ROLE OF ANGIOTENSIN II AND THE SUBFORNICAL ORGAN IN THE PHARMACOLOGICAL ACTIONS OF ETHANOL

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Abstract — Aims: The current study was designed to evaluate if angiotensin II mediates the hypothermic effects of ethanol, and to determine if the effects of angiotensin are mediated centrally. We also tested the hypothesis that the subfornical organ (SFO) is a site responsible for the alterations in body temperature and aerial righting reflex mediated by ethanol and for the modulation of ethanol consumption in rats. Methods: Male Sprague-Dawley rats were used in a series of experiments to evaluate the role of both peripheral and central administration of losartan, a selective angiotensin type 1 receptor antagonist on ethanol-induced hypothermia. Subsequent studies were undertaken in SFO-lesioned rats to evaluate the effects of SFO-lesion on alcohol intake, the thermal response to alcohol and angiotensin, and the aerial righting reflex. Results: Selective antagonism of the angiotensin II type 1 receptor, administered either peripherally or centrally, attenuated not only the fall in colonic temperature but also attenuated the transient rise in tail skin temperature that was associated with administration of ethanol. The thermal responses to both angiotensin and ethanol were similarly attenuated in SFO-lesioned rats. Likewise the aerial righting reflex, which has previously been shown to be impaired by losartan treatment, was also significantly attenuated in SFO-lesioned animals. Alcohol intake, as determined by a 48 h, two-bottle preference test also revealed that SFO-lesioned animals consumed significantly less alcohol (ethanolic beer) than did controls. Conclusion: Collectively, the results demonstrate that ethanol-induced temperature responses are mediated by the renin–angiotensin system and that this interaction is mediated centrally. In addition, the results demonstrate that the SFO is a site that mediates several neurobiological effects of ethanol, possibly via the renin–angiotensin system.

INTRODUCTION

Angiotensin (Ang II) is a peptide hormone that plays a significant role in the vascular control of blood pressure regulation, water and sodium balance. More recently a role for Ang II has been ascribed to temperature regulation. Systemic administration of Ang II to the rat has been shown to induce a dose-dependent hypothermic response, which is manifested by a decrease in metabolic rate, a fall in colonic temperature (Tc), and a transient increase in tail skin temperature (tsT) (Wilson and Fregly, 1985a,b; Fregly and Rowland, 1993; Wright and Katovich, 1996). In rats, the hypothermic response is abolished by pretreatment with inhibitors of the renin–angiotensin system (RAS), and appears to be centrally mediated via the Ang II type 1 (AT1) receptor (Wilson and Fregly, 1985a,b; Fregly and Rowland 1992, 1996). In addition, deoxycorticosterone treatment, which increases central Ang II receptor number (Wilson et al., 1986), increases the thermal response to peripherally administered Ang II (Fregly and Rowland, 1992). This type of thermal response of Ang II is suggestive of a possible downward resetting of the central thermostat by Ang II, and supports the hypothesis that this is a centrally mediated event (Wilson and Fregly, 1985a,b; Fregly and Rowland, 1996).

