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Angiotensin and Captopril Increase Alcohol Intake

DOUGLAS A. FITTS

Department of Psychology, NI-25, University of Washington, Seattle, WA 98195

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FITTS, D. A. *Angiotensin and captopril increase alcohol intake.* PHARMACOL BIOCHEM BEHAV **45**(1) 35–43, 1993.—Reportedly both angiotensin II (ANG II) and angiotensin-converting enzyme (ACE) inhibitors reduce ethanol intake when they are injected SC into certain chronic experimental conditions in the rat. The ACE inhibitors are suggested to reduce ethanol intake by increasing ANG II synthesis in the brain. The present results show that several different methods can produce opposite effects of ANG II and the ACE inhibitor captopril on ethanol intake. Continuous intraventricular infusions of ANG II for 7 days or low doses of oral or SC-infused captopril for up to 12 days increased the intake of ethanol. The only reduction of ethanol intake resulted from a universal blockade of all ACE in both the brain and periphery, a condition in which ANG II could not possibly mediate the decrease. The results contradict the hypothesis that ethanol intake is suppressed by centrally acting or centrally synthesized ANG II. ACE inhibitors may reduce ethanol intake only when they affect the brain as well as the periphery.

Angiotensin-converting enzyme inhibitors Ethanol intake Thirst

PERIPHERALLY administered angiotensin II (ANG II) octapeptide has recently been reported to reduce intake of dilute ethanol solutions in rats [(10); reviewed in (18)]. Other drugs or treatments that increase ANG II synthesis peripherally, such as isoproterenol, fluoxetine, prostaglandin E2, histamine, sodium depletion, renal arterial obstruction, and certain strains of rats that have high rates of renin secretion have all been associated with a reduction of alcohol intake under certain experimental conditions (10–15,20–23,33). This effect of ANG II was reportedly eliminated by a lesion of a brain circumventricular organ, the subfornical organ [SFO; (17)], and by prior treatment with [Sar^1, Thr^8]-ANG II, a specific competitive inhibitor of ANG II (16). By contrast, lesions of another circumventricular organ, the area postrema, and injections of vasopressin or cholinergic agents did not affect ethanol intake (8,19,34).

The same laboratory reported that the angiotensin-converting enzyme (ACE) inhibitors captopril, enalapril, and abutapril also reduced ethanol intake under some circumstances (27,39). ACE inhibitors were postulated to reduce ethanol intake by indirectly increasing the synthesis of ANG II in the brain while synthesis in the periphery remained blocked (27). Therefore, the effects of both peripheral ANG II and peripheral ACE inhibitors were suggested to create their effects by activating ANG II receptors in the brain (18). This hypothesis, based upon rat work, has recently been tested in humans as a therapy for both alcoholism and associated hypertension (30), although the results of that study were negative. Unfortunately, the critical control experiment for the effects of ACE inhibitors on ethanol intake in rats, that is, injecting or infusing a high enough dose of the drug to block ACE in both the brain and periphery, has not explicitly been attempted. This failure is demonstrated by the fact that published experiments with ACE inhibitors showed increased water intake concurrent with reduced ethanol intake. The excess water ingestion in these conditions is completely dependent upon ANG II synthesized by unblocked ACE in the SFO (5,41). When the ACE in the brain as well as in the periphery becomes completely blocked by large doses of ACE inhibitors, water intake returns to normal (2,3). This unusual dose-response relationship relies upon a much higher concentration of ACE in the SFO than in the lung, kidneys, and other peripheral tissues that generate ANG II in the circulation [see (41) for review]. Low peripheral doses of captopril that block these peripheral beds of ACE do not completely block the SFO, which is open to the blood-brain barrier and free to convert ANG I to ANG II and generate drinking. Higher peripheral doses of captopril block both the peripheral ACE and the ACE in the SFO, thus eliminating the excess water intake. By this same logic, the "brain-ANG II-reduces-ethanol-intake" hypothesis must require that if all of the ANG II synthesis in the periphery and brain are simultaneously blocked then ethanol intake must not decrease for the same reason that water intake must not increase.

The present study tested the hypothesis that brain ANG II receptors mediate reduced ethanol intake. This was accomplished by chronically infusing ANG II or administering low doses of captopril either orally or by SC infusion to produce a sustained activation of ANG II receptors in the brain. The chronic captopril infusion experiment included a group that was blocked both in the periphery and brain to test whether the effects of ACE inhibitors are specific to conditions when ANG II is being synthesized in the brain.

GENERAL METHOD

SUBJECTS

Male Long-Evans rats weighing 300–500 g were used. They were housed in hanging wire mesh cages with Wayne laboratory chow and tapwater continuously available. The lights were on 12 h per day. Temperature in the room was constant at 23–25°C. Sample sizes are given in the individual experiments.

