Intracerebroventricular infusion of angiotensin II increases water and ethanol intake in rats

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Weisinger, R. S., J. R. Blair-West, P. Burns, and D. A. Denton. Intracerebroventricular infusion of angiotensin II increases water and ethanol intake in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R162–R172, 1999.—The influence of prolonged ingestion of ethanol on thirst mechanisms in rats, evidence consistent with disturbance of the thirst mechanism (8) and related to taste preference. In conclusion, chronic consumption of ethanol solution did not appear to adversely effect ethanol stimulation of water intake. The intake of ethanol solution during infusion of ANG II was inhibited by a direct effect of ingested ethanol and/or by indirect effect from metabolized ethanol.

Studies in humans have shown that the prolonged ingestion of excessive amounts of alcohol may be detrimental to health. Individuals that abuse alcohol or are addicted to alcohol suffer from numerous ailments that may be due to imbalances in body fluid homeostasis (4, 5). As part of our investigation concerned with the effects of prolonged ingestion of ethanol on physiological mechanisms responsible for body fluid and energy homeostasis in mammals, experiments have been conducted on sheep (2) and mice (3) trained to drink ethanol solutions. The animals had access to ethanol solution as their only source of fluid during intracerebroventricular infusion of ANG II evaluated in rats. Animals were maintained for 5–6 mo with either 10% ethanol solution or water as their only source of fluid. In both groups of rats, infusion of ANG II caused a large increase in water intake (7-fold) and a lesser increase in 10% ethanol intake (2-fold). The effect of ANG II on the volume of ethanol solution ingested, however, was inversely related to the concentration of the ethanol solution. As the concentration of ethanol solution was decreased, frequency and duration of drinking bouts increased. The intake of sweetened 10% ethanol solution or commercially produced wine during infusion of ANG II was similar to the intake of 10% ethanol and not related to taste preference. In conclusion, chronic consumption of ethanol solution did not appear to adversely affect ANG II stimulation of water intake. The intake of ethanol solution during infusion of ANG II was inhibited by a direct effect of ingested ethanol and/or by indirect effect from metabolized ethanol.

Intracerebroventricular infusion of angiotensin II caused a substantial increase in water intake but no increase in intake of ethanol solutions. The failure of the intracerebroventricular infusion of ANG II to stimulate intake of ethanol in mice was not due solely to its aversive taste. When given only one fluid to drink, intracerebroventricular infusion of ANG II increased intake of a potassium chloride solution that under free access conditions was shown to have an aversive taste relative to the ethanol solution.

Although there have been no studies directly examining the influence of prolonged ethanol intake on thirst mechanisms in rats, evidence consistent with disturbance of these mechanisms has been reported in rats chronically maintained on ethanol (1, 7, 13, 14, 16, 32). For example, Essig (7) reported that rats chronically maintained with access to 20% ethanol had an exaggerated intake of water when it was made available. Altered sensitivity to vasopressin (32) and altered plasma levels of vasopressin, aldosterone, atrial natriuretic peptide, and brain natriuretic peptide have also been reported (13, 14). Also, changes in central nervous system mechanisms involved in the regulation of body fluid homeostasis have been observed. Chronic ingestion of ethanol decreases the number of neurons in the supraoptic nucleus (16) and alters the sensitivity of the cholinergic neurons (1).

The purpose of the present series of experiments was to evaluate the influence of the prolonged ingestion of ethanol on mechanisms involved in body fluid homeostasis in rats. Specifically, the ability of intracerebroventricular infusion of ANG II to stimulate water intake was determined. In addition, the ability of intracerebroventricular infusion of ANG II to alter ethanol intake was assessed. Although ANG II is a potent stimulus to water intake, its influence on intake of ethanol is controversial. Evidence that intracerebroventricular infusion of ANG II stimulates (8) or does not alter (11) ethanol intake has been reported. The influence of intracerebroventricular infusion of ANG II on intake of solutions of varying ethanol concentration, sweetened ethanol solution and a commercially available wine, was determined to assess the role of flavor factors in modulating the influence of ANG II on fluid intake.

**METHODS AND RESULTS**

**Animals**

Preexperimental period. The rats used were male Sprague-Dawley rats, 6–7 wk of age at the start of the preexperimental period. The rats were maintained in sawdust-lined group boxes 70 (length) x 40 (width) X

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15 (height) cm in a temperature-controlled room on a 12:12-h light-dark cycle. They were divided into two groups. For 5 mo, one group of rats had free access to rat chow (GR2+ food pellets, Clarke-King) and 10% ethanol solution (ETOH rats, n = 32), whereas the other group had free access to rat chow and water (Water rats, n = 32).

Experimental period. After 5 mo, the rats were moved to individual stainless steel metabolism cages [25 (length) × 18 (width) × 18 (height) cm]. During this experimental period, the rats had access to rat chow and, unless specified otherwise, their maintenance fluid. Intake of fluids was recorded continuously via an automated drinking system, unless otherwise stated.

