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The Reduction in Alcohol Drinking by Peripherally Injected Angiotensin II Is Selectively Mediated by the AT_1 Receptor Subtype

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GRUPP, L. A. AND S. HARDING. *The reduction in alcohol drinking by peripherally injected angiotensin II is selectively mediated by the AT_1 receptor subtype.* PHARMACOL BIOCHEM BEHAV **47**(3) 385-392, 1994.—We investigated which of the two angiotensin (ANG) receptor subtypes mediates the reduction in alcohol intake produced by peripheral injections of ANG II. Adult male Wistar rats were trained to self-administer alcohol (6% w/v) using a procedure that, by limiting access to a brief daily availability period (40 min), fosters a bout pattern of alcohol drinking and a pharmacodynamic effect. Water was continuously available. Once intake stabilized, groups received daily injections either 200 μg/kg ANG II SC or the control vehicle saline immediately prior to alcohol availability. Alcohol consumption was attenuated and water intake elevated in the groups receiving ANG II and was unaffected by the vehicle injections. Following this, different groups were pretreated with ascending doses (0.25, 0.5, 1.0 mg/kg) of either PD123319, the selective AT_2 receptor antagonist, Sar^1,Thr^8-ANG II (0.25 mg/kg), the nonselective ANG II antagonist, or DuP753 (0.25, 0.5, 1.0 mg/kg), the selective AT_1 receptor antagonist. Control groups received antagonist pretreatment followed by the ANG II vehicle. Neither PD123319, DuP753, or Sar^1,Thr^8-ANG II had any effect of their own on alcohol or water intake. Pretreatment with PD123319 did not alter the suppressive effect of ANG II on alcohol intake. DuP753 produced a dose-dependent attenuation in the suppressive effect of ANG II on alcohol intake and antagonized the dipsogenic effect of ANG II on water intake. The effect of Sar^1,Thr^8-ANG II was similar to that of DuP753. These findings suggest that the reduction in alcohol intake produced by ANG II is mediated through the AT_1 receptor subtype.

Angiotensin II Alcohol drinking Renin-angiotensin system Angiotensin antagonist PD123319
DuP753 Sarthran Angiotensin receptor subtypes Losartan AT_1 receptor AT_2 receptor

LIKE vasopressin (7-9), oxytocin (8), ACTH (6), and a number of other peptides that have behavioral (30) as well as endocrinological functions (34), angiotensin (ANG) II is identified with the regulation of blood pressure and electrolyte balance, yet it can also influence learning and memory [e.g., (1,31)] and has been shown to be a potent inhibitor of alcohol drinking [see (21) for a review]. Normative data have indicated that this suppressive effect of peripherally injected ANG II on alcohol intake is evident in both moderately drinking wild type rats (17,23) and in selectively bred heavy drinking alcohol-preferring P rats (15). This suppression is not secondary to ANG II's hypertensive (18) or dipsogenic properties [(13), Grupp, unpublished observations], is not the result of a nonspecific reduction in the intake of any sapid solution (17), and does not come about because ANG II alters the pharmacokinetics of alcohol (17).

While ANG II can produce a conditioned taste aversion (CTA) when it is administered *following* the consumption of a novel and distinctively flavored solution (16), the reduction in alcohol intake produced by ANG II injections that *precede*

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the consumption of alcohol is not likely the result of a CTA. Once developed, a CTA tends to persist in the absence of the aversion-inducing agent (12), yet we have found that alcohol intake rapidly recovers following suspension of the ANG II injections (15), and that alcohol intake that is suppressed by ANG II can be rapidly reinstated if the animals are cotreated with an ANG II antagonist (19).

The central administration of ANG II into either the lateral or third ventricles causes a profound increase in water intake and blood pressure, but does not produce a reduction in alcohol intake [(13), Grupp, unpublished observations]. This finding suggests that the change in alcohol intake is not a secondary effect of the dipsogenic property of ANG II. Furthermore, lesions to the subfornical organ attenuate but do not completely reverse the effect of ANG II on alcohol intake (20), suggesting that angiotensin-sensitive sites in the periphery such as the liver, kidney, or adrenals may also be involved.

