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BRIEF COMMUNICATION

Systemic Angiotensin II Acts at the Subfornical Organ to Suppress Voluntary Alcohol Consumption

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GRUPP, L A , E PERLANSKI AND R B STEWART *Systemic angiotensin II acts at the subfornical organ to suppress voluntary alcohol consumption* PHARMACOL BIOCHEM BEHAV **34**(1) 201–205, 1989 —The subfornical organ plays a role in a number of the effects of blood-borne angiotensin II (ANG II) including the increase in water drinking and blood pressure and the release of vasopressin from the pituitary Recently it has been shown that systemically administered ANG II also reduces voluntary alcohol intake The present study assessed the role of the SFO in alcohol consumption by examining the effects of SFO lesions on voluntary alcohol intake and on the suppression of voluntary alcohol intake by ANG II Whereas the lesion did not alter alcohol consumption per se, it did significantly attenuate the ability of ANG II to reduce alcohol intake This effect was not due to a lesion-induced change in the pharmacokinetics of alcohol and was observed only in those animals whose lesions produced a functional deficit, i e , abolishing the increase in water drinking produced by ANG II These results indicate that the SFO mediates the effect of systemically administered ANG II on alcohol intake but does not otherwise affect the regulation of alcohol consumption

Alcohol intake Renin-angiotensin system Alcohol Ethanol Subfornical organ Circumventricular organ Angiotensin II

VOLUNTARY alcohol intake appears to be inversely related to activity in the renin-angiotensin system (3,19). Among the findings supporting this conclusion is the demonstration that systemic administration of the peptide angiotensin II (ANG II) produces a dose-dependent decrease in alcohol consumption (4,5) which can be blocked by an angiotensin antagonist such as Sar-1 Thr-8 angiotensin II (5) This effect is not related to an increase in blood pressure per se produced by ANG II since alcohol intake in hypertensive rats can be elevated (7), suppressed (6) or unchanged (6) depending on whether the hypertension is associated with a decrease, an increase, or no change in the activity of the renin-angiotensin system ANG II does not pass the blood-brain barrier (2), and therefore is likely to act at circumventricular sites such as the area postrema (AP), the nucleus of the solitary tract (NTS) or the subfornical organ (SFO) where ANG II-stained cell bodies and specific ANG II binding have been noted [e.g., (8,20)] Since the SFO has been identified as one of the most sensitive sites mediating the various actions of blood-borne ANG II (12, 13, 15–17), the present study assessed the role of the SFO in the regulation of alcohol intake by examining the general effect of SFO lesions on voluntary alcohol intake and the effect of these lesions on the ability of ANG II to suppress alcohol intake.

METHOD

Subjects

The subjects were 55 naive male Wistar rats weighing 300–430 g at the beginning of the experiment. They were individually housed in cages with unlimited access to food and tap water and kept on a reverse 12 hr/12 hr light-dark cycle with lights off at 7 00 a m The animals were always run during the dark cycle

Procedure

Surgery Stereotaxic surgery was performed on all animals while anesthetized with sodium pentobarbital (50 mg/kg). For the

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animals that were to receive SFO lesions (n = 39), a 250 μ nichrome wire totally insulated except for 0 5 mm at the tip was angled 6° from the vertical and positioned at the coordinates 7 55 mm anterior to the interaural line, 0 6 mm lateral to bregma and 4 45 mm below the dura Anodal DC current of 1 mA was passed for 20 sec between the tip of the electrode and an ear bar which served as the indifferent The sham-operated controls (n = 16) were treated identically except that no current was passed

Postsurgery drinking One to two weeks following surgery, when all the rats appeared healthy, a choice between alcohol and water was offered using the limited access drinking procedure (10,11) Each day during the dark cycle the animals were removed from their home cages, weighed, and then placed for 1 hr 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 1 hr had elapsed, the amounts of water and alcohol consumed were recorded and the animals were returned to their home cages The positions of the two fluids in the drinking cages were alternated daily to control for position preferences For the first two weeks the alcohol solution was 3% (w/v) then increased to 6% (w/v) for a further 14 days During the next and final two-week period both groups continued to have a choice between the 6% (w/v) alcohol and water, but in addition were given daily subcutaneous injections of Val-5 ANG II (Hypertensin-Ciba) at a dose of 200 μg/kg just prior to being placed in the drinking cages The peptide was prepared fresh daily, dissolved in saline and injected in a volume of 0 1 ml per 100 g body weight

