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Angiotensin II-Induced Suppression of Alcohol Intake and its Reversal by the Angiotensin Antagonist Sar-1 Thr-8 Angiotensin II

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GRUPP, L. A., E. PERLANSKI AND R. B. STEWART. *Angiotensin II-induced suppression of alcohol intake and its reversal by the angiotensin antagonist Sar-1 Thr-8 angiotensin II.* PHARMACOL BIOCHEM BEHAV 31(4) 813–816, 1988.—The effects of three doses of angiotensin II (AII) on alcohol consumption using the limited access procedure were studied. Fifty and 100 μg/kg AII administered subcutaneously (SC) did not alter alcohol intake while 200 μg/kg suppressed alcohol intake. These findings confirm previous work and show that AII begins to be effective in reducing alcohol intake in the range of 200 μg/kg. In the second part of the study, the AII antagonist Sar-1 Thr-8 AII (500 μg/kg SC) was given immediately prior to the administration of either saline or 200 μg/kg AII. In the control group treated with saline, the antagonist had no effect of its own on intake but completely blocked the suppressive effect of the 200 μg/kg dose AII on alcohol consumption. These findings indicate that the reduction in alcohol intake produced by AII is mediated by events occurring at the receptor level.

Alcohol intake Renin-angiotensin system Angiotensin II Angiotensin antagonist Sar-1 Thr-8 angiotensin II

PREVIOUS research has demonstrated an inverse relationship between manipulations which alter activity in the renin-angiotensin (r-a) system and voluntary alcohol intake. For example, a low salt diet with a diuretic, high doses of the synthetic mineralocorticoid, desoxycorticosterone acetate, serotonin uptake inhibitors, renal artery stenosis and, under certain conditions, angiotensin converting enzyme inhibitors can elevate r-a activity and have been shown to suppress alcohol intake (2–5, 7, 8, 11, 14). Conversely, a high salt diet, the salt sensitive Dahl line of rats or lesions to the angiotensin-receptor-rich area postrema in the medulla all elevate alcohol intake (4, 6, 12, 13). These manipulations are known to be correlated with a decrease in r-a activity. Recently, we have shown that subcutaneous (SC) injections of angiotensin II (AII) can produce a dose-dependent decrease in alcohol consumption (1). In that experiment 20 μg/kg of AII had no effect but 200 μg/kg and 1000 μg/kg produced graded reductions in alcohol intake. These reductions were selective since AII did not alter the intake of a highly palatable glucose solution (1). The purpose of the present study was two-fold. First, to explore more closely the AII dose range between 20 μg/kg and 200 μg/kg in order to identify the lowest dose which would produce a significant reduction in alcohol intake. Second, to examine the effect of an AII antagonist on the AII-mediated reduction in alcohol intake. A reinstatement of alcohol drinking by the antagonist would indicate that the effect of AII on alcohol intake is a receptor-mediated phenomenon and not one mediated by nonspecific effects of the peptide.

METHOD

Subjects

The subjects were 35 naive male Wistar rats weighing 270–310 g at the beginning of the experiment. They were individually housed in cages equipped with a water bottle and food hopper and kept on a reverse 12 hr/12 hr light-dark cycle with lights off at 7:00 a.m.

Procedure

A limited access drinking procedure was used (9,10). This procedure was chosen because it fosters intake in excess of the rat's metabolic capacity (approx. 300 mg/kg/hr), yields detectable blood alcohol levels (9) and therefore produces pharmacologically relevant central nervous system effects. The pattern of intake is such that most of the alcohol is consumed during the first 15–20 min of the 40-min access period and in some ways may be described as a bout-like pattern.

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Each day during the dark cycle (approximately 10:00 a.m.) the animals were removed from their home cages, weighed, and then placed for 40 min in individual "drinking cages" which had two graduated drinking tubes at the front, one containing a solution of alcohol and water, the other containing water. No food was available in the drinking cage. After the 40 min had elapsed, the amounts of water and alcohol consumed were recorded and the animals were returned to their home cages where water and pelleted rat chow were always available. The positions of the two fluids in the drinking cages were alternated daily to control for position preferences. A stock solution of alcohol was prepared fresh weekly. Alcohol to fill the graduated tubes was drawn daily from the stock solution. For two weeks a 3% (w/v) alcohol solution was offered followed by a 6% (w/v) solution for a further 46 days. The data to be reported are based on the experimental manipulations that were carried out during the 46-day period when the 6% alcohol solution was available. The alcohol intake of seven animals was low and could not have consistently exceeded the metabolic rate of 300 mg/kg/hr. These animals were not included in the data analysis. The experiment consisted of three phases: a Baseline phase followed by an Angiotensin Dose-Response phase and finally an Angiotensin-Antagonist phase.