In a previous study, the potent ANG II receptor antagonist Sar^1,Thr^8-ANG II completely reversed the reduction in alcohol intake produced by ANG II (19). This particular antagonist, however, is nonselective for the two subtypes of the ANG II receptor (AT_1 and AT_2) that have recently been identified (2,4). Selective nonpeptide antagonists displaying differences in their ability to block these two receptors have been developed: DuP753 (Losartan®) is a nonpeptide AT_1 receptor antagonist [(36) for a review]; PD123319 is a nonpeptide AT_2 receptor antagonist (11). The present study examines the ability of each of these antagonists to block the reduction in alcohol intake produced by ANG II and compares these with the effect of the nonselective ANG II antagonist Sar^1,Thr^8-ANG II. The results provide no evidence for a role of the AT_2 receptor subtype but do indicate that the AT_1 receptor subtype mediates the inhibitory effect of ANG II on alcohol intake.

METHOD

Subjects

The subjects were 64 naive male Wistar rats (Charles River, Montreal) weighing an average of 375 g at the start of the experiment. Animals were individually housed and maintained on a reverse 12L : 12D cycle with lights off at 7 a.m. Purina rat chow and tap water were available in home cages ad lib.

Procedure

The limited access drinking procedure was used (29) to promote alcohol consumption to levels that produce a pharmacodynamic effect (28). The daily schedule was to weigh the rats, then transfer them to individual hanging wire cages (30 × 20 × 15 cm) equipped with two 15-ml graduated tubes (0.1 ml gradations), one containing an alcohol solution, the other tap water. The position of these tubes was alternated daily and no food was available in the drinking cage. After 40 min elapsed the animals were returned to their home cages and the amounts of alcohol and water consumed were recorded. All this took place during the dark cycle when the animals were awake and most active.

The limited access procedure employs a gradual exposure of the animals to the taste and effects of alcohol. Initially, the animals were offered a choice between 3% (w/v) alcohol and water for 12 days and then a choice between 6% (w/v) alcohol and water for 17 days. Once intake had stabilized, they were divided into four experimental groups (n = 8 per group) and four control groups (n = 8 per group), all matched for their 6% alcohol intake during the final 4 days of this baseline phase when consumption was at asymptote. In the next phase (8 days), the four experimental groups were first pretreated with saline injections, then immediately injected with a SC dose of 200 μg/kg ANG II and placed in the drinking cages. The four control groups received two SC injections of the saline vehicle. Injections were given daily with the pretreatment injections occurring 15 min prior to the treatment injection. Once the ANG II-induced reduction in alcohol intake had reached its maximum and stabilized, three of the four experimental groups were pretreated sequentially in the following three phases (6 days per phase) with all three doses of either DuP753 (250 μg/kg, 500 μg/kg, and 1.0 mg/kg), the nonpeptide AT_1 receptor antagonist, PD123319 (250 μg/kg, 500 μg/kg, and 1.0 mg/kg), the nonpeptide AT_2 receptor antagonist, or Sar^1,Thr^8-ANG II (250 μg/kg), the nonselective AT_1/AT_2 receptor antagonist. This latter antagonist had previously been demonstrated to antagonize the effect of ANG II on alcohol intake (19). The fourth experimental group was pretreated with the antagonist saline vehicle. Like the experimental groups, three of the four control groups were also pretreated in the three subsequent phases with the same doses of either DuP753, PD123319, or Sar^1,Thr^8-ANG II. The fourth control group received two injections of the saline vehicle. Thus, each of the eight groups received two injections prior to being placed into the drinking cage: a pretreatment injection of one of the receptor antagonists or vehicle followed by a treatment injection of ANG II or vehicle immediately prior to entering the drinking cage.

Drugs

All drugs were dissolved in 0.9% saline and prepared fresh daily. ANG II (Val^5-ANG II, Hypertensin, Ciba) was a gift of Ciba-Geigy Canada Ltd. DuP753 (Losartan®) was a gift of DuPont Merck Co. Ltd., and PD123319 was a gift of Parke-Davis Warner Lambert. Sar^1,Thr^8-ANG II was purchased from Sigma Chemical Co.

Data Analysis

Alcohol and water intake for each rat was averaged over the 6 days of each phase. One-way analyses of variance (ANOVA) were performed followed by post hoc Duncan's tests, which examined relevant pairwise group comparisons. In some cases t-tests were used to examine between-group differences. For all analyses alpha was set at 0.05.

