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Activity Stress Effects on Voluntary Ethanol Consumption, Mortality and Ulcer Development in Rats

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ROCKMAN, G E AND G B GLAVIN. *Activity stress effects on voluntary ethanol consumption, mortality and ulcer development in rats* PHARMACOL BIOCHEM BEHAV 24(4) 869–873, 1986 —The present study was designed to investigate the relationship between activity stress, alcohol consumption and ulcer proliferation. Ethanol consuming rats were initially divided into low, medium or high ethanol preferring groups on the basis of daily ethanol intake (g/kg/day). Following a habituation period in activity cages, animals were fed for 1 hr per day Access to both water and ethanol remained ad lib. Yoked control home cage animals were fed the same amount of food consumed by their wheel-housed partners This procedure continued until wheel-housed animals died, at which time they and their yoked home cage control partners were examined for ulcers Results indicated that in contrast to the yoked controls, only the high ethanol-preferring rats reduced their ethanol consumption Although no differences were apparent in ulcer frequency (mean number of ulcers per rat) or severity (mean cumulative ulcer length in millimeters), animals exposed to ethanol had a lower ulcer incidence (number of rats per group developing ulcers) and mortality rate than non-ethanol exposed animals

Activity stress Ethanol Ulcer

DESPITE considerable research designed to examine the interaction between stress and alcohol, much of these data are contradictory [15]. One area of investigation concerns the effects of ethanol on the pathophysiological consequences of exposure to stress. For example, while ethanol has been shown to have a preventative effect against stress-induced alterations in plasma corticosterone [3], brain monoamines [10], and plasma non-esterified fatty acids [16], other studies have demonstrated either a potentiation or an absence of stress-induced gastric mucosal injury [11, 18, 19]. Another aspect of research designed to elucidate the potential interaction between stress and ethanol is that of studying the effects of various stressors on ethanol intake in animals. In this area as well, the results have been equivocal For example, while it was originally demonstrated that stress increased ethanol consumption in cats [12], few subsequent studies have found similar findings [6], and in addition, numerous studies found no relationship between stress and ethanol consumption (for a comprehensive review see [15]). The present study was designed to investigate the interaction between long-term voluntary ethanol intake and chronic activity-wheel stress in rats. This procedure has been consistently and reliably shown to be an effective procedure for inducing gastric ulcer and has become a standard technique for producing chronic stress responses [13]. The long term ethanol free-choice drinking paradigm was employed as a more physiological (approximating human consumption) means of ethanol administration.

METHOD

Animals

Eighty-six male Wistar rats (Holtzman Co., Madison, WI) weighing 200±10 g on delivery were used. Animals were housed individually in standard lab cages with food and water available ad lib and with a 12·12 L/D cycle (lights on 0700 hr)

Procedure

After 7 days of adaptation, the ethanol screening procedure began. Two calibrated drinking tubes were attached to the front of each animal's cage. One contained water while the other initially contained ethanol in a concentration of 3% (v/v). This concentration of ethanol was presented every other day for 8 days; that is, every other day the animals received two tubes of water and on alternate days, they received one tube of ethanol and one tube of water. The position of the ethanol tube was changed upon each presentation to eliminate the possibility of a position preference by the

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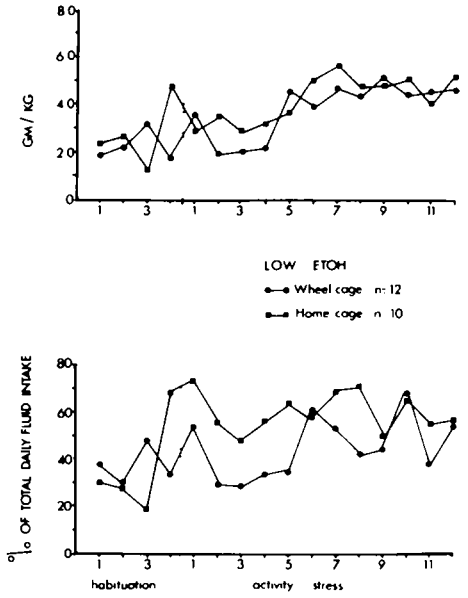

FIG 1 Ethanol consumption in terms of mean grams per kilogram and percent of total daily fluid intake for low-ethanol consuming animals

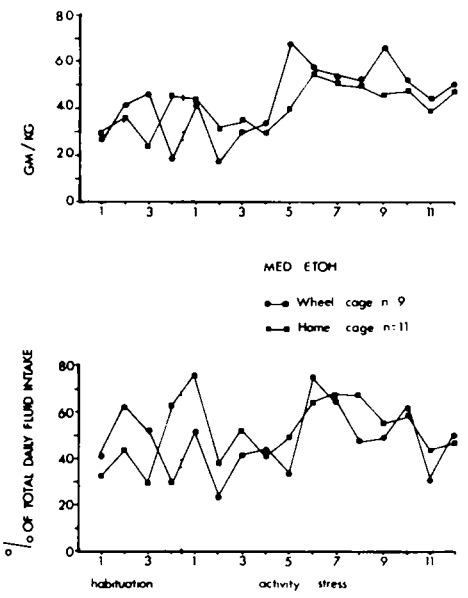

FIG 2 Ethanol consumption in terms of mean grams per kilogram and percent of total daily fluid intake for medium ethanol consuming animals

