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Effect of β-Adrenergic Antagonists on Experimentally Induced Drinking in Female Rats

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KATOVICH, M. J. AND C. C. BARNEY. *Effect of β-adrenergic antagonists on experimentally induced drinking in female rats.* PHARMACOL BIOCHEM BEHAV **22**(4) 553–558, 1985.—The nonspecific β-adrenergic antagonist d,*l* propranolol, the specific β_1-adrenergic antagonist atenolol, and the specific β_2-adrenergic antagonist butoxamine were administered intraperitoneally (IP) to ovariectomized female rats in order to determine the role of β-adrenergic receptors in drinking. D,*l* propranolol and atenolol administered at doses of 6, 12, and 18 mg/kg significantly attenuated the one-hour water intakes of rats administered angiotensin II (200 μg/kg, SC) and the water intakes of rats deprived of water for 24 hours. D propranolol, which has little β-adrenergic blocking ability, administered at doses of 6 and 12 mg/kg, and butoxamine, administered at doses of 25 and 35 mg/kg, had no significant effects on the water intakes of angiotensin II treated or water deprived rats. Regardless of the dose, d,*l* propranolol, atenolol, and butoxamine failed to significantly alter the water intakes of rats administered 1.0 M NaCl (10 ml/kg, IP) The results provide evidence that β_1-adrenergic receptors, but not β_2-adrenergic receptors, are involved in mediating the increased water intakes induced by angiotensin II and water deprivation. On the other hand the increased water intake due to administration of hypertonic saline does not appear to mediated by β-adrenergic receptors.

β-Adrenergic antagonists d,*l* Propranolol d Propranolol Atenolol Butoxamine Water deprivation Angiotensin II Hypertonic saline Thirst Water intake

THE β-adrenergic agonist isoproterenol has often been used to mimic the increase in water intake observed in rats following reductions in plasma volume [15]. Isoproterenol has been reported to increase water intake in rats following systemic administration [7, 25, 27, 28, 29, 34]. Current evidence indicates that at least a portion of the isoproterenol-induced water intake may be due to the dipsogenic action of angiotensin II (AII). Isoproterenol has been shown to increase both plasma renin activity [27] and plasma AII concentration [1, 22, 34]. AII is known to be a potent dipsogen following either peripheral or central administration [15,35]. However, the relevance of these observations to physiological drinking is not universally accepted [26, 40, 41]. We [23] and others [11] have shown that isoproterenol-induced drinking can be blocked by prior administration of captopril, an angiotensin converting enzyme inhibitor. Saralasin, an AII receptor blocker, and teprotide, another angiotensin converting enzyme inhibitor, have also been reported to block isoproterenol-induced drinking [7]. Thus AII formation appears to be important in the development of isoproterenol-induced drinking. It is possible, however, that under some circumstances some other factor, such as venous or arterial baroreceptors sensing the isoproterenol-induced hypotension, may be involved in isoproterenol dipsogenesis [34].

That isoproterenol induces water intake by its β-adrenergic stimulatory action was shown by experiments in which prior administration of d,*l* propranolol, a β-adrenergic antagonist, blocked the water intake induced by isoproterenol [7, 25, 28, 29]. In addition, other β-adrenergic agonists such as salbutamol and quinterenol cause increases in water intake in rats [12,25]. Prior administration of propranolol also reduced the increase in plasma renin activity observed following administration of isoproterenol [27]. It is interesting to note that d,*l* propranolol also reduced the increases in plasma renin activity and water intake that accompanied removal of rats from a cold to a warm environment ([17,24] unpublished data). This thermogenic drinking has been shown to be dependent on the formation of AII [24]. More specifically, both isoproterenol-induced and thermogenic drinking have been reported to be at least partially mediated via β_2-adrenergic receptors [17,25].

