Acute hypertension inhibits thirst stimulated by ANG II, hyperosmolality, or hypovolemia in rats

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Acute hypertension inhibits thirst stimulated by ANG II, hyperosmolality, or hypovolemia in rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R214–R224, 2001.—The present study sought to determine whether increases in arterial blood pressure inhibited drinking behavior evoked by ANG II, hyperosmolality, or hypovolemia in rats. Cumulative water intakes in 60- or 90-min tests and latency to the first lick were recorded as indexes of thirst. During intravenous infusions of 100 ng·kg⁻¹·min⁻¹ ANG II, attenuation of the induced increases in arterial pressure with the arterial vasodilator diazoxide resulted in greater water intakes and shorter latencies to drink. Drinking behavior stimulated by intravenous infusion of hypertonic saline was significantly inhibited by increases in arterial pressure caused by intravenous infusion of phenylephrine or endothelin-1, and this inhibition of drinking was proportional to the induced increase in pressure. Upon termination of the phenylephrine infusion, mean arterial pressure returned to basal values, and drinking was restored. Phenylephrine-induced increases in arterial pressure also inhibited drinking behavior in response to hypovolemia that could not be explained by changes in plasma renin activity, plasma protein concentration, or plasma osmolality. Thus increases in arterial pressure inhibit water drinking behavior in response to each of these three thirst stimuli in rats.

METHODS

Animals

Adult male Sprague-Dawley rats (Zivic Laboratories, Zelienople, PA) weighing 350–400 g were housed individually in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle (lights on at 8:00 AM). Tap water and Purina Laboratory Chow (no. 5001) were available ad libitum except where noted. All experiments began between 10:00 AM and 2:00 PM. At least 24 h before experiments, catheters were implanted in the left femoral artery (PE-50 tubing) and vein

INCREASES IN ARTERIAL BLOOD pressure (AP) evoked by an intravenous infusion of ANG II have been suggested to inhibit the thirst that is also stimulated by ANG II (6, 8, 19, 23). The strongest support for this hypothesis is provided by a series of experiments in which the water intake evoked by an intravenous infusion of ANG II was greater in rats when the increase in AP was prevented by treatment with one of three vasodilators [isoproterenol, diazoxide (DZX), or minoxidil] at doses selected to hold AP at or near basal levels (8, 23). To ensure that the circulating ANG II resulted from the intravenous infusion and not the decrease in AP, rats were pretreated with captopril (CPT) to inhibit the angiotensin-converting enzyme and thereby block endogenous ANG II production. With each dose of ANG II tested and with all three vasodilators, prevention of the ANG II-induced increase in AP was associated with an increase in water intake. Furthermore, the inhibitory effect of increased AP on water intake evoked by ANG II appeared to be graded; small reductions in the ANG II-induced increase in AP resulted in small increases in water intake, whereas larger reductions in AP resulted in larger increases in water intake (23). Thus it appears that increased AP reduces water intake evoked by ANG II.

In contrast to ANG II-evoked water intake, whether increases in AP reduce water intake evoked by other thirst stimuli has not been determined. Furthermore, if an increase in AP inhibits thirst, then, in addition to reducing cumulative water intakes, an increase in AP should also inhibit the initiation of the behavior, thereby resulting in a longer latency to drink; however, this has not been tested previously. The initial purpose of the present experiments was to confirm and extend the previous findings that an increase in AP inhibits drinking behavior evoked by an intravenous infusion of ANG II (23) to provide data that allowed direct comparisons with the effects of increased AP on drinking behavior in response to other stimuli. In these experiments, we found that graded reductions in AP resulted in graded increases in water intake and shortened latencies to drink when thirst was stimulated by ANG II. We then sought to determine whether increases in AP similarly inhibit drinking behavior evoked by other thirst stimuli. Water drinking was evoked in rats either by an intravenous infusion of hypertonic saline (HS) to raise plasma osmolality or by a subcutaneous injection of polyethylene glycol (PEG) solution to lower plasma volume. Subsequently, AP was raised by intravenous infusion of either phenylephrine (PE) or endothelin-1 (ET).

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Effects of Increases in AP on Drinking Behavior Evoked by Infusions of ANG II

After a 20-min recording of baseline MAP and HR, rats were injected with CPT (3 mg/kg iv) and then infused continuously with CPT (0.33 mg/min at 25 μl/min iv). Ten minutes later, rats were injected with DZX (5, 10, or 20 mg/kg iv; n = 8 for each dose) or isotonic saline (SLN, 1 ml/kg iv; n = 8). Approximately 1 min later, rats were infused intravenously with 100-ng·kg⁻¹·min⁻¹ ANG II together with 0.33 mg/min of CPT. These groups will be referred to as “ANG II plus CPT and the respective dose of DZX” (e.g., ANG II + CPT + DZX-5). In a similar treatment regimen, CPT has been demonstrated previously to block every water intake evoked by administration of several vasodilators (5, 7, 23). Furthermore, we have observed that CPT administered using this protocol markedly reduces drinking in response to treatment with DZX (20 mg/kg iv); rats treated with CPT and DZX drank 0.5 ± 0.2 ml (n = 6) in a 2-h test, whereas we have reported that this dose of DZX without CPT treatment resulted in an ingestion of 8.5 ± 0.8 ml (n = 11) of water (30). Additional rats were infused simultaneously with ANG II + CPT together with the α-adrenergic receptor agonist PE (4 μg·kg⁻¹·min⁻¹; ANG II + CPT + PE-4; n = 5). Control rats for the CPT treatment were infused only with 100-ng·kg⁻¹·min⁻¹ ANG II (ANG II; n = 8).