A variety of neuropeptides and transmitters have been implicated both in the control of alcohol intake and in the physiological responses associated with consumption of ethanol. The RAS has been implicated in control of alcohol intake. Much of the early work in this area demonstrated a significant reduction in alcohol intake by Ang II in rodents (Grupp et al., 1989, 1991; Grupp and Harding, 1994, 1995; Blair-West et al., 1996; Kulkosky et al., 1996). However, there also are numerous studies that have demonstrated that Ang II is stimulatory to ethanol intake (Hubbell et al., 1992; Fitts, 1993a; Weisinger et al., 1999a,b). There are major differences in these protocols that may account for the opposite results. In studies that suggested an inhibitory role for Ang II on alcohol intake, the design was for acute studies with only a limited time (40 min) access to alcohol per day. Those protocols are designed to produce acutely high blood alcohol levels with a pharmacodynamic effect (Linesman, 1987). Conversely, many of the studies that suggest a stimulatory role for Ang II administered drugs centrally and/or looked at chronic preference testing. Recently, Maul et al. (2001), utilizing transgenic mice, demonstrated that mice expressing a rat angiotensinogen transgene displayed an increase in alcohol consumption when offered a choice between water and 10% (vol./vol.) ethanol. This increase in alcohol consumption was not caloric driven, and transgenic mice lacking the angiotensinogen transgene drank significantly less alcohol than did wild-type controls. In addition, when the transgenic mice harbouring the rat angiotensinogen transgene were administered an ACE inhibitor that penetrates the blood brain barrier, there was a significant reduction of alcohol consumption. These studies by Maul et al. (2001) demonstrated a direct correlation between endogenous Ang II levels and voluntary alcohol intake in genetically altered mice. Collectively, these data may be reconciled by the hypothesis that only a prolonged increase in central Ang II and/or an upregulated central RAS is stimulatory to ethanol intake.

Thus, Ang II may have a role in alcohol intake and may also be involved with the physiological actions of alcohol. The thermoregulatory response to Ang II is similar to that induced by ethanol (Meyers, 1981; York and MacKinnon, 1999). Core temperature is significantly reduced following administration of ethanol, but the skin temperature response has not been well characterized. Several reports also have suggested that the RAS is involved in some of the behavioural responses induced

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by ethanol. Ang II impairment and ethanol’s inhibition of long term potentiation (LTP) can both be blocked by pretreatment with losartan, a selective AT₁ receptor antagonist (Wayner et al., 1993). Tracy et al. (1996) reported that the impairment in aerial righting caused by ethanol also was reversed by losartan in a dose-dependent manner. These same authors later demonstrated that ethanol-induced cognitive deficits are mediated by Ang II via the AT₁ receptor (Tracy et al., 1997), and that losartan reduces the intoxicating effects of ethanol in the rat (Wayner et al., 1994). A variety of studies have suggested that the subfornical organ (SFO) and other adjacent areas are critical for the central actions of Ang II (Fitzsimons, 1998; Rowland, 1999), and we (Fregly and Rowland, 1996) previously demonstrated that lesion of the SFO (SFO-X) resulted in abolished Ang II-mediated temperature response in rats. Collectively, these observations led us to hypothesize that the thermoregulatory effect of ethanol might be mediated by endogenous Ang II release, and therefore also should be attenuated by losartan. Further, we evaluated if this ethanol–Ang II interaction was mediated centrally, by studies in which the RAS was inhibited by central administration of antagonists and by the studies utilizing SFO-lesioned animals.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Harlan Sprague–Dawley) were used in all studies. The University of Florida’s institutional animal care and use committee (IACUC) approved all protocols. Unless otherwise stated, 300–400 g rats were housed individually in hanging wire cages, with free access to food (500 l Purina Rat Chow) and water. The cage rack was housed individually in hanging wire cages, with free access to food (500 l Purina Rat Chow) and water. The cage rack was kept in an animal room maintained at 24 ± 1°C and illuminated from 05.00 to 17.00 hours daily.