SURGERY. Under general anesthesia (Equithesin, 3 ml/kg ip), a 23-gauge cannula was surgically implanted into a lateral cerebral ventricle. Briefly, the procedure involved fixation of the cannula to the skull with dental acrylic and stainless steel screws. With the use of a stereotaxic frame, the rats’ heads were positioned such that the interaural line was 5 mm below the level of the upper incisor bar and the coordinates used were 1.5 mm lateral to the midline, 0.2 mm caudal to bregma, and 4.5–4.9 mm ventral to dura. Correct placement of the cannula in the lateral ventricle was substantiated before and between experiments by observing water intake (>5 ml/20 min) after intracerebroventricular injection of ANG II (100 ng).

INFUSIONS. After the surgical recovery period, intracerebroventricular infusions were performed using a miniosmotic pump (model 2001, Alza; rate = 1 µl/h, maximum 7 days) placed subcutaneously on the animal’s back and connected to the lateral ventricular cannula via a SV45 polyvinyl cannula. Minipump implantation and removal was performed under general anesthesia (Brietal sodium, Eli Lily; 40–60 mg/kg ip). The procedure required ∼5 min to complete, and the rats were active again within 1 h. The pumps were implanted/removed after daily measurements were completed. Infusates used were artificial cerebrospinal fluid [aCSF; (in mM) 149 Na, 2.9 K, 131.5 Cl, 1.1 Ca, 0.9 Mg, 0.5 HPO4; Ref. 23] and ANG II (50 ng/h) made up in aCSF. Each rat had between one and four infusions with at least 7–10 days between infusions.

Experimental Design and Statistical Analysis

During the experimental period, intakes (e.g., food, ethanol solution, water) and body weight were measured daily. The experiments were divided into three periods: baseline, treatment, and posttreatment periods. For each rat, the mean of the values obtained during the period (3 or 4 days) before treatment was used as a baseline value. A two-way analysis of variance (Figs. 1 and 4–7), repeated measures on one variable (e.g., time) and independent measures on one variable (e.g., maintenance condition), and subsequent least-significant differences test (Statistica, Statsoft) were used to compare the values obtained during the various periods. All values (except in Figs. 2 and 3) are presented as means ± SE.

Experiment 1: Effect of prolonged ethanol intake on ANG II stimulation of water or 10% ethanol solution intake. METHODS. During a 4-day period, rats were infused intracerebroventricularly with ANG II at 50 ng/h (n = 5 Water rats, n = 8 ETOH rats) or aCSF (n = 5 Water rats, n = 6 ETOH rats) with only water available. During another 4-day period, rats were infused intracerebroventricularly with ANG II at 50 ng/h (n = 7 Water, n = 8 ETOH rats) or aCSF (n = 5 Water rats, n = 10 ETOH rats) with only 10% ethanol solution available.

The appropriate maintenance fluid only, i.e., 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after the infusion period.

RESULTS. A significant two-way interaction between infusion and days [F(4,80) = 38.87, P < 0.001] and subsequent analyses indicated that relative to baseline, intracerebroventricular infusion of ANG II caused a large increase (P < 0.001) in water intake in both ETOH and Water rats (Fig. 1, top). The intake increased on the first day and reached a plateau from the second day of infusion. No change in intake occurred during intracerebroventricular infusion of aCSF. Average water intake (ml/100 g body wt) of the combined groups (ETOH and Water, differences between the
groups were not significant) during the last day of infusion was 40.2 ± 4.6 (ANG II) and 6.2 ± 0.5 (aCSF).

In both ETOH and Water rats, body weight decreased by 2.5–3.5% (P < 0.001) during the intracerebroventricular infusion of ANG II. Food intake was decreased (P < 0.01) during the first day of infusion of either ANG II or aCSF.

A significant two-way interaction between infusion and days [F(4,104) = 22.45, P < 0.001] and subsequent analyses indicated that relative to baseline, intracerebroventricular infusion of ANG II caused an increase (P < 0.001) in ethanol intake from the first day of infusion in ETOH and Water rats (Fig. 1, bottom, mean intake of the first 3 days has been reproduced in Fig. 7). A small but significant decrease (P < 0.01) in ethanol intake occurred during the first 2 days of intracerebroventricular infusion of aCSF (Fig. 1, bottom). Average intake of 10% ethanol (ml/100 g body wt) of the combined groups (ETOH and Water, differences between the groups were not significant) during the last day of infusion was 10.0 ± 0.8 (ANG II) and 5.0 ± 0.4 (aCSF).

In both ETOH and Water rats, body weight decreased by 2.0–3.0% (P < 0.001) during intracerebroventricular infusion of ANG II. Neither infusion altered food intake.

