Effect of DuP 753, a Nonpeptide Angiotensin II Receptor Antagonist, on the Drinking Responses to Acutely Administered Dipsogenic Agents in Rats

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Abstract. **The present studies examine the effect of the nonpeptide angiotensin II (AII) type 1 receptor antagonist, DuP 753, on water intake in rats treated with dipsogenic stimuli, which are thought to induce drinking via release of renin and subsequent formation of AII. Subcutaneous administration of DuP 753 in doses that are known to inhibit drinking induced by AII failed to inhibit the water intake of rats following subcutaneous administration of the β-adrenoceptor agonist isoproterenol. The peptide antagonist ^{1}Sar, ^{8}Ileu-AII, which blocks both AII type 1 and AII type 2 receptors, also failed to inhibit isoproterenol-induced drinking, suggesting that neither subtype is involved in this drinking response. Additional studies verified previous reports that acute subcutaneous administration of both the β-adrenoceptor antagonist propranolol and the angiotensin I-converting enzyme inhibitor captopril could block the drinking response to subcutaneous administration of isoproterenol. Subcutaneous administration of DuP 753 also failed to inhibit the drinking responses to subcutaneous administration of serotonin, 5-hydroxytryptophan, hypertonic saline, and polyethylene glycol. However, central intraventricular administration of DuP 753 inhibited the drinking response to subcutaneous administration of isoproterenol. The results are discussed in terms of the importance of AII in mediating isoproterenol-, serotonin-, and 5-hydroxytryptophan-induced water intake and suggest a need to readdress this mechanism.** [P.S.E.B.M. 1992, Vol 199]

Earlier studies from this and other laboratories have assessed the effect of DuP 753, a nonpeptide agent that appears to be an effective inhibitor of angiotensin II type 1 (AII_1) receptors, on angiotensin II-related drinking (1, 2). The results of these experiments revealed that subcutaneous administration of DuP 753 inhibited the characteristic dipsogenic response to subcutaneous administration of angiotensin II (AII; 1, 2). The dipsogenic response to subcutaneous administration of angiotensin III was also inhibited by subcutaneous administration of DuP 753 (1). Furthermore, central (intracerebroventricular) administration of DuP 753 also inhibited the dipsogenic response to intracerebroventricular administration of AII (1).

The objective of the present series of experiments was to assess the effect of DuP 753 on the drinking responses to other dipsogenic stimuli, a number of which have been thought to induce drinking via the release of renin and the subsequent formation of AII (3). These include the β-adrenoceptor agonist isoproterenol, the vasoactive indole serotonin (5HT), its precursor, 5-hydroxytryptophan (5HTP), and the hyperoncotic colloid polyethylene glycol. Additional studies were carried out to assess the effect of serile (the peptide AII receptor blocker), propranolol (the β-adrenoceptor antagonist), and captopril (the angiotensin I (AI)-converting enzyme inhibitor) on isoproterenol-induced drinking. Furthermore, the effect of DuP 753 on hypertonic saline-induced drinking, which is mediated by osmoreceptors, was also tested.

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Methods

Two slightly different protocols were used in these studies. In the first protocol, male rats of the Sprague-Dawley (Harlan) strain, weighing initially from 425 g to 450 g, were kept three to a cage in a windowless vivarium maintained at 25 ± 2°C and illuminated from 7 AM to 7 PM. Food (Purina Laboratory Chow 5001) and tap water were allowed *ad libitum* prior to all studies. At 9 AM on the day of each study, the rats were weighed, injected with the appropriate drug or vehicle, and placed alone in a stainless steel metabolic cage. A preweighed bottle of water (26°C) was placed in each cage and water intake was measured at 0.5, 1.0, and 2.0 h later. The water bottles were infant nursing bottles with cast bronze spouts, as described by Lazarow (4). No food was available during the study. The animals were used in several studies, at 3- to 4-day intervals, and, in all studies, the treatments were randomly assigned.

In the second protocol, adult male rats (400–450 g) of the Sprague-Dawley (Harlan) strain were housed individually in stainless steel cages under conditions similar to those described above, except that lights were on from 8 AM to 8 PM. Drinking tests were conducted in the middle of the light period and in the home cage, from which food and water were removed. The rats were administered the agent(s) as specified in each study, and tap water was presented (26°C) at the front of the cage from graduated burettes (±0.1 ml) with sipper tubes attached. The volume consumed was recorded after 0.5, 1.0, and 2.0 hr, although for brevity, only the 2-hr measures will be presented.

