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Angiotensin II Reduces Voluntary Alcohol Intake in the Rat

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GRUPP, L A , M KILLIAN, E PERLANSKI AND R B STEWART *Angiotensin II reduces voluntary alcohol intake in the rat* PHARMACOL BIOCHEM BEHAV **29**(3) 479–482, 1988 —The voluntary intake of alcohol has been shown to be attenuated by a variety of manipulations which increase activity in the renin-angiotensin system In the present study we examined the effects of peripheral injections of the peptide angiotensin II on alcohol drinking The peptide produced a dose-dependent decrease in alcohol intake with 20 μg/kg having little effect, 200 μg/kg reducing intake by approximately 50% and 1 mg/kg virtually abolishing all alcohol drinking This decrease was not due to a peptide induced motor deficit, or state of sickness, and could also not be accounted for by the increased water intake, or by a change in pharmacokinetics and taste function These data provide direct evidence that angiotensin II can modulate voluntary alcohol drinking The possibility that the level of angiotensin II serves as a satiety signal in alcohol drinking is discussed

Alcohol drinking Angiotensin II Renin-angiotensin system Satiety

OVER the past few years, our laboratory has demonstrated that manipulations which are known to change activity in the renin-angiotensin (r-a) system will markedly alter the voluntary intake of alcohol For example, when r-a activity is elevated by the use of a low salt diet and diuretic [6,7], by the administration of certain drugs [4,15], or by constricting one renal artery (Two-Kidney, One-Clip procedure) [3], alcohol drinking is reduced On the other hand, when r-a activity is suppressed as is the case in the genetically selected salt-sensitive line of Dahl rats [5], by the use of a high salt diet [4], or if the angiotensin "signal" from the periphery is decreased by lesioning the angiotensin-receptor-rich area postrema [16], voluntary alcohol drinking is enhanced

The following experiments examine the involvement of the bioactive component of the r-a system, angiotensin II, on voluntary alcohol drinking These experiments demonstrate that subcutaneous injections of angiotensin II produce a potent and dose-dependent attenuation of voluntary alcohol drinking

METHOD

Subjects

The subjects were 35 naive male Wistar rats weighing 270–300 g at the start 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

The animals were randomly assigned to three groups, one designated to receive 20 μg/kg (n=12) of angiotensin II (AII) (asparagine-1 valine-5 angiotensin II–Hypertensin, Ciba), one to receive 1 mg/kg angiotensin II (n=12) and the control group to receive the saline vehicle (n=11) The agents were administered by the subcutaneous route

A limited access drinking procedure was used [13,14]. Each day during the dark cycle, the animals were removed from their home cages, weighed, injected and then placed for 1 hr in separate "drinking" cages which had two graduated tubes, one containing alcohol, the other containing water No food was available in the drinking cage, and positions of the two fluids were alternated daily After the hour had elapsed, the amounts consumed of both fluids were recorded and the animals were then returned to their home cages Since the half-life of AII in the circulation of the rat is less than one minute, a 1 hr session should be sufficient to be able to observe all of the effects of the peptide on intake

For 14 days the alcohol concentration was 3% w/v and was then increased to 6% w/v for the next 14 days For a further 14 days the alcohol concentration remained at 6%, but the group that was previously treated with 20 μg/kg AII now received 200 μg/kg AII The doses of the other two groups remained unchanged Following this, and in order to probe for any group differences in taste sensitivity, the drug injections continued and a 14% w/v glucose solution was substituted for the alcohol during the one hour drinking period This test was carried out for 4 consecutive days Finally, at the end of the experiment all rats were first injected with their respective doses of AII and then with an intraperitoneal dose of 2 5 g/kg alcohol (12 5%) Blood samples were taken from the cut tip of the tail at 20 min intervals during the first hour after the injection and thereafter at hourly intervals for the next 5 hours These blood samples were used to examine the effect of AII on the disposition and metabolism of alcohol

One way analyses of variance were used to analyze group differences in consumption of alcohol and water Post hoc multiple comparisons were done with the Duncan's test

RESULTS

3% Alcohol-Water Choice

Figure 1A shows the mean alcohol and water intake across the 14 days of choice between 3% alcohol and water Significant differences in the intake of alcohol, $F(2,32)=11\ 5$, $p<0\ 01$, and of water, $F(2,32)=4\ 44$, $p<0\ 02$, were found among the three groups of animals The group receiving 20 μg/kg AII did not differ significantly in its intake of either alcohol or water from the control group However, the group receiving 1 mg/kg AII showed a very significant reduction in alcohol intake along with a significant elevation in water intake compared to the control group

6% Alcohol-Water Choice

When the concentration of alcohol was increased to 6% (Fig 1B), significant group differences in the intake of alcohol, $F(2,32)=19\ 2$, $p<0\ 01$, persisted, with the 1 mg/kg group continuing to show a dramatic suppression in alcohol intake Again, the 20 μg/kg group did not show a change in intake compared to control