Baseline

This phase lasted 14 days at the end of which the animals were divided into 4 groups matched for alcohol intake and designated to receive either the saline vehicle (n=7), 50 μg/kg AII (n=7), 100 μg/kg AII (n=7), or 200 μg/kg AII (n=7) in the following two phases.

Angiotensin Dose-Response

This phase lasted 14 days during which each group received its respective daily dose of AII or saline by the SC route immediately prior to the 40-min access period to 6% alcohol and water.

Angiotensin-Antagonist

This phase lasted 18 days during which only the saline and the 200 μg/kg AII groups continued to be tested. Both groups continued to receive their respective daily doses of either AII or saline immediately prior to the alcohol access period. In addition, using an "A-B-A'" design, both groups were pretreated with saline during the first 6 day cycle (A) and the last 6 day cycle (A') of the phase, while the AII antagonist, Sar-1 Thr-8 angiotensin II (500 μg/kg), was the pretreatment during the intermediate (B) 6-day cycle. Both the saline and the antagonist pretreatments were given by the SC route 10 min prior to the administration of the AII or saline.

Drugs

Both AII (val-5 angiotensin II—Hypertensin, Ciba) and the AII antagonist (Sar-1 Thr-8 angiotensin II, Sigma) were dissolved in saline and prepared fresh daily just prior to their administration. The concentrations were adjusted so as to permit the administration of 0.1 ml/100 g body weight.

RESULTS

Effect of Low Doses of AII on Alcohol and Water Intake

Figure 1A shows the mean alcohol intake for the three AII groups and the saline control group during the Baseline and Dose-Response phases. The drinking for each animal was averaged across the 14 days of each of the two phases. A two-way analysis of variance of these means with Dose as the between subjects factor and Phase as the within subjects factor yielded a nonsignificant effect of Dose, $F(3,24)=1.97$, n.s., but a significant effect of Phase, $F(1,24)=4.38$, $p=0.04$, and the interaction of Dose with Phase, $F(3,24)=5.09$, $p=0.007$. Post hoc Duncan's tests indicated that all four groups drank similar amounts of alcohol in the Baseline phase and that only the group receiving 200 μg/kg AII showed a significant reduction in alcohol intake compared to the saline control.

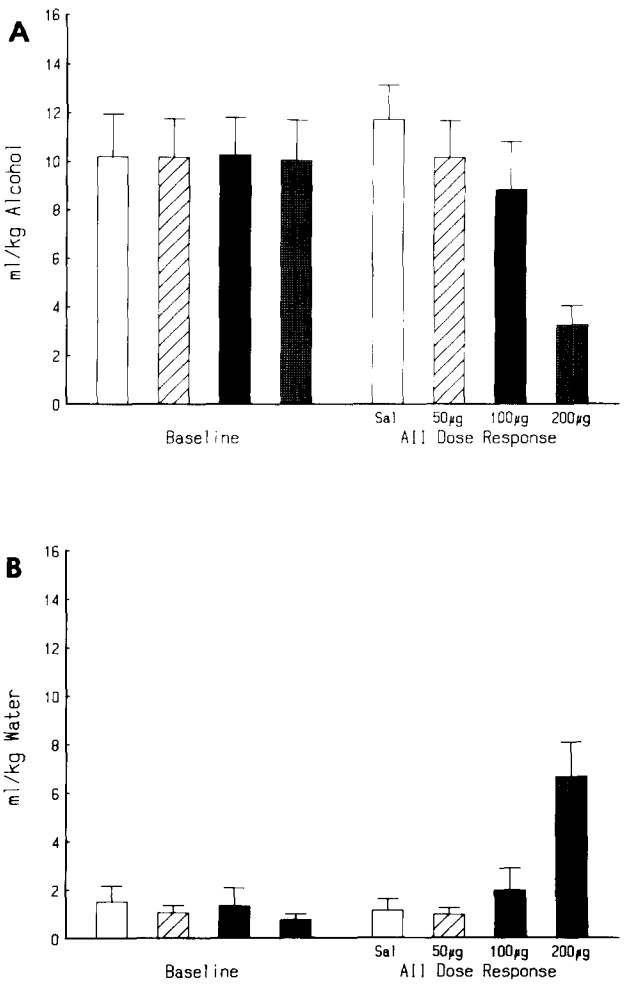

FIG. 1. Mean 6% (w/v) alcohol (A) and water (B) intake before (Baseline) and during (Treatment) administration of saline (open bar), 50 μg/kg (hatched bar), 100 μg/kg (black bar) and 200 μg/kg (checked bar) angiotensin II. Each dose was given to a separate group of animals immediately prior to the access to alcohol. Bars represent ±standard error of the mean.