FLUID INTAKES

Water and 6–8% (v/v) ethanol intakes were measured using 100-ml graduated cylinders fitted with drinking spouts. Intakes of the fluids were recorded to the nearest milliliter. The positions of the water and ethanol tubes were alternated daily to control for position preferences.

These concentrations of alcohol were equivalent to 4.68–6.24% (w/v). Other experimenters using mainly Wistar and Sprague-Dawley rats typically use higher concentrations of ethanol, but the experience of this laboratory is that Long-Evans rats have lower preferences for ethanol and sodium than white rats. A lower responsiveness of the Long-Evans strain to a variety of thirst-related stimuli has been documented (7).

SURGERY

Stereotaxic surgery was conducted under Equithesin anesthesia (0.35 ml/100 g, IP), with gentamicin (0.2 ml, IM) and topically applied betadine to control postsurgical infection. One 23-ga stainless steel cannula was implanted into the left lateral ventricle at coordinates AP −0.6, L +1.4, and DV −4.0 relative to bregma and a flat skull. The cannula was affixed to the skull with stainless steel screws and methyl methacrylate cement. In the ANG II infusion experiment, the cannula was connected by a 5-cm length of PE-90 tubing to an Alzet 2001 miniosmotic pump calibrated to deliver 1.0 μl/h for 7 days. In the captopril infusion experiment, rats were recovered for 1 week after implantation of the 23-ga guide cannula, and a 31-ga injector cannula was then inserted under brief halothane anesthesia. This cannula was connected by concentrically fitted PE-10, PE-50, and PE-100 tubing to an Alzet 2002 miniosmotic pump calibrated to deliver 0.5 μl/h for 14 days. In the same surgery, rats also received an SC-implanted Alzet 2ML2 miniosmotic pump delivering 5.0 μl/h for 14 days.

DRUGS

Angiotensin was purchased from Bachem and was mixed in sterile isotonic saline immediately before being injected into the reservoirs of the minipumps. Captopril was a gift from S. J. Lucania of the Bristol-Myers Squibb Pharmaceutical Research Institute and was mixed fresh daily. For infusions, each was mixed in sterile isotonic saline immediately before being injected into the reservoirs of the minipumps. Ethanol solutions were mixed fresh daily using 95% USP ethanol in tapwater.

Oral captopril treatments were delivered into the water at 0.1 mg/ml. Thus, the daily dose of the captopril was derived from the intake of water on the treatment days. This method of dosing rats with captopril produces reliable thirst and salt appetite, the intakes at this dose are not affected by the taste of the captopril (40), and captopril in either low or high doses delivered either peripherally or centrally does not alter food intake (29,42).

Experimental Design and Statistical Analysis

Data were analyzed using analysis of variance (ANOVA). Planned comparisons used Fisher's least significant difference test if the *F*-ratio was significant and the Bonferroni correction if it was not. A probability of less than 0.05 was required for significance.

EXPERIMENT 1

The purpose of this experiment was to examine the effect of intraventricular infusions of ANG II on alcohol intake.

METHOD

Rats received lateral ventricular cannulae connected to osmotic minipumps delivering either the saline vehicle or 5 or 20 pmol/h ANG II for 7 days in different groups to test the effects of ANG II infusions in the brain on alcohol intake. Rats that did not significantly increase water intake during the infusion of ANG II were excluded from the analysis, resulting in sample sizes of 6, 4, and 4 in the sham, 5-, and 20-pmol/h groups, respectively.

Before surgery, rats were given 3 days of acclimation to drinking 6% (v/v) alcohol, followed by 5 days of measured baseline. After surgery, intake measurements of both water and 6% (v/v) ethanol continued for the 7 days of infusion and an additional 5 days of recovery after the minipumps were empty.

RESULTS

The water and alcohol intake data for the 17 days of the experiment in the three infusion groups are presented in Fig. 1. Water and alcohol intakes were similar in the three groups before surgery, and both fluid intakes were depressed on the day immediately following anesthesia and surgery. The intakes quickly recovered to normal in the control group. The water intake of the 5-pmol/h group rose to approximately double the group's baseline intake, but alcohol intake was not consistently affected. The water intake of the 20-pmol/h group quadrupled compared with the group's baseline intake, and alcohol intake also increased significantly on 4 of the 7 days of infusion, with the average intake on these days being at least double the baseline intake, interaction $F(34, 187) = 4.27, p < 0.001$.

After the last day of infusion, all groups, including the control group, gradually increased alcohol intake to the level achieved by the 20-pmol/h group during the infusion. The 20-pmol/h group first reduced its alcohol intake at the end of the infusion to a level equal both to its own baseline and to the intake of the control group before rising again at the end of the recovery period. This indicates that the elevated alcohol intakes during the infusion resulted from an activation of central ANG II receptors and not from a simple acclimation to alcohol with time.

EXPERIMENT 2

This experiment examined the effects of oral captopril administration on alcohol intake in rats.

