Role of renin-angiotensin system in hypotension-evoked thirst: studies with hydralazine

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Received 9 September 1999; accepted in final form 20 March 2000

Stocker, Sean D., Alan F. Sved, and Edward M. Stricker. Role of renin-angiotensin system in hypotension-evoked thirst: studies with hydralazine. Am J Physiol Regulatory Integrative Comp Physiol 279: R576–R585, 2000.—Injection of rats either with diazoxide (25 mg/kg iv), isoproterenol (0.33 mg/kg sc), or hydralazine (HDZ) (10 mg/kg ip) decreased arterial blood pressure from ~120 to 70–80 mmHg and stimulated renin secretion. However, diazoxide and isoproterenol treatments each stimulated water ingestion, whereas HDZ treatment did not. HDZ treatment did not reduce water intake evoked by systemic injection of hypertonic saline or 20% polyethylene glycol solution or by 24-h water deprivation, suggesting that HDZ treatment did not interfere with drinking behavior. In contrast, HDZ treatment markedly reduced water intake evoked by injection of diazoxide or isoproterenol or by intravenous infusion of renin. Furthermore, a highly significant correlation was observed when plasma ANG II levels were plotted as a function of plasma renin activity after intravenous infusion of renin and after diazoxide and isoproterenol treatments. However, values obtained after HDZ treatment alone or in combination with intravenous infusion of renin did not fall near the 99% confidence interval of the regression line, suggesting that HDZ treatment blocks ANG II production and/or promotes its clearance. Thus rats apparently do not increase water intake after HDZ treatment, because this drug interferes with the renin-angiotensin system. These results provide further evidence that arterial hypotension evokes thirst in rats predominantly by activation of the renin-angiotensin system.

METHODS

Animals

Adult male Sprague-Dawley rats (Zivic Laboratories, Zelienople, PA), weighing 275–400 g, were individually housed in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle. Tap water and Purina Laboratory Chow pellets were available ad libitum, except where noted. All experiments were performed during the light portion of the light-dark cycle. All surgery was performed in rats anesthetized with halothane (2–3% in 100% O2).

Effects of Hypotensive Agents on Water Intake

An initial experiment was conducted to replicate previous reports (6, 21) that HDZ treatment does not increase water intake in rats despite substantial hypotension. Dia and Isop also were studied for purposes of comparison. Doses and routes of drug administration used in previous studies were selected to produce comparable reductions in mean arterial blood pressure (MAP). Animals to be given Dia had a catheter (PV-3 tubing filled with 40 U/ml heparin in isotonic saline) placed into the right femoral vein at least 1 day before testing. The free end of the catheter was passed subcutaneously up the back and exited between the scapulae.

On the day of experiments, all animals were weighed and food was removed from the cages. One hour later, rats were injected with either HDZ (10 mg/kg iv; n = 11), Isop (0.33 mg/kg sc; n = 11), Dia (25 mg/kg iv; n = 11), or an isotonic saline vehicle (Sal, 1 mL/kg ip; n = 11). Rats then were returned to their cages, where access to water was delayed for 10 min to allow the drugs to exert their effects. Cumulative water intakes (±0.5 ml) were measured every 15 min for the first hour and then again 1 h later. Urine volumes (±0.1 ml) were monitored hourly during the 2-h test.

The initial purpose of the present study was to determine whether HDZ treatment actually does disrupt drinking behavior in rats. HDZ treatment was combined with various paradigms known to evoke thirst in rats. The results indicated that HDZ-treated rats drank normally when plasma hyperosmolality, water deprivation, or hypovolemia stimulated thirst; however, HDZ-treated rats did have markedly reduced water intakes when evoked by injection of Dia or Isop. These observations led us to examine the effects of HDZ treatment on the renin-angiotensin system (RAS).