Cumulative water intakes (±0.5 ml) were monitored every 15 min for 60 min. In addition, latency from the initiation of the ANG II infusion to the first lick of water was recorded. Urine outputs (±0.1 ml) also were monitored every 15 min for 60 min and then were analyzed for Na⁺ and K⁺ concentrations (System E2A Electrolyte Analyzer; Beckman Instruments, Brea, CA).

Effect of Increases in AP on Drinking Behavior Evoked by Infusion of HS

After water was removed from the cages and MAP and HR were recorded for 20 min, rats were infused intravenously with HS (1 M NaCl, 2 ml/h) for 2 h. At the end of this infusion period, the infusion was switched to one of three concentrations of PE (2, 4, or 8 μg·kg⁻¹·min⁻¹; n = 7, 8, and 8, respectively) or ET (250 ng·kg⁻¹·min⁻¹ for 10 min, then 50 ng·kg⁻¹·min⁻¹ for the remainder of the test; n = 6) given at a rate of 25 μl/min for 90 min. Control rats received an infusion of SLN (n = 8) instead of PE or ET. These groups were referred to as “HS plus the subsequent treatment” (e.g., HS + PE-2). A separate group of rats (n = 6) was infused with HS and then PE (4 μg·kg⁻¹·min⁻¹) as already described, except at 30 min the infusion was switched from PE to SLN; this group will be referred to as “HS + PE-430.” A second group of HS + SLN rats (n = 6) was studied along with the HS + PE-430 group.

Water access was allowed 10 min after the onset of the PE, ET, or SLN infusions. At the time the water bottle was returned to the cages, a few drops of water were placed on the rat’s snout to make them aware of the water bottle. Cumulative water intakes (±0.5 ml) were monitored every 15 min, and latency to the first lick was also recorded. Urine outputs (±0.1 ml) were monitored before and every 15 min after access to water during the test and later were analyzed for Na⁺ and K⁺ concentrations. In addition, blood samples (0.3 ml) were collected via the arterial line from HS + PE-430 rats and from the second group of HS + SLN rats; these samples were taken 10 min before the infusion of HS, 5 min before water access, and 30 and 90 min after water access. Samples were placed in microcentrifuge tubes containing heparin (5 units), immediately centrifuged (10,000 g, 1 min), and then analyzed for plasma Na⁺ and K⁺ concentrations as already described. In this and subsequent experiments, the first blood sample was replaced with an equal volume of SLN, whereas subsequent samples were replaced with red blood cells from the previous sample resuspended in heparinized saline (40 μl/ml). No difference was detected in water intakes, MAP, or HR between HS + SLN rats that had undergone blood sampling and rats that had not, as compared by independent t-tests. Therefore, the data from these two groups were combined.

Effect of Increases in AP on Drinking Behavior Evoked by Hypovolemia

After a 20-min baseline recording of MAP and HR, rats were injected with a 30% (wt/wt) solution of PEG (5 ml sc, Compound 20-M; Carbowax) and were returned to their cages without access to food or water. After allowing 5 h for hypovolemia to develop, rats were infused with PE (24 μg·kg⁻¹·min⁻¹; n = 8) for 60 min; in preliminary experiments, it was found that this large dose of PE was needed to raise MAP. Control rats were infused with SLN (25 μl/min; n = 8) instead of PE. Again, groups will be referred to as “PE plus the subsequent infusion” (e.g., PEG + PE-24). Water access was allowed 10 min after onset of the PE or SLN infusion, with a few drops of water placed on the rat’s snout to make them aware of the water bottle. Cumulative water intakes, urine outputs, and the latency to the first lick were monitored. In addition, blood samples (0.5 ml) were collected from the arterial line in microcentrifuge tubes containing heparin (5 units), immediately centrifuged (10,000 g, 1 min), and then analyzed for Na⁺ and K⁺ concentrations as already described. In addition, red blood cells from the previous sample were resuspended in heparinized saline (40 μl/ml) and then analyzed for plasma renin activity (PRA) as described previously (30).

Statistical Analysis

All data are expressed as means ± SE. Water intakes were analyzed by ANOVA (Systat; SPSS) at each time, and directional planned comparisons were made between a group and the next highest dose of DZX (for ANG II experiments) or PE (for HS or PEG experiments). MAP and mean latencies to drink were analyzed similarly. Significant F values were followed by a layered Bonferroni analysis for the repeated-measures variable. The relationship between water intake or latency to drink and MAP was evaluated by fitting the data to the algorithm...
commonly used to describe baroreflex responses (3, 12). Water intakes at 15 min were plotted as a function of MAP averaged across the first 15 min of the drinking test. MAP values for scatterplots of latency to drink as a function of MAP were taken 30 s before the first lick, since the act of drinking was observed to elevate AP and HR as previously reported (13). For purposes of comparison, rats that did not drink during the tests were assigned values of 30 min for ANG II experiments and 15 min for HS or PEG experiments, since every rat that drank did so within these respective times. MAP values for these animals were simply averaged over these times. The curves generated by the baroreflex algorithm for latency to drink as a function of MAP were similar to those previously generated for the heart period (12).