Figure 1B shows the mean water intake for the four groups during the Baseline and Dose-Response phases. As was the case for alcohol, water drinking for each animal was averaged across the 14 days of each of the two phases. A two-way analysis of variance of these means yielded a significant effect of Dose, $F(3,24)=4.2$, $p=0.02$, Phase, $F(1,24)=12.29$, $p=0.002$, and a significant Dose × Phase in-

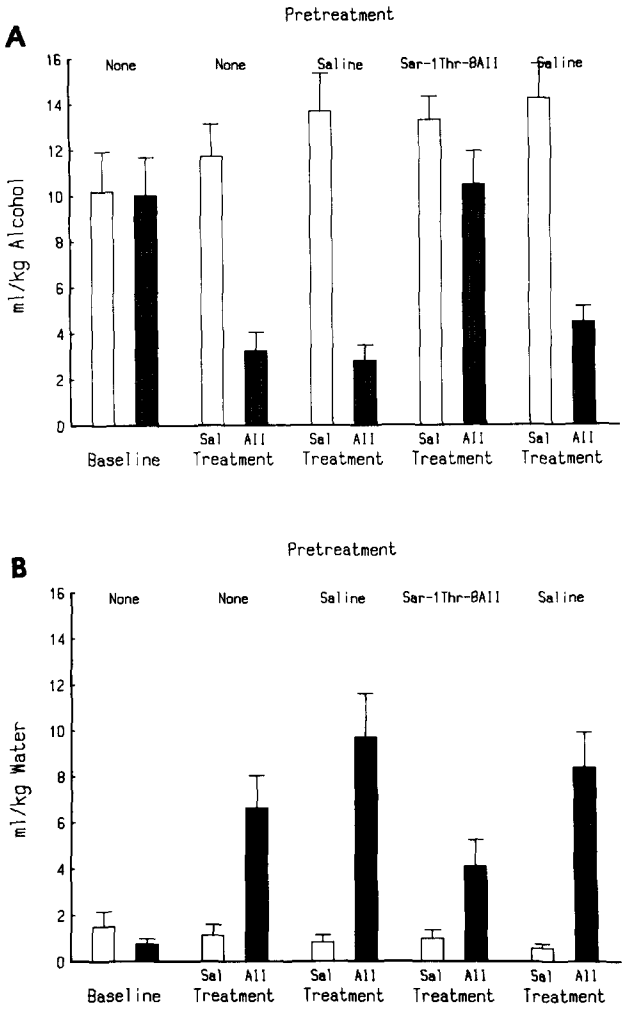

FIG. 2. Mean 6% (w/v) alcohol (A) and water (B) intake for the two groups receiving either saline or 200 μg/kg angiotensin II. Baseline—no drugs administered. Treatment—200 μg/kg angiotensin II or saline administered prior to access to alcohol. Pretreatment—either no injection, saline injection or injection of the antagonist Sar-1 Thr-8 angiotensin II immediately before the administration of angiotensin II or saline. Bars represent ±standard error of the mean.

teraction, $F(3,24)=11.77$, $p<0.001$. Post hoc Duncan's test indicated that all four groups drank similar amounts of water in the Baseline phase and that only the group receiving 200 μg/kg AII drank significantly more water than the saline control group.

Effect of the AII Antagonist, Sar-1 Thr-8 AII, on AII-Induced Changes in Alcohol and Water Intake

Figure 2A gives the compete profile of the alcohol intake for the 200 μg/kg and the saline groups across all three phases of the experiment. Drinking for each animal was averaged across the 14 days of each of the Baseline and Angiotensin Dose-Response phases and across each of the three 6-day cycles of the Angiotensin-Antagonist phase. A two-way analysis of variance with Drug (AII vs. Saline) as the between subjects factor and Phase (Saline or no injections vs. Antagonist) as the within subjects factor indicated a significant effect of Drug, $F(1,12)=23.2$, $p<0.001$, a significant effect of Phase, $F(4,48)=5.34$, $p=0.001$, and a significant interaction of Drug and Phase, $F(4,48)=9.98$, $p<0.001$. Post hoc Duncan's tests showed that 200 μg/kg AII either alone or when combined with saline pretreatment (first and last cycles of the Antagonist phase) produced a significant decrease in alcohol intake compared to the intake during the Baseline phase. Pretreatment with the AII antagonist Sar-1 Thr-8 AII completely blocked the ability of AII to reduce alcohol consumption, significantly elevating intake above the levels seen with the saline pretreatments and indeed bringing alcohol intake back to the same level observed during the Baseline phase. Post hoc tests also showed that alcohol intake in the saline control group did not change significantly across the three phases of the experiment and in particular that pretreatment with Sar-1 Thr-8 did not by itself produce any change in alcohol intake.