Group differences in water intake, on the other hand, were marginally significant, $F(2,32)=2\ 85$, $p<0\ 07$, largely because the enhanced water intake of the 1 mg/kg group seen was slightly lower than its level during the first 14 days of the experiment (i e , from approximately 3 ml to 2 ml)

During the final 14 days of the experiment, the group of animals that previously received 20 μg/kg AII, now began to receive a dose of 200 μg/kg AII The other two groups still received their respective agents and a choice between 6% alcohol and water continued to be offered Figure 1C shows that the 200 μg/kg dose significantly reduced the consumption of alcohol compared to control, while the 1 mg/kg dose continued to suppress alcohol drinking, $F(2,32)=41\ 99$, $p<0\ 01$ Group differences in water intake were also obtained, $F(2,32)=8\ 01$, $p<0\ 01$, with both the 200 μg/kg and 1 mg/kg groups showing an enhanced intake relative to control

Glucose-Water Choice

The control, 200 μg/kg and 1 mg/kg groups drank similar amounts of the glucose solution across the four day testing period (mean=44 4 ml/kg, 41 2 ml/kg and 35 3 ml/kg, respectively) and statistical analysis indicated no significant group differences, $F(2,32)=2\ 6$, n s Although mean water intake for these groups was minimal (1 2 ml/kg, 0 85 ml/kg and 3 9 ml/kg, respectively) the 1 mg/kg group did consume significantly more than the other two groups, $F(2,32)=4\ 0$, $p<0\ 01$

Drug Handling

Figure 2 shows the mean blood alcohol levels for the three groups at the eight sampling times following the 2 5 g/kg injection of alcohol The last four points on the descending portion of the curves were used to calculate the slopes which represent the rate of alcohol metabolism Statistical analysis showed a significant difference in metabolism among the three groups, $F(2,31)=3\ 29$, $p<0.05$, and post hoc tests indicated that the 1 mg/kg group had a faster rate than control This accelerated rate of metabolism, however, could not account for the reduced alcohol drinking in this group because an accelerated rate would be expected to permit a greater but not a lesser alcohol intake Extension of the linear portion of the curves back to the ordinate yields an estimate of the concentration at time zero from which the volume of distribution is calculated There were no significant group differ-

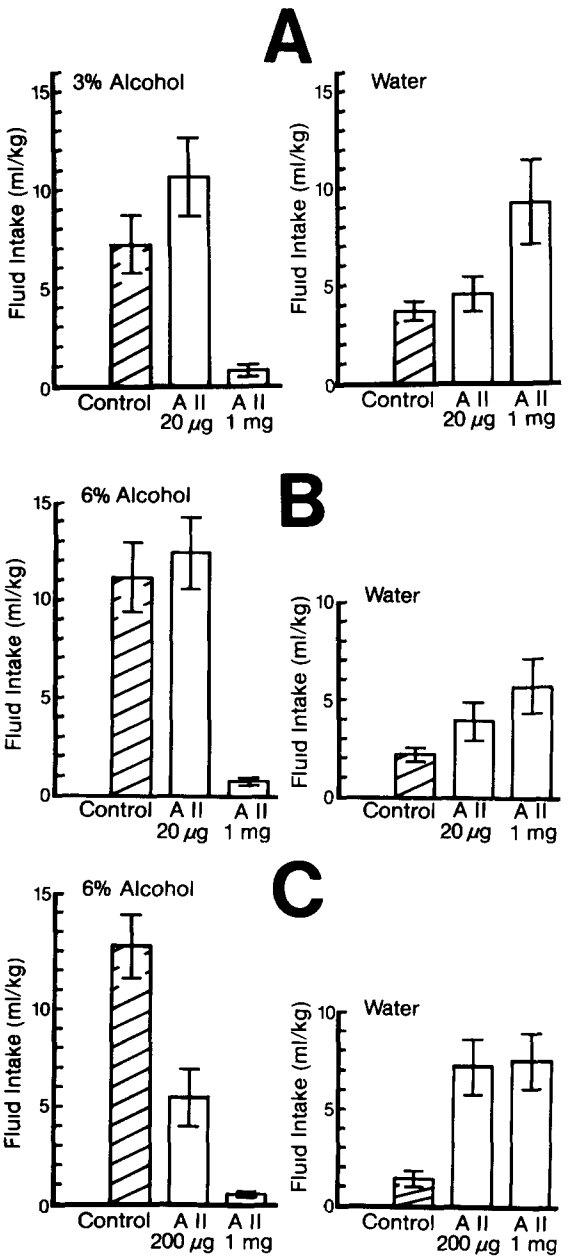

FIG 1 (A) Mean 3% (w/v) alcohol and water intake for the two angiotensin II groups and the control group across the first 14 days of the limited access procedure Bars represent ±standard error of the mean (B) Mean 6% (w/v) alcohol and water intake for the two angiotensin II groups and the control group across the next 14 days of the limited access procedure Bars represent ±standard error of the mean (C) Mean 6% (w/v) alcohol and water intake for the two angiotensin II groups and the control group across the next 14 days of the limited access procedure The group that received 20 μg/kg angiotensin II now received 200 μg/kg angiotensin II Bars represent ±standard error of the mean