Urine volume, urine Na⁺ and K⁺ loss, and overall water balance were compared by an ANOVA. Significant \( F \) values were followed by post hoc testing using a layered Bonferroni analysis. Water intakes and MAP of HS + ET rats were compared with those of HS + SLN and HS + PE-4 rats by an ANOVA and were followed by post hoc testing using Fisher’s tests. Plasma osmolality, Na⁺ and K⁺ concentrations, plasma protein concentration, and PRA were compared by repeated-measures ANOVA and were followed by correlated \( t \)-tests or independent \( t \)-tests. In all statistical comparisons, a \( P \) value <0.05 was considered to be significant.

RESULTS

Effect of Increases in AP on Drinking Behavior Evoked by ANG II

An intravenous infusion of 100 ng·kg⁻¹·min⁻¹ ANG II increased water intake and MAP above baseline values (Fig. 1, A and B). Seven of eight rats infused with ANG II drank during the 60-min test, and in these seven rats the latency to drink was 15.4 ± 2.9 min. The intravenous infusion of ANG II + CPT did not significantly affect drinking behavior or MAP compared with an intravenous infusion of ANG II (Fig. 1, A and B);

\[ \text{Fig. 1. Cumulative mean } \pm \text{ SE water intakes (A) and mean arterial blood pressure (MAP; B) of rats infused with ANG II only (ANG II Only; 100 ng·kg}^{-1} \cdot \text{min}^{-1}) \text{ or with captopril (ANG II + CPT; 0.33 mg/kg iv) and injected either with diazoxide (DZX; 5, 10, 20 mg/kg iv) or isotonic saline (SLN; 1 ml/kg) or infused with phenylephrine (PE; 4 mg·kg}^{-1} \cdot \text{min}^{-1}). \text{ Attenuation of the ANG II-induced increase in MAP by DZX resulted in an increase in water intake and did so in a graded manner; the only exception for water intakes was at 30 min for rats given 5 and 10 mg/kg DZX. Injection of DZX resulted in a significantly lower MAP at every time; however, MAP of rats receiving 5 and 10 mg/kg DZX did not differ at 5 and 45 min. *Significant difference from rats infused with ANG II (P < 0.05). †Significant difference from the next smaller dose of DZX in rats infused with ANG II (P < 0.05).} \]

Scatterplot either of 15-min water intake (C) or latency to drink as a function of MAP (D) for each rat infused with ANG II. Significant correlations were found for 15-min water intake and latency to drink when the data were fit to the algorithm commonly used to describe baroreflex responses (*r = -0.77 and 0.70, respectively; both \( P < 0.001)\). Not shown are the changes in heart rate (HR) evoked by these treatments. As expected, rats infused with ANG II displayed a significant bradycardic response; HR dropped from 375 ± 11 to 338 ± 7 beats/min (\( P < 0.05)\) within 3 min after initiation of the infusion of ANG II and remained significantly below baseline values for the remainder of the test. In rats treated with DZX, the ANG II-evoked bradycardia was prevented, and, in rats treated with ANG II + CPT and 20 mg/kg DZX, HR was elevated significantly by 15 min (from 375 ± 5 to 452 ± 19 beats/min; \( P < 0.05)\).
Similarly, seven of eight rats infused with ANG II + CPT drank, and the latency to drink of these seven rats was 17.9 ± 2.4 min.

To produce graded changes in AP, rats were treated with various doses of DZX or 4 μg·kg⁻¹·min⁻¹ of PE together with the infusion of ANG II + CPT. Attenuation of the ANG II-induced increase in MAP with DZX resulted in an increase in water intake and a shorter latency to drink compared with rats infused only with ANG II + CPT (Fig. 1). As the dose of DZX was increased, rats displayed a lower MAP and ingested more water (Fig. 1, A and B); however, water intakes did not differ significantly between rats treated with the two highest doses of DZX (Fig. 1, A and B). All rats treated with 5, 10, and 20 mg/kg of DZX drank, and with increasing doses of DZX the latency to drink decreased (11.8 ± 2.0, 8.0 ± 1.2, and 5.1 ± 1.2 min with 5, 10, and 20 mg/kg, respectively; P < 0.05 compared with the next highest dose of DZX). Infusion of PE in rats treated with ANG II + CPT further increased MAP but did not significantly affect drinking behavior (Fig. 1, A and B). Four of five rats infused with ANG II + CPT + PE drank, and the latency to drink of these four rats (17.3 ± 1.6 min) was not significantly different from that of rats infused with ANG II + CPT (P > 0.05).

The relationship between increases in MAP and 15-min water intake or latency to drink for rats infused with ANG II is shown in Fig. 1, C and D. Acute increases in MAP were correlated strongly with reductions in water intake (r = −0.77, P < 0.001; Fig. 1C) and longer latencies to drink (r = 0.73, P < 0.001; Fig. 1D) when the data were fit to the baroreflex algorithm.

As previously reported (8, 21), attenuation of the ANG II-induced increase in AP led to a dose-related increase in positive water balance (Table 1). No statistically significant differences were found in urinary excretion of Na⁺ or K⁺ (Table 1).

