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Ethanol diuresis in rats: possible modifying factors

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Ethanol has been shown to produce a biphasic dose-dependent effect on urine output in rats. Experiments were carried out to examine factors which may influence ethanol diuresis. Immobilization stress (30 min) decreased and ethanol (2·5 g kg^{-1}) increased urine output of intragastrically hydrated rats. In stressed rats, ethanol had a more pronounced diuretic effect compared with home cage control rats. This increased sensitivity to ethanol disappeared when rats were immobilized daily for four days, indicating development of tolerance. The diuretic action of ethanol was not influenced by adrenalectomy.

Ethanol has a pronounced diuretic effect in man and in experimental animals (Murray 1932; Nicholson & Taylor 1938; Linkola 1974). It is thought that ethanol produces diuresis by inhibition of vasopressin release (Beard & Sargent 1979), the mechanism of this effect has not been established.

Research from my laboratory indicates that ethanol has a differential effect in stressed animals (Pohorecky et al 1980; Brick & Pohorecky 1982, 1983). Low doses of ethanol decrease several biochemical indices of stress while large doses of ethanol potentiate these stress-induced changes. The experiments presented here were designed to examine whether ethanol also has a biphasic effect on urine output in stressed animals. Furthermore, we examined possible mediation of the effect of ethanol on urine output by other pituitary factors.

Methods

Male Holtzman Sprague-Dawley rats (Charles River Breeding Labortories, Inc., Wilmington, MA), ca 250 g, were individually housed on a stainless steel rack equipped with metabolic cages (Wahmann Manufacturing Co., Timonium, MD), in a room at 21 °C + 1·0 °C and with a 12 : 12 h light : dark cycle, lights on at 0 7 h). Rats had free access to Purina rat chow and water except where indicated. All were implanted with gastric cannulae made of PE-100 (Clay Adams, Division of Becton Dickinson Co, Parsippany, NJ) which were exteriorized at the scapular region. One week was allowed for recovery from surgery. Beginning four days after surgery, animals were handled daily for about 1 min to adapt them to human contact and the injection procedure. On the afternoon before the experiment, they were weighed and the patency of the cannulae checked. Experiments began at about 9.00 am with the rats being injected intragastrically with a fluid load, corresponding to 27·0 ml kg^{-1} (Linkola, 1974) consisting of 0·9% NaCl (saline) for the controls, and of an appropriate dilution of 95% ethanol in saline for the test group. Urine was collected in conical 15 ml graduated tubes for 3 h. Water and food were withdrawn for the duration of the experiment.

Diuresis is defined as an increase in urine output in the test group greater than that seen in the corresponding controls.

Adrenalectomy or sham adrenalectomy was performed on rats at the time they were implanted with gastric cannulae. Adrenalectomized rats were given saline to drink. One week later half of the animals in each group were infused with ethanol (2·5 g kg^{-1}) or an equivalent volume of saline. Since adrenalectomized animals metabolize ethanol at a slightly lower rate, their dose of ethanol was 15% less (Pohorecky & Newman 1978).

To test rats at different ambient temperatures, animals were placed in a Tenney Benchmaster Environmental Chamber (Tenney Engineering Inc., Union, New Jersey). The chamber was calibrated to preset temperatures (15°, 22°, or 30 °C ± 1 °C). Animals received a gastric load of either saline or ethanol (2·5 g kg^{-1}) 30 min after being placed in the chamber, and were returned to the environmental chamber for 3 h.

Rats were stressed by immobilization in 7·0 cm diameter tubes made of 7·5 mm wire mesh closed off at one end with a perforated metal cup and at the other with two restraints that were adjusted to allow a snug fit without discomfort. After 30 min, the restrained group was returned to their home cages. Control rats remained in their home cages. Half an hour later the animals in the stressed and non-stressed groups were infused with either saline or ethanol (2·5 g kg^{-1}) and urine was collected for 3 h.

Breath ethanol concentration was determined in a 1 ml sample of equilibrated rebreathed air as previously described (Brick & Pohorecky 1982).

Results

Results are presented as total urine volume. The mean responses (n = 7 rats/group) of control and experimental groups were compared for statistical significance using Student's *t*-test (Fisher 1950). In addition, the tolerance data were subjected to ANOVA analysis with repeated measures. Differences between means were considered statistically significant when $P<0·05$.