RESULTS

Effect of the AT_1 Antagonist DuP753 on Alcohol and Water Intake

Figure 1 (top left) illustrates the effect of pretreatment with three doses of DuP753 on mean alcohol and water intake in the control group that was given the saline vehicle immediately prior to the limited access session. Neither alcohol or water intake appeared to be altered by pretreatment with this antagonist and the ANOVA confirmed that, throughout the experiment, DuP753 did not significantly alter alcohol, $F(4, 28) = 0.35$, NS, or water, $F(4, 28) = 0.72$, NS, consumption at any of the doses tested.

Effect of the AT_2 Antagonist PD123319 on Alcohol and Water Intake

Figure 1 (top right) illustrates the effect of pretreatment with three doses of PD123319 on mean alcohol and water

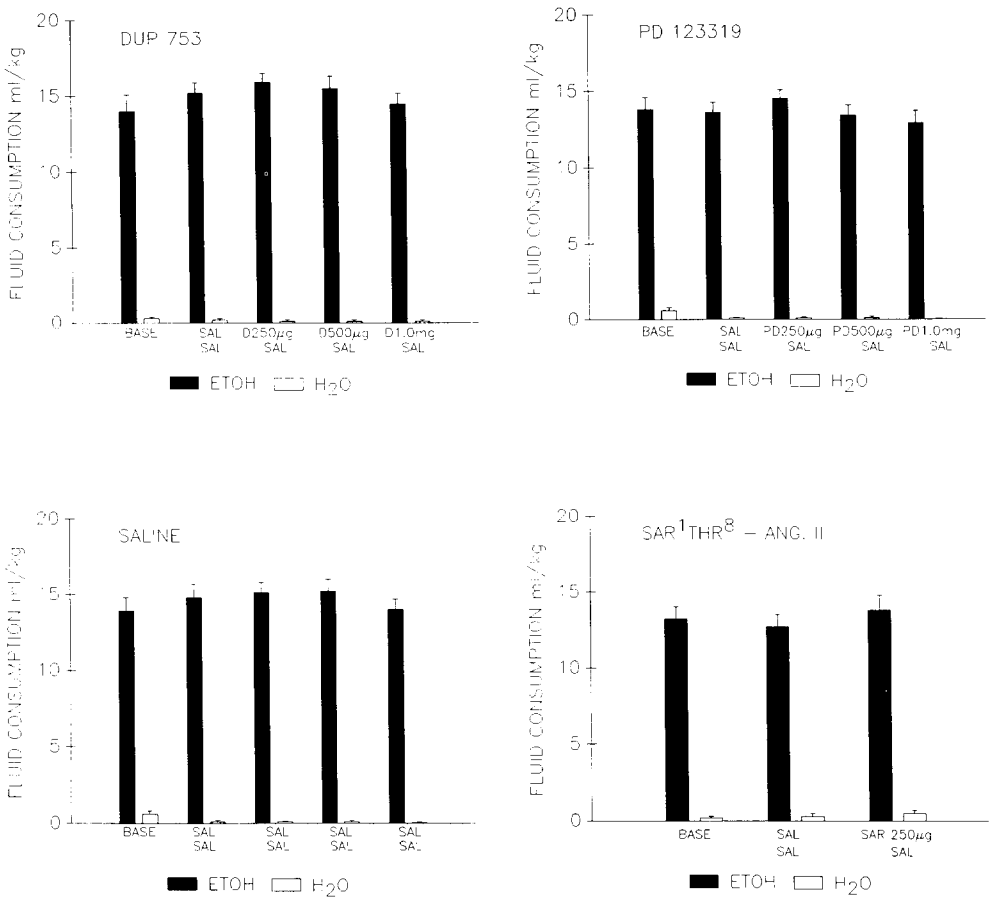

FIG. 1. Mean 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for each of the groups receiving the ANG II antagonists or saline. In the baseline phase (Base) no injections were given. In the following phase, two saline (SAL) injections were given just prior to the limited access period. In the subsequent three phases, ascending doses (/kg) of either DuP753 (D) or PD123319 (PD) were given followed immediately by a saline injection. The saline control group received only saline and the Sar[1],Thr[8]-ANG II (SAR) group received only one dose (250 µg/kg) of the antagonist. Bars represent ± SEM.

intake in the control group that was given the saline vehicle immediately prior to the limited access session. As was the case with the AT_1 antagonist, alcohol intake was not altered by pretreatment with the AT_2 antagonist. The ANOVA confirmed this with a nonsignificant effect of phase, $F(4, 28) = 0.75$, NS, indicating that PD123319 did not alter alcohol consumption at any of the doses tested. Water intake was low across all phases—not exceeding 1 ml/kg, but it did show a drop of approximately 1 ml/kg (0.5 ml) compared to baseline in all subsequent phases, including the phase in which two injections of saline were administered. While this reduction was statistically significant, $F(4, 28) = 3.63$, $p < 0.02$, it is unlikely to be a direct drug effect since it was also seen in the control group that did not receive any drug (see below).