METHOD

Eighteen rats were used in the experiments. They had access to 4% (v/v) alcohol in choice with water for 12 days

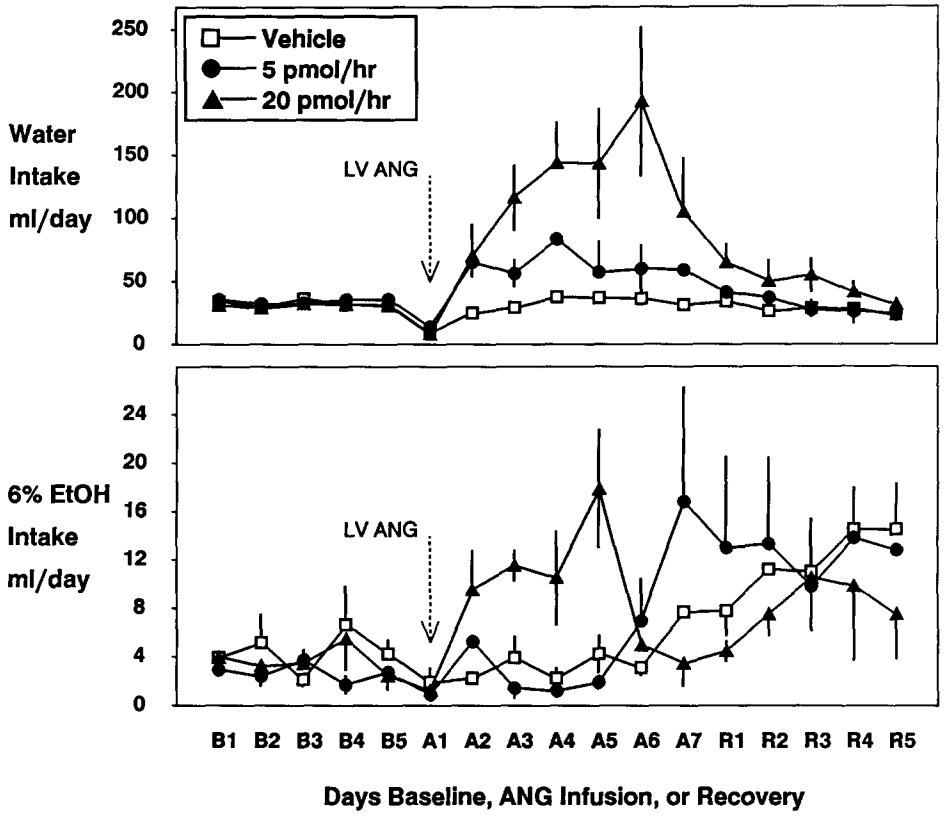

FIG. 1. Daily water and alcohol intakes during lateral ventricular infusions of ANG II. B, presurgical baseline days; A, postsurgical angiotensin infusion days; R, recovery days. Water intake was elevated at both doses of ANG II. Alcohol intake was significantly elevated on days A2–A5 in the 20-pmol/h group. Mean and SE.

before any captopril was given. They then received captopril in the water for 4 days as a pilot experiment. Fifteen of 18 rats increased alcohol intake during the pilot. They received two additional days of alcohol at 4% without captopril, during which alcohol intake did not decline.

Immediately following the recovery phase of the pilot test, the concentration of alcohol was increased to 8% (v/v) for 6 days. Rats were randomly divided into two groups of nine rats each with captopril administered at different times in the two groups so that the effects of captopril could not be attributed simply to an acclimation to alcohol with time. On the following 6 days, nine rats had captopril in the water as above while the other nine were untreated. On the next 6 days the treatments were reversed, with the formerly captopril-treated rats receiving no captopril and the formerly untreated rats receiving captopril. All rats were then monitored for an additional 6 days of baseline intakes.

RESULTS

The data were analyzed using a mixed-model design with repeated measures comparing the means of the 6-day baseline, captopril, and recovery phases and the groups representing the different orders. The results for the two orders (captopril earlier in the sequence or later in the sequence) are shown in Fig. 2. Analysis revealed significant effects of captopril on both water, $F(2, 34) = 30.05$, $p < 0.001$, and alcohol, $F(2, 34) = 6.92$, $p < 0.01$, intakes. The water and alcohol intakes were both significantly higher during the captopril treatments than during either the baseline or recovery phases. The overall intakes of water and alcohol were not significantly different between the two orders, although the main effect for water was marginal: for water, $F(1, 16) = 3.71$, $0.05 < p < 0.10$; for alcohol, $F(1, 16) = 0.73$, NS. Thus, the increase of alcohol intake must have resulted from the captopril itself rather than from an acclimation to alcohol with time.

EXPERIMENT 3

The purpose of this experiment was to examine the effects of sustained infusions of captopril either peripherally alone or peripherally in combination with another ACE-blocking infusion into the lateral ventricles.