Temperature studies

On the day of the temperature studies, tail skin (tsT) and colonic (Tc) temperatures were measured with the rats restrained in wire mesh cylinders, the diameter of which could be adjusted to fit the rat snugly, and to which the rats had been adapted beforehand. tsT was measured from a copper–constantan thermocouple taped to the surface of the tail near its base, while Tc was measured by a thermocouple inserted ~6 cm into the rectum as previously described (Wright and Katovich, 1996; Katovich et al., 2001). Temperatures were measured simultaneously at 5 min intervals with a data acquisition and control system (CYBORG) interfaced to an Apple IIe computer. Animals were allowed to equilibrate to the experimental conditions for 30 min prior to recording temperatures. At that time, the AT₁ receptor antagonist, losartan (5, 10 or 20 mg/kg), or the saline vehicle was administered subcutaneously (s.c.). After a 30 min control period, all animals were administered ethanol (2 g/kg as a 40% vol./vol. solution in 0.9% saline, 5 ml/kg, i.p.) via a port in the bottom of the restraining cage. Temperatures were recorded at 5 min intervals for an additional 180 min. Twelve animals were studied on a given day. The data were analysed as change from the last baseline reading and as the integrated area under the 180 min temperature curve (AUC), using the trapezoid method. In the initial study six animals were administered 20 mg/kg losartan and six animals were administered the saline/kg prior to injection with ethanol. In a subsequent dose–response study, animals were randomized for the dose of losartan. These experiments were performed once per week for 4 consecutive weeks. Animals ranged from 380 to 410 g at the start of these studies and body weights were similar between groups for all the temperature studies.

ICV cannulations

In a subsequent study 20 Sprague-Dawley male rats (280–300 g) were fitted with an intracerebroventricular (i.c.v.) cannula. Animals were anaesthetized with a ketamine (50 mg/ml) plus xylazine (5 mg/ml) cocktail mixture (0.7 ml/kg, i.p.). Animals were placed in a Kopf stereotaxic apparatus with the incisor bar set at −2.5 mm. Animals were implanted with a 10 mm cannula made from 23 gauge stainless steel tubing (Small Parts, Miami, FL). The coordinates for the lateral ventricle cannula were obtained from the stereotaxic atlas of Pellegrino et al. (1979) and were 1.5 mm lateral to the midsagittal line, 2.8 mm below the skull surface and at bregma. After insertion of three stainless steel anchoring screws, dental acrylic was applied to secure the cannula in place. A wire stylet was fitted into the outer guide cannula and remained in place throughout the remainder of the experiment. All cannulation material was pyrogen free. Following a 2 week recovery period animals’ dipsogenic response to angiotensin II (10 ng/5 µl i.c.v.; Sigma, St Louis, MO) was determined. Only animals with a positive dipsogenic response were utilized in the subsequent temperature studies. The temperature studies were conducted as described above, except that the losartan was administered i.c.v. at dosages of 0, 4.5 or 10 µg/5 µl, 30 min prior to administration of ethanol. Animals were randomized on the day of the study. The study was repeated weekly for 3 weeks.

Subfornical organ lesions

Twenty Sprague–Dawley male rats (250–275 g) were anaesthetized using the ketamine–xylazine cocktail and placed in the stereotaxic device. The subfornical organ (SFO) was then lesioned using electrocoagulation in 12 rats. This method involved the insertion of a recording electrode to determine the exact dorsal-ventral depth of the SFO at 0.4, 0.8 and 1.4 mm posterior to the bregma. This procedure was followed by the insertion of the lesioning electrode to the same coordinates. Lesions were produced by passing a current of 800 µA through the lesioning electrode for 20 s at each of the three sites (Rowland et al., 1994). Control rats (n = 8) were similarly anaesthetized, but only the recording electrode was inserted into the brain, to a depth of 1.5 mm ventral of the dura mater. The skull was patched using a small surgical sponge, and the skin sewn shut. Rats were allowed 1 week to recover from the surgery while housed in their home cages. These rats were then used to measure the temperature response to Ang II and ethanol, and for the ethanol drinking preference test and the aerial righting reflex test.

Subcutaneous angiotensin II

SFO-lesioned (SFO-X) rats and their controls were instrumented with tsT and Tc thermocouple temperature probes as previously described. The rats were acclimated to

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their restraint cages for 30 min before the experiment was started. Baselines were established by recording $T_c$ and $tsT$ every 5 min for 30 min. Angiotensin II (200 μg/kg, s.c.) was administered and temperatures recorded every 5 min for 60 min. Three days later, the animals were similarly instrumented and challenged with ethanol (2 g/kg as a 40% vol./vol. in 0.9% saline, 5 ml/kg, i.p.) and the $tsT$ and $T_c$ temperatures monitored at 5 min intervals for 180 min.