Blood alcohol levels At the end of the experiment, all animals were injected first with 200 μg/kg ANG II and then intraperitoneally with 2 5 g/kg alcohol Fifty μl blood samples were drawn from the cut tip of the tail at 20-min intervals during the first hr and thereafter at 1-hr intervals for the next 5 hr The samples were analyzed by gas-liquid chromatography

Histology At the conclusion of the experiment all rats were anesthetized and perfused intracardially with isotonic saline followed by 10% formalin The brains were removed, stored in formalin and then frozen sectioned at 50 μ and mounted for light microscope examination

RESULTS

Alcohol Consumption

Histological examination revealed that 12 of the 39 lesioned rats sustained some damage to the SFO The alcohol and water intake for each of these 12 rats and each of the 16 sham animals was averaged across each of the three two-week periods (3%, 6%, and 6%-ANG II) An analysis of variance of these means with lesion as the between subjects factor and testing period as the within subjects factor did not yield a significant effect of lesion or a significant interaction of lesion with testing period for either alcohol or water intake suggesting that the SFO lesions did not modify the intake of alcohol or water The significant effect of testing period reflected the suppression in alcohol intake, $F(2,52) = 30\ 8$, $p<0\ 001$, and elevation in water intake, $F(2,52) = 16\ 4$, $p<0\ 001$, produced by ANG II administration

The apparent inability of the SFO lesion to attenuate the stimulatory effect of ANG II on water intake was unexpected in light of the numerous reports in the literature showing that SFO lesions attenuate ANG II-induced dipsogenesis (15–17) The apparent failure to replicate this effect in the present experiment prompted a reexamination of the water intake data Figure 1A plots the water intake for each rat in the SFO group during the 6% phase against its own intake during the 6%-ANG II phase This analysis revealed a bimodal distribution in consumption with one

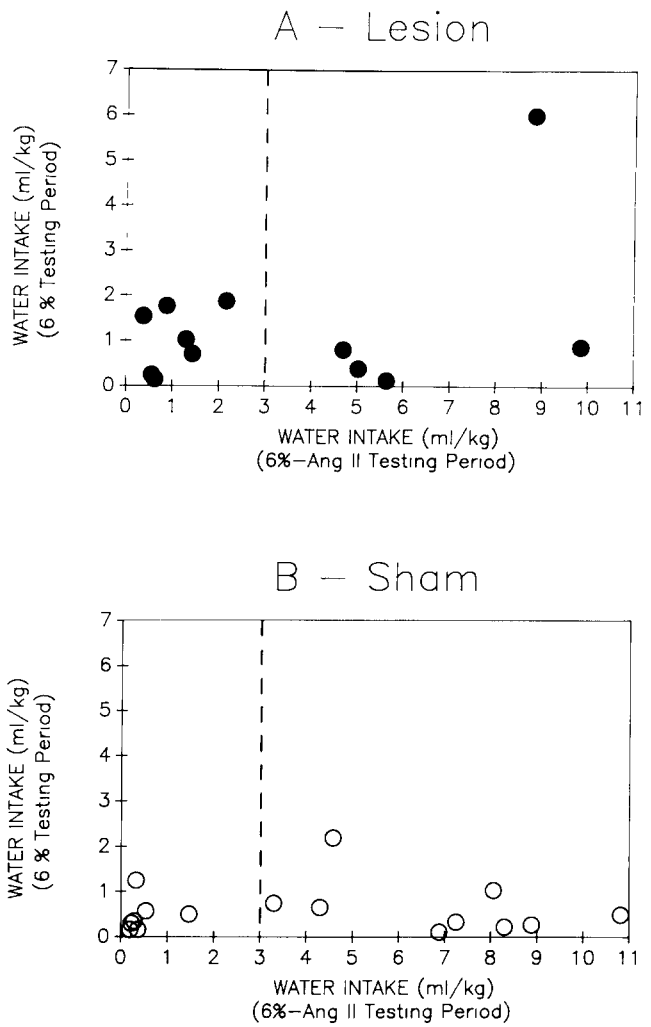

FIG 1 Scatter plot of water intake for each rat during the 6% testing period against its own intake during the 6%-ANG II testing period A—SFO lesion group, B—Sham group Vertical line represents criterion of 3 ml/kg