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Effects of Hypotensive Agents on MAP, Heart Rate, Plasma Osmolality, and Plasma Renin Activity

A separate group of rats was used for recording MAP and heart rate (HR) as well as for collecting blood samples for assay of plasma renin activity (PRA). At least 1 day before these experiments, catheters were implanted into the right femoral artery (PE-50 tubing filled with 500 U/ml heparinized saline) and vein (PV-3 tubing filled with 40 U/ml of heparinized isotonic saline). The catheters were tunneled subcutaneously to exit between the scapulae. On the day of the experiments, animals were weighed and the arterial and venous lines were connected to a tether and swivel system (Harvard Apparatus, South Natick, MA) to allow for blood sampling, drug administration, and continuous recording of MAP and HR. Rats were returned to their cages, and food and water were removed 1 h before beginning the experiment. Baseline MAP and HR were measured for at least 20 min before drug administration and for 2 h thereafter. Rats were treated with HDZ (n = 5), Dia (n = 6), Isop (n = 6), or Sal (n = 6) in the doses used in the investigation of drinking behavior. Blood samples (1.5 ml) were taken from the arterial catheter just before drug administration and 10, 30, and 120 min later. Samples were collected in cold centrifuge tubes containing 2 μl of heparin (10,000 U/ml) and immediately centrifuged (10,000 g, 1 min). Plasma aliquots of 0.1 ml were stored at −80°C until they were used for determination of plasma osmolality and radioimmunoassay of PRA. Plasma osmolality was measured from two 20-μl aliquots by freezing-point depression using a micro-osmometer (model 3360, Advanced Instruments, Norwood, MA). In this and subsequent experiments, the first blood sample was replaced with an equal volume of isotonic saline, whereas subsequent samples were replaced with red blood cells from the previous sample resuspended in heparinized saline (40 U/ml).

Effect of HDZ Treatment on Thirst in Different Paradigms

Subsequent experiments were conducted to determine whether HDZ treatment disrupted drinking stimulated by one of several MAPs known to evoke thirst in rats. In one group, HDZ (10 mg/kg ip; n = 11) was administered 2 h after injection of 2 M NaCl (1 ml/100 g ip). In a second group, rats were dehydrated of water overnight and given HDZ (10 mg/kg ip; n = 11) the next morning. In a third group, rats were injected with 20% (wt/wt) solution of polyethylene glycol (PEG; 5 ml sc; Carbowax, Compound 20-M) and returned to their cages without access to water or food. Such PEG treatment gradually withdraws protein-free plasma from the intravascular space and sequesters it in an edema at the injection site; water intake stimulated by this treatment is proportionate to the induced plasma volume deficit (31). After allowing 3 h for hypovolemia to develop, HDZ (10 mg/kg ip; n = 11) was administered. In all three experiments, Sal (1 ml/kg sc; n = 11) was administered instead of HDZ in control rats. Water access was delayed for 10 min after injection. Cumulative water intakes and urine outputs were measured every 15 min for the first hour and 1 h thereafter.

Additional rats were pretreated with HDZ (10 mg/kg iv) 10 min before injection of Dia (25 mg/kg iv; n = 11) or Isop (0.33 mg/kg sc; n = 11). Other groups of catheterized rats were pretreated with HDZ (10 mg/kg ip; n = 8) or Sal (1 ml/kg iv; n = 8) 10 min before infusion of renin (0.8 U/kg · min⁻¹, 25 μl/min iv for 2 h) or ANG II (250 ng · kg⁻¹ · min⁻¹, 25 μl/min iv for 1 h). Water intakes and urine outputs were measured every 15 min throughout these experiments. Measurements of MAP and HR were taken before each drug administration and every 15 min during the experiments. In addition, blood samples (0.5 ml) were collected at baseline and 30, 60, and 120 min after initiation of the infusion of renin. Plasma was collected in cold microcentrifuge tubes containing 1 mM EDTA (5 μl) and immediately centrifuged (10,000 g, 1 min). Plasma aliquots were stored at −80°C until they were used later for determination of PRA and ANG II levels.