Figure 2 shows the patterns of intake of a Water (Fig. 2, top) and an ETOH (Fig. 2, bottom) rat during intracerebroventricular infusion of ANG II with either water (Fig. 2A) or 10% ethanol solution (Fig. 2B) available. It is clear that the patterns of intake were similar for the Water and the ETOH rats. For comparison, the fluid intake patterns over 6 days of four Water rats drinking water (Fig. 3A) and four ETOH rats drinking 10% ethanol (Fig. 3B) are shown. The drinking patterns are very similar; both ETOH and Water rats consumed most of their daily fluid during the night hours, presumably with food. It did appear, however, that the ETOH rats drank a greater percentage of their daily fluid intake during the daytime hours than did the Water rats. Relative to the patterns of normal daily intakes (Fig. 3, A and B), the pattern of water drinking during infusion of ANG II (Fig. 2A) was continuous throughout the day and night, whereas ethanol drinking (Fig. 2B) continued to occur episodically. During the infusion of ANG II, intake of water was four to six times greater than that of ethanol.

**Experiment 2:** Effect of prolonged ethanol intake on ANG II stimulation of intake of solutions of decreasing ethanol concentration. **Methods.** Over 7 days, rats were infused intracerebroventricularly with ANG II at 50 ng/h (n = 7 Water rats, n = 7 ETOH rats) or aCSF (n = 8 Water rats, n = 8 ETOH rats). During the infusion, a decreasing concentration of ethanol solution was available: 10% ethanol (days 1 and 2), 8% ethanol (day 3), 6% ethanol (day 4), 4% ethanol (day 5), 2% ethanol (day 6), and 0% ethanol, i.e., water (day 7). The maintenance fluid only, i.e., 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after the infusion period.

Subsequent to the completion and analysis of experiment 2, an additional four Water rats were given access to solutions of decreasing ethanol concentration during intracerebroventricular ANG II as above. From these rats, a blood sample for measurement of ethanol concentration (BAC) was taken from the tail during the morning (1100–1200) and evening (2200–2300).

In addition, a blood sample for BAC was taken from the tail of ETOH rats (n = 8) during the morning (1100–1200) and evening (2200–2300) under baseline conditions. BAC was determined with the enzymatic measurement PAP 150 (bioMerieux, Mercy-l’Étoile, France) using a Beckman Synchron CX5, Clinical System (Beckman, Brea, CA).

**Results.** A significant two-way interaction between infusion and days [F(7,182) = 76.35, P < 0.001] and subsequent analyses indicated that there was an inverse relationship between the concentration of ethanol solution offered and the magnitude of the fluid intake (ml/100 g body wt) caused by the intracerebroventricular infusion of ANG II (Fig. 4A, top). Without exception, as the concentration of ethanol solution decreased intake increased. With intracerebroventricular infusion of aCSF, a small decrease (P < 0.01) in intake...
Fig. 3. A: water intake patterns of 4 individually housed Water rats. Hourly water intake was measured over 6 consecutive days. B: 10% ethanol intake patterns of 4 individually housed ETOH rats. Hourly ethanol intake was measured over 6 consecutive days.
Fig. 4. A: effect of intracerebroventricular infusion of ANG II or aCSF on intake of decreasing concentrations of ethanol [top: intake of ethanol in ml·100 g body wt (bwt)⁻¹·day⁻¹; bottom: intake in g ethanol·100 g body wt⁻¹·day⁻¹]. After a baseline period (3 days), rats were infused over 7 days with ANG II at 50 ng/h (•, Water rats, n = 7; ○, ETOH rats, n = 7) or aCSF (■, Water rats, n = 8; ●, ETOH rats, n = 8). During first 6 days of infusion, a decreasing concentration of ethanol solution was available: 10% ethanol (days 1 and 2), 8% ethanol (day 3), 6% ethanol (day 4), 4% ethanol (day 5), 2% ethanol (day 6). On last day of infusion, water was available. Maintenance fluid only, 10% ethanol solution (ETOH rats) or water (Water rats), was available during baseline period. Statistical analysis described in METHODS AND RESULTS: *P < 0.05, **P < 0.01, ***P < 0.001 (vs. baseline); +++P < 0.001 (ANG II vs. aCSF); ♦P < 0.01, ♦♦P < 0.001 (vs. previous day’s intake).

B: effect of intracerebroventricular infusion of ANG II on 24-h fluid intake patterns of 1 Water rat (top) and 1 ETOH rat (bottom). Rats were infused over 7 days with ANG II at 50 ng/h. During first 6 days of infusion, a decreasing concentration of ethanol solution was available as described for A. On last day of infusion, water was available. Hourly fluid intake was measured over 9 consecutive days. On days 1 and 9, maintenance fluid only, 10% ethanol solution (ETOH rats) or water (Water rats), was available and no infusion was given.
occurred during the first day of infusion. The changes in intake caused by the intracerebroventricular infusion of ANG II were similar for ETOH and Water rats (Fig. 4A, top). Average intakes of fluid (ml/100 g body wt) of the combined groups (ETOH and Water) during the infusion of ANG II were 9.0 ± 0.5 (10% ethanol, first and second days), 13.0 ± 0.5 (8% ethanol), 15.7 ± 0.8 (6% ethanol), 19.3 ± 1.0 (4% ethanol), 27.3 ± 1.7 (2% ethanol), and 30.1 ± 2.0 (water), whereas intakes during the infusion of aCSF were 6.1 ± 0.7.