**Ethanol drinking preference**

SFO-X rats and their controls were housed in their home cages with free access to food (500 g Purina rat chow). Two drinking tubes were presented, one with de-carbonated non-alcoholic beer (Coors) mixed with ethanol, and one with tap water, both at room temperature. Beer was used as a vehicle for the alcohol because it is consumed by rats without extensive fading or training procedures and it is a vehicle with extensive relevance to human alcohol consumption (Lancaster et al., 1989). Volumes of each liquid were measured once every 24 h for 7 days, and tube placement (left/right) was alternated each day. The rats were presented with a 5% ethanol-containing beer for 3 days, and 10% ethanol (v/v) for the last 4 days. Preference data were obtained by dividing ethanol consumed by total liquids consumed on days 2 and 3 [for 5% (vol/vol.) ethanol beer], and days 6 and 7 [for 10% (v/v) ethanol beer].

**Aerial righting test**

SFO-X rats and their controls were injected with ethanol (1.25g/kg in a 25% vol./vol. solution in 0.9% saline, 5 ml/kg, i.p.) and housed in their home cages. At 0.5, 1.0, 1.5, 2.0 and 3.0 h post-injection, the rats were individually removed from their cages, held inverted 30 cm above a foam rubber landing pad, and released as previously described by Tracy et al. (1996). The minimum height needed to perform an accurate aerial righting behaviour was determined by lowering the dropping height until the rat could no longer correct its orientation before contacting the landing pad. This experiment was repeated using 40% ethanol solution (2 g/kg, i.p.).

**Histology**

SFO-lesioned rats were anaesthetized using an overdose of sodium pentobarbital (100 mg/kg, i.p.), then perfused with 10% formaldehyde solution, and their brains were removed and sectioned coronally as previously described (Rowland et al., 1994). Sections were stained using thionin, and examined under a microscope for anatomically successful lesion and to confirm the i.c.v. cannulation site. Data from rats with incomplete or misplaced lesions or incorrect cannulation were not used in the data analysis.

**Statistics**

All data were expressed as mean ± 1 standard error of the mean (SEM), and differences between groups were analysed by ANOVA followed by Fisher’s PLSD post hoc tests with significance set at the 95% confidence level. For the temperature studies, data were analysed using integrated area under temperature curves, and the maximal changes in temperature from baseline, irrespective of time. For the aerial righting test, data were analysed by two-way ANOVA with significance set at the 95% confidence level. All statistical analysis was performed with a StatView program from SAS.

Figure 1 summarizes the effects of losartan (20 mg/kg, s.c.) on the $tsT$ (A) and the $T_c$ (B) response to a bolus injection of ethanol (2 g/kg, 40% vol./vol., i.p.) at time zero. Skin temperatures were similar between the control and losartan treated groups at the time of ethanol administration (25.3 ± 0.2 and 24.9 ± 0.1°C, respectively). The maximal rise in $tsT$, irrespective of time, was 3.2 ± 0.9°C in controls and 1.6 ± 0.6°C in the losartan pretreated group ($P < 0.02$). This transient increase in $tsT$ was short lived, and baseline levels were restored over the next 30 min. Colonic temperatures also were not different between the control and losartan treated groups at the time of ethanol administration (37.4 ± 0.1 and 37.1 ± 0.1°C, respectively). The fall in $T_c$, however, was more robust (−1.7 ± 0.1°C) and significantly greater ($P < 0.02$) in the control animals than in the losartan pretreatment group (−0.7 ± 0.3°C). Area under the $T_c$ curve also reflected this significant difference ($P < 0.01$) between the control and losartan-treated groups in response to administration of ethanol (−196.2 ± 27.9°C/min versus −49.5 ± 32.9°C/min, respectively).