Additional male rats (400–450 g), tested in the second protocol, were fitted with indwelling lateral cerebroventricular cannulae. This stereotaxic surgery was performed with the rats under Equithesin anesthesia (3 ml/kg). A 10-mm long, 23-gauge stainless steel tube was implanted into the right lateral ventricle (flat skull coordinate: 1.5 mm lateral on bregma and 4.0 mm down from the skull surface) and fixed to the skull with jeweler's screws and dental acrylic. The cannula was closed with a wire obturator. Tests were started 7–10 days after surgery. For injections, rats were held gently in a towel, the obturator was removed, and a 10.5-mm long, 27-gauge injector needle was attached via PE20 tubing to a microliter syringe, which was used to deliver the drug intraventricularly. Ten microliter volumes were used. The obturator was then replaced and rats were put back into their cages. Water intakes were recorded as before.

Statistical analysis of experimental data was carried out by a one-way analysis of variance. The post hoc Duncan's Multiple Range Test was used to test the significance of the difference between two individual means.

Experiment 1: Effect of Graded Doses of DuP 753 on the Dipsogenic Responsiveness to Acute Administration of Isoproterenol

Study 1. Forty-eight male rats weighing 400–450 g were used in accordance with the first protocol. At the beginning of the experiment the rats were divided into four equal groups. The first group served as controls and received a subcutaneous injection of isotonic saline (1 ml/kg). The second group received DuP 753 (5 mg/kg, sc); the third and fourth groups received DuP 753 at 10 and 20 mg/kg, sc, respectively. Fifteen minutes later, all rats were injected with isoproterenol (25 μg/kg, sc). Water intakes were then measured at 0.5, 1.0, and 2.0 hr after injection.

Study 2. The above study was repeated under the conditions of the second protocol. Three groups of six rats each received subcutaneous injections of either the vehicle or DuP 753 at 3 or 10 mg/kg, and 45 min later, each group received an injection of isoproterenol (25 μg/kg, sc). Water intakes were then measured for 2 hr.

Experiment 2: Effect of a Peptide Angiotensin II Receptor Antagonist on the Dipsogenic Responsiveness to Acute Administration of Isoproterenol

Study 1. Two groups of six rats each that had been used in Experiment 1 were administered either isotonic saline (1 ml/kg, sc) or ^{1}Sar-^{8}Ile-AII (sarile; 600 μg/kg, sc). Sarile is a peptide antagonist of both AII_1 and AII_2 (type 2) receptors (5). Fifteen minutes later, each rat was administered isoproterenol (25 μg/kg, sc). The rats were placed in individual cages without food and were given a preweighed bottle of water. Water intakes were then measured at 0.5, 1.0, and 2.0 hr thereafter.

Study 2. Twenty-four rats that had not been used previously in drinking experiments were divided into four equal groups, half of which received a subcutaneous injection of the vehicle (saline, 1 ml/kg) while the other half received sarile (500 μg/kg). Fifteen minutes later, half of the rats in each pretreatment group received vehicle (saline, 1 ml/kg, sc), while the other half was injected with isoproterenol (25 μg/kg, sc). Water intakes were measured for 2.0 hr, as in Protocol 2.

Experiment 3: Effect of Either Propranolol or Captopril on the Dipsogenic Responsiveness to Acute Administration of Isoproterenol

This experiment was carried out in the same fashion as Experiment 2. The propranolol study used another 12 of the rats that had been used in Experiment 1. Six of the rats were administered isotonic saline (1 ml/kg, sc), while the remaining six rats received the β-adrenoceptor antagonist propranolol (1.0 mg/kg, ip). Fifteen minutes later, each rat was administered isoproterenol (25 μg/kg, sc). Water intakes were measured at 0.5, 1.0, and 2.0 hr thereafter.

Two studies were performed with captopril, each using 12 of the rats that had been used in Experiment 1. The procedure was also identical to that described in Experiment 2, except that six rats were pretreated with the angiotensin I-converting enzyme inhibitor captopril (either 35 [first study] or 60 [second study] mg/kg, sc) while the second group received the saline vehicle (1 ml/kg, sc). Fifteen minutes later, each rat was administered isoproterenol (25 μg/kg, sc). Intakes were measured thereafter for 2 hr.