Figure 2B gives the complete profile of the water intake for the 200 μg/kg and the saline groups across all three phases of the experiment. As was the case for alcohol, drinking for each animal was averaged across the 14 days of each of the Baseline and Angiotensin Dose-Response phases and across each of the three 6-day cycles of the Angiotensin-Antagonist phase. A two-way analysis of variance with Drug as the between subjects factor and Phase as the within subjects factor indicated a significant effect of Drug, $F(1,12)=22.6$, $p<0.001$, a significant effect of Phase, $F(4,48)=8.6$, $p<0.001$, and a significant interaction of Drug and Phase, $F(4,48)=12.0$, $p<0.001$. Post hoc Duncan's tests showed that 200 μg/kg AII either alone (Dose-Response phase) or when combined with the saline pretreatments (first and last cycles of the Antagonist phase) produced a significant increase in water intake compared to the intake during the Baseline phase. Pretreatment with the AII antagonist Sar-1 Thr-8 AII significantly attenuated the ability of AII to increase water consumption, bringing intake back to the same level achieved during the Baseline phase. Post hoc tests also showed that water intake in the saline control group did not change significantly across the three phases of the experiment and that pretreatment with Sar-1 Thr-8 AII did not by itself produce a change in water intake.

DISCUSSION

The present experiment adds two new findings about the effects of AII on alcohol intake. First, the results show that both 50 μg/kg and 100 μg/kg AII do not reduce alcohol intake while the 200 μg/kg dose produces a robust decrease in alcohol intake. This finding together with previous work (1) suggests that 200 μg/kg, administered by the subcutaneous route, defines the lower end of the dose range of val-5 AII that is effective in reducing voluntary alcohol intake in the limited access procedure.

The 200 μg/kg dose also appears to be the minimally effective dose for elevating water intake and blood pressure (unpublished observations), and while an increase in thirst is the hallmark of AII, in the present experiment the increased thirst is directed specifically to water and not expressed as a general increase in the motivation for all available fluids including alcohol which is also present and initially preferred. On the contrary, alcohol intake is suppressed at the same time that water intake is increased and this suppression cannot be secondary to the increase in water intake because fluid intake in the limited access procedure is not of a regulatory nature given that animals have full access to water (and

food) in their home cages. These considerations indicate that the effect of AII on alcohol intake is independent of its effect on water intake.

The second new finding is that the AII receptor antagonist, Sar-1 Thr-8 AII, can counteract the suppressive effect of AII on alcohol drinking. Whereas the 200 μg/kg dose of AII produced a marked decrease in alcohol drinking, pretreatment with the AII antagonist promptly reversed this suppression and restored alcohol intake to baseline (i.e., pre-AII) levels. Furthermore, when pretreatment with the antagonist was suspended, AII administration again decreased alcohol intake. Taken together these findings indicate that the effect of AII on alcohol intake is a receptor-mediated phenomenon and that the biological events occurring at the level of the AII receptor are important in the regulation of alcohol drinking.

There did not appear to be any "savings" or carry-over from the 28-day episode of AII-induced suppression of alcohol intake because the AII antagonist produced a prompt and complete reversal of the reduction in alcohol intake produced by AII. Examination of the raw data revealed that recovery of alcohol intake was present in four of the seven animals on the very first day of antagonist pretreatment and was virtually complete in all animals by the second day. These considerations tend to argue against an interpretation of the effect of AII on alcohol intake which is based on learning. Such an interpretation would assume that AII has some aversive properties which are incompatible with the intake of alcohol (but not water) and that these properties come to be conditioned to and elicited by drinking cage cues. But if this type of conditioning has taken place, the ability of these cues to elicit the incompatible aversive reaction should persist for some time even when the AII receptor antagonist prevents AII from binding to the receptor. The rapid recovery in alcohol intake following pretreatment with the AII antagonist, however, is more reflective of the interplay of agonists and antagonists at a receptor site than it is of a longer term associative effect. Future research in this area should determine whether other hormones, such as vasopressin or aldosterone whose activity is directly stimulated by angiotensin II, also play a role in the regulation of alcohol intake.

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