In a subsequent study, the effects of graded doses of losartan on the ethanol-induced hypothermia were evaluated. Body weights and basal $tsT$ and $T_c$ were similar in the four experimental groups (Table 1). Losartan treatment had no effect on any of these parameters. Maximal changes in $tsT$ were nearly 50% lower in the losartan-treated groups compared with controls, although these changes did not reach statistical significance in this study. The maximal change in $T_c$ was greatest in the control group, and significantly attenuated
Table 1. Effects of peripheral administration of graded doses of losartan on the ethanol-induced changes in tail skin (tsT) and colonic (Tc) temperature in male SD rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight (g)</th>
<th>Basal tsT (°C)</th>
<th>Maximal change in tsT (°C)</th>
<th>Basal Tc (°C)</th>
<th>Maximal change in Tc (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 14)</td>
<td>397.6 ± 7.7</td>
<td>25.5 ± 0.1</td>
<td>2.2 ± 0.6</td>
<td>37.3 ± 0.1</td>
<td>−1.6 ± 0.1</td>
</tr>
<tr>
<td>5 mg losartan (n = 9)</td>
<td>406.7 ± 11.4</td>
<td>26.1 ± 0.3</td>
<td>1.6 ± 0.6</td>
<td>37.3 ± 0.2</td>
<td>−1.4 ± 0.2</td>
</tr>
<tr>
<td>10 mg losartan (n = 8)</td>
<td>402.4 ± 9.1</td>
<td>25.8 ± 0.2</td>
<td>1.0 ± 0.6</td>
<td>37.0 ± 0.1</td>
<td>−1.2 ± 0.2</td>
</tr>
<tr>
<td>20 mg losartan (n = 14)</td>
<td>400.1 ± 6.9</td>
<td>25.4 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>37.1 ± 0.1</td>
<td>−0.9 ± 0.1a,b</td>
</tr>
</tbody>
</table>

Values represent mean ± one SEM.

* p < 0.01 between control and 20 mg losartan group.

b p < 0.03 between 5 and 20 mg losartan groups.

Fig. 2. Area under the 180 min colonic temperature (Tc) curve following administration of ethanol (2 g/kg, 40% vol./vol., i.p.) in male Sprague–Dawley rats pretreated with either saline (n = 14), 5 (n = 8), 10 (n = 8) or 20 (n = 14) mg/kg losartan, 30 min prior to ethanol. The control and the 20 mg/kg losartan group were significantly different (*P < 0.001), and the 5 and 20 mg losartan groups were significantly different from each other (†P < 0.02).

Fig. 3. Area under the 180 min colonic temperature (Tc) curve following administration of ethanol (2 g/kg, 40% vol./vol., i.p.) in male Sprague–Dawley rats pretreated with central administration of either saline (n = 12), 4.5 (n = 6) or 10 µg/5 µl (n = 9) of losartan, 30 min prior to ethanol. The control and the 10 µg losartan group were significantly different (P < 0.01).

in the 20 mg/kg losartan-treated group (Table 1). The area under the 180 min Tc curve summarizes the reduced hypothermic response in the group administered the highest dose of losartan (Fig. 2).

Ethanol-induced hypothermia was likewise attenuated by central administration of losartan (Fig. 3). Although the lowest dose of losartan did not significantly alter the hypothermic response, the higher, 10 µg/5 µl dose of losartan significantly (P < 0.005) reduced the ethanol-induced hypothermia. A higher dose (20 µg/5 µl) was also utilized; however, the reduction was no greater than that of the 10 µg dose (P < 0.04, data not shown) when compared to controls.

