO (n = 7) male mice were injected subcutaneously with PEG (0.5 mL of 30% PEG). Licking activity was measured by using a 2-bottle choice, water or 2% NaCl. Food was removed during the test period to eliminate an alternative source of sodium.

Cardiovascular Surgery and BP Measurements

For cardiovascular measurements, mice were chronically implanted with carotid arterial catheters.22,23 The system uses a micرونethylene catheter covered with a metal spring that is attached to a swivel at the top of the cage. A heparinized saline infusion (80 U/mL, 25 μL/h) is used to maintain catheter patency. The recovery period was at least 6 days, after which drinking and eating patterns had returned to normal. BP and heart rate (HR) were measured continuously (87 Hz) for 24 hours in control (n = 6) and OTKO (n = 6) mice (model MP100 WSW, BIOPAC Systems). Data were processed by calculation of 10-minute means, with determination of dark and light means (3.7×10⁴ samples/12-hour period).

Central Injection of Ang II

Experiments were performed to test the effect of central stimulation with Ang II on salt and water intakes and BP (n = 6 to 8 per group). Control and OTKO mice were chronically implanted with lateral ventricular cannulas by using stereotaxic surgery. The injections were made through the intracerebroventricular (ICV) guide cannula (30 gauge, 8-mm total length; Small Parts). Artificial cerebrospinal

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time After PEG, h</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>46±0.4</td>
</tr>
<tr>
<td>Plasma osmolality, mOsm/kg</td>
<td>280±5</td>
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C57/BL6 mice (n = 6 for all groups) were injected with 0.5 mL of 30% PEG subcutaneously and humanely killed at 0, 1, and 4 hours. Data are mean±SEM. *P < 0.01, †P < 0.001 vs time 0.

fl uid (aCSF, 3 μL) or Ang II (5, 50, and 200 ng/3 μL, aCSF) was injected ICV, with measurement of licking activity 2 hours after injection. Animals were given a choice of water or 2% NaCl to drink, with no access to food. To determine thepressor response to Ang II, control and OTKO mice were prepared with ICV and carotid catheters. Ang II (200 ng/3 μL) was injected ICV, with measurement of the peak BP response.

Licking Activity

Licking activity was measured by using a drinkometer system (Columbus Instruments) interfaced to a data acquisition system. The cages were wired to the drinkometers, with the positive input connected to the metal tips on the water bottles and the negative input to the metal grid flooring of the cage. When the mice made simultaneous contact with the floor and water bottle, a lick was recorded. The volume of salt and water consumed was previously confirmed to be correlated to licking activity.20 The data were analyzed with acquisition software, which converted the digital data to numerical form. Incidental contacts with the water bottles, registered as single data points, were excluded from the data.

Statistical Analysis

All data were analyzed by 1-or 2-way ANOVA for repeated measures or a t test, where appropriate, followed by the Newman-Keuls test or the least significant difference test. A value of P < 0.05 was considered significant.

Results

Volume Depletion with PEG

To establish the method for PEG-induced volume depletion in mice, we performed a time-course study of hematocrit and plasma osmolality changes after subcutaneous PEG injection (Table 1). In C57BL/J mice, there was an increase in hematocrit after 1 and 4 hours when compared with time zero (P < 0.0002). Plasma osmolality did not change. These data illustrate that the colloid produces an iso-osmotic fluid contraction, ie, an increase in hematocrit without a change in osmolality.

Volume Depletion and Salt Appetite

OTKO mice consumed more salt than did controls, 306±85 versus 111±44 licks/24 h (OTKO vs control, P < 0.05) in response to the volume depletion induced by PEG (Figure 1). Water intake was not significantly different between the groups (OTKO, 413±75 vs control mice, 384±71 licks/24 h).

ICV Ang II–Stimulated Salt Appetite

Injection of Ang II (5, 50, and 200 ng) into the lateral ventricle produced a dose-related increase in water and salt intakes (P < 0.0001 and P < 0.0005 for main effect of dose for water and salt intake, respectively), with no differences observed between the groups (Figure 2). Water intake was 303±54 versus 251±102 licks (control vs OTKO), whereas
salt intake was 169±34 versus 126±34 licks (control vs OTKO) after the 200-ng dose. Even the lowest dose of Ang II (5 ng) produced an increase in water consumption compared with the vehicle, aCSF (139±37 vs 22±8, P<0.01).

Cardiovascular Parameters
BP and HR were measured continuously in OTKO and control mice. Results showed a modest hypotension in OTKO mice (P<0.0001 for main effect of genotype). There was a significant diurnal rhythm in mean arterial pressure and HR (P<0.0001 for main effect of day phase) in both experimental groups (Table 2). Central stimulation with 200 ng Ang II produced an increase in BP, which was not different between the groups. The increase was 14±3 mm Hg in OTKO mice compared with 17±3 mm Hg in controls.

Discussion
Results provide evidence that in mice, (1) OT is important in regulating salt intake in response to volume stimuli; (2) OT’s effects on salt intake are not mediated by the central angiotensin system; and (3) OT plays a role in the regulation of basal BP but not in Ang II–induced pressor responses. These conclusions are based on results obtained in an OT gene disruption model,19 a mouse strain that lacks the ability to synthesize and secrete the hypothalamic peptide.