Effect of HDZ Treatment on the RAS

Experiments were conducted to determine PRA and plasma ANG II levels during arterial hypotension and during intravenous infusion of renin. Blood samples for measurement of PRA in rats treated with HDZ, Dia, Isop, or Sal were collected while measuring MAP and HR as described earlier. Blood samples for plasma ANG II levels after these same treatments were collected from other rats that were implanted with arterial and venous catheters as described above. After a 20-min baseline recording of MAP and HR, animals were injected with HDZ (10 mg/kg ip; n = 5), Dia (25 mg/kg iv; n = 6), Isop (0.33 mg/kg sc; n = 6), or Sal (1 ml/kg iv; n = 5). At 5 min before drug injection (baseline), and then 10, 30, and 120 min after drug administration, blood samples (0.9 ml) were collected in cold microcentrifuge tubes containing 0.3 mM EDTA (25 μl) and 20 mM 1,10-phenanthroline (75 μl) and immediately centrifuged (10,000 g, 1 min). Plasma aliquots were stored at −80°C until they were used for determination of ANG II levels. In addition, blood samples collected during the infusion of renin were analyzed for PRA and plasma ANG II levels.

PRA was measured as described previously (27, 34), except that incubation times were 15 min. ANG II levels were determined in duplicate 200-μl plasma aliquots by radioimmunoassay after extraction using C₁₂ Sep-Pak Vac Cartridges (1 ml, 50 mg; Waters, Milford, MA) as previously described for measurement of oxytocin (26). After plasma samples and standard solutions of synthetic ANG II (Bachem, Torrence, CA) were extracted, dried using a Speed Vac (Savant Instruments, Hicksville, NY), and reconstituted in 200 μl of buffer (50 mM NaPO₄, 25 mM EDTA, 0.9% NaCl, 0.5% bovine serum albumin, 0.1% sodium azide, pH 7.4), samples were incubated overnight at 4°C with a rabbit anti-serum to ANG II (1,300,000) generously provided by Dr. Ian Reid. On the next day, 125I-labeled ANG II (New England Nuclear-DuPont, Boston, MA) was added to each sample, and samples were again incubated overnight at 4°C. Goat anti-rabbit IgG and normal rabbit serum were then added, and, after an overnight incubation, samples were centrifuged (3,000 g, 20 min). The supernatant was aspirated, and pellets were counted. The sensitivity of the assay is 0.6 pg/ml plasma. In a separate set of experiments, varying concentrations of HDZ, up to 0.1 mg/ml, were added directly to plasma samples to determine whether HDZ interferes with either PRA or plasma ANG II radioimmunoassays. At each concentration, HDZ was found not to alter values from either assay.

Statistical Analysis

All data are expressed as means ± SE. Cumulative 2-h water intakes and urine outputs were compared between treatments by ANOVA (Systat, SSPS). Significant F values were followed by post hoc testing using a layered Bonferroni analysis. MAP and HR measurements were analyzed by ANOVA with repeated measures and were followed by post hoc testing with layered Bonferroni test when significant F values were obtained. PRA and ANG II levels were log transformed and analyzed by ANOVA with repeated measures. Post hoc testing was performed with the layered Bonferroni.
ferroni test. Ratios of ANG II to PRA from intravenous renin infusion were analyzed via Mann Whitney-\textit{U} test. A \( P \) value <0.05 was considered to be statistically significant. A linear regression analysis of the relation between PRA and plasma ANG II levels was computed by the method of least squares. A linear regression analysis of the relation between PRA and plasma ANG II was altered in HDZ-treated rats.

**RESULTS**

As expected, rats did not increase water intake significantly after HDZ treatment; HDZ-treated rats drank 2.2 ± 0.7 ml in 2 h, whereas rats injected with Sal drank 1.1 ± 0.2 ml in 2 h (Fig. 1). In contrast, Dia and Isop treatments each increased water intake substantially during the 2-h test period, with rats consuming 9.5 ± 0.9 and 8.7 ± 0.8 ml, respectively (Fig. 1). Cumulative 2-h urine volumes did not differ significantly in rats after HDZ, Sal, Dia, or Isop treatments (0.5 ± 0.2, 1.5 ± 0.3, 2.2 ± 0.8, 1.6 ± 0.4 ml, respectively; \( P > 0.1 \) for overall ANOVA).