With regard to the intake of ethanol (g ethanol/100 g body wt; Fig. 4A, bottom), a significant main effect of ANG II infusion [F(1,26) = 210.90, P < 0.001] indicated that ethanol intake was increased by 0.4–0.5 g/100 g body wt during the intracerebroventricular infusion of ANG II compared with infusion of aCSF. Intakes were similar for ETOH and Water rats, but a significant two-way interaction between maintenance condition and infusion days [F(5,130) = 3.93, P < 0.01] and subsequent analyses indicated that the ethanol intake of the Water rats was greater (P < 0.001) than that for the ETOH rats during the first day of intracerebroventricular infusion. The maximum intake of ethanol (g/100 g body wt) occurred during access to 10% (second day of infusion) and 8% ethanol solution.

In the four Water rats bled for BAC during intracerebroventricular infusion of ANG II with various concentrations of ethanol solution available, intakes were similar to those shown in Fig. 4A (although not included in the figure). BAC levels measured during the morning (2.3 ± 0.4 mmol/l) and evening (5.9 ± 1.7 mmol/l) were quite low. These results are similar to those seen in ETOH rats under maintenance conditions (morning, 2.1 ± 1.3 mmol/l; evening, 5.8 ± 1.6 mmol/l).

In both ETOH and Water rats, body weight decreased by 2.0–2.5% (P < 0.05) during intracerebroventricular infusion of ANG II and during the first 2 days of intracerebroventricular infusion of aCSF. Food intake was decreased (P < 0.01) during the first 5 days of infusion of ANG II and during the first 3 days of infusion of aCSF.

Figure 4B shows the patterns of intake of a Water (Fig. 4B, top) and an ETOH (Fig. 4B, bottom) rat during infusion of ANG II with decreasing concentrations of ethanol available. It is clear that the patterns of intake were similar for the Water and the ETOH rats. During intracerebroventricular infusion of ANG II, as the concentration of ethanol solution offered decreases, both frequency and duration of drinking bouts increase. The drinking patterns observed when 2% ethanol solution was available are indistinguishable from those observed when water is offered.

Experiment 3: Flavor preferences and effects of intracerebroventricular infusion of ANG II. FLAVOR PREFERENCES.

METHODS. From glass drinking columns hung on the front of the cage, group 1 rats (n = 5 Water rats, n = 5 ETOH rats) were offered 10% ethanol and three commercially available white wines with a concentration of alcohol =10% (wine R, De Bortoli, Gold Seal Riesling, concentration of glucose + fructose ~230 mM; wine M, De Bortoli, Gold Seal Moselle, concentration of glucose + fructose ~290 mM; and wine S, Lindemans, porphyry sauterne, concentration of glucose + fructose ~450 mM). These four solutions were available for 4 days. On day 5, the most preferred solution was removed and the three remaining solutions were offered for a further 4 days. On day 9, the most preferred solution was removed and the two remaining solutions were offered for a final 4 days. The maintenance fluid only, i.e., 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after this 12-day period. Glucose and fructose concentrations in wine were measured using a Beckman Synchron CX5, Clinical System.

From glass drinking columns hung on the front of the cage, group 2 rats (n = 7 Water rats, n = 4 ETOH rats) were offered 10% ethanol and sweetened (5% sucrose) 10% ethanol. These two solutions were available for 3 days. The maintenance fluid only, i.e., 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after this 3-day period.

RESULTS. In group 1 rats, there was a significant main effect of solution [F(3,24) = 298.08, P < 0.001] and of maintenance condition [F(1,8) = 15.28, P < 0.01], and subsequent analyses indicated that the intake of 10% ethanol was greater (P < 0.001) than the intake of any of the commercially produced wines offered and that total fluid intake by the ETOH rats was greater (P < 0.001) than that by the Water rats (Fig. 5A). Subsequent analyses indicated that the least-preferred beverage was wine R; the order of preference was 10% ethanol > wine S > wine M > wine R, with no difference between ETOH and Water rats (Fig. 5B and C). This order of wine preference was related to the glucose + fructose content.

In group 2, Both ETOH and Water rats showed a significant preference for sweetened 10% ethanol over 10% ethanol [F(1,9) = 100.89, P < 0.001; Fig. 5D]. Total fluid intake (10% ethanol plus sweetened 10% ethanol) of the ETOH rats was greater than that of the Water rats [F(1,9) = 5.94, P < 0.05].