METHOD

Twenty-one rats were used in the experiment. They received continuous access to 6% (v/v) alcohol for 4 weeks prior to stereotaxic implantation of the guide cannulae in the lateral ventricles and another week of access during the week of recovery from surgery. During recovery, both the body weights and fluid intakes recovered to presurgical levels. All rats were then given SC infusions of saline vehicle or 0.25 mg/h captopril by implanted minipumps under brief halothane anesthesia. Some rats simultaneously had lateral ventricular injectors inserted into their guide cannulae pumping 25 μg/h of capto-

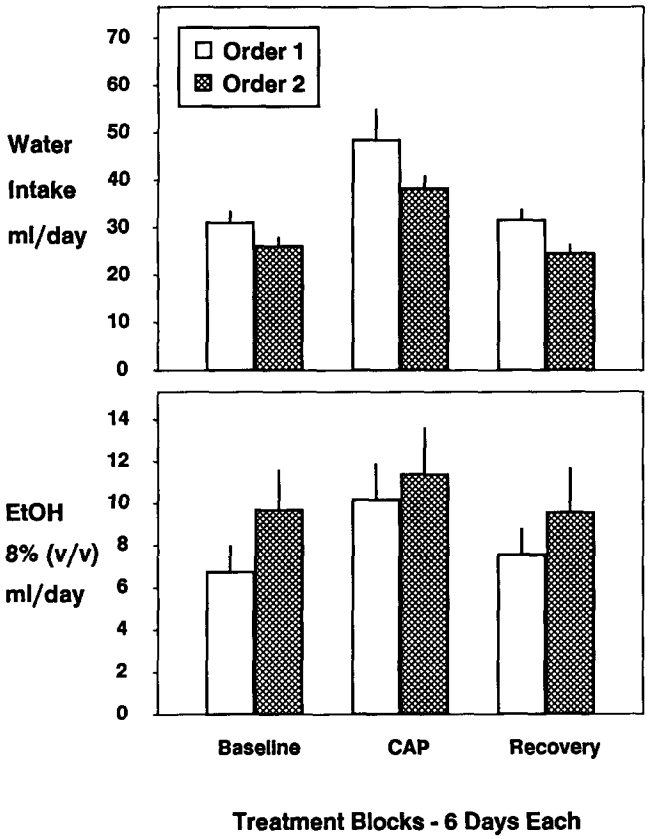

FIG. 2. Daily water and alcohol intakes during 6-day blocks of baseline (tapwater), captopril (CAP, in the water at 0.1 mg/ml), and recovery (tapwater) phases. Order 1 rats received captopril 6 days earlier in the sequence than order 2 to control for acclimation to ethanol with time. Both groups drank more water and alcohol during the captopril phase than during either of the tapwater phases. Mean and SE.

pril or saline vehicle. Of the eight rats in the vehicle control group, four randomly selected rats received injectors infusing saline vehicle and the other four received no injector at all. Thus, the control group was also a control for the effects of having an injector or not. The five rats in the peripheral captopril infusion group did not have lateral ventricular injectors although they did have guide cannulae implanted. The eight rats in the combined peripheral and central blockade group, referred to as the universal captopril group, all received injectors to deliver the captopril.

The intakes of water and alcohol were recorded for the 12 days of infusions. After recording the data for the 12th day, all rats were weighed and then rapidly anesthetized with halothane for the withdrawal of 5.0 ml blood by cardiac puncture using heparinized needles. Hematocrit was determined by the microhematocrit method; 100 μl blood was pipetted into 0.9 ml dilute trichloroacetic acid to stop enzymatic activity and the mixture was frozen for later blood ethanol assay by the enzymatic method. The blood was centrifuged at 5,000 rpm for 5 min to extract plasma, and the plasma osmolality and protein were determined immediately by freezing point depression and refractometry. The residual plasma was frozen for later assay of sodium concentration by flame photometry.

RESULTS

Behavioral Results

The behavioral data are shown in Fig. 3. The intakes of the four vehicle controls receiving lateral ventricular saline infusions and the other four not receiving any infusions were statistically similar, so the two groups have been combined in the remaining analysis. This indicates that any differences between the universal captopril rats receiving infusions and the peripheral captopril rats not receiving infusions was a function of the central captopril and not a function of merely having an infusion.

The analysis included fluids and days as within-subjects factors and groups as a between-subjects factor in a mixed-model ANOVA. A baseline measurement shown as day B in Fig. 3 represents the mean of the last 2 days of stable baseline intakes after the recovery of the fluid intakes from the stereotaxic surgeries. The triple interaction was significant, $F(24, 216) = 1.81, p < 0.01$, indicating that the treatments caused different effects on water and alcohol intakes on different days. This effect is most easily described as a large increase from baseline in the intakes of both water and alcohol in the peripheral infusion group and not in the other groups, with the increases in this group occurring immediately for water intake and on a delayed basis for alcohol intake.