As shown in Fig. 2, baseline levels of MAP did not differ among groups of rats receiving the different treatments. After HDZ treatment, MAP decreased abruptly from \(-120\) to 65–75 mmHg and remained well below baseline levels throughout the 2-h test period. Dia and Isop treatments produced comparable effects in the onset, magnitude, and duration of the reduction in MAP. Rats displayed a significant tachycardia after each drug treatment; HR rose rapidly from baseline values of 375–400 to 475–550 beats/min within 15 min and stabilized at 450–525 beats/min during the remainder of the test (\( P < 0.05 \); data not shown). There were no significant differences observed in HR at any time between rats given HDZ, Dia, or Isop treatments (\( P > 0.1 \) from overall ANOVA). The Sal treatment had no significant effect on MAP or HR. Plasma osmolalities did not differ between treatment groups at any time (data not shown), as previously reported (1, 26).

**Effect of HDZ Treatment on Thirst in Different Paradigms**

**Hypertonic saline.** Sal-treated rats drank 10.3 ± 1.0 ml of water after intraperitoneal injection of 2 M NaCl. Similarly, HDZ-treated rats drank 11.5 ± 1.2 ml in 2 h (Fig. 3A). The urine volumes of these two groups did not differ significantly during the 2-h period before water access (8.9 ± 1.2, 9.3 ± 1.1 ml, respectively), but HDZ-treated rats excreted less urine during the drinking test than did Sal-treated rats (0.7 ± 0.1, 1.6 ± 0.2 ml, respectively; \( P < 0.01 \)).

**Water deprivation.** Similar results were obtained when water intake was evoked by 24-h water deprivation. Sal-treated control rats drank 12.7 ± 0.7 ml of water in 2 h, while HDZ-treated rats drank 14.7 ± 0.7 ml (Fig. 3B). Urine volumes during the test did not differ significantly between the two groups (1.9 ± 0.5, 1.2 ± 0.4 ml, respectively).

**PEG treatment.** HDZ treatment also did not reduce water intake stimulated by subcutaneous injection of 20% PEG. In fact, HDZ-treated rats drank 9.4 ± 1.2 ml in 2 h, which was unaccountably higher than the 6.0 ± 0.4 ml consumed by Sal-treated rats. However, this difference in water intake was not seen at the 1-h time point (Fig. 3C). The urine volumes of the two groups did not differ significantly either in the 3-h period before water access (1.0 ± 0.3, 1.0 ± 0.2 ml, respectively) or during the 2-h drinking test (0.4 ± 0.2, 0.6 ± 0.1 ml, respectively).

**HDZ plus Dia or Isop.** In contrast to the treatments above, HDZ treatment reduced water intakes evoked either by Dia or Isop. Rats given Dia and Isop treatments drank 9.5 ± 0.9 and 8.7 ± 0.8 ml in 2 h, respectively, whereas rats drank only 3.3 ± 0.2 ml when HDZ was combined with Dia and 4.5 ± 0.2 ml when HDZ was combined with Isop (Fig. 4A). Most of
the drinking by rats given Dia or Isop treatment alone occurred during the first 45 min of the test, by which time rats pretreated with HDZ had consumed only 0–2 ml of water. Urine volumes of rats given HDZ combined with Dia or Isop were very low (each was 0.4 ± 0.1 ml in 2 h; both P values <0.05 compared with rats given Dia or Isop alone). MAP of HDZ-pretreated rats given Dia or Isop were stable at 60–70 mmHg, 10–15 mmHg below those of rats given Dia or Isop alone (Fig.

4B), whereas their elevated HR resembled those of rats given Dia or Isop alone (450–525 beats/min).

Renin infusion. HDZ markedly reduced water intake stimulated by intravenous infusion of renin (Fig. 5A). Sal-pretreated rats ingested 14.4 ± 1.8 ml of water in 2 h, whereas rats pretreated with HDZ drank only 4.9 ± 1.2 ml (P < 0.01). In fact, only one of the eight HDZ-treated rats drank within the first 45 min of the test, whereas every Sal-pretreated rat drank within the first 15 min. HDZ-treated rats also excreted much less urine in 2 h than did Sal-pretreated rats (1.3 ± 0.6, 12.3 ± 1.7 ml, respectively; P <0.001).