EFFECTS OF INTRACEREBROVENTRICULAR INFUSION OF ANG II. METHODS. Group 1 rats were infused intracerebroventricularly over 3 days with ANG II at 50 ng/h (n = 7 Water rats, n = 7 ETOH rats) or aCSF (n = 7 Water rats, n = 8 ETOH rats) with wine R (least preferred) available. The maintenance fluid only, 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after the infusion period.

Group 2 rats were infused over 3 days with ANG II at 50 ng/h (n = 5 Water rats, n = 8 ETOH rats) or aCSF (n = 5 Water rats, n = 6 ETOH rats) with sweetened (5% sucrose) 10% ethanol available. The maintenance fluid only, i.e., 10% ethanol solution (ETOH rats) or water (Water rats), was available before and after the infusion period.

RESULTS. In group 1 rats, there was a significant two-way interaction between infusion and days [F(3,87) = 10.30, P < 0.001], and subsequent analyses indicated that intake of wine R was increased (P < 0.001) during the intracerebroventricular infusion of ANG II.
ANG II, relative to intake of water or 10% ethanol during the baseline period or during intracerebroventricular infusion of aCSF (Fig. 6, top). Intake of wine was decreased (P < 0.05 or greater) during the first 2 days of intracerebroventricular infusion of aCSF. Intakes were similar for ETOH and Water rats.

In ETOH and Water rats, body weight decreased by 1.5–2.5% (P < 0.001) during intracerebroventricular infusion of ANG II. Neither infusion altered food intake.

In group 2 rats, there was a significant two-way interaction between infusion and days [F(3,60) = 11.13, P < 0.001], and subsequent analyses indicated that intake of the sweetened ethanol solution was increased (P < 0.01 or greater) during the intracerebroventricular infusion of ANG II relative to intake of water or 10% ethanol during the baseline period or during intracerebroventricular infusion of aCSF (Fig. 6, bottom). No change in intake occurred during intracerebroventricular infusion of aCSF. The increase in sweetened ethanol intake caused by the intracerebroventricular infusion of ANG II was similar for ETOH and Water rats.

In both ETOH and Water rats, body weight decreased by 3.0–4.0% (P < 0.001) during intracerebroventricular infusion of ANG II. Food intake was decreased (P < 0.001) during the intracerebroventricular infusion of ANG II or aCSF.

Intake of alcohol solution (10% ethanol, sweetened 10% ethanol, and wine R) was higher during intracerebroventricular infusion of ANG II than during infusion of aCSF [F(1,75) = 51.15, P < 0.001; Fig. 7]. Average intakes of fluid (ml/100 g body wt) of the combined groups (ETOH and Water) during the 3 days of infusion of ANG II were 8.6 ± 0.5 (10% ethanol), 10.0 ± 0.6 (sweetened 10% ethanol), and 7.8 ± 0.8 (wine R), whereas intakes during the infusion of aCSF were 4.5 ± 0.4, 6.5 ± 0.6, and 4.3 ± 0.4, respectively. The animals drank more of the sweetened ethanol (P < 0.05
5.37, P 10% ethanol (to maintain body fluid balance of ETOH rats) may be spite the prolonged acceptance of the ethanol solution during intracerebroventricular infusion of ANG II (decrease differences in ethanol and water intake observed a daily source of fluid. One possible explanation for the experienced in the ingestion of 10% ethanol solution as (2). The findings were the same in rats either naive or result is in agreement with observations made in sheep smaller than the increase in water intake (7-fold). This increase in intake of 10% ethanol solution (2-fold) was during intracerebroventricular infusion of ANG II, the loss of neurons in a number of brain areas (1) and influences membrane structure and membrane-related processes, e.g., cAMP-dependent protein kinase activity in rat brain (25), and profound changes in ANG II sensitivity have been reported (17), the intake of water caused by the intracerebroventricular infusion of ANG II observed in the present studies, as well as in previous studies in sheep (2) and mice (3), was not affected. It has been shown in alcoholic humans that there is a blunting of the effectiveness of ANG II to stimulate aldosterone secretion or thirst (4). No such blunting effects on thirst were observed in the ETOH rats, and it is likely that other factors are involved in the human or that species differences exist.

The results of this study also demonstrate that during intracerebroventricular infusion of ANG II, the increase in intake of 10% ethanol solution (2-fold) was smaller than the increase in water intake (7-fold). This result is in agreement with observations made in sheep (2). The findings were the same in rats either naive or experienced in the ingestion of 10% ethanol solution as a daily source of fluid. One possible explanation for the large differences in ethanol and water intake observed during intracerebroventricular infusion of ANG II (despite the prolonged acceptance of the ethanol solution to maintain body fluid balance of ETOH rats) may be that the postigestional effects of ethanol activate mechanisms inhibitory to further intake. Then, as the intoxicating or toxic effects of ethanol lessen, the inhibition decreases and the animals resume drinking. That is, the pattern of 10% ethanol intake observed would result from the drive to drink, caused by the intracerebroventricular infusion of ANG II, interrupted because of the inhibitory mechanisms activated by the ingested ethanol. This proposal is consistent with the episodic pattern of ethanol intake, different from the almost continuous pattern of intake observed when water was available, resulting in the smaller consumption of 10% ethanol than of water.