Within-groups comparisons showed that infusion day 2 was the only day that was not significantly different from the peripheral captopril group's water intake baseline, and infusion days 1, 2, 5, 7, and 9 were not significant for alcohol intake. Among the other groups, the only group showing a significant difference in water intake from its baseline was the decrease in the universal captopril group on day 4. Alcohol intakes did not change significantly in the within-groups analysis for either the control or universal groups.

Between-groups comparisons showed that all groups were equal in both water and alcohol intakes during baseline. The peripheral captopril group drank significantly more water than both other groups on all 12 days of infusion; the group drank significantly more alcohol than both other groups on all infusion days except 1 and 2, when it was not different from either group, and days 9, 10, and 12, when it was significantly higher than the universal captopril group but not the vehicle control group. The lack of significance on these days near the end of the experiment appeared to be due more to a rise in the intake of the control group rather than to any reduction in the intake of the peripheral group.

This overall analysis was conducted despite the obvious heterogeneity of the variances because there is no other way to provide even a rough significance test of these complex interaction effects. However, the inflated error mean square for the interaction term that resulted from the heterogeneity may have obscured real effects occurring within and between the alcohol intakes of the groups with small variances. That is, the universal captopril group may have decreased alcohol intake relative to the vehicle control group without being detected statistically among the noisy variability produced by the peripheral captopril group. This interesting a priori hypothesis based upon the predictions of other investigators (18) was not given a fair test in the original analysis. Nevertheless, there were hints that the groups might differ: a) The universal captopril group was the only group to show a significant decrease in intake from its baseline alcohol intake on any day; and b) the vehicle control group elevated its intake on 3 of the last 4 days of the test such that it was no longer significantly different from the peripheral captopril group, whereas the universal

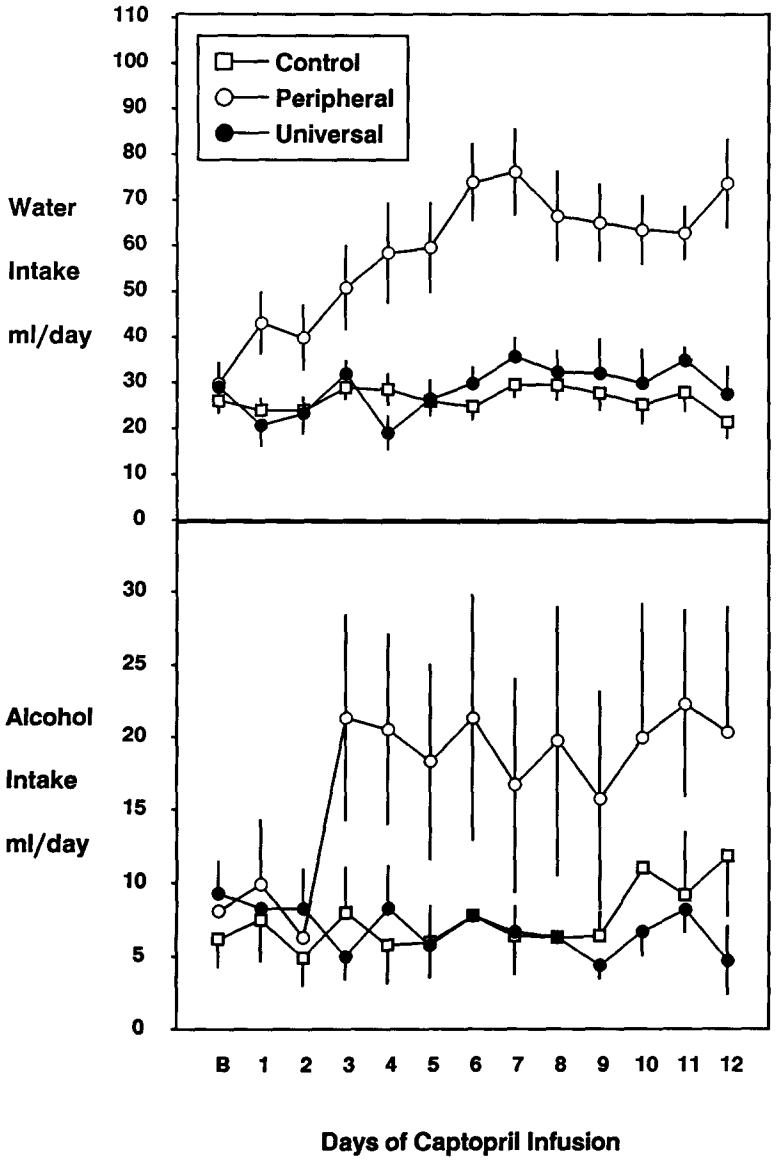

FIG. 3. Daily water and alcohol intakes during peripheral and intraventricular captopril (CAP) infusions with SC-implanted minipumps. B, mean of 2 days of presurgical baseline; control, no captopril; peripheral, 0.25 mg/h CAP SC; universal, 0.25 mg/h CAP SC and 25 μg/h CAP in lateral ventricle. Peripheral CAP enhanced water and alcohol intake. Universal CAP eliminated the excess water intake and gradually reduced alcohol intake relative to control. Mean and SE.

captopril group remained significantly lower than the peripheral captopril group throughout the experiment.