Since there seems to be some ability of β-adrenergic antagonists to attenuate drinking associated with AII we hypothesized that the dipsogenic response to AII itself may be manifested, in part, by a β-adrenergic-mediated pathway. Thus we designed experiments to examine the effects of several β-adrenergic antagonists on the water intake caused by peripheral administration of AII. Additional studies were

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designed to determine if the β-adrenergic system is involved in water deprivation-induced drinking, which may also involve AII [1, 3, 4, 15, 32] or in hypertonic saline (NaCl)-induced drinking, which probably does not involve AII [1, 3, 15, 37]. To examine the role of the β-adrenergic system in these types of thirst three β-adrenergic blocking drugs were used: d,l propranolol, a non-selective β-adrenergic antagonist [10], atenolol, a selective β_1-adrenergic antagonist [10], and butoxamine, a selective β_2-adrenergic antagonist [10]. In addition, experiments were performed using d propranolol which has less than 1% of the β-adrenergic receptor blocking activity of *l* propranolol but has the same potency as *l* propranolol as a local anesthetic [20].

METHOD

Seventy-two female rats of the Blue Spruce Farms (Sprague-Dawley) strain weighing from 200 to 330 g were used. Each rat was ovariectomized at least three weeks prior to the first experiment. Rats were ovariectomized in order to eliminate the effects of the estrous cycle on drinking [14]. The rats were housed 3 per cage in a room maintained at 26±1°C and illuminated from 6:00 a.m. to 6:00 p.m. All rats were provided with Purina laboratory chow and tap water ad lib except during periods of water deprivation when food but not water was available. Rats were used at random except that no rat was used more than once every two weeks. All experiments began between 9:00 a.m. and 10:00 a.m. All animals were familiarized with the experimental protocol on two separate occasions at least one week prior to the first experimental procedure. Isotonic saline (1 ml/kg) was used as the administered agent in these adaptation sessions.

Experiment 1: Effect of β-Adrenergic Antagonists on Angiotensin II-Induced Drinking

In this experiment the effects of d,l propranolol, d propranolol, atenolol, and butoxamine, administered in separate trials, on drinking induced by peripheral administration of AII were studied. For each study the rats were divided into 4 or 5 groups of 6 rats each. Each rat was placed in an individual stainless steel metabolism cage with a water bottle. One hour later each rat was given an intraperitoneal (IP) administration of either a β-adrenergic blocking drug or the vehicle. The vehicle was isotonic saline (0.15 M NaCl) for all antagonists except butoxamine which was dissolved in dimethylsulfoxide. Thirty minutes later each rat was administered a subcutaneous (SC) injection of either isotonic NaCl or AII (Beckman). Following the second injection each rat was given a 100 ml graduated spill-proof water bottle (Bio Serv, Inc.) with the water temperature at 25±1°C. Water intake was determined by the volume difference during the first hour after administration of AII (200 μg/kg) or NaCl (1 ml/kg).

For the d,l propranolol study the first group of rats (control) received 1 ml/kg isotonic NaCl followed by 1 ml/kg isotonic NaCl. The second group (0 propranolol) received isotonic NaCl followed by AII. The third through fifth groups received 6, 12, and 18 mg/kg of d,l propranolol (Inderal®, Ayerst Laboratories) respectively followed by AII. The same experimental design was used for the atenolol (Stuart-Pharm Division of ICI American, Inc.) study and the d propranolol (provided by Ayerst Laboratories) study except the 18 mg/kg dose was not used in the d propranolol study.

For the butoxamine study the first group (control) received dimethylsulfoxide (1 ml/kg) followed by isotonic NaCl. The second group (0 butoxamine) received dimethylsulfoxide followed by AII. Groups three and four received 25 and 35 mg/kg of butoxamine (Burroughs Wellcome Co.) respectively followed by AII.