cluster of 7 animals showing the expected attenuation in ANG II-induced water intake and a second cluster of 5 animals which did not The point of differentiation appeared to be around 3 ml/kg Applying the same analysis to the sham group, Fig 1B shows that 9 of the 16 animals responded to the administration of 200 μg/kg ANG II with an increase in water intake above the 3 ml/kg level, while 7 failed to show an appreciable increase This analysis suggested a subdivision of the original lesion and sham groups into four subgroups along functional lines, i e , the presence or absence of a dipsogenic response to ANG II The groups thus formed were lesion with dipsogenic response (l-d, n = 5), lesion without dipsogenic response (l-nd, n = 7), sham with dipsogenic response (s-d, n = 9) and sham without dipsogenic response (s-nd, n = 7) The mean alcohol and water intake for the animals in each of these four groups was recalculated for the three two-week periods and is illustrated in Fig 2 An analysis of variance of the alcohol means with group as the between subjects factor and testing period as the within subjects factor did not show an overall group difference in alcohol consumption but did reveal a significant effect of testing period, $F(2,48) = 48\ 5$, $p<0\ 001$,

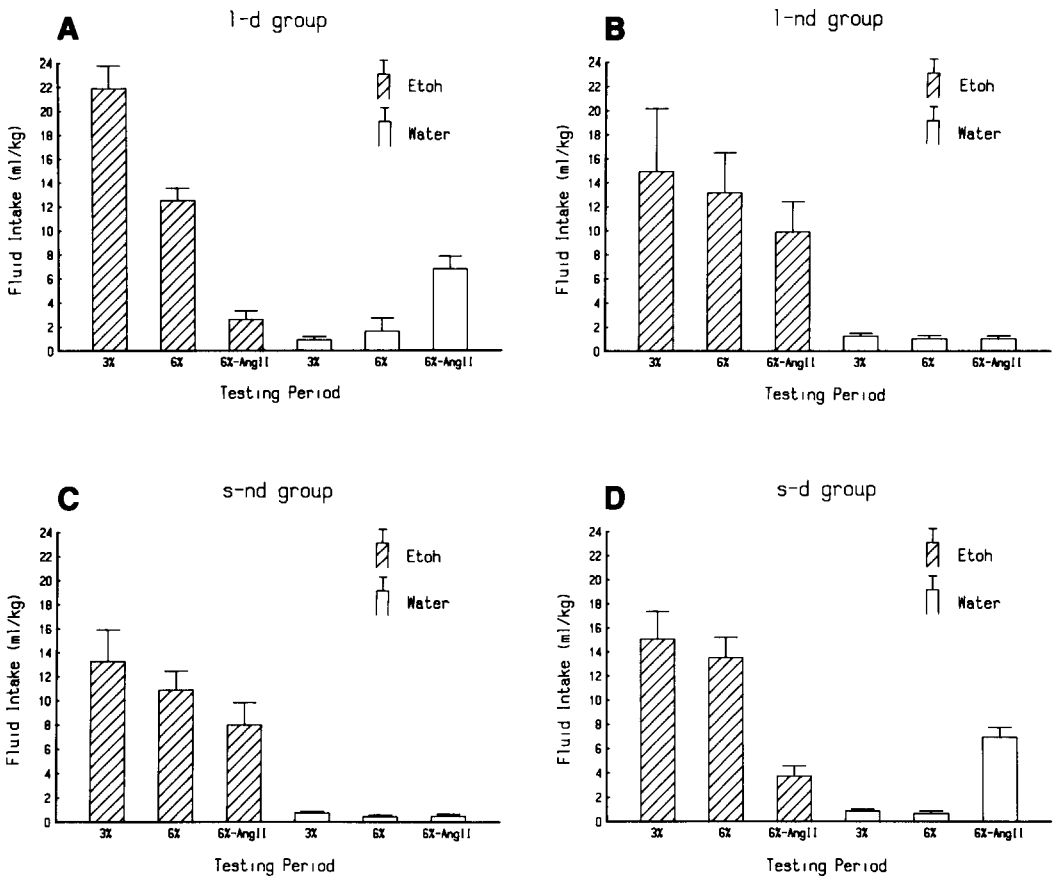

FIG 2 Mean alcohol and water intake for the four subgroups across the three testing periods of the experiment A—lesion with dipsogenic response (1-d) group, B—lesion without dipsogenic response (1-nd) group, C—sham without dipsogenic response (s-nd) group, D—sham with dipsogenic response (s-d) group