This study also showed that during intracerebroventricular infusion of ANG II, larger volumes of ethanol solution were ingested when the concentration of the ethanol solution was lowered. Thus, unlike the results obtained in mice (3), increased intake of ethanol solution was observed during intracerebroventricular infusion of ANG II in rats. The actual amount of ethanol ingested during intracerebroventricular infusion of ANG II, however, was reasonably constant, although the concentration of the ethanol in solution varied. The level consumed was 7–8 g·kg⁻¹·day⁻¹, which is about the maximum level that a rat can metabolize in a 24-h period (11).

The amount of ethanol ingested (g/100 g body wt; Fig. 4A, bottom) when 2, 4, or 6% ethanol solution was available was less than that when 8% ethanol was available (maximum ethanol intake recorded), irrespective of infusion. During intracerebroventricular infusion of ANG II it is intriguing that with access to the less concentrated solutions of ethanol, ethanol consumption (g/100 g body wt) did not increase to the same level achieved when 8% ethanol solution was available. This result is inconsistent with the idea that ANG II causes animals to ingest an alcohol solution until a maximal amount of ethanol is ingested. The decreasing ethanol intake with decreasing ethanol concentrations does not appear to be due to progressive inebriation over the infusion period. During intracerebroventricular infusion of ANG II, BAC was always very low before offering the next day’s solution (1100–1200), consistent with the low ethanol intake that occurred during the morning period. These BACs were comparable, if not identical, to those measured during basal conditions in ETOH rats. There does not appear to be any carryover of ethanol from one day to the next, although it is possible that the rats did, on occasion, consume enough ethanol to become intoxicated during the night. The fact that the amount of liquid ingested is increasing regularly each day (albeit in association with the decreasing concentration of ethanol) is not consistent with the idea of a strengthening conditioned aversion. Although further work will be required to understand the mechanisms involved, it is possible that the episodic pattern of ethanol is at least part of the explanation. The findings with graded concentrations of ethanol solution showed no difference in the daily drinking patterns of experienced and naive rats infused intracerebroventricularly with ANG II. The gradual increase

DISCUSSION

Our model tests whether animals habituated to high ethanol intake show any difference in water drinking or ethanol drinking responses to the dipsogen ANG II compared with the responses of ethanol-naive animals. The present results, similar to results in sheep and mice, are in the negative. Prolonged exposure of rats to ethanol for months had no effect on ANG II-induced intakes of water or ethanol compared with ethanol-naive rats. Although some evidence exists that maintaining rats with prolonged access to ethanol causes the loss of neurons in a number of brain areas (1) and influences membrane structure and membrane-related processes, e.g., cAMP-dependent protein kinase activity in rat brain (25), and profound changes in ANG II sensitivity have been reported (17), the intake of water caused by the intracerebroventricular infusion of ANG II observed in the present studies, as well as in previous studies in sheep (2) and mice (3), was not affected. It has been shown in alcoholic humans that there is a blunting of the effectiveness of ANG II to stimulate aldosterone secretion or thirst (4). No such blunting effects on thirst were observed in the ETOH rats, and it is likely that other factors are involved in the human or that species differences exist.

The results of this study also demonstrate that during intracerebroventricular infusion of ANG II, the increase in intake of 10% ethanol solution (2-fold) was smaller than the increase in water intake (7-fold). This result is in agreement with observations made in sheep (2). The findings were the same in rats either naive or experienced in the ingestion of 10% ethanol solution as a daily source of fluid. One possible explanation for the large differences in ethanol and water intake observed during intracerebroventricular infusion of ANG II (despite the prolonged acceptance of the ethanol solution to maintain body fluid balance of ETOH rats) may be that the postigestional effects of ethanol activate mechanisms inhibitory to further intake. Then, as the intoxicating or toxic effects of ethanol lessen, the inhibition decreases and the animals resume drinking. That is, the pattern of 10% ethanol intake observed would result from the drive to drink, caused by the intracerebroventricular infusion of ANG II, interrupted because of the inhibitory mechanisms activated by the ingested ethanol. This proposal is consistent with the episodic pattern of ethanol intake, different from the almost continuous pattern of intake observed when water was available, resulting in the smaller consumption of 10% ethanol than of water.

This study also showed that during intracerebroventricular infusion of ANG II, larger volumes of ethanol solution were ingested when the concentration of the ethanol solution was lowered. Thus, unlike the results obtained in mice (3), increased intake of ethanol solution was observed during intracerebroventricular infusion of ANG II in rats. The actual amount of ethanol ingested during intracerebroventricular infusion of ANG II, however, was reasonably constant, although the concentration of the ethanol in solution varied. The level consumed was 7–8 g·kg⁻¹·day⁻¹, which is about the maximum level that a rat can metabolize in a 24-h period (11).