Significant differences in MAP and HR were observed between renin-infused rats pretreated with HDZ and those pretreated with Sal. The intravenous infusion of renin in Sal-pretreated rats increased MAP significantly from 120 ± 2 to 166 ± 6 mmHg within 15 min, and MAP remained elevated throughout the infusion (Fig. 5B). These rats also displayed a significant
bradycardia; HR dropped from 382 ± 8 to 340 ± 8 beats/min at 15 min and remained significantly lower than baseline values throughout the 2-h test (350 ± 9 beats/min at 2 h; *P* < 0.05). In contrast, HDZ initially lowered MAP from 120 ± 4 to 80 ± 3 mmHg, and intravenous infusion of renin never did elevate MAP above control levels during the test (Fig. 5B). HR increased significantly from 396 ± 9 to 521 ± 10 beats/min and remained elevated during the infusion of ANG II (*P* < 0.05).

**Effect of HDZ Treatment on RAS**

PRA levels increased significantly from baseline after HDZ, Dia, and Isop treatments, and remained

Fig. 5. Cumulative mean ± SE water intakes (A) and MAP (B) of rats treated either with HDZ (10 mg/kg iv; *n* = 8) or with Sal (1 ml/kg iv; *n* = 8) 10 min before infusion of renin (0.8 U · kg⁻¹ · min⁻¹ iv). The first arrow in B indicates treatment with HDZ or Sal, the second arrow indicates onset of renin infusion. HDZ treatment significantly reduced water intakes (*P* < 0.01) and MAP evoked by renin at each time (*P* < 0.025).

**ANG II infusion.** When HDZ treatment was combined with intravenous infusion of ANG II, rats drank water steadily throughout the test, consuming 9.2 ± 1.7 ml in 1 h. These intakes did not differ significantly from those of Sal-treated rats, which drank 10.4 ± 1.8 ml in 1 h (Fig. 6A). Urine outputs also did not differ significantly between these groups (4.2 ± 0.8, 4.0 ± 0.9 ml, respectively, in 1 h).

Striking differences were observed in MAP and HR in Sal- and HDZ-treated rats infused with ANG II (Fig. 6B). As expected, in Sal-treated rats MAP markedly increased during the intravenous infusion of ANG II, from 118 ± 2 to 162 ± 3 mmHg at 15 min, and it remained elevated throughout the test (*P* < 0.01; Fig. 6B). In contrast, MAP was lowered by HDZ treatment, from 117 ± 2 to 76 ± 3 mmHg, and, although it increased initially during the intravenous infusion of ANG II, it then fell below baseline values and remained low during the balance of the test. Sal-treated rats also displayed a significant bradycardia in response to intravenous infusion of ANG II, as HR dropped from 398 ± 8 to 357 ± 14 beats/min and remained below baseline values throughout the test (*P* < 0.05). In contrast, HDZ-treated rats displayed a significant tachycardia, as HR increased from 396 ± 9 to 521 ± 10 beats/min and remained elevated during the infusion of ANG II (*P* < 0.05).

Fig. 6. Cumulative mean ± SE water intakes (A) and MAP (B) of rats treated either with HDZ (10 mg/kg iv; *n* = 8) or with Sal (1 ml/kg iv; *n* = 8) 10 min before infusion of ANG II (250 ng · kg⁻¹ · min⁻¹, 25 μl/min iv). The first arrow in B indicates treatment with HDZ or Sal, the second arrow indicates onset of ANG II infusion. HDZ did not significantly affect ANG II-induced water intakes but did blunt the induced increase in MAP at 30, 45, and 60 min of the test period (*P* < 0.02).
above baseline during the 2-h test (Fig. 7A). No statistically significant differences were found between the effects of HDZ, Dia, and Isop treatments at any times except 30 min; then, PRA levels were significantly higher after HDZ and Isop treatments than after Dia treatment (Fig. 7A). Sal treatment had no effect on PRA levels (P > 0.2 from overall ANOVA; Fig. 7A).

Similarly, plasma ANG II levels after Dia and Isop treatments increased significantly and remained elevated throughout the 2-h test (Fig. 7B). In contrast, plasma ANG II levels after HDZ treatment did not differ significantly from baseline values at 10 or 30 min, but were significantly higher at 120 min (Fig. 7B). Furthermore, plasma ANG II levels in HDZ-treated rats were not significantly different from those of Sal-treated rats at 10 min (Fig. 7B). However, no statistically significant differences were observed between the effects of HDZ and Dia treatments on plasma ANG II levels (Fig. 7B). Sal treatment had no effect on PRA or plasma ANG II levels at any time (Fig. 7, A and B).

