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Effects of Angiotensin II and an Angiotensin Converting Enzyme Inhibitor on Alcohol Intake in P and NP Rats

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GRUPP, L. A. *Effects of angiotensin II and an angiotensin converting enzyme inhibitor on alcohol intake in P and NP rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 105–108, 1992.—While it is known that randomly bred normotensive Wistar stock and hypertensive rats alter their alcohol consumption when activity in the renin-angiotensin (R-A) system is modified, the effect of manipulations to the R-A system on alcohol intake in genetically selected alcohol-preferring P and -nonpreferring NP rats has not been assessed. In Experiment 1, nine P rats and 8 NP rats were injected with the saline vehicle and offered limited access to 10% (v/v) alcohol for 40 min each day for 7 days. When intake stabilized both groups were given daily intraperitoneal injections of the angiotensin converting enzyme inhibitor, ceranapril (20 mg/kg) 45 min prior to alcohol access for 11 days. Ceranapril (SQ 29,852) reduced alcohol intake in both the P and NP animals, while saline had no effect. In Experiment 2, these same two groups of P and NP rats were injected with three doses of angiotensin II (ANG II) (100, 200, 400 μg/kg) immediately prior to alcohol access. Each dose was tested for 10 consecutive days, with a 14-day period of no drug preceding and following the ANG II treatments. ANG II reduced alcohol intake in the NP rats and produced a dose-dependent reduction in the alcohol consumption of the P rats. These findings indicate that the renin-angiotensin system can modify alcohol consumption in rats selectively bred for high and low alcohol intake.

Alcohol intake P and NP rats Renin-angiotensin system Angiotensin II
Angiotensin converting enzyme inhibitor SQ 29,852

PREVIOUS work with randomly bred normotensive Wistar stock as well as hypertensive animals has shown that the renin-angiotensin (R-A) system participates in the control of alcohol consumption (3, 5, 7, 8). Peripheral injections of angiotensin II (ANG II) produce a dose-dependent, antagonist reversible reduction in alcohol intake (6). Similarly, the class of drugs known as the angiotensin converting enzyme (ACE) inhibitors, used to treat hypertension and heart failure in humans, also produce a dose-dependent reduction in alcohol intake (9, 11, 18). The effect of these agents has been evaluated with the two-bottle 24-h choice and limited access procedures. While both these procedures can produce a baseline of alcohol consumption which yields pharmacologically relevant blood alcohol levels (2,12), the doses self-administered are typically much lower than those achieved by rats which have been genetically selected to consume alcohol, such as the alcohol-preferring P rats. Earlier work with the P and the alcohol-nonpreferring NP rats demonstrated that alcohol consumption to be inversely related to plasma renin activity and suggested that the R-A system could modulate alcohol consumption in these genetically selected animals (4). The present experiments further evaluated the role and assessed the vigor of the R-A system in the modulating alcohol consumption by evaluating the effects of ANG II and the ACE inhibitor, ceranapril (SQ 29,852), on alcohol intake in the high and low alcohol consuming P and NP rats.

METHOD

Subjects

Ten naive male P and 10 naive male NP rats, obtained from the colony at the Indiana University School of Medicine, were housed individually in a temperature-controlled environment on a 12-h light/dark cycle with lights on at 7:00 a.m. The animals had continuous access to food and water in their home cages except where noted and weighed 374–486 g at the beginning of the experiment. Prior to testing, the animals were screened in the standard way for alcohol consumption (13) by first exposing them to forced 10% v/v alcohol for 4 days followed by 4 weeks on two-bottle 24-h choice between 10% v/v alcohol and water. At the end of this period, the P rats drank a daily average of 8 g/kg while the NP rats drank 1.2 g/kg. Two NP rats and 1 P rat died during the course of the Experiment 1 and an additional NP rat died during Experiment 2. Their data will not be presented.

Procedure

The animals were submitted to the limited access procedure in which, on a daily basis, they were first weighed and then transferred to drinking cages which had two graduated drinking tubes, one containing a 10% (v/v) alcohol solution, the other,

water. After 40 min had elapsed, the animals were returned to their home cages and alcohol and water consumption were measured to the nearest 0.1 ml. The position of these tubes was alternated daily and no food was available in the drinking cage.