The SFO lesion had no significant effect on body weight or basal tsT and Tc (Table 2). As we have reported before (Fregly and Rowland, 1996), SFO-X rats showed significantly reduced thermal responses to Ang II compared with controls (Table 2). The temporal pattern was similar between the two groups (Fig. 4). When the animals were challenged with ethanol (2 g/kg) a similarly reduced response was observed in SFO-X rats, with a reduced rise in tsT and a reduced fall in Tc compared with the sham-lesioned controls (Table 2; Fig. 5). It should be noted that the area under the tsT curve is for 60 min, similar to that used in the Ang II study, but the area for the Tc curve is over the entire 3 h of measurement. We feel that the colonic temperature changes observed are a better indicator of the hypothermic effects of both Ang II and ethanol than are the more transient changes in tsT. The temporal responses also were similar between the two groups, although the maximal changes were prolonged when compared to animals challenged with Ang II. The maximal rise in tsT occurred about 20 min after administration of ethanol and the fall in Tc maximized around 2 h.

A 48 h, two-bottle preference test between water and either 5 or 10% ethanol added to non-alcoholic beer was determined for the sham control and SFO-X-lesioned groups. SFO-X rats consumed significantly less 10% ethanolic beer that did the sham controls (P < 0.002) (Fig. 6). The consumption of 5% ethanolic beer also was reduced in SFO-X rats compared with controls, but this effect was not significant. SFO-X rats have reduced ethanol consumption as well as the physiological responses to Ang II.

In a subsequent set of SFO-X animals, the aerial righting reflex was examined and the results are summarized in Fig. 7. Without administration of alcohol, all animals displayed a righting reflex when held supine 5–10 cm above the foam pad. The maximal impairment of the reflex was observed at 90 min in controls and 120 min in SFO-X animals. In the SFO-X group, a reduced degree of impairment was observed (two-way ANOVA revealed time and treatment effects of P < 0.0001 with no significant interaction). When a one-way ANOVA was performed at individual time points the SFO-X...
physiological responses associated with consumption of ethanol. The results of the current study suggest that Ang II may be an important peptide that is involved with ethanol intake and the physiological responses mediated by ethanol. In addition, the results suggest that these Ang II-mediated responses are most probably centrally mediated.

When Ang II is injected peripherally in rats, a rise in tail skin temperature ($tST$) and a subsequent fall in colonic temperature ($Tc$) occurs (Wilson and Fregly, 1985a, b; Wilson et al., 1986; Fregly and Rowland, 1992, 1993, 1996; Wright and Katovich, 1996), mediated by AT$_1$ receptors. This group displayed a significantly ($P < 0.01$) reduced impairment at all time points except at 120 min. At a higher concentration of ethanol (40% vol./vol.), no differences were observed between the two groups (data not shown).

### DISCUSSION

A variety of neuropeptides and transmitters has been implicated both in the control of alcohol intake and in the physiological responses associated with consumption of ethanol. The results of the current study suggest that Ang II may be an important peptide that is involved with ethanol intake and the physiological responses mediated by ethanol. In addition, the results suggest that these Ang II-mediated responses are most probably centrally mediated.

When Ang II is injected peripherally in rats, a rise in $tST$ and subsequent fall in $Tc$ occurs (Wilson and Fregly, 1985a, b; Wilson et al., 1986; Fregly and Rowland, 1992, 1993, 1996; Wright and Katovich, 1996), mediated by AT$_1$ receptors. This
conducted at each time interval revealed a significant difference in treatment ($P < 0.001$) effects with no interaction. One-way ANOVA conducted at each time interval revealed a significant difference ($P < 0.01$) between control and subfornical-lesioned (SFO-X) groups at all time points except 120 min.

Fig. 6. 48 h bottle preference test between water and either 5 or 10% ethanol added to non-alcoholic beer in control and subfornical lesioned (SFO-X) adult Sprague–Dawley rats. The SFO-X rats consumed significantly less ($P < 0.002$) of the 10% ethanolic mixture than did the controls.