Effects of the Saline Vehicle and the Nonselective AT_1/AT_2 Antagonist Sar[1],Thr[8]-ANG II on Alcohol and Water Intake

Figure 1 (bottom left panel) shows that the pretreatment and treatment injections of saline, which are the control vehicles for both the antagonist drugs and for ANG II, had no significant effect on alcohol intake, $F(4, 28) = 0.76$, NS. Water intake was low and tended to decrease over the course of the experiment, $F(4, 28) = 5.76$, $p < 0.002$. Figure 1 (bottom right panel) gives the mean alcohol and water intake in the group receiving Sar[1],Thr[8]-ANG II, the nonselective ANG II antagonist. As was the case with the selective AT_1 and AT_2 antagonists, this nonselective antagonist had no effect of its own on either alcohol, $F(2, 14) = 0.28$, NS, or water intake, $F(2, 14) = 0.58$, NS. This finding confirms previous work reporting no intrinsic activity of Sar[1],Thr[8]-ANG II with respect to either alcohol or water intake (19).

Effect of the Saline Vehicle and the Nonselective AT_1/AT_2 Antagonist Sar[1],Thr[8]-ANG II on the ANG II-Induced Reduction in Alcohol Intake

Figure 2 (top panel) shows that the 200 µg/kg dose of ANG II was active throughout the entire experiment, producing a significant reduction in alcohol intake, $F(4, 28) = 15.13$, $p < 0.0001$. Although there was a tendency for the alcohol consumption to recover compared to baseline (first phase), Duncan's test indicated that alcohol intake was significantly reduced in all of the subsequent four phases. Alcohol intake

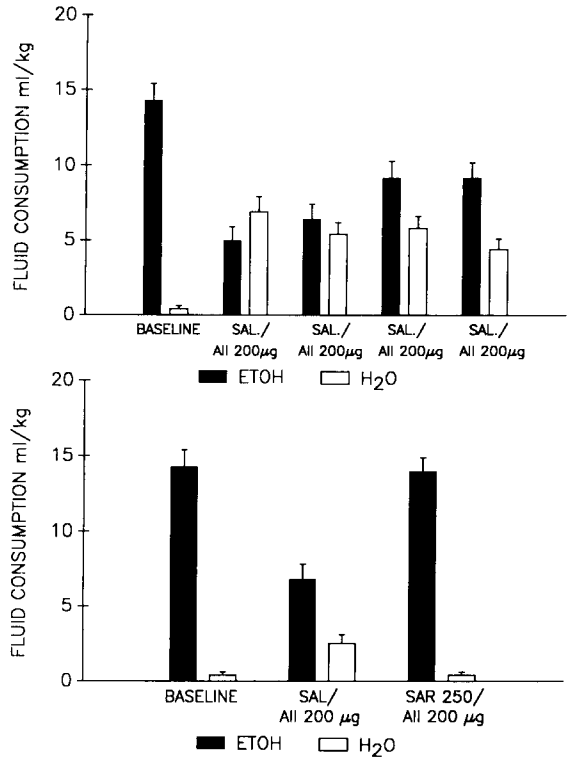

FIG. 2. Top panel: mean 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for the group receiving no antagonist pretreatment. In the baseline phase no injections were given. In the following four phases, a saline (SAL) injection preceded the injection of a 200 μg/kg dose of ANG II (AII). Bars represent ± SEM. Bottom panel: mean 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for the group receiving the Sar[1],Thr[8]-ANG II (SAR) pretreatment. In the baseline phase no injections were given. In the following phase, a saline (SAL) injection precede the injection of ANG II (AII) 200 μg/kg. In the final phase, Sar[1],Thr[8]-ANG II (SAR) 250 μg/kg was administered just prior to the injection of ANG II. Bars represent ± SEM.

in this group during the final phase of the experiment was statistically similar, $t(14) = 0.92$, NS, to that of the group receiving pretreatment with PD123319 during the same phase (Fig. 3, top panel). ANG II also produced a significant elevation in water intake, $F(4, 28) = 6.91$, $p < 0.0005$, which persisted throughout all phases of the experiment. These findings once again demonstrate the ability of ANG II to exert an inhibitory effect on alcohol intake and a stimulatory effect on water intake. Pretreatment injections of saline prior to ANG II did not alter the course of its action on either alcohol or water intake.