Experiment 1

This experiment examined the effect of the ACE inhibitor, ceranapril (SQ 29,852), on alcohol intake. In the first phase (7 days) all animals were injected intraperitoneally (IP) with the ceranapril vehicle, saline, 45 min prior to alcohol availability. In the second phase (11 days), ceranapril (20 mg/kg) was administered to both groups 45 min prior to alcohol availability. The third phase (10 days) represented a return to the conditions of phase 1. Ceranapril was dissolved in saline, made fresh daily and administered in a volume of 0.2 ml/100 g body weight.

Experiment 2

Fourteen days following Experiment 1, the effect of the peptide Val^5 angiotensin II (ANG II) on alcohol consumption in the same P and NP rats was examined. During these 14 intervening days no alcohol was offered to the animals. In phase 1 (14 days), the saline vehicle was injected immediately prior to the 40 min limited access to alcohol and water in the drinking cage. In phase 2 (10 days), phase 3 (10 days), and phase 4 (10 days), 100, 200 and 400 μg/kg ANG II respectively were injected subcutaneously immediately prior to the limited access session. In phase 5, all injections were suspended prior to the limited access session and recovery of alcohol intake from the effects of ANG II were monitored. ANG II was prepared fresh daily in saline and injected in a volume of 0.1 ml/100 g body weight.

Statistical Methods

Repeated measures one-way analyses of variance followed by Duncan's test for multiple comparisons were used to evaluate the results. The confidence level was set at .95.

RESULTS

Experiment 1

Intake for each of the P and NP rats was averaged for every phase and the mean group intake per phase is presented in Fig. 1. Panel A indicates and statistical analysis confirms that the daily administration of 20 mg/kg ceranapril 45 min prior to alcohol availability significantly reduced the consumption of alcohol in P rats, $F(2,16)=5.913$, $p=0.01$. Water consumption (Table 1) in these animals was slightly elevated, but not to any significant degree, $F(2,16)=3.6$, n.s. Suspension of ceranapril treatment in the third phase initiated a return to predrug levels of alcohol consumption suggesting that the reduction in alcohol intake produced by ceranapril did not result in a long-lasting change in the animals' pattern of alcohol ingestion.

NP rat consumption of alcohol as shown in Fig. 1B was considerably less than in the P rats. Ceranapril administration in phase 2 also resulted in a significant reduction in alcohol consumption in these rats, $F(2,14)=5.5$, $p=0.02$, with a tendency for alcohol consumption to recover when ceranapril injections were suspended in phase 3. Water consumption showed a greater increase in these rats following ceranapril compared to the P rats (Table 1), but this did not achieve statistical significance, $F(2,14)=2.15$, n.s.

Experiment 2

As in Experiment 1, intake for each of the P and NP rats was averaged for every phase and the mean group intake per phase was calculated. Figure 2A shows that ANG II produced a dose-dependent decrease in the alcohol consumption of the P rats, $F(3,24)=285.5$, $p>0.0001$, which recovered to baseline levels in phase 5 following suspension of ANG II injections. Post hoc tests indicated that all doses of the peptide significantly diminished alcohol consumption. Examination of the mean daily intake showed that recovery was complete by the 10th day of the final phase. Table 1 shows that water intake in the P rats was also elevated in a dose-dependent manner by ANG II, $F(3,24)=30.1$, $p>0.0001$, and that suspension of peptide administration led to a rapid return to baseline levels of intake. In contrast to the effects on alcohol intake, water intake was not affected by the lowest dose of ANG II but only by the two higher doses.

Figure 2B shows that ANG II significantly reduced alcohol intake in the NP rats as well. Compared to intake during the baseline phase 1, all 3 doses of the peptide significantly reduced intake, $F(3,18)=11.9$, $p=0.0003$. However, there was no progressively greater reduction in intake with increasing dose owing to the initial low levels of alcohol consumption in NP rats. Again, as was the case with the P rats, suspension of ANG II treatment resulted in a recovery in alcohol consumption to baseline levels. Table 1 also shows that ANG II produced a dose-dependent increase in water intake of the NP rats, $F(3,18)=$

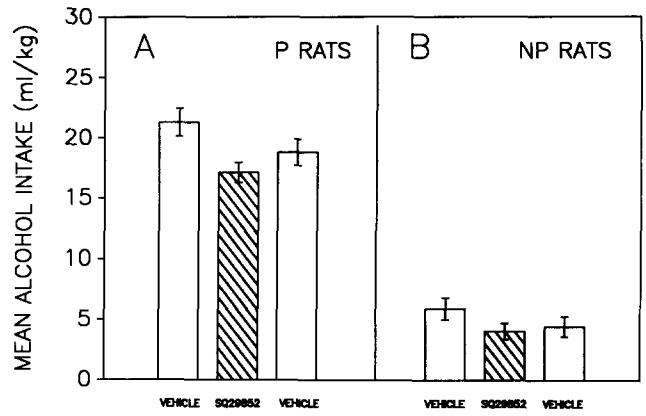

FIG. 1. Mean alcohol consumption of the P rats (A) and NP rats (B) before (VEH), during (SQ 29,852) and after (VEH) daily treatment with 20 mg/kg ceranapril. This ACE inhibitor produced a modest but significant decrease in alcohol intake of both groups. Bars represent ± standard error of the mean.