Fig. 7. Effects of lesioning the subfornical organ (SFO) in adult male Sprague–Dawley rats on the righting reflex for 180 min after the administration of ethanol (1.25 g/kg, 25% v/v, i.p.) at time zero. Results from a two-way ANOVA revealed significant time ($P < 0.001$) and treatment ($P < 0.001$) effects with no interaction. One-way ANOVA conducted at each time interval revealed a significant difference ($P < 0.01$) between control and subfornical-lesioned (SFO-X) groups at all time points except 120 min.

The SFO-X studies reported herein, as well as the i.c.v. administration of losartan, would support the hypothesis that the thermoregulatory actions of Ang II are mediated centrally. Ang II is known to act at a unique set of brain regions known as the circumventricular organs (Fitzsimons, 1998; Rowland, 1999). These structures lack the normal blood brain barrier, and are thought to participate in the central processing of neuroendocrine signals. In particular, the SFO is a known site of action of the effects of Ang II (Mangiapane and Simpson, 1980; Ferguson and Wall, 1992; Johnson and Gross, 1993). Peripheral injection of Ang II induces drinking in many species, and this is abolished by lesion of the SFO (Fitzsimons, 1998). Likewise, the thermal responses attributed to Ang II in rats are abolished with lesions of the SFO (Fregly and Rowland, 1996). The primary transducing role for the SFO has also been shown in studies of induction of c-fos (or Fos protein) in brain by Ang II, which occurs in all of the above-mentioned areas and is abolished in all of them after lesion of the SFO (Rowland, 1999). Several investigators have demonstrated that losartan can enter the brain to inhibit central actions of Ang II (Palmer et al., 1994; Culman et al., 1999). Recently Collister and Hendel (2003) demonstrated that the SFO is necessary for the complete expression of the hypotensive effects of losartan. The SFO projects to the median preoptic nucleus, the organum vasculosum of the lamina terminalis, the nucleus tractus solitarius, and both the paraventricular and supraoptic nuclei of the hypothalamus (Ferguson, 1992). Insofar as the SFO is a key transduction site for circulating Ang II, these data complement the losartan results in implicating the circulating RAS in the response to ethanol. Further, they suggest that the effects of Ang II in the brain (and perhaps the SFO) may be critical in mediating the effects of ethanol.

Ethanol impairs the aerial righting reflex (Mattucci-Schiavone and Ferko, 1986), and losartan has been shown to block this ethanol-induced impairment in a dose-dependent manner (Tracy et al., 1996), as well as other behavioural and physiological effects of ethanol (Wayner et al., 1993, 1994; Tracy et al., 1997). Likewise, hippocampal dentate granule cell long term potentiation (LTP) induction, which is mediated by Ang II and the AT$_1$ receptor (Wayner et al., 1993), is inhibited by ethanol in relatively low doses. Collectively, these findings suggest that effects of ethanol can be mediated by the
RAS. Our findings demonstrate that SFO-lesioned animals respond to ethanol in a manner similar to animals treated with losartan, which suggests that central components (particularly those components residing in the SFO) of the RAS may be involved in mediating the behavioural and thermoregulatory effects of ethanol.

Our data suggest that impaired Ang II transduction attenuates two effects of ethanol, but they do not address whether the avidity for, or self-administration of, ethanol might be affected. If the hypothesis that Ang II stimulates the intake of ethanol is true, then impairment of Ang II signalling should result in a decrease in ethanol intake. The results in the SFO lesion rats given an ad libitum choice between water and 5% or 10% alcoholic beer (nonalcoholic beer with ethanol added), each for several days demonstrated that SFO lesion rats consumed less beer than the controls, especially at 10% ethanol. These data support the hypothesis that the SFO is integrally involved in preference for and/or the reinforcing effects of ethanol, but are not conclusive, as this paradigm can not easily dissect the consequences of ethanol ingestion from the consequences of the caloric content of the ethanol consumed. More detailed ingestive studies need to be conducted in order to validate this hypothesis.