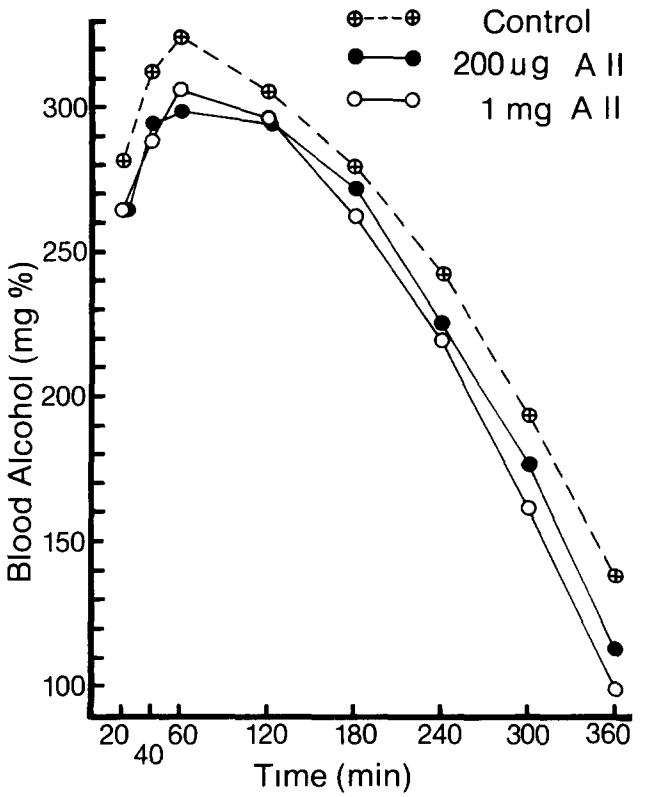

FIG 2 Mean blood alcohol levels (mg/decalitre) for the two angiotensin II and the control groups at various times after a 2 5 mg/kg IP injection of alcohol (12 5% w/v)

ences in volume of distribution, $F(2,31)=0.55$, n s Finally a two-way analysis of variance of the blood alcohol levels measured at the first three intervals following the alcohol injection did not show any group differences in absorption, $F(2,31)=0$ 44, n s , or group×interval interaction, $F(2,31)=$ 0 25, n s A significant effect of interval, $F(2,31)=13$ 28, $p<0$ 01, reflected the increasing blood alcohol levels during the absorption phase These findings suggest that AII did not alter alcohol intake through a change in drug disposition or metabolism

DISCUSSION

This experiment has shown that angiotensin II can significantly reduce voluntary alcohol drinking in a dose-dependent fashion. A 20 µg/kg dose was ineffective, a 200 µg/kg dose produced a 50% reduction in intake and a 1 mg/kg dose almost completely abolished all alcohol intake The effect of AII on alcohol intake lasted at least 1 hr because there was no obvious recovery within that period of time A time-response study would delineate the duration of action of the peptide on intake

At the same time that alcohol drinking was suppressed, water intake was elevated From casual observations the effect on water intake was over within the first 15–30 min following the injection Since angiotensin II is a dipsogen, this increase in water intake is a reflection of the peptide-induced increase in thirst. This thirst, however, appears to be linked specifically to water rather than a general increase in the motivation for both available fluids It is not merely that animals are "thirstier" for water and indifferent to alcohol Rather they are "thirstier" for water while at the same time avoid the alcohol. Furthermore, this suppression of alcohol drinking cannot be the result of a regulatory compensation secondary to the enhanced water intake because regulatory considerations are not relevant in a limited access procedure where animals are not weight reduced, have full access to food and water in their home cages and are in the drinking cage for only one hour every day Finally, the fact that angiotensin II stimulated consummatory behavior directed to water and did not change glucose intake rules out the possibility that the peptide-induced decrease in alcohol intake was related to a non-specific decrement in the animals' ability to approach or consume the available fluids Taken together these findings suggest that the effect of angiotensin II on alcohol intake is independent of its effects on water intake.