The first experiment examined the interaction of ethanol and stress on urine output of hydrated rats. Restraint stress produced a significant decrease in urine output of control rats (Table 1). The 2·5 g kg^{-1} dose of

ethanol produced significant diuresis (62% higher) in non-stressed rats while the 0·6 g kg^{-1} dose of ethanol had no effect on urine output. In stressed rats, both doses of ethanol had a significantly greater diuretic effect (Table 1). The low dose of ethanol (0·6 g kg^{-1}), which by itself did not produce a statistically significant diuresis, elevated urine output in stressed rats by 55% ($P<0·01$). The larger dose of ethanol (2·5 g kg^{-1}) increased urine output by 158% in stressed rats.

Table 1. Effect of ethanol (0·6 or 2·5 g kg^{-1} intragastrically) on urine output of non-stressed and stressed (30 mins of immobilization) hydrated (27 ml kg^{-1}, intragastrically) rats. Mean urine volume (ml/3 h) ± s.e.m. for groups of 7 rats is presented.

Treatment	Non-stress	Stress
	Urine volume (ml/3 h)	
Saline	4·01 ± 0·25	3·10 ± 0·29[1]
Ethanol (g kg^{-1})		
0·6	4·63 ± 0·31	4·82 ± 0·35[2]
2·5	6·5 ± 0·49[2]	8·50 ± 0·81[1,2]

[1] $P<0·05$ compared with the corresponding non-stress group.
[2] $P<0·01$ or less compared with corresponding saline-treated group.

However, the greater efficacy of ethanol in stressed rats decreased when animals were stressed repeatedly (Table 2). Animals stressed daily for 90 min for 1 or 4 days had a lower urine output compared with non-stressed rats. Ethanol increased urine volume by 69% in 4 day-stressed rats compared with 187% in acutely stressed rats and 59% in non-stressed rats. These results indicate development of tolerance ($F = 9·9$, $df = 2,24$; $P<0·01$).

The diuretic effect of ethanol in cold stressed animals was also examined. The rationale was twofold. The first aim was to examine the generality of the effect of stress as observed in restrained rats. The second, was to determine whether ethanol-induced changes in temperature might affect the diuretic response itself. Ethanol produces marked hypothermia (Freund 1973; Pohorecky & Jaffe 1975), and cold can affect diuresis (Talso et al 1948), therefore it was necessary to determine whether the ethanol-induced hypothermia could alter the diuretic effect of ethanol. Ethanol-induced hypothermia can be modified by environmental temperature (Table 3, Pohorecky & Rizek 1981). The hypothermia can be blocked by exposing rats to an environmental temperature of approximately 30 °C. Conversely the hypothermia can be exacerbated by exposing rats to environmental temperatures below room temperature.

Table 2. Development of tolerance to the effects of ethanol (2·5 g kg^{-1}) on urine output of intragastrically hydrated non-stressed rats, and rats stressed by immobilization (30 min/day) once or four times. Data represents mean total urine volume (ml) for a 3 h collection period ± s.e.m. for groups of 7 rats.

	Saline	Ethanol (2·5 g kg^{-1})
	Urine volume (ml/3 hours)	
Non Stress	4·21 ± 0·30	6·70 ± 0·59[1]
Stress—1 day	3·11 ± 0·25[2]	8·92 ± 0·75[1,2]
Stress—4 days	3·75 ± 0·29	6·35 ± 0·55[1]

[1] $P<0·01$ or less compared with corresponding saline treated group.
[2] $P<0·01$ compared with corresponding non-stressed group.

In saline-treated animals, compared with the rats kept at 22 °C, cold (15 °C) but not heat (30 °C) stressed animals showed a significant increase in diuresis in response to the fluid load. Urine volume of ethanol-treated rats and not significantly affected by temperature. Compared with saline-treated rats, ethanol diuresis was less marked in heat-stressed rats then in rats kept at 22 °C and 15 °C (Table 3). It is possible that rats kept at 30 °C had less of an effective fluid load since they may have lost fluid through evaporative loss (e.g. saliva) which was not controlled.

Ethanol produced statistically significant hypothermia in rats kept at 15 ° and 22 °C, but not in rats kept at 30 °C. However, there was no apparent relation between the effect of ethanol on temperature and diuresis. Thus the greatest effect of ethanol on diuresis was seen at 22 °C, while the greatest hypothermia was noted at 15 °C. On the other hand no hypothermia, and the least degree of diuresis, was found at 30 °C. These results suggest that it is unlikely that ethanol-induced changes in body temperature have a major influence on the control of urine output in ethanol-treated rats.