Experiment 4: Effect of DuP 753 on the Dipsogenic Responses to Acute Administration of L-5-Hydroxytryptophan, Serotonin, Polyethylene Glycol, or Hypertonic NaCl Solution

The study with 5HTP used 12 of the rats that had been used in Experiment 1-Study 1. Six rats received a pretreatment with DuP 753 (10 mg/kg, sc) and another six received the saline vehicle. Fifteen minutes later, each rat was administered the serotonin precursor L-5-hydroxytryptophan (5HTP, 25 mg/kg, sc). Intakes were measured thereafter as described in Experiment 2 and Protocol 1.

In the other studies, rats used in Experiment 1-Study 2, were tested according to the second protocol. Groups of six rats received either vehicle or DuP 753 (3 or 10 mg/kg, sc) and 45 min later received either hypertonic NaCl (1 ml 2*M* NaCl/rat, sc, in 0.2% lidocaine), or serotonin (4.4 mg/kg, as creatinine sulfate salt, sc). In another study, polyethylene glycol (4 ml/rat of a 30% w/v solution in saline, mol wt 20,000) was injected subcutaneously and, after 4 hr without food or water, either DuP 753 (10 mg/kg) or saline was injected subcutaneously, 45 min later water was presented as before. In each study, the 2-hr water intake was measured.

Experiment 5: Effect of Cerebroventricular Administration of DuP 753 on the Drinking Response to Peripheral Administration of Isoproterenol

One group of six rats received intraventricular DuP 753 (100 μg) at the same time as peripheral (subcutaneous) injection of isoproterenol (25 μg/kg). The second group (six rats) received an equal volume of saline and isoproterenol subcutaneously. Water was given immediately after the injections and intakes were measured for 2 hr.

Results

Experiment 1. *Studies 1 and 2.* Acute administration of graded doses of DuP 753 either 15 or 45 min prior to administration of isoproterenol failed to affect water intake measured at either 0.5, 1.0, or 2.0 hr (Study 1, Fig. 1A; Study 2, Table I).

Experiment 2. *Study 1.* Acute administration of the peptide AII receptor blocker sarile 15 min prior to administration of isoproterenol also failed to affect water intake measured at 0.5, 1.0, or 2.0 hr (Fig. 1B).

Study 2. The unexpected results of Study 1 (above) were replicated in this study (Table I). Sarile itself was not dipsogenic.

Experiment 3. Acute administration of propranolol 15 min prior to administration of isoproterenol reduced water intake significantly ($P < 0.05$) at 1.0 and 2.0 hr (Fig. 2). Likewise, acute administration of a high dose of captopril (60 mg/kg) inhibited isoproterenol-induced water intake after 0.5 and 1.0 hr (Fig. 3B), although the lower dose of captopril was ineffective (Fig. 3A).

Experiment 4. Acute administration of DuP 753 15 min prior to administration of 5HTP had no significant effect on water intake at any time after treatment (Fig. 4). Likewise, DuP 753 had no significant inhibi-