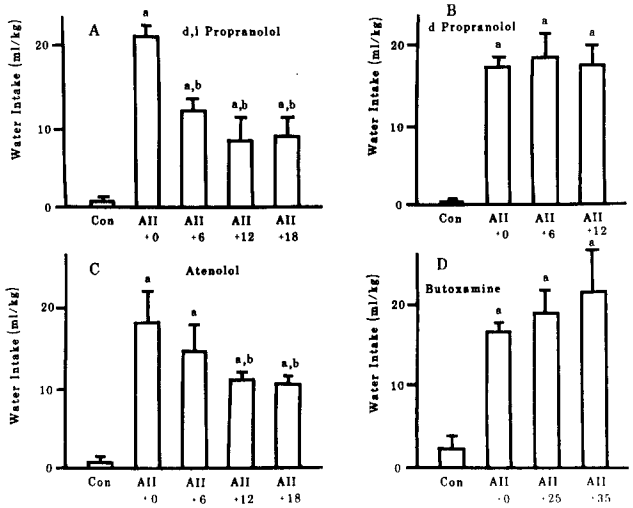

FIG. 1. Mean one hour water intakes of rats administered angiotensin II following pretreatment with d,l propranolol (A), d propranolol (B), atenolol (C), and butoxamine (D). One standard error of the mean is set off at each bar. Con=control rats. AII+=rats administered 200 μg/kg angiotensin II following the dose of pretreatment drug given in mg/kg. a=Significantly different from the control group of $p<0.05$. b=Significantly different from the angiotensin II+0 dose of pretreatment drug group at $p<0.05$.

Experiment 2: Effect of β-Adrenergic Antagonists on Hypertonic-Saline Induced Drinking

The effects of d,l propranolol, atenolol, and butoxamine, administered in separate trials, on drinking induced by IP administration of hypertonic NaCl (1.0 M NaCl) were studied in this experiment. For each study the rats were divided into four groups of six rats each. Following the one hour adjustment period in the metabolism cage each rat was administered (IP) either a β-adrenergic blocking drug or the vehicle (1 ml/kg). Thirty minutes later each rat was administered (IP) either isotonic NaCl (10 ml/kg, 37°C) or hypertonic NaCl (10 ml/kg, 37°C). Water intake was then measured as in Experiment 1.

For both the d,l propranolol and the atenolol studies the first group of rats (control) received isotonic NaCl followed by isotonic NaCl. The second group (0 antagonist) received isotonic NaCl followed by hypertonic NaCl. The third and fourth groups received 6 and 12 mg/kg of the β-adrenergic antagonist respectively followed by hypertonic NaCl. For the butoxamine study the first group (control) received dimethylsulfoxide followed by isotonic NaCl. The second group (0 butoxamine) received dimethylsulfoxide followed by hypertonic NaCl. Groups three and four received 25 and 35 mg/kg of butoxamine respectively followed by hypertonic NaCl.

Experiment 3: Effect of β-Adrenergic Antagonists on Water Deprivation-Induced Drinking

In this experiment the effects of d,l propranolol, d pro-

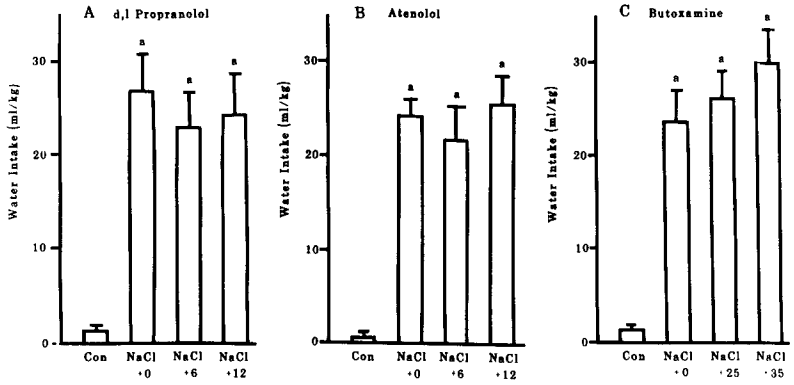

FIG. 2. Mean one hour water intakes of rats administered hypertonic NaCl solution following pretreatment with d,*l* propranolol (A), atenolol (B), and butoxamine (C). One standard error of the mean is set off at each bar. Con=control rats. NaCl+=rats administered 10 ml/kg 1.0 M NaCl following the dose of the pretreatment drug given in mg/kg. a=Significantly different from the control group at $p<0.05$.