Figure 2 (bottom panel) shows that the effect of ANG II on alcohol intake can be completely blocked by pretreatment with the nonselective AT_1/AT_2 ANG II receptor antagonist Sar[1],Thr[8]-ANG II. A one-way ANOVA revealed a significant effect of phase for alcohol consumption, $F(2, 14) = 9.53$, $p < 0.002$, and post hoc Duncan's tests indicated that alcohol intake was significantly reduced by ANG II and then returned to baseline levels when the pretreatment with the ANG II antagonist was instituted. In this group of rats the ANG II enhancement of water intake, while present, was less pronounced and did not reach statistical significance, $F(2, 14) = 2.25$, NS.

Effect of the AT_2 Antagonist PD123319 on the ANG II-Induced Reduction in Alcohol and Water Intake

Figure 3 (top panel) shows that the ANG II-induced reduction in alcohol intake was not attenuated by pretreatment with any of the three doses of the selective AT_2 antagonist PD123319. The ANOVA showed a significant effect of phase, $F(4, 28) = 12.68$, $p < 0.0001$, reflecting the reduction in intake following ANG II administration. However, post hoc Duncan's test showed that alcohol intake was significantly reduced in all of the subsequent four phases, including the final three phases where ascending doses of PD123319 were administered. Alcohol intake during these PD123319 phases was statistically similar to that in the second phase, where no antagonist was administered prior to ANG II. The ANG II-induced stimulation in water intake also did not appear to be attenuated by PD123319, as water intake was significantly elevated, $F(4, 28) = 6.41$, $p < 0.0009$. Duncan's test showed that compared to the baseline intake in phase 1, water consumption was significantly elevated by ANG II regardless of the presence or absence of the AT_2 antagonist.

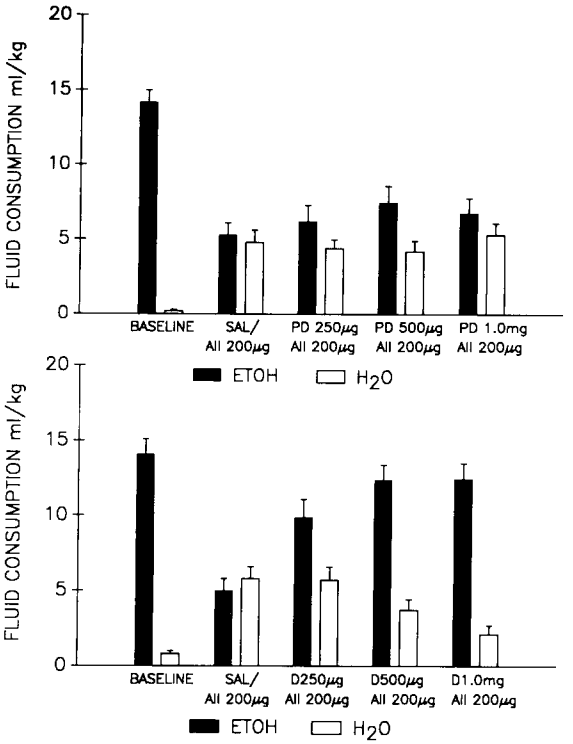

FIG. 3. Top panel: mean 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for the group receiving the PD123319 (PD) pretreatment. In the baseline phase no injections were given. In the following phase, saline (SAL) was injected just prior to the injection of ANG II (AII 200 μg/kg). In the final three phases, ascending doses of PD123319 (PD) were administered prior to the AII injections. Bars represent ± SEM. Bottom panel: mean 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for the group receiving the DuP753 (D) pretreatment. In the baseline phase no injections were given. In the following phase, saline (SAL) was injected just prior to the injection of ANG II (AII 200 μg/kg). In the final three phases, ascending doses of DuP753 (D) were administered prior to the AII injections. Bars represent ± SEM.