Table 1. Urine volumes, urine Na⁺ and K⁺ loss, and overall water balance for rats infused with ANG II

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume, ml</th>
<th>Na⁺, meq</th>
<th>K⁺, meq</th>
<th>Overall Water Balance, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG II + PE-4</td>
<td>5.1 ± 0.5*</td>
<td>0.17 ± 0.04</td>
<td>0.36 ± 0.09</td>
<td>−1.4 ± 0.9*</td>
</tr>
<tr>
<td>ANG II</td>
<td>2.6 ± 1.2</td>
<td>0.10 ± 0.04</td>
<td>0.27 ± 0.08</td>
<td>+2.5 ± 0.9</td>
</tr>
<tr>
<td>ANG II + CPT</td>
<td>3.5 ± 0.9</td>
<td>0.09 ± 0.05</td>
<td>0.30 ± 0.09</td>
<td>+2.2 ± 1.4</td>
</tr>
<tr>
<td>ANG II + CPT + DZX-5</td>
<td>3.2 ± 1.1</td>
<td>0.05 ± 0.01</td>
<td>0.24 ± 0.04</td>
<td>+4.8 ± 1.6</td>
</tr>
<tr>
<td>ANG II + CPT + DZX-10</td>
<td>2.6 ± 0.5</td>
<td>0.09 ± 0.06</td>
<td>0.19 ± 0.03</td>
<td>+8.4 ± 1.4</td>
</tr>
<tr>
<td>ANG II + CPT + DZX-20</td>
<td>2.1 ± 0.4</td>
<td>0.08 ± 0.02</td>
<td>0.17 ± 0.05</td>
<td>+8.5 ± 0.9†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rats were infused with ANG II (100 ng·kg⁻¹·min⁻¹) either alone or with captopril (CPT, 0.33 mg/min), phenylephrine (PE, 4 μg·kg⁻¹·min⁻¹), or CPT plus diazoxide (DZX, 5, 10, or 20 mg/kg iv). †Significant difference from rats treated with ANG II and ANG II + CPT (P < 0.05). *Significant difference from rats treated with ANG II + CPT + DZX-5 (P < 0.05).

Effects of Increases in AP on Drinking Behavior Evoked by HS Infusions

Effects of PE-induced increases in AP on drinking behavior. To determine whether an increase in AP inhibits drinking behavior evoked by hyperosmolality, water intake and latency to drink were measured after an intravenous infusion of HS, whereas AP was raised by intravenous infusions of PE. The intravenous infusion of HS caused an increase in water intake; all 14 HS + SLN rats drank with a short latency (3.2 ± 0.3 min) and ingested substantial amounts of water during the 90-min test (Fig. 2A). Although MAP was elevated significantly by ~10 mmHg at the end of the 2-h infusion of HS (Fig. 2B), MAP of HS + SLN rats had returned to baseline values by the time water access was allowed and was not statistically different from baseline values thereafter (Fig. 2B).

Each of the three doses of PE tested caused significant elevations in MAP and significantly inhibited drinking behavior (Fig. 2). Furthermore, these effects of PE on both drinking behavior and MAP were dose dependent. However, HS + PE-8 rats ingested amounts of water comparable to HS + PE-4 rats even though HS + PE-8 rats had a significantly higher MAP (Fig. 2, A and B). Infusions of PE also increased the latency to drink. All seven HS + PE-2 rats drank, but the latency to drink (5.1 ± 0.9 min) was significantly longer than observed in HS + SLN rats (3.2 ± 0.3 min; P < 0.05). Furthermore, only four of eight HS + PE-4 rats and five of eight HS + PE-8 rats drank, and they did so with significantly longer latencies (8.0 ± 1.2 and 7.2 ± 1.2 min, respectively) than HS + SLN rats (P < 0.05).

Water intake at 15 min or latency to drink plotted as a function of MAP for each rat infused with HS is presented in Fig. 2, C and D. When these data were fit to the baroreflex algorithm, acute increases in MAP were found to be strongly correlated with the reductions in water intake (r = −0.77, P < 0.001; Fig. 2C) and longer latencies to drink (r = 0.70, P < 0.001; Fig. 2D).

Urine volume, Na⁺, and K⁺ did not differ among treatment conditions before the drinking test (Table 2). Similarly, urine volume and K⁺ did not differ statistically during the drinking test, although a small difference was found in urine Na⁺ between HS + SLN and HS + PE-4 treatment groups. However, significant differences in overall water balances were found among treatment conditions; HS + PE-4 and HS + PE-8 groups each had a significantly greater negative water balance compared with the HS + SLN group.

Effects of terminating PE-4 infusion at 30 min. If the PE-induced increases in AP inhibit drinking behavior evoked by hyperosmolality, then termination of the PE infusion should remove this inhibition. As expected, HS + PE-4₃₀ rats drank little water and displayed significantly elevated MAP during the 30-min PE infusion (Fig. 3, A and B). Only four of six HS + PE-4₃₀ rats drank, and these four rats displayed significantly longer latencies to drink than HS + SLN rats (HS + PE-4₃₀, 6.9 ± 1.6 min; HS + SLN, 3.2 ± 0.3 min;
Upon termination of the intravenous infusion of PE, MAP returned to baseline values within 5 min, and each of the six HS \(1\) PE-430 rats drank during the remainder of the test (Fig. 3, A and B). Cumulative 90-min water intakes of HS \(1\) PE-430 rats were not significantly different from those of HS \(1\) SLN rats, but they were significantly higher than rats infused with PE-4 for the entire test (HS \(1\) PE-4; Fig. 3A).