Table 3. Comparison of ethanol (2·5 g kg^{-1} intragastrically) diuresis in rats exposed to different ambient temperatures in environmental chambers. Urine volume was collected for 3 h post-treatment. Rectal temperature was measured in the same group of animals given the same intragastric ethanol or saline treatments 3 days after the diuresis experiment. Data are presented as means ± s.e.m. for a group of 7 rats.

	Total urine output (ml)		Change	Rectal temp. (°C)	
Temp.	Saline	Ethanol	%	Saline	After ethanol
15 °C	7·1 ± 0·4[2]	13·6 ± 1·8[1]	91·5	37·7 ± 0·2	−2·6 ± 0·2[1]
22 °C	4·7 ± 0·9	9·8 ± 0·8[1]	108·8	37·5 ± 0·2	−1·0 ± 0·2
30 °C	6·4 ± 1·2	10·6 ± 1·2[1]	65·3	38·4 ± 0·3[2]	−0·1 ± 0·1

[1] $P < 0·01$ or less compared with the corresponding saline group.
[2] $P < 0·01$ compared with the corresponding group of rats at 22 °C.

The next study was to delineate other possible mediators of the action of ethanol on urine output. Ethanol markedly elevates adrenocortical hormones (Ellis 1966; Pohorecky & Jaffe 1975). Adrenocortical hormones have been reported to influence plasma vasopressin levels in rats (Seif et al 1978). I therefore examined ethanol diuresis in rats deprived of adrenal hormones. Adrenalectomy had no significant effect on urine output in response to saline infusion. In sham operated animals, ethanol significantly elevated urine output, as we had seen before. Total urine output was elevated 96% by ethanol. In adrenalectomized rats the effect of ethanol on urine volume was similar to that in sham operated rats (Table 4). Blood ethanol levels of the adrenalectomized and sham operated animals given ethanol injections were not statistically different (235·3 ± 12·9 mg % and 245·0 ± 15·1 mg % respectively, 1 h post treatment). It may be concluded from this study that adrenal hormones do not significantly influence ethanol-induced diuresis.

Table 4. Effect of adrenalectomy or sham operation on urine output in response to ethanol (2·5 g kg^{-1} sham and 2·12 g kg^{-1} for adrenalectomized) or saline given gastrically. Urine was collected for 3 h, mean ml ± s.e.m. for groups of 7 animals is presented.

Treatment	Urine volume (ml/3h)
Saline/sham	4·81 ± 0·33
Ethanol/sham	9·46 ± 0·84[1]
Saline/adrenalectomized	4·23 ± 0·25
Ethanol/adrenalectomized	10·01 ± 0·67[1]

[1] $P < 0.001$ compared with the corresponding saline-injected control group.

Discussion

The experiments in stressed rats support earlier findings in man. Bennett et al (1964) and Kozlowski et al (1967) reported that ethanol ingestion inhibited the antidiuresis produced by stress of pain or of physical exercise. The dose-dependent effect of ethanol on urine output in stressed animals was similar to the interaction of ethanol and stress (Pohorecky et al 1980; Brick & Pohorecky 1982, 1983). While ethanol produced diuresis, stress, on the other hand, decreased urine volume. This suggests that stress and ethanol affect urine output through different mechanisms. In fact, in contrast to the inhibition of AVP produced by ethanol, stress has been found to elevate blood AVP levels (Konzett et al 1971; Knepel et al 1982; Zbuzek et al 1983). This is surprising since others reported that adrenal cortical hormones and the potent synthetic glucocorticoid, dexamethasone, exert a tonic inhibitory input on factors controlling AVP release (Ahmed et al 1967; Knepel et al 1982). Furthermore, it is apparent that the action of ethanol does not involve adrenal hormones (Table 3).

Ethanol-induced hypothermia is known to alter biochemical and behavioural effects of ethanol (Pohorecky & Rizek 1981). However, the diuretic effect of ethanol did not appear to be greatly influenced by ethanol-induced hypothermia or by changes in environmental temperature. Since these ethanol-related factors did not play a major role in the diuretic action of ethanol, other mechanisms must be explored.

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