reflecting the tendency for dose consumed to increase with the increasing concentration of alcohol, but more importantly a significant interaction of group with testing period, $F(6,48) = 5\ 6$, $p<0.001$, indicating the tendency for the lesion and sham groups to differ in alcohol intake at certain testing periods Post hoc analysis using the Duncan's test showed that there was no difference in alcohol intake among any of the four subgroups during the 3% and 6% alcohol testing periods indicating that the lesion had no effect on voluntary alcohol consumption per se There was, however, a significant and selective difference in the effect of ANG II on alcohol intake. Figure 2A shows that the 1-d group which showed no functional deficit in ANG II-induced water intake also showed a significant ANG II-mediated reduction in alcohol intake However, ANG II failed to reduce alcohol intake in the 1-nd group (Fig 2b) which had a functional deficit in ANG II-induced water intake. The two sham subgroups followed a similar pattern to that seen in the two lesion subgroups, i e , that a functional deficit to ANG II with respect to water intake in the s-nd group was coincident with a failure of ANG II to suppress alcohol intake (Fig 2C) while animals in the s-d group which showed the typical dipsogenic response to ANG II with respect to water intake also manifested an ANG II-mediated suppression in alcohol intake (Fig 2D) Susceptibility to the dipsogenic effect of ANG II or the lack thereof either by reason of physical damage to the area sensitive to the peptide or by reason of natural variation among animals in their response to the peptide, appears to be an indicator of the effect of ANG II on alcohol intake.

Drug Handling

Figure 3 shows the mean blood alcohol levels for the four groups at the eight sampling times following the sequential injections of 200 μg/kg ANG II and 2 5 g/kg alcohol The last four points on the descending portion of the curves were used to calculated the slopes which represent the rate of metabolism There were no significant differences among the groups in rate of metabolism, $F(3,17) = 0.8$, n.s. Extension of the linear portions of the curves yields an estimation of the concentration at time zero from which the volume of distribution is calculated There were no significant group differences in volume of distribution, $F(3,17) = 0.9$, n s. A comparison of blood alcohol levels at the first point on the rising portion of the curves when absorption would be taking place did not yield any significant groups differences, $F(3,17) = 0\ 9$, n s Taken together these findings suggest that the reduced ANG II effect in the 1-nd and s-nd groups was not brought about by altered pharmacokinetics of alcohol

DISCUSSION

The results of this study suggest that the SFO plays a role in mediating the suppressive effect of systemic ANG II on voluntary alcohol intake SFO lesions that resulted in a failure to elicit water

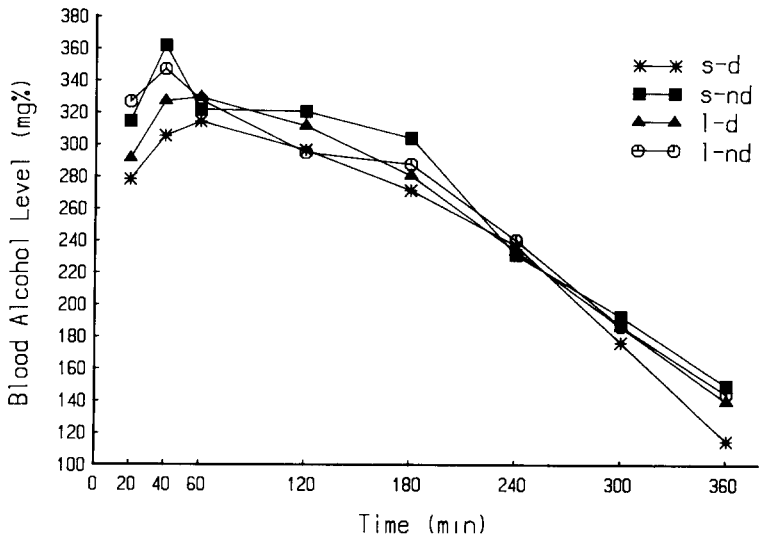

FIG 3 Mean blood alcohol levels (mg/decalitre) for the four groups at the various times following the 200 μg/kg ANG II and 2 5 g/kg alcohol injections