This inhibition of drinking behavior due to the infusions of PE occurred despite significant elevations in plasma Na\(^+\). The intravenous infusions of HS significantly raised plasma Na\(^+\) in both groups (Table 3); however, plasma Na\(^+\) of rats treated with HS + SLN returned to basal values by 30 min into the drinking test. In contrast, plasma Na\(^+\) of HS + PE-430 rats remained significantly elevated above baseline values at 30 min despite access to water and were significantly higher than those of HS + SLN rats (Table 3).

However, once the PE infusion was terminated and HS + PE-430 rats ingested water, plasma Na\(^+\) returned to baseline values.

Urine volumes and Na\(^+\) and K\(^+\) concentrations did not differ among HS + PE-430, HS + PE-4, and HS + SLN rats before or at the end of the drinking test (Table 2). HS + PE-430 rats were in a smaller negative water balance than rats infused with PE-4 for the entire 90-min, but they were in a greater negative water balance than HS + SLN rats (Table 2).

Effects of ET-induced increases in AP on drinking behavior. To examine whether increases in AP induced by a pressor agent other than PE would inhibit drinking behavior, rats were infused with HS followed by ET. Similar to the results obtained with intravenous infusions of PE, HS + ET rats displayed a significantly elevated MAP throughout the test and drank considerably less water compared with HS + SLN rats (Fig. 4, A and B). Furthermore, five of six HS + ET rats...
Table 2. Urine volumes, urine Na⁺ and K⁺ loss, and overall water balance before and 90 min after water access

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume, ml</th>
<th>Na⁺, meq</th>
<th>K⁺, meq</th>
<th>Overall Water Balance, ml</th>
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<tr>
<td>Before water access</td>
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<td></td>
<td></td>
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<tr>
<td>HS + SLN</td>
<td>9.5 ± 0.7</td>
<td>2.17 ± 0.20</td>
<td>0.64 ± 0.03</td>
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<tr>
<td>HS + PE-2</td>
<td>10.5 ± 1.1</td>
<td>2.05 ± 0.18</td>
<td>0.55 ± 0.06</td>
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<tr>
<td>HS + PE-4</td>
<td>11.6 ± 1.3</td>
<td>2.41 ± 0.23</td>
<td>0.62 ± 0.06</td>
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<tr>
<td>HS + PE-8</td>
<td>10.9 ± 0.7</td>
<td>2.31 ± 0.11</td>
<td>0.66 ± 0.03</td>
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<tr>
<td>HS + PE-4₀₈₀</td>
<td>12.3 ± 1.4</td>
<td>2.53 ± 0.24</td>
<td>0.66 ± 0.06</td>
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<tr>
<td>HS + ET</td>
<td>9.2 ± 0.8</td>
<td>1.89 ± 0.20</td>
<td>0.70 ± 0.09</td>
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<tr>
<td>After water access</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>HS + SLN</td>
<td>6.4 ± 1.0</td>
<td>1.12 ± 0.08</td>
<td>0.26 ± 0.02</td>
<td>+1.1 ± 1.2</td>
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<tr>
<td>HS + PE-2</td>
<td>5.9 ± 1.8</td>
<td>1.15 ± 0.27</td>
<td>0.25 ± 0.06</td>
<td>−3.4 ± 2.4</td>
</tr>
<tr>
<td>HS + PE-4</td>
<td>7.0 ± 1.1</td>
<td>1.77 ± 0.28*</td>
<td>0.31 ± 0.04</td>
<td>−8.7 ± 1.5*</td>
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<tr>
<td>HS + PE-8</td>
<td>7.5 ± 0.6</td>
<td>1.57 ± 0.09</td>
<td>0.25 ± 0.02</td>
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<tr>
<td>HS + PE-4₀₈₀</td>
<td>5.3 ± 0.4</td>
<td>1.33 ± 0.13</td>
<td>0.19 ± 0.02</td>
<td>−4.2 ± 1.2†</td>
</tr>
<tr>
<td>HS + ET</td>
<td>5.3 ± 0.4</td>
<td>1.17 ± 0.34</td>
<td>0.22 ± 0.06</td>
<td>−4.2 ± 2.4‡</td>
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Values are means ± SE. Rats were infused with 1 M NaCl (2 ml/h) for 2 h followed by a 90-min infusion of PE (2, 4, or 8 μg·kg⁻¹·min⁻¹), endothelin-1 (ET, 250 ng·kg⁻¹·min⁻¹ for 10 min, then 50 ng·kg⁻¹·min⁻¹ for the remainder of the test), or isotonic saline (SLN, 25 μl/min). These groups are referred to as hypertonic saline (HS) plus the subsequent treatment (e.g., HS + PE-2). Another group was infused with PE (4 μg·kg⁻¹·min⁻¹) for 30 min and then switched to SLN (HS + PE-4₀₈₀). *Significant difference from HS + SLN group (P < 0.05). †Significant difference from HS + PE-4 group (P < 0.05).

The relationship between increases in MAP and 15-min water intake or latency to drink for rats injected with PEG is shown in Fig. 5, C and D. As with ANG II and HS experiments, acute increases in MAP were strongly correlated with the reductions in water intake (r = −0.76; P < 0.001; Fig. 5C) and longer latencies to drink (r = 0.84, P < 0.001; Fig. 5D) when fit to the baroreflex algorithm.

As expected, subcutaneous injection of PEG significantly raised PRA and plasma protein values in both groups. Throughout the experiment, no statistically significant differences were detected between the groups except that PEG + SLN rats had lower plasma

drunk, and the latencies were significantly longer compared with HS + SLN rats (6.6 ± 0.9 and 3.2 ± 0.3 min, respectively; P < 0.05). Interestingly, HS + ET rats displayed similar MAP to HS + PE-4 rats, and the associated water intakes and latencies to drink did not differ significantly between these two groups (Figs. 2 and 4).