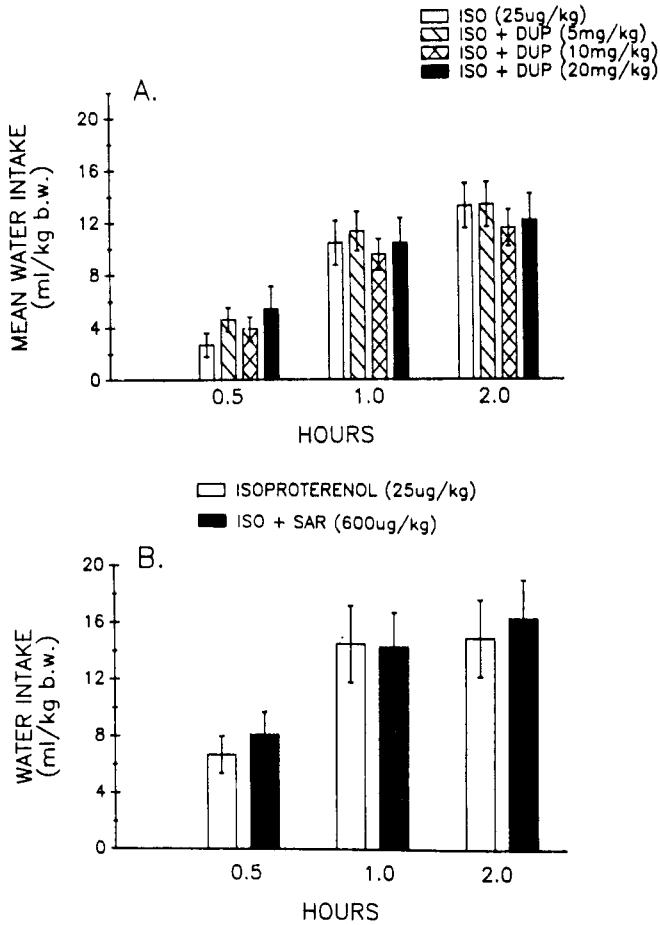

Figure 1. (A) Effect of acute subcutaneous administration of graded doses of DuP 753 on water intake of rats administered isoproterenol acutely. Accumulative intakes are shown. (B) Effect of acute subcutaneous administration of ^{1}Sar-^{8}Ileu-AII (SAR) on water intake induced by subcutaneous administration of isoproterenol. In both panels, accumulative intakes are shown at 0.5, 1.0, and 2.0 hr after administration of isoproterenol. Groups, drugs, and doses are designated in each panel. One SE is set off at each mean.

Table I. Effect of Peripheral Administration of Angiotensin II Receptor Antagonist on the Dipsogenic Responses to Peripheral Administration of Certain Dipsogens[a]

Treatment	Body wt (g)	2-Hr water intake (ml)	2-Hr water intake (ml/kg body wt)
Experiment 1, Study 2			
Vehicle + vehicle	429 ± 13	1.5 ± 0.4	3.5 ± 0.9
Vehicle + isoproterenol (25 μg/kg, sc)	471 ± 9	5.4 ± 0.6[b]	11.4 ± 1.2[b]
DuP (3 mg/kg, sc) + isoproterenol	439 ± 12	7.6 ± 1.5[b]	17.1 ± 3.0[b,c]
DuP (10 mg/kg, sc) + isoproterenol	451 ± 17	6.7 ± 0.5[b]	14.8 ± 0.8[b]
Experiment 2, Study 2			
Vehicle + vehicle	388 ± 14	0.3 ± 0.2	0.9 ± 0.4
Vehicle + isoproterenol (25 μg/kg, sc)	429 ± 7	7.2 ± 0.3[b]	16.9 ± 0.8[b]
Sarile (500 μg/kg, sc) + vehicle	483 ± 20	0.7 ± 0.3	1.5 ± 0.7
Sarile + isoproterenol	411 ± 9	7.7 ± 1.0[b]	18.8 ± 2.4[b]
Experiment 4			
Vehicle + serotonin (4.4 mg/kg, sc)	462 ± 12	4.2 ± 0.7[b]	9.1 ± 1.5[b]
DuP (10 mg/kg, sc) + serotonin	457 ± 12	5.5 ± 0.7[b]	12.0 ± 1.5[b]
Vehicle + 2 *M* NaCl	486 ± 5	8.0 ± 1.5[b]	16.5 ± 3.1[b]
DuP (10 mg/kg, sc) + 2 *M* NaCl	449 ± 16	8.6 ± 0.9[b]	19.2 ± 2.0[b]
Vehicle + PEG	418 ± 7	5.3 ± 0.6[b]	12.7 ± 1.5[b]
DuP (10 mg/kg, sc) + PEG	427 ± 5	6.1 ± 0.5[b]	14.3 ± 1.2[b]

[a] Shown are means ± SE for six rats per group. PEG, polyethylene glycol.
[b] Significantly greater than vehicle-vehicle baseline intake ($P < 0.05$).
[c] Significantly greater than vehicle-isoproterenol-treated group ($P < 0.05$).