A variety of studies have supported the hypothesis that the RAS is involved in the control of alcohol intake. Although there is some controversy as to whether Ang II is stimulatory or inhibitory to ethanol intake, recent studies using continuous i.c.v. administration of Ang II to rodents demonstrate that Ang II is stimulatory to alcohol intake (Fitts, 1993a; Weisinger et al., 1999a,b). Inhibition of peripheral angiotensin converting enzyme (ACE) by administration of low doses of captopril increased ethanol intake in rats (Fitts, 1993a). Low doses of captopril act in the periphery only, and increase plasma Ang I levels, which then can enter the brain where ACE is still active, thus elevating central Ang II. The increased ethanol intake observed with low dose captopril was blocked in rats with SFO-X suggesting that the conversion of Ang I to Ang II in the SFO mediated the increase in fluid intake (Fitts, 1993b). In contrast, a high dose of captopril, which additionally blocks the ACE in the brain, has been shown to decrease alcohol consumption in rats (Hubbell et al., 1992). This effect may be a result of the complete blockade of both central and peripheral RAS (Fitts, 1993b) and/or by its actions on bradykinin, which has been shown to suppress alcohol intake (Robertson et al., 1993). Further evidence for a stimulatory role of the RAS on ethanol intake is the recent use of transgenic mice that over or under express components of the RAS and increase or decrease ethanol intake, respectively (Maul et al., 2001). Collectively, these results are consistent with the view that Ang II action in the brain is stimulatory to alcohol intake.

In contrast to these findings, several papers have suggested that increased Ang II decreases alcohol intake in rats (Grupp et al., 1989, 1991; Hubbell et al., 1992; Grupp and Harding, 1994, 1995; Blair-West et al., 1996; Kulakosky et al., 1996). These studies have been criticized (Weisinger et al., 1999a) on the grounds that they mostly used manipulations of peripheral Ang II and so do not negate an opposite effect in brain, or they use a limited time access paradigm to monitor ethanol intake. Kraly and Jones (1999) also could not support the hypothesis that Ang II provides an inhibitory physiological control of ingestion of ethanol, and in fact demonstrated that losartan significantly inhibited alcohol intake in a two-bottle drink test. Finally, the work of Grupp and colleagues that hypothesized that Ang II reduced alcohol intake was tested in alcoholic humans and the results of that study were negative (Naranjo et al., 1991). These data may be reconciled by the hypothesis that only a prolonged increase in central Ang II and/or an upregulated central RAS is stimulatory to ethanol intake in rats. Our data would support this latter hypothesis, and suggest that the effects of ethanol are mediated at least in part by central RAS components.

The mechanism(s) by which Ang II may mediate the responses observed in the current study may involve eventual stimulation of the central dopaminergic system. It is known that Ang II modulates dopamine release in the brain (Mounzih et al., 1994; Raghavendra et al., 1998), and angiotensin receptors are abundantly expressed in brain areas where dopaminergic transmission has been strongly implicated in alcohol sensitivity and self-administration (Jenkins et al., 1997; Daubert et al., 1999). It is interesting to note that mice overexpressing angiotensinogen and consuming larger amounts of alcohol displayed a decreased intake when administered either a central acting ACE inhibitor or fluphenazine, a dopaminergic antagonist (Maul et al., 2001). Likewise, mice lacking the D2 receptor gene were less sensitive to alcohol-induced ataxia than their wild type littermates (Phillips et al., 1998). Thus the RAS may mediate some of the effects of ethanol through central stimulation of dopaminergic pathways. This association will have to be further investigated. It is interesting that a correlation between the ACE DD genotype, which is associated with high ACE activity, and increased susceptibility to alcoholism in humans has been recently reported (Garrrib and Peters, 1998). Also of interest is the recent report that chronic ethanol ingestion increases expression of the angiotensin II, type 2 receptor (Bechara et al., 2003). Collectively, the results support a role of Ang II in the actions of alcohol and in alcohol consummatory behaviour, and may prove of interest in future therapeutic approaches for treating alcoholism.

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