Urine volumes and Na⁺ and K⁺ concentrations did not differ between HS + ET, HS + SLN, and HS + PE-4 groups before and at the end of the drinking test (Table 2).

Effects of Increases in AP on Water Intake Evoked by Hypovolemia

To determine whether increases in AP inhibit drinking evoked by stimuli other than ANG II or HS, rats were made hypovolemic by subcutaneous injection of PEG, and AP was subsequently raised by an intravenous infusion of PE. As expected, subcutaneous injection of PEG increased water intake in PEG + SLN rats and had no effect on MAP compared with baseline values (Fig. 5, A and B). In contrast, PEG + PE-24 rats had elevated MAP and ingested significantly less water than PEG + SLN rats (Fig. 5, A and B). In the PEG + PE-24 group, seven of eight rats drank during the test, and these seven rats had significantly longer latencies to drink than PEG + SLN rats (7.2 ± 2.5 and 2.0 ± 0.4 min, respectively; P < 0.05). Urine volumes did not differ significantly between PEG + PE-24 and PEG + SLN groups (1.7 ± 0.9 and 1.0 ± 0.3 ml, respectively).
protein and lower plasma osmolality than PEG + PE-24 rats did at the end of the drinking test (Table 4).

**DISCUSSION**

Previous studies have demonstrated that attenuation of ANG II-induced increases in AP results in a greater increase in water intake stimulated by ANG II (6, 8, 19, 23). The present data confirm and extend this relationship between AP and drinking behavior by showing that increases in AP also inhibit drinking stimulated by hyperosmolality or hypovolemia. Increases in AP reduced cumulative water intakes and lengthened the latency to drink with each thirst stimulus. Thus it appears that an acute increase in AP provides a signal that generally inhibits thirst in rats.

**Increases in AP Inhibit Drinking Behavior Evoked by ANG II**

Evered and colleagues (8, 23) previously reported that prevention of ANG II-induced increases in AP resulted in a greater increase in water intake. Furthermore, progressive reductions in the ANG II-induced increase in AP resulted in graded increases in water intake (8, 23), as if the elevated AP was constraining thirst. The present findings also show that ANG II-evoked water intakes are greater when the associated increases in AP are prevented by coadministration of the vasodilator DZX, and these findings are remarkably consistent with those reported previously (8, 23).

Cumulative water intakes are only one measure of drinking behavior and may possibly confound the interpretations of experiments, since the ingestion of water will lead to the generation of inhibitory signals (e.g., gastric distension and osmotic dilution) that may act to mask the thirst stimulus. Therefore, we recorded another index of drinking behavior that is insensitive to the consequences of water consumption: the latency to drink. If increases in AP inhibit drinking behavior, then not only should cumulative water intakes be reduced but the latency to drink should be longer. In fact, the present results indicate that ANG II-induced increases in AP delay ANG II-induced drinking, whereas graded reductions of the ANG II-induced increases in AP shorten the latencies to drink. Collectively, the present findings confirm and extend previous observations (8, 19, 23) that increases in AP resulting from an intravenous infusion of ANG II inhibit the evoked thirst.

**Increases in AP Inhibit Drinking Behavior Evoked by Hyperosmolality**

A major goal of these experiments was to determine whether acute increases in AP also inhibit thirst induced by body fluid hyperosmolality or by hypovolemia, two signals for thirst other than ANG II. To examine whether increased AP inhibits drinking induced by hyperosmolality, vasoconstricting drugs were used to increase AP in rats that were treated systemically with HS. This inhibition of thirst was apparent both in the reduced amount of water ingested and in the increased latency to drink. Furthermore, this inhibitory effect of increased AP on drinking was graded, as when thirst was stimulated by ANG II. In addition, these effects were observed with two different pressor agents, PE and ET; indeed, HS + PE-4 and HS + ET rats exhibited approximately equal elevations in MAP, ingested

![Fig. 4. Cumulative mean ± SE water intakes (A) and MAP (B) of rats infused with 1 M NaCl (2 ml/h) for 2 h and then ET (250 ng·kg⁻¹·min⁻¹) for 10 min, then 50 ng·kg⁻¹·min⁻¹ for the remainder of the test; referred to as “HS + ET”). Cumulative water intakes and MAP for HS + SLN rats, presented in Fig. 2, A and B, are included here for purposes of comparison. HS + ET rats drank significantly less than HS + SLN rats and displayed significantly higher MAP throughout the entire 90-min test. *Significant difference from HS + SLN rats at every time during water access (P < 0.01). Rats infused with ET also displayed a bradycardic response as HR dropped from 390 ± 8 to 345 ± 10 beats/min at 0 min (P < 0.05) but did not remain significantly lower than baseline values at the end of the 90-min test (356 ± 14 beats/min).
equivalent amounts of water, and displayed similar latencies to drink. Most striking were the results of the experiment in which the PE infusion was terminated at 30 min. Water intakes of HS \textsuperscript{1}PE-430 rats were blunted significantly during the PE infusion despite elevated plasma Na\textsuperscript{+}, yet these rats ingested water in amounts comparable to control rats within 60 min after the PE infusion was terminated. These results provide strong support for the proposal that increases in AP inhibit drinking behavior stimulated by HS.