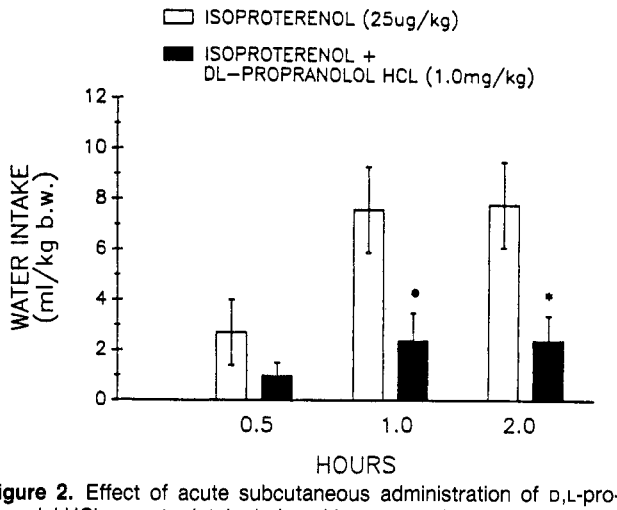

Figure 2. Effect of acute subcutaneous administration of D,L-propranolol HCl on water intake induced by acute subcutaneous administration of isoproterenol. Groups, drugs, and doses are designated in the figure. One SE is set off at each mean. * $P < 0.05$, compared with isoproterenol-treated group.

tory effect on water intake induced by either serotonin, 2 *M* NaCl or polyethylene glycol (Table I).

Experiment 5. Central administration of DuP 753 inhibited water intake induced by peripheral administration of isoproterenol (Table II).

Discussion

Our previous experiments with graded doses of DuP 753 showed that this AII receptor blocker can inhibit AII-induced (150 μg/kg) drinking in a dose-related fashion when the inhibitor is administered either 15 or 45 min prior to the dipsogen (1). The dose for 50% inhibition is about 3 mg/kg. Our expectations were that DuP 753 would inhibit isoproterenol-induced drinking, since it has been assumed for some time that such drinking was actually induced by AII (3). Thus, isoproterenol is known to induce the release of renin from the kidneys, with a subsequent resultant increase in the concentration of AII in blood (6). AII was believed to initiate drinking after stimulation of its receptors in the subfornical organ and other regions of the brain containing a "leaky" blood-brain barrier (7). Evidence for this assumption was based, in part, on the fact that both propranolol, a β-adrenoceptor blocker that inhibits the release of renin, and captopril, an angiotensin I-converting enzyme inhibitor that prevents the formation of AII from AI, prevented isoproterenol-induced drinking (8). In addition, others showed that bilateral nephrectomy prevented isoproterenol-induced drinking (9). The fact that DuP 753, administered either 15 or 45 min prior to isoproterenol, and at doses that completely antagonized AII-induced drinking, does not block isoproterenol-induced drinking suggests that the drinking response is not mediated by AII. This conclusion is tentative, however, in that DuP 753 inhibits only one subtype of AII receptors, AII_1. A second subtype of AII receptors, AII type 2, also exists and is blocked by another class of nonpeptide AII receptor antagonists, among them, PD 123177 (2). It is possible that this compound may block the drinking response to isoproterenol, although this seems unlikely, since it