Effect of the AT_1 Antagonist DuP753 on the ANG II-Induced Reduction in Alcohol and Water Intake

A completely different picture emerged with respect to the effect of DuP753 on alcohol and water intake. Figure 3 (bottom panel) shows that before any antagonist pretreatment, ANG II produce a robust reduction in alcohol intake, $F(4, 28) = 11.17$, $p < 0.0001$. Post hoc Duncan's test demonstrated that compared to baseline, alcohol intake in phase 2 was significantly reduced by ANG II. Pretreatment with ascending doses of DuP753 attenuated the ability of ANG II to reduce alcohol intake in a dose-dependent fashion. Alcohol intake in the subsequent three phases with active pretreatment was significantly elevated compared to phase 2, where no pretreatment was given. Furthermore, alcohol intake during the last two phases, with the highest doses of DuP753, was not significantly different from the level of consumption occurring in the baseline phase, where no pretreatment drug was administered. The lowest dose of DuP753 produced some attenuation but was not great enough to be significantly different from phase 2. Water intake was also significantly altered throughout the course of the experiment, $F(4, 28) = 6.34$, $p < 0.0009$. The ANG II-induced increase evident in phase 2 was antagonized by DuP753. The lowest dose did not have any effect, while the intermediate dose showed a nonsignificant tendency to antagonize water intake. Only the highest (1.0 mg/kg) dose significantly attenuated the ability of ANG II to elevate water consumption.

Topography of the Effect of ANG II and the ANG II/ANG II Antagonist Interaction on Alcohol Intake

Figure 4 illustrates the mean daily intake of alcohol and water throughout the entire experiment for the saline control group, and the three ANG II groups pretreated with either saline the AT_1 or the AT_2 antagonist. Prior to antagonist pretreatment, ANG II produced a reduction in alcohol intake that was close to maximal after the first daily injection for the PD123319 and saline pretreatment groups (Fig. 4A and C) and maximal by the third daily injection in the DuP753 pretreatment group (Fig. 4B). ANG II produced a more gradual rise in water intake. DuP753, but not PD123319, produced a dose-dependent attenuation in the effect of ANG II on both alcohol and water intake over days. Compared to the immediately preceding phase, the 250 μg/kg dose of DuP753 pro-