TABLE 1

EFFECT OF THE ANGIOTENSIN CONVERTING ENZYME INHIBITOR, SQ 29,852 (EXPERIMENT 1) AND VAL^5 ANG II (EXPERIMENT 2) ON WATER INTAKE IN THE ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS

Experiment 1			Experiment 2			
	P	NP			P	NP
Veh	0.31	1.8	Veh		0.1	0.5
SQ 29,852	0.56	3.5	100 mg/kg	ANG II	0.3	0.8
Veh	0.21	2.2	200 mg/kg	ANG II	3.0*	2.0*
			400 mg/kg	ANG II	5.4*	8.5*
			No Injection		0.089	1.1

Statistical significance determined with Duncan's, multiple range test, *$p<0.05$.

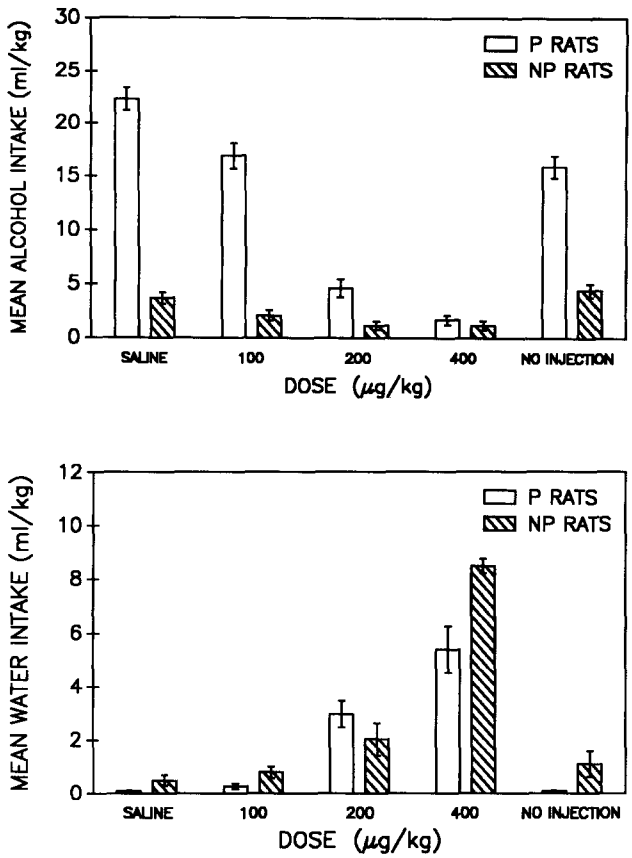

FIG. 2. Mean alcohol consumption of the P rats (A) and NP rats (B) before (saline), during and after (No injection) consecutive daily treatment with each of three doses of Val^5 ANG II. In the P rats, all doses produced a significant reduction in alcohol intake with the highest dose producing almost a complete suppression. ANG II also suppressed alcohol intake in the NP rats. Bars represent ± standard error of the mean.

112.2, $p>0.0001$. Again, as was the case with the P rats, the 100 μg/kg dose which did reduce alcohol intake was ineffective in altering water consumption, with the two higher doses stimulating water consumption. When ANG II injections were no longer given in phase 5, water intake returned to pre-ANG II levels.

DISCUSSION

Both the agonist Val^5 ANG II and the angiotensin converting enzyme inhibitor, ceranapril (SQ 29,852), reduced alcohol intake in the genetically selected alcohol-preferring P and the non-preferring NP rats. Thus manipulations of the R-A system which are known to reduce alcohol intake in animals which do not voluntarily ingest large amounts of alcohol (i.e., stock Wistar and renal hypertensive rats) are also very effective in decreasing alcohol intake in the P rats which do voluntarily consume large amounts of alcohol and satisfy most of the criteria for an animal model of alcoholism (10). The fact that the P and NP response to ANG II and the ACE inhibitor was qualitatively alike suggests that alcohol consumption in these two lines of rats is governed by similar mechanisms.