**Increases in AP Inhibit Drinking Behavior Evoked by Hypovolemia**

Increases in AP also inhibited drinking behavior stimulated by hypovolemia. Subcutaneous PEG treatment is known to gradually withdraw protein-free plasma from the intravascular space and sequester it in an edema at the injection site; stimulated water intake is proportionate to the induced plasma volume deficit (31). Compared with rats injected with PEG solution followed by infusion of SLN, PEG + PE-24

### Table 4. PRA, plasma protein, and plasma osmolality of PEG + SLN and PEG + PE-24 rats

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 min Before H\textsubscript{2}O Access</th>
<th>60 min After H\textsubscript{2}O Access</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRA, ng ANG I·ml plasma\textsuperscript{−1}·h\textsuperscript{−1}</strong></td>
<td></td>
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<td></td>
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<tr>
<td>PEG + SLN</td>
<td>2.0 ± 0.3</td>
<td>19.5 ± 2.6\textsuperscript{*}</td>
<td>17.4 ± 5.1\textsuperscript{*}</td>
</tr>
<tr>
<td>PEG + PE-24</td>
<td>1.4 ± 0.4</td>
<td>18.4 ± 2.9\textsuperscript{*}</td>
<td>14.2 ± 1.4\textsuperscript{*}</td>
</tr>
</tbody>
</table>

| **Plasma protein, g/dl**                     |          |                                        |                                       |
| PEG + SLN      | 5.7 ± 0.1 | 7.0 ± 0.1\textsuperscript{*}          | 6.7 ± 0.2\textsuperscript{*}          |
| PEG + PE-24    | 5.9 ± 0.1 | 7.2 ± 0.3\textsuperscript{*}          | 7.4 ± 0.2\textsuperscript{†}         |

| **Plasma osmolality, mosmol/l**              |          |                                        |                                       |
| PEG + SLN      | 295 ± 1  | 294 ± 2                                | 287 ± 1\textsuperscript{*}           |
| PEG + PE-24    | 297 ± 2  | 296 ± 1                                | 294 ± 3\textsuperscript{†}           |

Values are means ± SE. Rats were injected subcutaneously with 30\% (wt/wt) solution of polyethylene glycol (PEG). Five hours later, rats were infused with PE (24 μg·kg\textsuperscript{−1}·min\textsuperscript{−1}; \(n = 8\)) or SLN (25 μl/min; \(n = 8\)) for 60 min. Blood samples were obtained at baseline conditions (before PEG injection), 5 min before the drinking test, and 60 min after water access. \*Significant difference from baseline values within the same group (\(P < 0.05\)). †Significant difference from PEG + SLN at the same time (\(P < 0.05\)).

![Fig. 5. Cumulative 60-min water intakes (A) and MAP (B) in response to a subcutaneous injection of polyethylene glycol (PEG) and an intravenous infusion of either PE (24 μg·kg\textsuperscript{−1}·min\textsuperscript{−1}) or SLN (25 μl/min). The arrow indicates the time of subcutaneous injection, and the infusions of PE or SLN began 5 h later. PEG + PE-24 rats drank less water (\#significant difference at each time; \(P < 0.01\)) and had a higher MAP (\#significant difference at each time during drinking test except at 60 min; \(P < 0.05\)) compared with PEG + SLN rats. Scatterplots either of 15-min water intake (C) or latency to drink (D) as a function of MAP for individual rats injected with PEG. Significant correlations were found for both 15-min water intake and latency to drink when the data were fit to the baroreflex algorithm (\(r = 0.76\) and 0.84, respectively; both \(P < 0.001\)). The subcutaneous injection of PEG significantly raised HR in both treatment groups from baseline values of 378 ± 10 and 386 ± 8 beats/min to values of 424 ± 11 and 440 ± 14 beats/min, respectively, just before intravenous infusions (\(P < 0.05\)). HR remained significantly elevated in PEG + SLN rats throughout the remainder of the test (\(P < 0.05\)). In contrast, PEG + PE-24 rats initially displayed a bradycardic response as HR dropped to 341 ± 14 beats/min at 0 min, but this response was not sustained throughout the remainder of the test.](http://ajpregu.physiology.org/)
ACUTE HYPERTENSION INHIBITS THIRST IN RATS

Rats exhibited an elevated MAP, ingested less water, and displayed a longer latency to drink.

Although these data clearly show that PE-induced increases in AP inhibit hypovolemia-induced water intake, the interpretation of this experiment may not be straightforward. Unlike the case with drinking stimulated by intravenous infusion of ANG II or HS where increased AP did not directly alter the strength of the stimulus for drinking, increased AP may decrease the stimulus for hypovolemic thirst. For example, decreases in intravascular fluid volume are thought to be detected by stretch receptors located in the venoatrial junction (9), and increases in AP may diminish this afferent signal by increasing intracardiac pressure (11). In addition, hypovolemia increases renin secretion, leading to increases in circulating ANG II that might contribute to the drinking under conditions of low intravascular fluid volume (9, 14, 28), but increases in AP may interfere with hypovolemia-induced renin secretion (17) and thereby also interfere with PEG-evoked drinking. Nonetheless, measurements of PRA in the present experiments indicate that, in PEG-treated rats, an infusion of PE did not significantly reduce PRA. Because these PRA measurements were taken 60 min after the start of the PE infusion when AP had almost returned to baseline levels, it is conceivable that sampling blood at earlier time points when AP was much higher may have revealed a PE-evoked reduction in PEG-stimulated renin secretion. Regardless, the present findings indicate that increases in AP inhibit drinking behavior stimulated by hypovolemia and by ANG II and HS.