pranolol, atenolol, and butoxamine, administered in separate trials, on drinking induced by water deprivation were studied. For each study the rats were divided into four groups of six rats each. Twenty-four hours prior to the initiation of each study the rats were weighed and placed into the metabolism cages. Three of the four groups were given food but not water and the other group was given food and water (control). Twenty-three and one-half hours later each animal was weighed and administered (IP) either a β-adrenergic blocking drug or the vehicle. Thirty minutes later each rat was given a water bottle and water intake was determined as in Experiment 1.

For each study the first group of rats were not water deprived and were administered the vehicle (1 ml/kg), either isotonic NaCl or dimethylsulfoxide. The second group of rats (0 antagonist) were water deprived and were administered the vehicle. In all but the butoxamine study, groups three and four received 6 and 12 mg/kg of the antagonist respectively. For the butoxamine study groups three and four received 25 and 35 mg/ml of butoxamine respectively.

Statistical analysis for each study of each experiment was by means of a one-way analysis of variance with significance set at the 95% confidence limit [9]. Individual comparisons were made by means of the Newman-Keuls test with significance set at the 95% confidence limit [9].

RESULTS

Experiment 1: Effect of β-Adrenergic Antagonists on Angiotensin II-Induced Drinking

The effects of d,*l* propranolol, d propranolol, atenolol, and butoxamine on drinking due to peripheral administration of AII are shown in Fig. 1. Analysis of variance showed a significant ($p<0.01$) effect in each study (d,*l* propranolol—$F(4,29)=19.72$; d propranolol—$F(3,23)=17.62$; atenolol—$F(3,23)=8.16$; butoxamine—$F(3,23)=7.07$). In the first study (Fig. 1A), d,*l* propranolol acted to significantly ($p<0.05$) inhibit the water intake of rats administered AII. The higher doses of d,*l* propranolol tended to reduce the water intake only slightly more than the lowest dose. However, even at the highest dose of d,*l* propranolol the AII-induced drinking was not completely blocked. On the other hand, d propranolol (Fig. 1B) had no effect on the water intake induced by AII. Atenolol (Fig. 1C) significantly reduced the AII-induced drinking at the higher doses used. Again the water intake induced by AII was attenuated but not completely blocked. Butoxamine, the selective β-adrenergic agonist did not have any significant effect on AII-induced water intake (Fig. 1D).

Experiment 2: Effect of β-Adrenergic Antagonists on Hypertonic NaCl-Induced Drinking

Figure 2 summarizes the results of the studies with d,*l* propranolol, atenolol and butoxamine on hypertonic NaCl-induced drinking. Analysis of variance showed a significant ($p<0.01$) effect in each study (d,*l* propranolol—$F(3,23)=21.03$; atenolol—$F(3,23)=20.32$; butoxamine—$F(3,23)=18.36$). In each study rats treated with hypertonic NaCl alone or hypertonic NaCl following pretreatment with a β-adrenergic antagonist had significantly ($p<0.05$) greater water intakes than the control rats. All doses of d,*l* propranolol (Fig. 2A), atenolol (Fig. 2B), and butoxamine (Fig. 2C) were without significant effect on hypertonic NaCl-induced water intake.