For this reason, the alcohol data were reanalyzed with only the control and universal groups in the analysis. A complex contrast tested the interaction effect between the baseline and last days of the experiment, and simple contrasts tested the individual group differences on these days. A Bonferroni test of the interaction was significant, $t(168) = 2.58$, $p < 0.05$, suggesting that the trends in alcohol intake from the baseline condition to the last day were opposite in the two groups. The intakes were not different on the baseline day but did differ significantly by the end of the experiment, with the universal group drinking less alcohol, $t(168) = 2.54$, $p < 0.05$. Trend analysis showed a correlation of days with mean alcohol intakes of $r(10) = +0.65$ in the control group and $r(10) = -0.54$ in the universal group. The difference between these correlations was significant (Fisher's $Z = 3.08$, $p < 0.01$).

Blood and Plasma Results

Blood and plasma data collected at the end of the experiment are shown in Table 1. A one-way ANOVA revealed no significant differences in body weight, hematocrit, or plasma protein concentrations in the three groups. Effects were signif-

TABLE 1

BLOOD AND PLASMA VALUES AT THE END OF 12 DAYS OF CHRONIC SC (0.25 mg/h) AND ICV (25 μg/h) MINIPUMP INFUSIONS WITH OR WITHOUT CAP

	Type of Infusion		
SC	Saline	CAP	CAP
ICV	Vehicle/None	None	CAP
n	4/4	5	8
Body weight	463.3	460.6	450.9
(g)	14.9*	22.4	14.7
Hct	43.8	43.7	44.1
(%)	0.9	0.7	1.0
PP	5.9	5.7	5.9
(g/dl)	0.1	0.1	0.1
P_{Osm}	306.3	296.6†	309.1
(mOsm/kg)	1.1	2.0	4.0
P_{Na}	133.8	130.2†	134.4
(mmol/l)	0.7	1.3	0.7
BEC	0.0	6.4†	0.6
(mg/dl)	0.0	2.8	0.6
(freq.)	0/8	3/5	1/8

Hct, hematocrit; PP, plasma protein; P_{Osm}, plasma osmolality; P_{Na}, plasma sodium; BEC, blood ethanol concentration.

*Mean and SE.

†$p < 0.05$ vs. saline control.

icant for plasma osmolality, $F(2, 17) = 4.98$, $p < 0.05$, and sodium concentrations, $F(2, 17) = 6.18$, $p < 0.01$, with the peripheral captopril group showing significant dilution because of their high daily intakes of water and alcohol. Similarly, the peripheral captopril group showed significantly elevated blood ethanol concentrations compared with the other two groups, which combined had only 1 of 16 rats with a detectable quantity of ethanol in the blood, Kruskal-Wallis test, $\chi^2(2) = 7.53$, $p < 0.05$. Although the amounts of ethanol found in the blood of the peripheral group were small at the time of sampling, it confirms that the group, contrary to the hypothesis that captopril reduces alcohol intake, actually consumed more alcohol than the other groups. In addition, the blood data and behavioral data combined suggest that the universally blocked captopril group was not demonstrably different from the control group in any hydration-related variable measured except for alcohol intake.

GENERAL DISCUSSION

Circulating ANG II normally regulates extracellular sodium and water content, blood pressure, and thirst during dehydration. Exogenous ANG II reliably produces drinking in many species after either peripheral or central administration, and the neural elements of the brain, including circumventricular organs, that are responsible for this thirst are reasonably well established (37). Centrally applied ANG II causes an appetite for sodium solutions as well as thirst, and ANG II synthesis and receptors in the brain control the behavioral expression of salt appetite (36,42). Experiment 1 of this study shows that alcohol intake can be modestly increased by high doses of central ANG II infusions.

ACE inhibitors such as captopril and enalapril block the conversion of inactive ANG I to the bioactive form ANG II and prevent many of the peripheral effects of ANG II during dehydration. However, low doses of captopril that completely block all ACE activity in the lung enhance rather than suppress thirst during dehydration (1,2,24). Experiment 2 of this study shows that low-dose, chronic oral captopril treatments can enhance alcohol intake as well as water and saline intakes. Chronic SC infusions of captopril in Experiment 3 enhanced alcohol intake, and a concurrent central blockade of ACE abolished this effect. Thus, the enhanced alcohol intake relies upon a central conversion of ANG I to ANG II. In addition, alcohol intake in the universally blocked group declined compared with the control group, the only circumstance in the present series of experiments in which captopril-treated rats decreased alcohol intake.