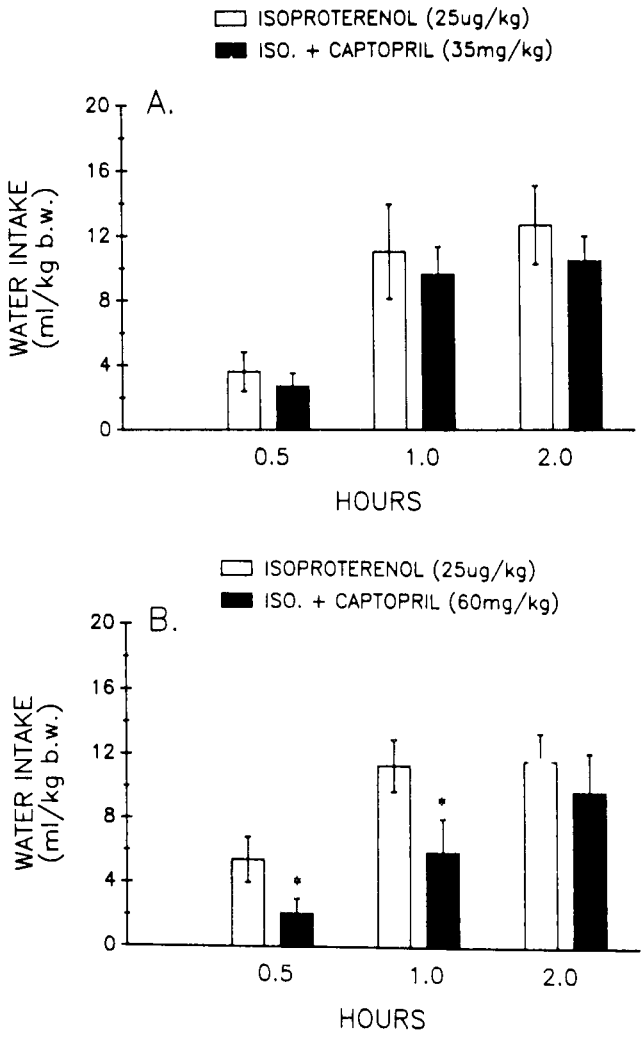

Figure 3. Effect of acute subcutaneous administration of captopril at (A) 35 mg/kg or (B) 60 mg/kg on water intake induced by acute subcutaneous administration of isoproterenol. Accumulative intakes are shown at 0.5, 1.0, and 2.0 hr after administration of isoproterenol. Groups, drugs, and doses are designated in each panel. One SE is set off at each mean. * $P < 0.05$, compared with isoproterenol-treated group.

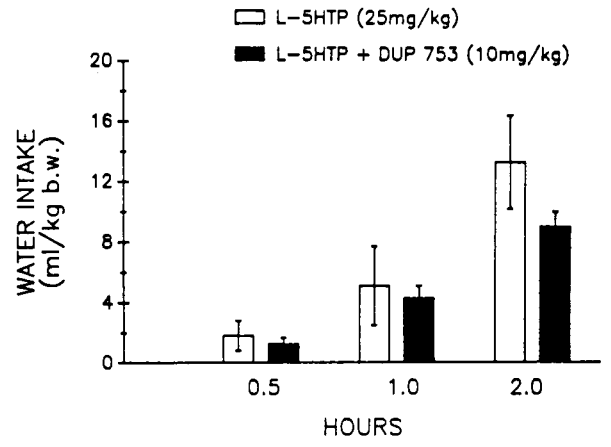

Figure 4. Effect of acute subcutaneous administration of DuP 753 on water intake of rats acutely administered L-5-hydroxytryptophan subcutaneously. Groups, drugs, and doses are designated in the figure. One SE is set off at each mean.

Table II. Effect of Intracerebroventricular Administration of DuP 753 on the Drinking Response to Subcutaneous Administration of Isoproterenol[a]

Co-treatments	*n*	Water intake (ml/2 hr)
Isoproterenol (25 μg/kg, sc)		
+ ICV vehicle	6	3.1 ± 0.8
+ ICV DuP 753 (100 μg)	6	0.6 ± 0.6[b]

[a] Values are means ± SE. ICV, intracerebroventricular.
[b] $P < 0.01$, compared with vehicle-treated group.

does not block drinking to peripherally administered AII (2).

To test further the possible mediation of isoproterenol-induced drinking by AII, the peptide AII receptor antagonist sarile was administered at a dose shown by an earlier study from this laboratory to block the drinking response to AII (150 μg/kg, sc) (10). Sarile failed to inhibit the drinking response to isoproterenol. This confirms the report by Tang and Falk (11) that ^{1}Sar-^{8}Ala-AII also failed to block isoproterenol-induced drinking. It also supports the results of the studies with DuP 753 and extends them one step further, in that sarile is known to block both subtypes of AII receptors (5). It is also of interest that intracerebroventricular administration of DuP 753 inhibited the drinking response to a subcutaneous injection of isoproterenol. This finding suggests that isoproterenol-induced drinking may be mediated indirectly by central AII-related systems that are independent of those activated by peripheral AII.

The inhibition of isoproterenol-induced drinking by propranolol may not be related specifically to its inhibition of the release of renin from the kidneys. Instead, it may be related specifically to its general blockade of β-adrenoceptors throughout the body, including those in the end organs mediating the isoproterenol-induced drink. The central membrane stabilizing effect of L-propranolol is unlikely to explain its inhibitory effect on isoproterenol-induced drinking, since D-propranolol, which exhibits similar membrane-stabilizing effects in the central nervous system, does not inhibit drinking (12).