Experiment 3: Effect of β-Adrenergic Antagonists on Water Deprivation-Induced Drinking

Water intake was increased by 24 hr of water deprivation (Fig. 3). Analysis of variance indicated a significant ($p<0.01$) effect in the studies with d,*l* propranolol, $F(3,23)=80.34$, d propranolol, $F(3,23)=117.99$, atenolol, $F(3,23)=43.40$, and butoxamine, $F(3,23)=55.43$. In each study all of the water deprived groups showed significantly ($p<0.05$) greater water intakes than did the control group. Water intake of the water deprived rats was significantly ($p<0.05$) reduced by prior administration of d,*l* propranolol (Fig. 3A). The higher dose of d,*l* propranolol had a significantly ($p<0.05$) greater effect on water intake than the lower dose of propranolol. However, d propranolol (Fig. 3B) did not significantly alter water deprivation-induced water intake at either high or low doses. Atenolol (Fig. 3C) significantly ($p<0.05$) reduced water intake in the water deprived rats although this effect was not dose dependent at the doses used. Neither d,*l* propranolol nor atenolol completely abolished the water deprivation-induced water intake. Butoxamine (Fig. 3D) was ineffective in altering the water deprivation-induced water intake.

DISCUSSION

As in previous experiments, administration of AII to water replete rats gave rise to a significant increase in water intake. Angiotensin II has been reported to cause drinking in several species of animals when administered either centrally or systemically [15,35]. It is generally accepted that the dipsogenic effect of AII is centrally mediated although the localization of the exact brain area or areas responsible is still under investigation [15,31]. Although some contradictory evidence exists [40,41], it is likely that the increased water intake observed following β-adrenergic stimulation with isoproterenol is due, at least in part, to generation of AII [1, 7, 11, 23].

As shown in the present study, AII-induced drinking, like isoproterenol-induced drinking [25] and thermogenic drinking [17], can be attenuated in the female rat by the β-adrenergic antagonist d,*l* propranolol. Six mg/kg of d,*l* propranolol reduced AII-induced drinking by 44% and 12 and 18 mg/kg of d,*l* propranolol reduced this dipsogenic response by about 61%. In comparison 1 mg/kg of d,*l* propranolol reduced isoproterenol-induced drinking by 77% [25] and 6 mg/kg of d,*l* propranolol reduced thermogenic drinking by 60% [17]. Neither isoproterenol-induced drinking [25] nor AII-induced drinking was significantly affected by d propranolol. This rules out a nonspecific anesthetic-like effect of d,*l* propranolol as a cause of the reductions in water intake. Thus, the decrease in water intake of the d,*l* propranolol treated rats is most likely due to blockade of β-adrenergic receptors. This is supported by the results of the atenolol study in which the specific β_1-adrenergic antagonist [10] decreased AII-induced water intake in a dose dependent manner. When butoxamine was administered at doses which have previously been shown to attenuate both isoproterenol-induced drinking [25] and thermogenic drinking [17], it failed to have a significant effect on water intake induced by AII. These data indicate that blockade of β_1- but not β_2-adrenergic receptors is effective in attenuating the thirst induced by peripheral administration of AII.

The site(s) of the β_1-adrenergic receptors involved in AII-induced drinking is not known. The β_2-adrenergic receptors involved in isoproterenol-induced and thermogenic drinking are most likely located at the kidney. Both isoproterenol-induced and thermogenic drinking are associated with renin release which, along with the drinking, can be blocked by d,*l* propranolol ([17, 24, 27] unpublished data). In addition renin release in the rat is reported to depend on activation of β_2-adrenergic receptors [6]. Of course, β-adrenergic antagonists may also attenuate isoproterenol-induced drinking by antagonizing the dipsogenic effect of the AII formed as a result of β-adrenergic stimulation [1, 7, 11, 23] or some blood pressure effect of β-adrenergic stimulation [34].