Previous studies tested the potential of OT to regulate salt appetite in mice. With OTKO mice, results showed an increase in sodium intake under need-free conditions or after water deprivation.20–21 OTKO animals consumed large quantities of an aversive salt solution under a voluntary paradigm. The OTKO mice also showed elevated intakes after 24 hours of water restriction, a condition that increases both hematocrit and plasma osmolality.21 These results are supportive of studies in rats that showed that PEG-induced salt intake was inhibited by central OT administration,9 whereas lesions of the brain OT system increased the response to hyperosmolality.13 In contrast, an OT antagonist had no effect on PEG-induced salt intake.9 Studies that compared the effect of volume expansion with hypertonic mannitol versus saline showed that saline intake was responsive to osmolality changes, rather than sodium.11

To proceed to the next stage, we determined whether a stimulus to volume receptors alters salt intake in mice and whether the response is changed in the OT gene-disruption model. Volume depletion with PEG has become a standard test model. This large-molecular-weight colloid is nontoxic and causes an iso-osmotic leaching of plasma from the vascular to the subcutaneous space. The end result is a slow, long-lasting hypovolemia, seen experimentally as an increase in hematocrit with no change in osmolality.25 These results were confirmed in the present study, which used mice instead of rats, and showed that subcutaneous injection of PEG increased hematocrit but did not change osmolality. This treatment is not a direct stimulus for either salt or water consumption but rather relies on interactions with the central nervous system to produce the efferent response to restore volume. Characterization time-course studies in rats showed that after volume depletion, water intake is continuously stimulated, whereas NaCl intake is intermittent.26,27 It is thought that centrally released OT mediates these bouts of inhibition followed by stimulation of saline intake. Because our protocol used licking activity as the dependent parameter, it was possible to observe whether there was a reciprocating rhythm of salt and water intake in mice. However, in contrast to the results in rats, there was no evidence for a rhythm of bouts of water and saline drinking (authors’ unpublished observation).

To evaluate the role of the RAS in OT’s actions in mice, we determined the effect of central stimulation with Ang II on fluid intake and BP. Because PEG-induced volume depletion activates the RAS, it was possible that the effect was mediated by central nervous system angiotensin systems.28 Central Ang II is a potent dipsogen, as well as a stimulus to salt intake12 and pituitary hormone release.29 Interactions with the OT system were observed in both adult and preweaning rats,12,13 because Ang II–induced salt intake was increased after treatment with an OT antagonist. In our studies, we tested a range of Ang II doses (5 to 200 ng) injected into the lateral ventricle of control and OTKO mice. Ang II increased water and salt intakes with no differences observed between the groups. The level of salt intake was proportionally higher than that observed in rats. The pressor response
was intact in the OTKO mice (increase of \(\pm 13\) mm Hg), verifying that the central angiotensin stimulus was active. These results suggest that in mice, the central angiotensin system is not a key transmitter in the OT inhibitory pathway. The differences in results compared with rats suggest that different mechanisms might come into play in different species.

Although OT receives most credit for its role in female reproductive function, there is increasing evidence that it is a cardiovascular peptide. This is seen by the localization of the peptide and its receptor in cardiovascular centers of the brain and the effects of OT on vascular reactivity, BP, and HR.\(^{30,31}\) In the absence of OT, we found that OTKO mice showed a modest hypotension and reduction in HR compared with controls. This suggests a role for OT in the regulation of baseline BP and HR and supports the idea that endogenous OT helps to maintain vascular tone and exerts a pressor action. A test of stress reactivity also showed that OTKO mice have an enhanced cardiovascular response.\(^{33}\) In rats, there is evidence for both pressor and depressor effects of OT injection.\(^{34–36}\) It is important to note that the method applied in these studies was different from many other approaches. The chronic arterial catheter preparation allows for complete recovery (> 5 days), at which time it is possible to measure BP continuously. This allows for measurement of nonstress levels in mice and provides information on day/night levels. The higher values of basal BP and HR observed in wild-type mice compared with OTKO mice might be associated with OT’s effect on the RAS. Increased plasma renin activity was found in dogs exposed to hemorrhage,\(^{37}\) and OT treatment prevented the fall in BP. OT neurons are also stimulated in response to hypotension\(^{38}\) and hypovolemia.\(^{39}\) Thus, pituitary OT secretion might serve to support BP by activation of the RAS through a \(\beta\)-adrenergic receptor–dependent mechanism.\(^{40}\)

In conclusion, our results demonstrate the importance of the brain OT system in the regulation of salt appetite and BP in mice. The pathway appears to link BP, salt appetite, and blood volume. Under conditions of OT deficiency, animals have a chronic, high-level salt drive, which is heightened by volume compromises. This change is likely not mediated by central angiotensin signaling.

Acknowledgments
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References

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**TABLE 2. Mean Arterial Pressure (MAP) and Heart Rate (HR) in OT+/+ and OTKO Mice as Functions of Time of Day**

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<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>HR, Beats/Min</th>
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<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>Control</td>
<td>111±2</td>
<td>106±2</td>
</tr>
<tr>
<td>OTKO</td>
<td>104±2*</td>
<td>100±1*</td>
</tr>
</tbody>
</table>

*There was a significant difference in blood pressure and HR in OTKO vs control mice (\(P<0.0001\), main effect of genotype) and diurnal rhythm (\(P<0.0001\), main effect of day phase) in both experimental groups. Data are mean±SEM. *\(P<0.05\) vs dark; †\(P<0.05\) vs OT+/+.\)