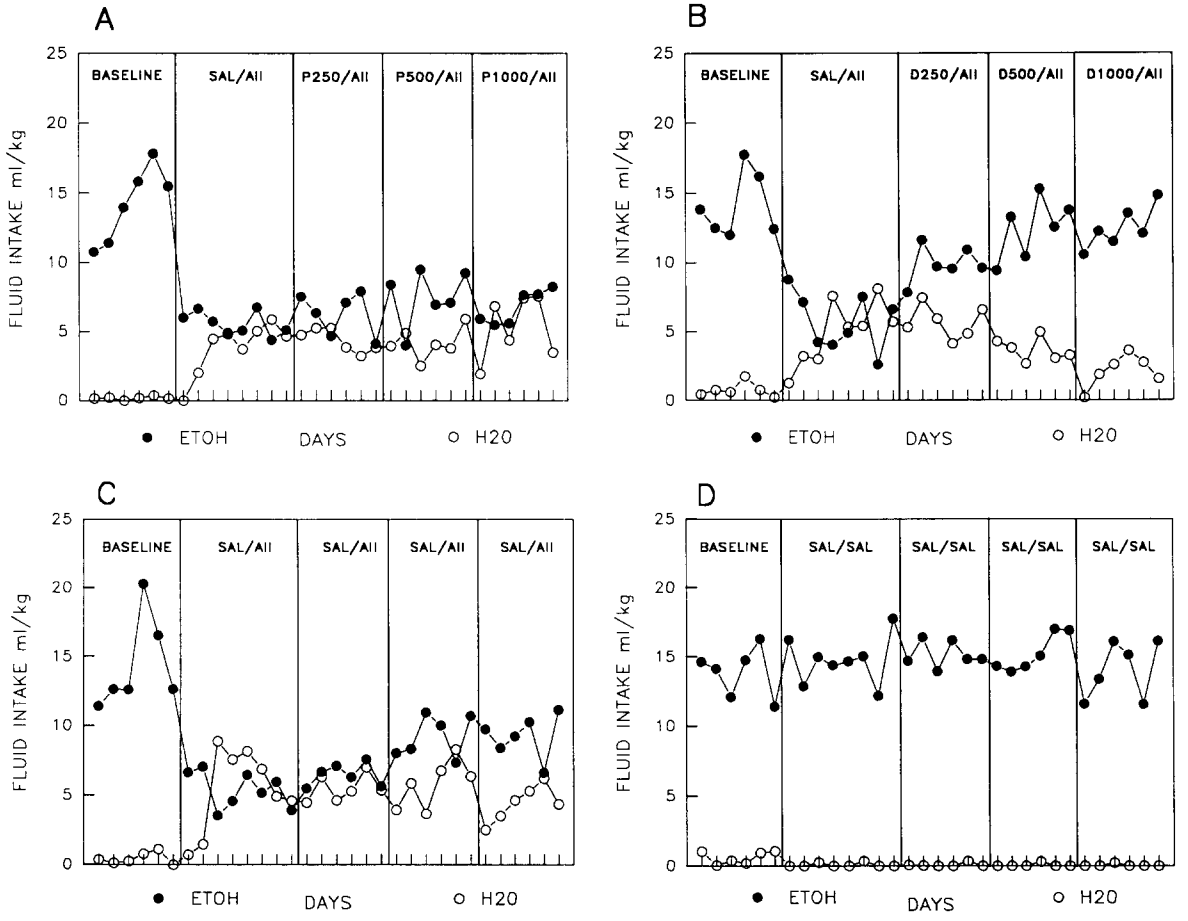

FIG. 4. Mean daily 6% alcohol (ETOH) and water (H_2O) intake during the 40-min limited access period for the groups receiving the PD123319 (P) pretreatment (A), the DuP753 (D) pretreatment (B), the vehicle (SAL) pretreatment (C), and the control group (D). All doses are in μg/kg. AII refers to angiotensin II at a dose of 200 μg/kg. This figure is an elaboration on some of the data presented in the previous figures and illustrates the topography of the intake across individual days of the experiment.

duced a clear attenuation of the ANG II-induced reduction in alcohol intake throughout the entire 6-day period of the phase. In contrast, the ANG II-stimulated water intake did not appear to be affected by this dose of the antagonist during this phase but was attenuated at the two higher doses. The control group receiving saline injections (Fig. 4D) showed a steady level of alcohol consumption throughout all phases of the experiment.

DISCUSSION

In agreement with other reports, we found that a selective AT_2 receptor antagonist such as PD123319 failed to antagonize the ANG II-induced increase in water intake (10,22,37) at any of the doses tested. The 1.0 mg/kg dose, which was the highest tested, was functional since a similar dose was shown to inhibit an increase in cerebral blood flow (37). The finding that PD123319 also failed to antagonize the ANG II-induced reduction in alcohol intake at any of the doses tested suggests that the AT_2 receptor subtype does not play a role in mediating the effect of ANG II on alcohol intake. Recently, Rowland et al. (35) have shown that centrally administered PD123319 at very high doses (10 × the DuP753 dose used here) can antagonize ANG II-induced water intake. The present data do not address the issue of whether very high doses of peripherally administered PD123319 might also have a similar effect on the ANG II-induced reduction in alcohol intake.