β_1-Adrenergic receptors in the brain may be involved in AII-induced drinking. Sumners *et al.* [38,39] have shown that AII will increase norepinephrine utilization in the hypothalamus and subfornical organ of the rat as well as in rat brain culture cells. Angiotensin II has also been shown to facilitate peripheral and central adrenergic nerves [42] which may play a role in the dipsogenic properties of the octapeptide. This potentiation of the sympathetic nervous system by AII can be inhibited by administration of propranolol [21]. Furthermore the central pressor but not the dipsogenic effect of AII can be blocked by α-adrenergic antagonists [5]. Both Gordon *et al.* [19] and Fitzsimons and Setler [16] reported that depletion of central nervous system catecholamines

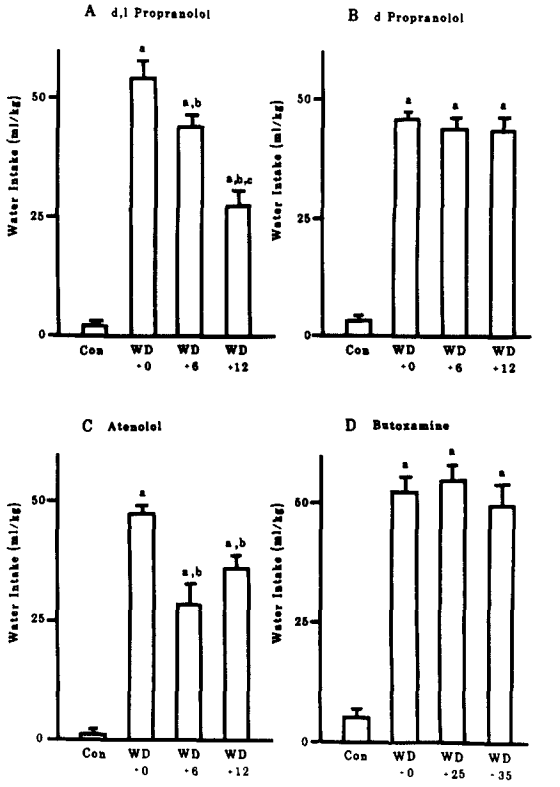

FIG. 3. Mean one hour water intakes of water deprived rats following pretreatment with d,- propranolol (A), d propranolol (B), atenolol (C), and butoxamine (D). One standard error of the mean is set off at each bar. Con=control rats. WD+=rats water deprived for 24 hr and administered the dose of the drug given in mg/kg. a=Significantly different from the control group at $p<0.05$. b=Significantly different from the water deprived + 0 dose of pretreatment drug group at $p<0.05$. c=Significantly different from the water deprived + 6 mg/kg dose of pretreatment drug group at $p<0.05$.

with 6-hydroxydopamine reduced AII-induced drinking but not carbachol-induced drinking in rats. Of course this may have occurred via effects on catecholamines other than those which stimulate β-adrenergic receptors.

Some investigators have looked at the role of β-adrenergic receptors in the brain in drinking behavior more directly. Leibowitz [30] injected isoproterenol centrally in rats and observed an increase in water intake although this has been attributed to leakage of isoproterenol into the periphery by other workers [28]. Cooling and Day [8] reported that in cats isoproterenol injected intracerebroventricularly (ICV) caused drinking and that d,*l* or *l* propranolol injected ICV attenuated the drinking due to ICV angiotensin II. Fitzsimons and Setler [16] reported a similar effect of propranolol in the rat but attributed the antidipsogenic action to a toxic effect of propranolol. Simon *et al.* [36] reported that in rats fed a propranolol-supplemented diet, the drinking response to an intraventricular injection of AII was reduced, which lends further support to the data presented here. Ohter investigators have failed to find a link between AII and the β-adrenergic system in drinking. Chronic propranolol treatment failed to alter the water intake of rats administered AII subcutaneously [18]. Lehr and Goldman [28] reported that neither central nor peripheral propranolol altered the

water intake due to central AII in rats and that low doses of isoproterenol injected centrally failed to induce water intake. Peripherally administered propranolol also failed to block drinking in cats following centrally administered AII [8]. And although AII alters brain norepinephrine release [38,39] norepinephrine injected IVT in rats failed to alter drinking [6]. These studies may indicate that the β_1-adrenergic receptors involved in drinking elicited by AII in female rats are not in the brain or that central administration of blockers and dipsogens do not act at the same brain sites as do peripherally administered agents. Further investigation is clearly needed to elucidate the site and mechanism at/by which β_1-adrenergic antagonists inhibit AII drinking.