The conclusion of the present experiments is that the principal effect of ANG II and captopril on alcohol intake is to enhance it. This conclusion contradicts numerous studies using different methods that found that ANG II or ACE inhibitors either directly or indirectly reduced alcohol intake (18). This contradiction will be discussed according to three major categories of experiments offered to support the hypothesis that ANG II reduces ethanol intake: a) the limited-access procedure; b) indirect evidence; and c) studies with ACE inhibitors.

"LIMITED-ACCESS" EXPERIMENTS

Many experiments supporting a role for ANG II in reducing alcohol intake used a procedure called the limited-access drinking test. In this procedure, rats were given alcohol and water for 1 h per night in a special drinking cage for several weeks, after which they reliably consumed considerable amounts of alcohol. Drugs were then injected SC before rats were placed into the drinking cages, and the effects of the drugs on alcohol intake were observed. Water was always available as an alternative; however, rats had water constantly available in their home cages so they drank little of it during the test.

Reductions in alcohol drinking have been found in the limited-access procedure after peripheral injections of different analogs of ANG II and ANG III, isoproterenol, histamine, and fluoxetine (15,18,21,35). Each of these either directly activates ANG II receptors or directly or indirectly causes renin secretion. Various procedures have been employed to interrupt the ANG II-induced effect on alcohol intake, such as prior administration of an ANG-receptor blocker (16), lesions of the presumed site of action, the SFO (17), and ACE blockade during fluoxetine administration (15).

A major correlate of virtually all of the experiments showing reductions of ethanol intake is an increase of water intake. The experimenters have argued that these effects of ANG II do not result from a simple competition of behaviors between water and alcohol drinking (16–18), but the hard evidence for this is weak. For instance, at some doses on particular dose-response curves generated from the limited access paradigm there is an apparent dissociation between the increased water drinking and reduced alcohol drinking responses. In their isoproterenol study (21), the lowest dose (2.5 μg/kg) reduced alcohol intake without significantly increasing water intake, and in a study of different ANG analogs (35) one dose of ANG III (200 μg/kg) increased water intake without signifi-

cantly decreasing alcohol intake. In both cases, the means of the nonsignificantly changed fluids were in the correct direction (higher water intake for isoproterenol and lower alcohol intake for ANG III) but the tests may have lacked the power to detect significance, so this is not a compelling argument. In addition, the correct control group was omitted for the isoproterenol study: The water intakes of rats drinking a reduced amount of alcohol after isoproterenol should have been compared with the intakes of normal rats pair fed the same reduced amount of alcohol to test the true thirst responses to the drug.

All procedures that inactivated the renin angiotensin system to demonstrate the specificity of reduced alcohol intake to ANG II also eliminated the water drinking correlated with the effect. For instance, SFO lesions or ANG II receptor blockers injected prior to ANG II (16,17) increased alcohol intake to normal, but they also removed the ANG II stimulus for thirst and promoted the rat's normal behavior in the drinking cage. For this reason, these experiments cannot establish definitively whether the effect of ANG II on alcohol intake is specific or merely secondary to its well-known effects on water intake.

The SFO lesion experiment (17) failed to provide evidence that any of the rats had complete SFO lesions. The putative SFO-lesioned and sham-lesioned rats had nearly equal proportions of responders and nonresponders after a dipsogenic screening dose of ANG II. It is so common to have several nonresponders among a sham-lesioned group that some studies of ANG II-induced thirst use a prelesion screening trial with ANG II to eliminate them (25,26). However, correctly identified SFO lesions always abolish drinking to angiotensin (38). Further subdividing this lesioned group into "drinkers" and "nondrinkers" says no more about the neurological status of the group than it does about the sham group, which produced identical results for its drinkers and nondrinkers. For this reason, the experiment must not be considered as demonstrating anything about the function of the SFO.

Another problem of interpretation with the limited-access procedure arises from the chronic nature of the protocol. The effects of ANG II, isoproterenol, ACE inhibitors, etc. to reduce alcohol intake do not occur immediately. The treatments must be repeated daily for as long as 2 weeks to achieve significance. This repetition is indistinguishable from a learning trial. The present experiments suggest that the first tendency of ANG II-treated rats would be to increase both alcohol and water intake. Increased alcohol intake relative to the already substantial drinking during these bouts would produce pharmacological consequences that rats ordinarily avoid. The result would be a learned shift to a water preference during the drug state. Any relief from the drug state, such as a control trial or a blocking trial with a receptor antagonist, would allow the rat to revert to its normal drinking pattern. This analysis applies equally to rats given only alcohol rather than alcohol in choice with water at some point during the procedure (23).

Such state conditioning does not occur during the sustained chronic treatments of the present study because rats are not limited to drinking the alcohol in a bout fashion that produces high blood ethanol levels (see Table 1).