Both PRA values (Fig. 8A) and plasma ANG II levels (Fig. 8B) increased significantly above baseline levels when Sal-treated rats were infused with renin. However, even larger increases in PRA were observed in HDZ-treated rats infused with renin (Fig. 8A), but the increases in plasma ANG II levels after HDZ treatment were markedly blunted compared with Sal treatment at each time (Fig. 8B). Furthermore, HDZ treatment significantly reduced the ratio of plasma ANG II levels to PRA below those observed during baseline and after Sal treatment at each time (Fig. 8C).

Figure 9 displays a linear regression analysis in which mean plasma ANG II values were plotted as a function of mean PRA obtained during intravenous infusion of renin and after Dia and Isop treatments. As expected, PRA and plasma ANG II levels were highly correlated with one another (Fig. 9). In contrast, values obtained from rats treated with HDZ, alone or during intravenous infusions of renin, did not fall close to the 99% confidence interval of this regression line (Fig. 9).

**DISCUSSION**

Dia and Isop treatments are well known to decrease arterial blood pressure, increase renin secretion, and increase water intake in rats (1, 3, 14, 17, 21–23). The biological basis of the induced drinking response has been attributed largely to increased activity of the RAS (1–3, 11, 13, 29). However, previous studies have observed that rats do not increase water intake after treatment with the selective arteriolar vasodilator HDZ (6, 21), which causes decreases in MAP (26) and increases in PRA (22). Those observations have now been replicated again, and it is clear from the present findings that different degrees of hypotension or renin secretion cannot account for observed differences in water intake. Nor does HDZ treatment disrupt drinking behavior, as suggested by Evered (1). Instead, HDZ appears to interfere with the RAS, and this unexpected effect likely underlies the failure of HDZ-treated rats to drink.

The general strategy used to determine whether HDZ treatment disrupts drinking behavior was to combine HDZ with other treatments known to evoke thirst in rats. During three such paradigms, drinking behavior clearly was not impaired by HDZ treatment. That is, after systemic injection of hypertonic saline or 20% PEG, or after 24-h water deprivation, HDZ treatment did not reduce, much less abolish, water intake. These findings indicate that the absence of drinking observed when HDZ alone is administered has some basis other than a general disruption of drinking behavior.

In contrast, water intakes stimulated by Dia and Isop treatments were reduced in HDZ-treated rats. However, it is difficult to interpret these findings. It has been suggested that drinking behavior in rats may
be impaired when MAP falls to 60 mmHg (9), and such low blood pressure occurred when HDZ was combined with Dia or Isop. This more severe hypotension may underlie the reduction in water intakes. Alternatively, HDZ treatment may selectively interfere with the signal for thirst evoked by these hypotensive drugs. To examine this latter issue more directly, we determined whether HDZ treatment interferes with drinking evoked solely by the RAS.

Similar to water intakes stimulated by Dia and Isop treatments but not by hypertonic saline, water deprivation, or PEG treatment, HDZ treatment markedly reduced water intakes evoked by intravenous infusions of renin. The stimulus of thirst during intravenous infusions of renin is certainly ANG II, whereas the stimulus of thirst evoked by hypertonic saline is not ANG II; in fact, renin secretion is suppressed by this treatment (15). Similarly, renin secretion is too mildly stimulated by 24-h water deprivation for ANG II to contribute much to the induced thirst (18). Although PRA and plasma ANG II levels are elevated by PEG treatment (12, 16), hypovolemic thirst is not notably affected when renin secretion is prevented by bilateral nephrectomy (7) or when ANG II production has been blocked pharmacologically by captopril (4). This segregation of findings, drinking unimpaired by HDZ treatment when thirst was independent of ANG II but markedly impaired when thirst was dependent on ANG II, raised the possibility that the effect of HDZ treatment on drinking results from interference with the RAS.