In contrast to the results with AII, none of the three β-adrenergic antagonists used, d,l propranolol, atenolol or butoxamine, had any significant effect on water intake stimulated by hypertonic NaCl administration. This indicates that neither β_1 nor β_2-adrenergic receptors are involved in hypertonic NaCl-induced drinking. This also indicates that the antagonists' effects on AII- and water deprivation-induced drinking cannot be attributed to a nonspecific toxic or depressant effect of these antagonists. Our results do not agree with those of Falk *et al.* [13] who reported that propranolol (isomer not identified) at a dose of 10 mg/kg attenuated the 3 hour water intake of rats administered 2 ml/100 g of 1.0 M NaCl solution. The reason for the contradictory results is not clear although numerous procedural differences between the prior study and the present investigation may account for the different results. Coupled with the results from Experiment 1, our data suggests that there is no AII-mediated component of hypertonic NaCl-induced drinking in rats. This is in agreement with earlier reports [3,37] in which saralasin and various angiotensin converting enzyme inhibitors failed to attenuate the water intake associated with administration of hypertonic NaCl solutions. Furthermore, plasma AII levels do not increase after administration of hypertonic saline solution to rats [1].

Water deprivation-induced drinking responded to β-adrenergic antagonists in a manner similar to AII-induced drinking. Both d,l propranolol and atenolol significantly attenuated water deprivation-induced drinking. Both d propranolol and butoxamine were without effect on the water intake observed after water deprivation. Thus the drinking induced by 24 hr of water deprivation also appears to involve β_1-adrenergic receptors. Acute [13] and chronic [18] peripheral administration of propranolol and acute central administration of propranolol [30] reduced the water intake of water deprived rats in earlier studies as well. The β-adrenergic agonists may attenuate drinking due to water deprivation via their effect on AII-induced dipsogenesis. Water deprivation increases plasma renin activity [3] and plasma AII levels [1,22]. Although in a recent study [26] saralasin was without effect on water deprivation-induced drinking in rats captopril has been shown to effectively reduce the water intake of water deprived rats [3,4]. Interestingly, the magnitude of the AII-dependent component of water deprivation-induced drinking was proportional to the length of water deprivation [4]. If AII is responsible for the extracellular component of drinking induced by 24 hr of water deprivation [4,33], then antagonizing AII induced water intake with β_1-adrenergic blockers would be expected to attenuate the water intake associated with this period of water deprivation. Further studies are needed to evaluate the role of the β-adrenergic system in more prolonged periods of water deprivation.

Taken as a whole the data indicate that β-adrenergic antagonists are only effective in attenuating water intake which involves the renin-angiotensin system. Isoproterenol-induced drinking, thermogenic drinking, AII-induced drinking and water deprivation-induced drinking all appear to involve components of the renin-angiotensin system and all are attenuated by β-adrenergic antagonists. Furthermore, Atkinson *et al.* [2] reported that both propranolol and saralasin reduced the drinking in rats due to renal artery constriction, a process which also elevates plasma AII levels. In our study β-adrenergic antagonists did not diminish drinking due to hypertonic NaCl administration, a process which does not appear to involve AII. However, the assumption that this response to hypertonic saline is equivalent to the dipsogenic responses to AII and 24 hr of water deprivation may not be valid and further studies are required to test this assumption. The control mechanisms which mediate drinking are indeed complex and may involve numerous receptor sites and pathways. The data presented here indicate that thirst stimuli that act through AII in the ovariectomized female rat are at least partially manifested by stimulating a component of the β-adrenergic system.

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