In this and in previous experiments, both ANG II and the ACE inhibitor, a drug which prevents the synthesis of ANG II, reduced alcohol intake. This paradoxical finding is hypothesized to be due to the common ability of both agents to raise activity in the R-A system, ANG II by a direct agonist effect in the periphery, the ACE inhibitor indirectly by increasing central ANG II activity (7,19). Experiment 1 showed that ceranapril, at a dose at which other ACE inhibitors are effective (i.e., 20 mg/kg), was also able to reduce alcohol consumption of both P and NP rats. In the P rats this was a 4 ml/kg (0.32 g/kg) reduction, while in the NP rats there was a much smaller 1.9 ml/kg (0.150 g/kg) reduction. Since the NP rats were already consuming very little alcohol, the smaller effect of ACE inhibitor in these animals compared to the P rats is likely to be due, in part, to an inability to drive an already low intake any lower.

In Experiment 2, the effect of ANG II on alcohol intake was quite robust. In the P rats, the 100, 200 and 400 μg/kg doses reduced consumption from approximately 1.8 g/kg per 40-min session in the saline vehicle phase to 1.4, 0.4 and 0.08 g/kg respectively. This decrease was not secondary to the increase in water intake since the additional amount of water consumed was much less than the amount by which alcohol consumption was reduced. For example, the 400 μg/kg dose reduced alcohol intake by 21 ml/kg, while at the same time increasing water intake by only 5 ml/kg. In the NP rats, alcohol intake was low at the outset (approximately 0.3 g/kg per 40-min session), yet even at this level, all doses of ANG II produced a significant reduction with the higher doses tending to produce larger effects. This effect of ANG II in NP rats is all the more impressive in light of the inability of other drug manipulations such as the calcium channel antagonist, verapamil, to reduce drinking in NP rats (17). In both the P and NP rats, the 100 μg/kg dose produced a significant decrease in alcohol consumption while at the same time failing to increase water intake. This finding further supports the suggestion that the effects of ANG II on alcohol and water consumption are not mirror images of each other and that the mechanisms controlling each enjoy a certain degree of autonomy from the other.

The administration of psychoactive agents such as amphetamine, morphine or cocaine following the ingestion of a novel and distinctively tasting substance can lead to the development of a conditioned taste aversion (CTA) which is expressed as an avoidance of that distinctive substance upon subsequent exposures to it. Rabin and Hunt (14) have shown that rats and cats would avoid a sweet tasting sucrose solution if ANG II injections followed the consumption of that solution. Given that the conditioning elements required for the development of a CTA were present in this experiment, albeit not in the standard sequence, could the drug-induced reduction in alcohol intake observed actually be a reflection of an acquired aversion to alcohol? A number of points argue against this interpretation. First, the conditions for the development of a taste aversion to alcohol were not ideal because the injection of ANG II preceded rather than followed the ingestion of alcohol. Backward conditioning of a taste aversion is poorly, if at all, expressed. Second, CTAs are poorly conditioned if the taste has been previously experienced by the animal and is therefore not a novel one (1,15). In the present experiment, the animals had consumed the 10% alcohol solution for many days before ANG II was administered, yet the ANG II-induced reduction in alcohol intake was very vigorous. Third, alcohol intake in both the P and NP rats recovered in the final phase following suspension of the ANG II treatment. In the P rats intake recovered daily until by the 10th day intake regained phase 1 (baseline) levels. Similarly, in the NP rats who drank less alcohol, intake recovered much more quickly and achieved baseline levels by the 2nd day following suspension of ANG II. Since extinction of CTAs usually occurs more slowly than extinction of the typical motor responses (16),

the reduction in alcohol intake by ANG II is unlikely to be the result of a conditioned taste aversion.

In summary, rats which prefer alcohol and drink large amounts of it on a daily basis, i.e., the genetically selected P rats, will show a reversal of this preference and avoid alcohol when pretreated with ANG II. This effect is specific to alcohol since water intake rises concomitantly and does not appear to be an associative effect related to a conditioned taste aversion. The ACE inhibitor, ceranapril, also reduces alcohol intake although its effect is not as robust as the peptide. NP rats show a similar profile of effects although to a much lesser degree owing to the initially small amounts of alcohol consumed. Taken together, these findings demonstrate that the renin-angiotensin system is a viable and potent modulator of alcohol consumption not only in the wild type Wistar stock rat but also in rats with genetically selected preferences for or against alcohol intake.

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