This study does show, however, that the reduction in alcohol intake produced by ANG II can be blocked in a dose-dependent manner by the selective AT_1 receptor antagonist, DuP753. The lowest dose of DuP753, 250 μg/kg, produced a significant attenuation in the suppression while the highest dose, 1.0 mg/kg, almost completely antagonized the effect of ANG II. In addition, and as has previously been reported (10,14,22,38), DuP753 dose-dependently attenuated the stimulation of water intake. These complementary changes might suggest that the reduction in alcohol intake is secondary to the increase in water intake, which is a classical property of ANG II. While this could be the case, several considerations argue against such an interpretation. Without the assumption that ANG II has acted directly on the processes that promote alcohol consumption, it is hard to explain why animals do not continue to drink alcohol even when their water intake has been augmented by administration of a thirst-inducing stimulus such as ANG II. Furthermore, water is not always the choice solution when ANG II is administered. Grupp et al. (17) showed that when animals are offered a choice between a preferred glucose solution and water, the administration of ANG II does not reverse their preference and cause them to drink more water than glucose solution. These animals continue to prefer and drink much more of the glucose solution than the water. In addition, other experiments have shown that the effect of ANG II on alcohol and water intake can sometimes be dissociated. For example, Fitts (13) has shown that ANG II chronically infused into the third ventricle will stimulate water intake but produce no change or a small increase in alcohol consumption. Our lab, using the limited access procedure, has confirmed these findings by showing that ANG II acutely infused both into the lateral and third ventricles increases water intake without changing alcohol intake (Grupp, unpublished observations). Finally, the daily intake data in the present experiment show that the lowest dose of the AT_1 antagonist (250 μg/kg) was able to significantly attenuate the suppressive effect of ANG II on alcohol intake without influencing water intake. Taken together, these findings suggest that the reduction in alcohol intake produced by ANG II and its subsequent reversal by a selective AT_1 and nonselective AT_1/AT_2 receptor antagonist, while associated with a change in thirst, may not necessarily be a result of enhanced water drinking per se. The ability of ANG II to alter alcohol consumption can sometimes be dissociated from its effect on water intake. This appears to be a behavioral property of the peptide that suggests associative influences of ANG II (1).

By way of comparison, the nonselective peptide antagonist Sar^1,Thr^8-ANG II was also administered in a separate group of rats. As previously reported (19), Sar^1,Thr^8-ANG II at the 250 μg/kg dose also reversed the effects of ANG II on both water and alcohol intake and to a degree comparable to that observed with the 1.0 mg/kg dose of DuP753. While the AT_2 antagonist had no effect, both the selective AT_1 and nonselective AT_1/AT_2 antagonists completely reversed ANG II's effects on both alcohol and water intake. Taken together, these findings suggest that in addition to playing no direct role in the effects of ANG II on alcohol and water consumption, the AT_2 receptor subtype also does not appear to act indirectly by modulating action at the AT_1 receptor.

By themselves, neither DuP753 (10) nor Sar^1,Thr^8-ANG II (19) reduce water intake. Similarly, except for high doses over long periods of time, these agents also do not reduce blood pressure in normotensive animals (36). It is only against a background of elevated ANG II activity, induced either by drugs (14,35,38), surgery (26), or genetic selection (3,5), that the cardiovascular effects of these antagonists become obvious. The results of the present experiment give a similar picture with respect to alcohol intake, since none of the antagonists exerted any change by themselves on alcohol intake. These same antagonist doses, however, actively inhibited the effects of ANG II on alcohol drinking. Failure to exert any effect of its own is, of course, one of the classical properties of a receptor antagonist that binds but has no intrinsic activity. This property of the AT_1 receptor antagonist with respect to alcohol intake stands in contrast to some other peptides and transmitters whose antagonists do have effects when administered alone. For example, while opiate peptide agonists and morphine will stimulate the intake of alcohol solutions, opiate antagonists will themselves suppress alcohol intake (24). A similar picture emerges with respect to the dopaminergic system, where agonists and antagonists can have opposite effects on alcohol drinking (27,33). Behavioral consequences arising from the administration of antagonists could suggest the involvement of a tonically active system. In the present context, where the antagonist alone did not increase alcohol intake, it appears that a tonically active system is not involved and that the role of ANG II may therefore pertain more to the cessation of alcohol drinking than to its initiation. In this sense, ANG II may be part of the mechanism that causes alcohol drinking to stop.

Recently, a second form of the AT_1 receptor, AT_{1B}, was sequenced and expressed from the rat pituitary (25). AT_{1A} mRNA appears to be expressed primarily by vascular smooth muscle (32), while adrenal and anterior pituitary appear to express AT_{1B} mRNA. Preliminary work in our laboratory has suggested that alcohol intake can be modified by manipulations that directly affect aldosterone activity. For example, injections of the synthetic mineralocorticoid, DOCA, can reduce alcohol drinking, and the aldosterone antagonist, spironolactone, can partially antagonize the ANG II-induced reduction in alcohol intake. The implication of these findings is that the adrenals may be involved in the effect of ANG II on alcohol intake. The development of selective antagonists for

these two forms of the AT_1 receptor will allow us to further refine and build on the main finding of this report; i.e., that the AT_1 receptor appears to be primarily involved in the inhibitory effect of ANG II on alcohol intake.

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