It is noteworthy that the dose of PE used to raise AP in PEG-treated rats was considerably larger than the doses used to increase AP in experiments when HS stimulated thirst. Preliminary experiments indicated that 4 or 8 µg·kg⁻¹·min⁻¹ infusion of PE did not alter MAP in rats injected with PEG. It seems likely that the effectiveness of a pressor substance such as PE to evoke an increase in AP would be blunted, since vascular tone is already elevated in hypovolemic animals to maintain AP (10, 24). Therefore, larger doses of PE were needed to increase AP above baseline values.

**Relationship Between Increases in AP and Drinking Behavior**

In summary, the present findings demonstrate that increased AP inhibits drinking behavior evoked by the following three thirst stimuli: ANG II, hyperosmolality, and hypovolemia. To compare the effect of increased AP on drinking across the various treatments, the mean ± SE percent change in water intake was plotted as a function of MAP for each of these groups. The data collected at 15 min were used in this analysis to limit the potential contribution of other inhibitory signals that may be generated by the ingestion of water (for example, gastric distension and osmotic dilution). As shown in Fig. 6, the induced increases in MAP reduced water intake comparably whether thirst was stimulated by ANG II, hyperosmolality, or hypovolemia. A similar relationship was observed with the effects of increased AP on latency to drink, as those rats with an elevated MAP displayed a longer latency to drink in the three groups (see Figs. 1D, 2D, and 3D).

It is noteworthy that the data in Fig. 6 relating water intake to MAP resemble the well-known baroreceptor-reflex curve relating decreases in HR (12) or sympathetic nerve activity (3, 15) to increases in MAP. First, graded increases in MAP reduce HR, sympathetic nerve activity, and water intake in a roughly linear manner in the range of MAP between 115 and 145 mmHg. Second, at MAP exceeding 160 mmHg, further reductions in HR, sympathetic nerve activity, and drinking do not occur. A similar effect is observed with latencies to drink, as a longer latency to drink was not observed in rats with MAP >160 mmHg (e.g., ANG II + CPT vs. ANG II + PE-4 in Fig. 1, HS + PE-4 vs. HS + PE-8 rats in Fig. 2). Furthermore, significant correlations were obtained with water intake and latency when the data were fit to the baroreflex algorithm (see Figs. 1, 2, 5, and 6).

The similarities between the effect of increased MAP on drinking behavior and HR suggest that they may be mediated by a common mechanism. The primary way in which the central nervous system detects changes in AP is through an afferent signal arising from stretch receptors located on vessel walls of the aortic arch and carotid sinus. Selective removal of these arterial baroreceptors eliminates the reflexive changes in sympathetic nerve activity (2, 3) and HR (26) to perturbations in AP. However, changes in AP might also influence venous return and thereby activate a second set of stretch receptors located on the walls of the vena cava and heart (cardiac baroreceptors; see Refs. 2 and 3).

With regard to drinking, when both arterial and cardiac baroreceptor afferents are eliminated by electrolytic lesions of the nucleus tractus solitarius, rats
drank sooner and ingested significantly more water than controls during an intravenous infusion of pressor doses of ANG II (25). Similarly, surgical removal of both arterial and cardiac baroreceptor afferents resulted in greater water intakes and shortened latencies to drink during intravenous infusions of ANG II in dogs (18). Although selective removal of arterial baroreceptor afferents has been reported not to enhance ANG II-induced water intakes in rats (16, 22), it is unclear whether animals in these two studies were denervated completely. Neither study examined reflex changes in HR to decreases in AP, and the importance of assessing baroreflex function by both increases and decreases in AP has been emphasized previously (27). Moreover, Rettig and Johnson (22) still observed small residual HR responses in their surgically denervated rats under anesthesia, which may further blunt these responses (27). In contrast, we have observed that rats with complete and selective removal of arterial baroreceptor afferents drink significantly more water than control rats, and also display shorter latencies to drink, in response to an intravenous infusion of pressor doses of ANG II (29). It remains to be determined whether arterial and/or cardiac baroreceptors mediate the inhibitory influence of increases in AP on thirst evoked by hyperosmolality and hypovolemia.

Perspectives

The baroreflex plays an important role in maintaining proper perfusion of tissues when animals are faced with acute perturbations in AP. These changes in AP are sensed by receptors on the vessel walls of the carotid sinus and aortic arch, integrated within various regions of the central nervous system, and lead to changes in the activity of the sympathetic and parasympathetic nervous systems as well as changes in the activity of hypothalamic neuroendocrine systems. These neural and endocrine responses act in concert to restore AP to the original set-point levels. The present findings demonstrate that acute increases in AP also inhibit drinking behavior. Therefore, discussions of baroreflex function should not only encompass neural and endocrine responses but should also include behavioral responses as well. Because baroreflex function involves reflex changes to both increases and decreases in AP, it would be interesting to examine whether decreases in AP enhance drinking in response to each of these stimuli for thirst.

Although the present data demonstrate that acute increases in AP inhibit drinking behavior, these results should not extend to chronic elevations in AP because baroreceptors are known to reset (3). In fact, chronically hypertensive rats typically exhibit normal daily water intakes and/or water balances (1, 4, 20, 21).

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