The blockade of isoproterenol-induced drinking by high doses of captopril has been taken as strong support that the drinking response is mediated by AII (8). If, however, the present data with DuP 753 imply that AII does *not* mediate isoproterenol-induced drinking, can actions of captopril other than its angiotensin I-con-

verting enzyme inhibitory activity account for the inhibition of drinking? Captopril is known to have α-adrenoceptor inhibitory activity (13, 14), to increase the half-life of bradykinin (15), as well as to inhibit plasma enkephalinase, a peptidase capable of degrading enkephalins (16, 17). Whether any of these possibilities can explain the results is unknown at present.

In order to examine the generality of the observations with isoproterenol, we examined the effect of DuP 753 on drinking to two other hypotensive congeners, serotonin and its precursor, 5HTP. Both of these compounds induce the release of renin and the formation of AII (18). We have suggested previously that 5HT-induced drinking may be more dependent on renal renin than 5HTP-induced intake, because acute bilateral nephrectomy inhibits water intake after administration of 5HT, but not after administration of 5HTP (19). In both cases, DuP 753 had no inhibitory effect, suggesting that the drinking responses to 5HT and 5HTP are not mediated by AII_1 receptors.

Drinking induced by intraperitoneal administration of hypertonic NaCl solution is generally considered to be mediated by osmoreceptors (20). Hence, the failure of DuP 753 to affect the drinking response to hypertonic NaCl solution is not unexpected. The drinking induced by polyethylene glycol is presumed to be induced by depletion of extracellular fluid volume and activation of the renin-angiotensin system (21). However, water intake following polyethylene glycol-induced hypovolemia is not reduced significantly by nephrectomy (21) and is not inhibited by DuP 753.

Hosutt *et al.* (22) have argued that isoproterenol can induce drinking in the absence of renal renin release, but the response may be obfuscated by hypotension, which is exacerbated under those conditions in which activation of the renin-angiotensin system has been compromised. In a similar vein, Evered *et al.* (23) have suggested that blood pressure and AII interact in determining drinking responses to hypotensive agents. We assume, but cannot prove, that DuP 753 exacerbates isoproterenol-induced hypotension, although it must be emphasized that the animals did not appear critically debilitated. Another possibility is that the high levels of plasma renin activity, and by inference AI and AII, induced by isoproterenol are able to penetrate the brain and overcome the blockade induced by DuP 753. A similar "spillover" hypothesis has been used to address the peculiar enhancement of drinking to administration of an angiotensin I-converting enzyme inhibitor (8). We are uncertain whether this may account for the enhancement of isoproterenol-induced water intake by low doses of DuP 753 (Table I).

Rettig *et al.* (24) presented evidence suggesting that moderate reductions in blood pressure induced by isoproterenol (i.e., arterial pressure maintained above 90 mm Hg) stimulated water intake via a renal-related factor, probably AII. With greater reductions in mean arterial pressure induced by a much larger dose of isoproterenol (i.e., arterial pressure reduced below 90 mm Hg), drinking was postulated to be induced by extrarenal mechanisms, such as arterial baroreceptors. Rettig *et al.* (24) used a continuous intravenous infusion, while our dose of isoproterenol was acutely administered subcutaneously. Although we did not measure blood pressure in the present study, it was measured in an earlier study under conditions identical to those used here (25). In that study, mean blood pressure decreased from 130 to 100 mm Hg within 3 min, after which it slowly increased toward control level. This suggests that the reduction in systemic pressure was well above the level identified by Rettig *et al.* (24) to mediate drinking by the release of a renal-related factor. Whatever the nature of the renal factor, its effect in initiating drinking is not blocked by administration of DuP 753.

The results of the studies presented here indicate a need to readdress the mechanisms mediating the drinking responses to a number of compounds, including isoproterenol, serotonin, and L-5HTP. The commonly held notion that the drinking responses to these compounds are mediated exclusively by the renin-angiotensin system may need to be modified. However, before any changes in mechanisms to explain the action of these compounds can be considered seriously, additional information concerning the inhibitors and their specificity, their half-life in the circulation, and their pertinent side effects under these conditions will be required.

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