Similar to water intakes stimulated by Dia and Isop treatments but not by hypertonic saline, water deprivation, or PEG treatment, HDZ treatment markedly reduced water intakes evoked by intravenous infusions of renin. The stimulus of thirst during intravenous infusions of renin is certainly ANG II, whereas the stimulus of thirst evoked by hypertonic saline is not ANG II; in fact, renin secretion is suppressed by this treatment (15). Similarly, renin secretion is too mildly stimulated by 24-h water deprivation for ANG II to contribute much to the induced thirst (18). Although PRA and plasma ANG II levels are elevated by PEG treatment (12, 16), hypovolemic thirst is not notably affected when renin secretion is prevented by bilateral nephrectomy (7) or when ANG II production has been blocked pharmacologically by captopril (4). This segregation of findings, drinking unimpaired by HDZ treatment when thirst was independent of ANG II but markedly impaired when thirst was dependent on ANG II, raised the possibility that the effect of HDZ treatment on drinking results from interference with the RAS. HDZ treatment clearly does not inhibit renin secretion, because PRA after HDZ administration was elevated to levels comparable to those observed after Dia and Isop treatments. Therefore, we sought other evidence that HDZ treatment might interfere with the RAS.

Plasma levels of renin and ANG II normally should be well correlated because the renin substrate, angio-
tensinogen, is present in very high concentrations in blood (15). To determine whether the relation between renin secretion and plasma ANG II levels was affected by HDZ treatment, we formed ratios of plasma ANG II levels to PRA during intravenous infusions of renin in Sal- and HDZ-treated rats. Any decrease in the ratio of plasma ANG II levels to PRA would suggest that HDZ treatment interferes with ANG II production or promotes its clearance. We found that HDZ treatment markedly reduced this ratio. Furthermore, a linear regression analysis of the mean values obtained after intravenous infusion of renin in Sal-treated rats and after Dia and Isop treatments confirmed that there is a very high correlation between PRA and circulating plasma ANG II levels. However, values obtained after HDZ treatment alone or in combination with intravenous infusion of renin were far from the 99% confidence interval of the regression line relating PRA and plasma ANG II levels. Despite elevated PRA, plasma ANG II levels did not increase proportionately in HDZ-treated rats. These data suggest that HDZ treatment in rats interferes with ANG II production and/or promotes its clearance and that a decrease in plasma ANG II levels at least partly accounts for the lack of drinking after HDZ treatment.

Surprisingly, plasma ANG II levels in rats given HDZ or Dia treatments were not significantly different from one another even though their water intakes differed markedly. These findings suggest that HDZ treatment may also interfere with ANG II receptors or have some postreceptor effect to block the dipsogenic actions of ANG II. Consistent with this hypothesis is the present observation that water intakes of HDZ-treated rats during intravenous infusions of ANG II were comparable to those of Sal-treated rats despite the differences in MAP. The ANG II-induced water intakes should have been potentiated in HDZ-treated rats due to the decreased inhibition of thirst resulting from the reduction in MAP. Previously, Evered and colleagues (2, 5, 25) suggested that increases in MAP inhibit water intake resulting from ANG II, because simultaneous administration of a hypotensive drug at a dose that clamps MAP at basal levels results in more water intake than occurs when rats have an elevated MAP. Furthermore, recent experiments from our laboratories indicate that ANG II-induced water intakes in rats are potentiated when baroreceptor afferents have been eliminated by electrolytic lesions of the nucleus of the solitary tract (28) or by sinoaortic denervation (30). Thus the observation that the water intakes of Sal- and HDZ-treated rats infused with ANG II were comparable suggests that HDZ treatment also disrupts the direct actions of ANG II.

Consistent with this possibility were the findings that PRA was generally higher after HDZ treatment than after Dia treatment although arterial blood pressures were comparable. Because circulating ANG II normally provides feedback control of renin secretion (15, 35), PRA should be elevated if HDZ reduces the level of circulating ANG II or blocks its postreceptor actions. A greater increase in PRA also was seen after Isop treatment, but it does not have a similar basis; instead, it is likely due to the direct actions of Isop on β-adrenergic receptors located on the renin-secreting cells in kidneys (15, 19, 35).