INDIRECT EVIDENCE

A second major category of experiments intended to support the hypothesis that ANG II suppresses alcohol intake consists of a number of indirect methods of manipulating the renin–angiotensin system. Examples of these include provision of a low-sodium diet combined with multiple injections of large doses of the diuretic furosemide (14,22), salt loading of the chow diet (13), interference with the renal arterial circulation (11,12), and selection of certain strains of rats with high or low activity of the renin–angiotensin system (9,20,31).

The manipulations of sodium are flawed because of the failure to measure food intake. Both intracellular and extracellular dehydration cause anorexia (4,32), and the low-sodium diet and multiple injections of high (60 mg/kg) doses of furosemide probably reduced calorie intake from chow as well as from alcohol. Liver damage from the large quantity of furosemide should also be considered.

In the complementary experiments, increasing the salt concentration in the chow to reduce renin secretion made the chow highly unpalatable and secondarily increased the intake of alcohol. Finally, the results of the experiments on circulatory interference or with different strains of rats are difficult to interpret in the absence of any definitive evidence that chronically elevated ANG II inhibits alcohol intake, and all experiments of this study cast doubts upon that assumption. Linkola et al. (28), for instance, attributed strain differences in plasma renin activity and alcohol intake in alcohol-preferring and -avoiding rats (Alko) to the salt preference and osmotic influences on calorie intake: Rats that disliked salt could be more likely to choose an alternative, salt-free calorie source such as alcohol, and vice versa. Thus, a genetic selection for alcohol preference was actually a selection for salt preference and the underlying hormonal substrates. This shows that alternative explanations are possible both for the salt-diet manipulations and for the rat strain observations.

ACE INHIBITORS

There is a large and convincing amount of support for the paradoxical assertion that high doses of converting enzyme inhibitors, like ANG II itself, reduce alcohol intake (23,27,39). Peripheral blockade of ACE with a low dose of inhibitor increases the synthesis of ANG II in circumventricular organs of the brain and provokes thirst and salt appetite (5,6,41). Hypothetically, this increased synthesis of ANG II in the brain also reduces alcohol intake. Some of these supportive data were collected using the limited-access procedure or other short-term drinking procedures and so may be limited to that procedure. However, one experiment used twice-daily injections of 50–200 mg/kg captopril and found a small reduction of daily ethanol intake along with an increase in water intake (39).

An exception to the foregoing model consisted of injections of the ACE inhibitor enalapril in the limited-access procedure (39). Enalapril in the dose used, 2 mg/kg, reportedly would not stimulate plasma renin activity (PRA), did not generate water intake, but did inhibit alcohol intake. This experiment was used as evidence that rats can reduce ethanol intake in the absence of increased water intake, an important point. However, the experiment raises a question about the validity of the investigators' own hypothesis about the central ANG II mediation of the effects of ACE inhibitors: If the inhibitor did not increase PRA, and consequently did not increase water intake that is dependent upon enhanced PRA and central ANG II synthesis, then how could ANG II have mediated this response? The proposed model (18) cannot account for this behavior.

Experiments 2 and 3 of this study show that different methods of administering the ACE inhibitors produce a radically

opposite result, increased alcohol intake, and show that this increase is dependent upon centrally generated ANG II. That is, the same model of paradoxical ANG II synthesis previously proposed to inhibit alcohol intake has now instead been demonstrated to increase alcohol intake. The only condition in the present study that reduced alcohol intake was a universal blockade of all ACE in the body, including in the brain. This finding is consistent with the enalapril experiment (39) in that an ACE inhibitor decreased ethanol intake without any apparent involvement of ANG II. Similarly, the captopril-injection experiment (39) showing decreased ethanol intake after SC captopril used doses of captopril that were all sufficient to completely block ANG II synthesis in the circumventricular organs as well as in the periphery for a period of a few hours (3). These high doses may also have reduced ethanol intake through a nonangiotensin-related effect of the drug on the brain in the same way that the universal captopril blockade in Experiment 3 reduced ethanol intake.

Thus, a common feature of those ACE-blocking experiments that did not use a limited-access procedure and did show a reduced ethanol intake is that they included a complete blockade of brain ACE for at least part of the duration of the experiment. The captopril experiment of Spinosa et al. (39) must not have sustained this universal blockade, however, because water intake was increased.

By contrast, the peripheral captopril-treated rats in Experiments 2 and 3 of this study received low doses of captopril that blocked the periphery without ever blocking the brain, and both of these experiments revealed increases in alcohol intake. The difference between protocols that increase or decrease alcohol intake may therefore depend upon the degree to which they affect the CNS rather than the periphery, and this effect therefore will not depend upon ANG II synthesis. This could explain why human alcoholics treated with enalapril failed to show reduced ethanol intake: Subjects could not tolerate doses of the drug that were high enough to affect the CNS (29).

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