The amount of ethanol ingested (g/100 g body wt; Fig. 4A, bottom) when 2, 4, or 6% ethanol solution was available was less than that when 8% ethanol was available (maximum ethanol intake recorded), irrespective of infusion. During intracerebroventricular infusion of ANG II it is intriguing that with access to the less concentrated solutions of ethanol, ethanol consumption (g/100 g body wt) did not increase to the same level achieved when 8% ethanol solution was available. This result is inconsistent with the idea that ANG II causes animals to ingest an alcohol solution until a maximal amount of ethanol is ingested. The decreasing ethanol intake with decreasing ethanol concentrations does not appear to be due to progressive inebriation over the infusion period. During intracerebroventricular infusion of ANG II, BAC was always very low before offering the next day’s solution (1100–1200), consistent with the low ethanol intake that occurred during the morning period. These BACs were comparable, if not identical, to those measured during basal conditions in ETOH rats. There does not appear to be any carryover of ethanol from one day to the next, although it is possible that the rats did, on occasion, consume enough ethanol to become intoxicated during the night. The fact that the amount of liquid ingested is increasing regularly each day (albeit in association with the decreasing concentration of ethanol) is not consistent with the idea of a strengthening conditioned aversion. Although further work will be required to understand the mechanisms involved, it is possible that the episodic pattern of ethanol is at least part of the explanation. The findings with graded concentrations of ethanol solution showed no difference in the daily drinking patterns of experienced and naive rats infused intracerebroventricularly with ANG II. The gradual increase
of volume intake (but not grams of ethanol) with decreasing ethanol concentration suggests that an inhibition of the evoked dipsogenic mechanism in the brain was progressively lessened. This inhibition may be due to a direct action of the concentration of alcohol ingested and absorbed, which may involve specific ethanol receptors (for which there is some recent evidence, Ref. 31), or the inhibition may be more indirect, by metabolic effect of the absorbed ethanol, e.g., inebriation, or the production or accumulation of some metabolic product. With either pathway, the regulatory inhibition must be evoked within minutes or perhaps hours, certainly not days.

The data obtained with commercially available wine and sucrose-flavored ethanol are quite novel. In apparent contrast to humans, both the ETOH and Water rats showed a clear preference for the plain 10% ethanol solution over all three commercial wines offered, although each contained ~10% ethanol. Wine R (Riesling) was the least preferred wine and was the least sweet (measurement of glucose/fructose and personal observations). Consistent with that, sucrose sweetened 10% ethanol solution was preferred to 10% ethanol solution. During intracerebroventricular infusion of ANG II, however, intake of either wine R or sweetened 10% ethanol solution was similar to that of 10% ethanol solution. That is, regardless of the solution offered and its relative preference, the amount of ethanol consumed (g) during intracerebroventricular infusion of ANG II did not rise above the 7–8 g·kg⁻¹·day⁻¹ level. Clearly, the hedonic flavor quality of the 10% ethanol solution is not the sole factor limiting its intake. This observation is consistent with results obtained in mice (3). The mice showed a clear preference for 4% ethanol over a bitter tasting solution of potassium chloride (personal observations) when both solutions were available; yet during intracerebroventricular infusion of ANG II, intake of the less preferred potassium chloride solution was increased but intake of the ethanol solution was not when only one solution was available. Again, it appears that the metabolic consequence of ingested ethanol, not flavor preference, was a major factor in determining the small increase in ethanol intake relative to water intake observed during intracerebroventricular infusion of ANG II.

On the basis of experiments in which rats were given a choice between 6% ethanol and water during a limited access period, Grupp and coworkers (11, 12, 24) suggested that the ethanol intake of rats is inversely related to the peripheral concentration of ANG II but independent of the central levels of ANG II. Systemic administration of ANG II caused a decrease, whereas intracerebroventricular injection of ANG II or ANG III did not alter the intake of ethanol. In contrast, Fitts (8) reported that intracerebroventricular administration of ANG II caused an increase in ethanol intake in rats given continuous access to water and 6% ethanol solution to drink. The present study did not examine preference for or stimulation to drink ethanol during intracerebroventricular infusion of ANG II in a free-choice situation. Rather, because ANG II is already well-established as a potent stimulus to the ingestion of water, we have chosen to use a single-bottle test to examine its influence on ethanol intake where only one unambiguous choice is involved, to drink or not to drink. Given the fact that ANG II is a powerful stimulus to the ingestion of water (2, 3, 10, 28), the results of single-fluid experiments and the increased intake of 6% ethanol solution in choice experiments (8) could be explained by the action of ANG II as a dipsogen and the adverse metabolic effects that occur as a consequence of ethanol intake.