intake in response to systemically administered ANG II (l-nd group), significantly attenuated the ability of ANG II to reduce alcohol consumption Since these same lesions did not alter alcohol consumption during the 3% and 6% testing periods, the SFO does not appear to play a role in the ongoing regulation of alcohol consumption per se, but would appear to exert an influence on intake only in response to stimulation of activity in the renin-angiotensin system

The l-d group, on the other hand, sustained lesions to the SFO that did not interfere with the ability of ANG II to elicit water intake Like the l-nd group, the l-d group also did not show a change in alcohol consumption during the 3% and 6% testing periods, but in contrast to the l-nd group, also failed to show a reduction in the ability of ANG II to attenuate alcohol intake This finding indicates that damage to the SFO per se is not a sufficient condition to blunt the effect of ANG II on alcohol intake Rather, the lesion must be such as to produce a functional deficit—exemplified in the present experiment by an attenuation of ANG II-induced water drinking—if an effect on alcohol intake is to ensue Simpson and Routtenberg (17) reported that partial lesions of the SFO (less than 50% destruction) did not alter daily water intake or water intake in response to systemically injected ANG II, while complete lesions (greater than 60% damage) also did not alter daily water intake, but did produce a significant attenuation in ANG II-induced dipsogenisis The results of the l-d and l-nd lesion groups with respect to alcohol intake are similar to the Simpson and Routtenberg data with water intake (17) and suggest that a difference in the degree or location of damage to the SFO might account for the difference between these two lesion groups in the effect of ANG II on alcohol intake presently noted Further studies with more circumscribed microlesions correlating extent of damage with reduction in function would shed light on this issue

The two sham subgroups (s-nd and s-d groups) also differed with respect to the effect of ANG II on alcohol intake The s-d animals showed both an ANG II-induced increase in water intake and reduction in alcohol intake, while the s-nd animals, which failed to show enhanced water intake to ANG II, also failed to show a significant ANG II-induced reduction in alcohol intake The s-nd group also showed a tendency to drink less alcohol than the s-d group during the 3% and 6% testing periods although this difference was not statistically significant Taken together, however, these findings highlight what appears to be a natural variation in the response of rats to ANG II and indicate that a lack of response to ANG II either as a result of a lesion to a site rich in ANG II receptors (l-nd group) or as a result of a low sensitivity to the dipsogenic effect of the peptide, can have similar consequences on the ability of ANG II to reduce alcohol intake This leads to the suggestion that a test for sensitivity to ANG II might turn out to be a useful predictor of, or marker for, the propensity to consume alcohol

Previous work has shown that area postrema (AP) lesions increased alcohol intake in rats that had continuous access to the drug (18) The present study using the limited access procedure showed no effect of SFO lesions on alcohol intake per se but an effect of the lesion on ANG II-induced reduction of alcohol intake This finding indicates that removal of angiotensin receptors is not in itself sufficient to cause a change in alcohol intake Both the SFO and the AP are devoid of a blood-brain barrier, rich in ANG II receptors and sensitive to circulating levels of ANG II They are connected via their common input to the parabrachial nucleus (9,14) and via the nucleus of the solitary tract (1,21) It is therefore possible that the circumventricular organs comprise an important subsystem that can modulate voluntary alcohol intake

In summary, lesions of the SFO have been found to attenuate the ANG II-induced reduction in alcohol intake but do not otherwise alter alcohol drinking This effect does not appear to be the result of changes in drug distribution or metabolism and occurs only when the lesions are such as to also attenuate the dipsogenic effect of ANG II Since alcohol intake and activity in the renin-angiotensin system are known to be inversely related (3,19), the SFO appears to be a site involved in the elaboration of this relationship

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