To summarize, the present findings confirm previous reports that rats do not increase water intake in response to HDZ treatment despite induced decreases in MAP and increases in PRA. This failure to drink does not result from a general disruption of drinking behavior, because HDZ-treated rats consumed as much water as Sal-treated rats did after systemic injection of hypertonic saline or 20% PEG and after 24-h water deprivation. In contrast, HDZ treatment did reduce drinking evoked by intravenous infusion of renin. Furthermore, HDZ treatment reduced the expected amount of circulating plasma ANG II for a given PRA, suggesting that HDZ interferes with ANG II production and/or promotes its clearance. Other findings suggest that HDZ may additionally blunt the dipsogenic actions of ANG II. Thus the failure of HDZ treatment to increase water intake in rats appears to result not because HDZ disrupts drinking behavior, but because it disrupts one or more elements of the RAS. Further experiments are needed to investigate the specific mechanism(s) by which HDZ treatment exerts its effects.

Perspectives

The present findings support accumulating evidence that water drinking evoked by arterial hypotension in rats is stimulated largely by ANG II. Strong support for this hypothesis may be found in numerous previous observations that water intake in rats given a hypertensive drug was abolished or markedly reduced when renin release was blocked by bilateral nephrectomy (3, 11), when ANG II production (1, 3, 13) or ANG II receptors (29) were blocked pharmacologically, or when the central site of the dipsogenic action of ANG II, the subfornical organ, was surgically destroyed (29). A direct and substantial role of extrarenal signals such as an afferent neural input from arterial baroreceptors, proposed previously to contribute to the stimulation of thirst (9, 20, 24, 32), was not supported by the present data because HDZ-treated rats did not drink despite the induced arterial hypotension.

The possible role of extrarenal signals in thirst during arterial hypotension has been based in part on evidence that PRA or circulating ANG II levels were not high enough to account for the induced water intake compared with the levels observed after systemic administration of renin or ANG II (10, 20, 32, 33). However, these studies had to be reinterpreted when later findings showed that drinking induced by administration of ANG II was inhibited by the induced hypertension (2, 5, 25), thus explaining why less ANG II was needed to elicit thirst when arterial hypertension was absent. However, the exact relation between plasma ANG II and water intake has never been defined under these circumstances.
The contribution of extrarebral signals to thirst during arterial hypotension also was suggested by observations that some drinking remained after elimination of the RAS either surgically or pharmacologically (9, 10, 24). However, this water intake usually amounted to only a few milliliters, thus suggesting that extrarebral signals did not contribute substantially to the induced thirst. Moreover, interpretation of these findings is confounded by the common choice of Isop as the hypotensive drug used in such investigations; for example, among its many actions, Isop dilates large capacitance venous beds (8) and thus it may provide a signal of thirst associated with a decrease in venous return that persists after elimination of the RAS. That signal would not be present when selective arteriolar vasodilators are administered.

Also relevant to these considerations are observations that water intake is not invariably evoked by drugs that strongly stimulate the RAS. Specifically, HDZ and sodium nitroprusside (and related hypotensive drugs) are known to stimulate renin secretion (15, 20, 22) but little or no water intake in rats (6, 20, 21). Those findings have raised questions about whether ANG II is a potent dipsogen under physiological conditions. However, for the findings to be compelling, it must be demonstrated that the animals were capable of drinking but did not ingest water because, presumably, they were not thirsty. Certainly water intake would not be expected in thirsty animals that were debilitated by an experimental treatment. In fact, the present studies suggest that the failure of rats to increase water ingestion after HDZ treatment is based on the drug's interference with the RAS. The lack of drinking by rats after administration of sodium nitroprusside seems likely to have a different basis; indeed, preliminary observations suggest that these animals actually are incapable of drinking.

The authors thank Ruwani Bandaranayake for technical assistance and Dr. Ian Reid for the generous gift of the ANG II antibody.

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This research was supported by National Institutes of Health (NIH) Grants MH-25140 (to E. M. Stricker) and HL-55687 (to A. F. Sved). S. D. Stocker was supported by a National Research Training Center grant (5-T32-NS-07433–01) from NIH.

A preliminary version of this report was presented at the national meetings of the Federated Society for Experimental Biology in San Francisco, CA, in April 1998, and in Washington, DC, in April 1999.