It is possible that angiotensin II reduced alcohol intake by inducing a general state of malaise or by altering taste function in such a way as to reduce palatability To examine this, all groups continued to receive their respective injections of either angiotensin II or vehicle and were run in the limited access procedure where they were offered a choice between a highly palatable glucose solution and water No significant group differences in glucose consumption were found suggesting that if the animals that received angiotensin II were feeling unwell, it was not reflected in the avidity with which they consumed the sweet solution. Furthermore, if angiotensin II reduced alcohol intake through a change in taste function, it would have to be a specific change in the acceptability of the taste of alcohol rather than a general effect on all taste sensations

While angiotensin II is a potent pressor agent, it is unlikely that these pressor effects per se mediated the reduction in alcohol drinking because previous work was shown that alcohol intake can be enhanced in certain types of hypertension For example, Grupp *et al* [4,5] have shown that Dahl salt-sensitive hypertensive rats. or Wistar rats rendered hypertensive by the chronic feeding of a high salt diet show an elevated and not a reduced alcohol intake

It is also unlikely that angiotensin II exerted its effect on alcohol drinking by altering the disposition or metabolism of alcohol An alcohol disappearance curve showed no group differences in the absorption or distribution of alcohol Furthermore, the group receiving 200 µg/kg angiotensin II did not differ significantly in its rate of alcohol metabolism from control The 1 mg/kg group had a significant accelerated rate of metabolism, however, even this cannot readily account for the ability of this dose to reduce alcohol drinking since an accelerated rate would, if anything, permit a greater not a lesser alcohol intake Taken together, these findings indicate that a change in drug handling cannot account for the effect of angiotensin II on alcohol intake

In the present experiment angiotensin II does not appear to alter alcohol intake because of motor impairment, general malaise, taste factors or pharmacokinetics, and appears to exert its effect independently of changes in water intake Angiotensin II is known to play a role in the control of fluid and electrolyte balance, blood pressure and thirst, but the suggestion that this peptide in particular or the renin-angiotensin system in general might also be involved in regulating alcohol intake is new and somewhat unusual relative to the biological functions normally associated with this peptide It may therefore be appropriate to outline, even if somewhat speculatively, how this peptide might be involved in voluntary alcohol intake Recently, it has been suggested

that angiotensin II may act as a satiety signal in controlling alcohol intake [2] This was based on two sets of findings First, it has been demonstrated in a number of different laboratories that alcohol ingestion can elevate angiotensin II levels [8, 10–12, 17] Second, manipulations which are known to elevate angiotensin II levels result in a reduction in voluntary alcohol drinking [2, 3, 6, 7] Putting these two sets of findings together led to the suggestion that the cessation of alcohol drinking might occur when circulating angiotensin II exceeds a "critical" level that signals satiety The following process is suggested Before alcohol drinking commences, the level of angiotensin II is below this "satiety" level Once drinking starts, and because alcohol itself elevates angiotensin II, the circulating level of angiotensin II would approach this satiety level Alcohol drinking would cease when enough alcohol has been consumed to cause the circulating levels of angiotensin II to exceed the satiety level

In the present experiment angiotensin II was administered by injection prior to alcohol drinking so that the circulating levels of angiotensin II would be elevated If this brought the animals closer to the satiety level prior to any alcohol intake, once alcohol drinking began, less could be consumed before the satiety level would have been surpassed Thus, angiotensin II injections may have reduced alcohol intake by bringing the animals closer to this satiety level beyond which no more alcohol would be consumed For the control animals not receiving angiotensin II, the satiety level would be reached only after sufficient and larger quantities of alcohol were consumed Such considerations await empirical validation by measurements of plasma angiotensin II levels during the 1 hr access period

This raises three other points First, that a role for angiotensin II as a satiety signal for stopping alcohol intake does not imply that angiotensin II is reducing alcohol intake by diminishing the positively reinforcing properties of alcohol Second, that this role for angiotensin II in alcohol drinking does not comment on or elucidate those factors that are responsible for the *initiation* of alcohol drinking Finally, the notion that angiotensin II does act as a satiety signal requires that the levels of this peptide for activating the satiety cut-off be established Future experiments will be directed towards determining this threshold

The effectiveness of angiotensin II (asp-1, val-5 angiotensin II Hypertensin, Ciba) in reducing alcohol intake raises the intriguing possibility that an analog or perhaps even a fragment of this peptide might be developed which can antagonize voluntary alcohol drinking without the attendant cardiac and thirst side-effects of Hypertensin In fact, fragments of angiotensin II are usually less potent than the parent peptide with respect to their effects on blood pressure (e g , [9]) and thirst (e g , [1]) Yet, if a fragment could be identified that still maintained biological activity vis a vis alcohol intake, such an agent could theoretically be very useful in the treatment of human alcohol abuse

In summary, we found that the subcutaneous administration of angiotensin II markedly reduces voluntary alcohol intake in a dose dependent manner This reduction does not appear to be related to an angiotensin-induced state of malaise, general motor deficit, change in taste function or change in drug handling and is independent of changes in water intake These results provide new and direct evidence for the involvement of the renin-angiotensin system in the control of alcohol drinking

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