The results obtained with peripheral administration of drugs [angiotensin-converting enzyme (ACE) inhibitors] that prevent the formation of ANG II are contradictory. For example, it has been reported that rats increased their intake of ethanol as well as water when captopril was added to the drinking water (8, 9), a treatment that blocks peripheral but not central formation of ANG II (29). This increased intake was not observed in rats with lesion of the subfornical organ, suggesting that the conversion of blood-borne ANG I to ANG II in the subfornical organ mediated an increase in fluid intake (9). On the other hand, evidence has shown that peripheral administration of ACE inhibitors (e.g., abutapril, captopril) decreased ethanol intake (15, 24). In these latter experiments, the reduction in ethanol intake caused by the ACE inhibitors was not altered by peripheral administration of ANG II or naloxone (15) but was attenuated by a bradykinin antagonist, suggesting a role for bradykinin (24). A role for ACTH has also been suggested (23). At present the explanation for these inconsistent results is not clear. It could be argued that the dose of captopril used in these latter experiments was high enough to block both peripheral and central formation of ANG II and therefore not stimulate thirst. Ethanol intake of alcoholics treated with ACE inhibitors was not altered (18).

Finally, the influence of the intracerebroventricular infusion of ANG II on food intake and body weight should be noted. In all of the experiments with ANG II, body weight decreased by 1–4%. Although significant, this decrease in body weight was smaller than that observed after 1 day of food or water deprivation (29). A decrease in body weight with chronic infusion of ANG II has been reported previously (26). In the present experiments, a decrease in food intake was not always associated with the decreased body weight. However, central administration of ANG II can cause both a natriuresis and a diuresis (10, 19), and it is possible that increased fluid loss accounts for the decrease in body weight. Another possibility is that an increase in ACTH and glucocorticoid hormones caused by the central administration of ANG II (19) causes the decrease in body weight (30).

Overall, the present experiments have shown that rats, similar to sheep (2) and mice (3), can readily adapt to a situation where they chronically ingest moderate to high amounts of ethanol. No evidence of any major disturbance in body fluid regulation was found. The ethanol-maintained rats in the present study consumed, on average, 15–20% of their daily intake of
calories as ethanol and appeared to remain healthy with no obvious signs of debilitation. These observations are consistent with earlier observations, e.g., rats maintained with access to 8% ethanol for 2 mo (27) or rats maintained with access to 8, 16, or 24% alcohol or table wine (12.5% ethanol) as their sole source of fluid for up to 18 mo (20–22) were reported as being in good health with no signs of addiction at the end of the time period. It seems clear that rats can ingest the 10% ethanol solution for extended periods of time and gain sufficient water to remain in water balance, with no obvious signs of ethanol-induced damage. Unlike humans who consume alcohol to frank intoxication, and in some instances become addicted, the chronically exposed animals examined thus far (Refs. 2 and 3 and present experiments) appear to have behavioral mechanisms that may serve to prevent prolonged intoxication, although short periods of elevated BACs and short periods of intoxication may occur. In contrast to human alcoholics that remain inebriated (BAC > 0.05%) for as much as 75% of the day (6), the BACs of rats, determined during morning or evening hours, were generally very low or not detectable, suggesting that the episodes of drinking were spaced far enough apart to minimize periods of inebriation. The failure to obtain consistently high BAC levels in the present studies is consistent with the failure to observe intoxicated rats in previous studies (22). Furthermore, the ETOH rats, similar to the Water rats, drank mainly during normal feeding hours, showing no evidence of a nonnutrient motivation to drink alcohol (6). However, small amounts of ethanol continued to be consumed throughout the day, perhaps in an effort to obtain sufficient fluid while minimizing the aversive/metabolic effects of ethanol.

Perspectives

These experiments in rats continue our investigation of the effect of prolonged ingestion of ethanol on mechanisms concerned with thirst. As in experiments in sheep and mice, the chronic intake of ethanol solution as the sole fluid source did not appear to impair health or the water intake evoked by intracerebroventricular infusion of ANG II. This infusion increased the intake of 10% ethanol solution in rats. The intake of 2–10% ethanol solutions (ml) was inversely proportional to the ethanol concentration; however, the average amount of ethanol ingested did not exceed 0.8–0.9 g·100 g body wt·day·1. ANG II infusion also caused almost equal intakes of 10% ethanol, 10% ethanol sweetened with 5% sucrose, and a commercial “dry” wine, although these fluids were not equally palatable.

New directions suggested by these results in rats include testing the effects of higher concentrations or longer duration of “forced” ethanol intake or increasing the consumption rate of ethanol by restricting the period of ethanol access. Effects on brain functions, particularly brain angiotensin mechanisms, and effects on liver function, particularly levels of alcohol dehydrogenase, would be assessed. The apparent limitation of ethanol intake to <0.8–0.9 g·100 g body wt in both alcohol experienced and naive rats suggests the operation of a post-ingestional effect of ethanol, possibly related to rates of alcohol metabolism. So far our evidence is that the central ANG II mechanism is not altered by months of 10% ethanol intake nor by the palatability of the 10% solution but higher or more prolonged ethanol intake may be influential.

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