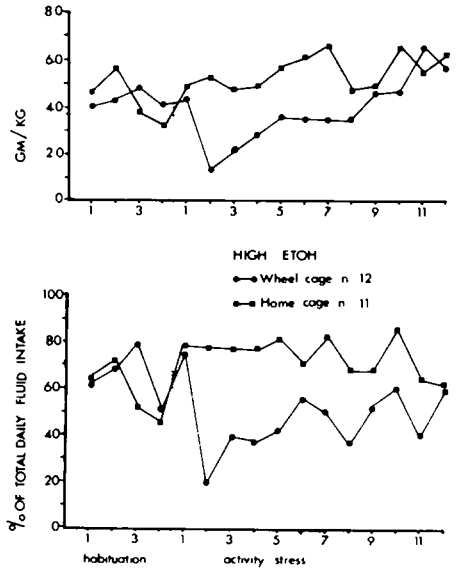

FIG 3 Ethanol consumption in terms of mean grams per kilogram and percent of total daily fluid intake for high ethanol consuming animals

rats The same alternate day presentation was continued for ethanol in increasing concentration of 5%, 7%, and finally 9% (v/v) At the end of screening procedure, all animals received the 9% (v/v) ethanol concentration (plus a tube of water) for seven consecutive days Therefore, the animals were exposed to ethanol for a total of 40 days prior to being placed in the wheel-cages Daily fluid consumption (both ethanol and water) and body weight were monitored and daily ethanol intake in grams per kilogram per day was calculated In this manner, four groups of rats were selected Non-ethanol exposed rats were never given ethanol, and had two tubes of water present throughout this phase of the study Low ethanol-preferring, medium ethanol-preferring, and high ethanol-preferring groups of rats were defined by their daily consumption of ethanol (1 5–2 5 g/kg/day, 2 5–4 5 g/kg/day, and 4 5–6 0 g/kg/day, respectively) It should be noted that these group designations were based upon average daily ethanol consumption throughout the entire 40 day screening period

Activity Stress

Experimental rats were individually housed in activity-wheel cages for a 4 day habituation period during which time food (granular Purina Rat Chow), water and ethanol (9%) were available ad lib The doors to the activity wheels were closed, preventing access to the running wheel In addition, at this time, control animals were assigned to similar ethanol groups (high, medium, low and non-ethanol exposed) but were housed individually in standard lab cages without activity wheels and placed in the same animal room These animals served as the food-yoked partners for the rats in the activity cages Following the habituation period, food was withdrawn at 0900 hr and the doors to the activity wheels were opened On the first day of the activity stress period

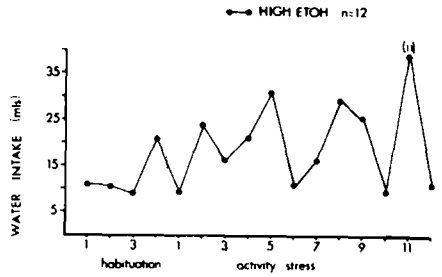

FIG 4 Water intake (ml) for high ethanol consuming animals in activity wheels

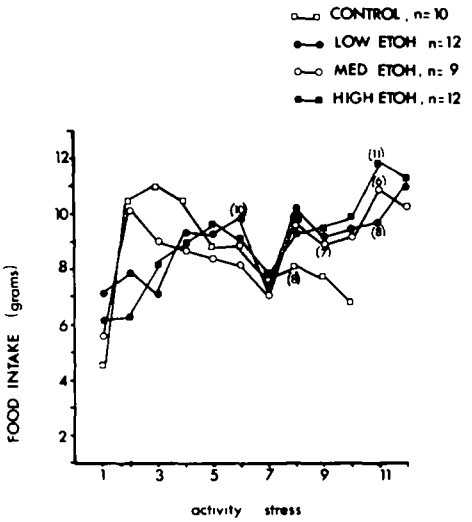

FIG 5 Food intake (g) for all animals during activity stress period

and on each succeeding day (until day 12), all wheel-housed rats were fed for 1 hr between 0900 and 1000 hr Body weight, 24 hr activity, water and ethanol consumption were recorded daily for each animal Each day following the 1 hr feeding period the amount of food consumed was measured and the home cage food-yoked controls were fed the exact amount of food consumed by their wheel cage partner In this manner, only activity, and not time-restricted feeding, differs between the groups This procedure continued for a total of 12 days. If and when a wheel-housed animal died during the 12 day activity-stress period, that animal and its home cage partner were examined by removing their stomachs and preserving them with 10% (v/v) formalin All animals remaining after the 12 day activity-stress procedure were sacrificed (along with their home cage partners) and their stomachs excised, preserved in formalin, and examined for damage by an observer who was naive with respect to treatment conditions. The number, location (rumenal or glandular) and cumulative length (in millimeters) of the ulcers were ascertained by examination under a dissecting microscope with an ocular micrometer Ulcer length (in mm) was determined by adding length plus width of each glandular ulcer This procedure has been widely employed in the past to determine ulcer "severity" [8]

Statistical Analysis

Ethanol consumption for both wheel-housed and home cage-housed animals was calculated both in terms of mean percent of total daily fluid intake (% TDF) and mean grams per kilogram per day (g/kg/day). For the sake of clarity, the low, medium and high ethanol-consuming groups are presented separately, with appropriate control groups All data were analyzed by repeated measures analysis of variance [group (wheel housed vs home-caged) × time period (habituation, days 1–4, days 5–8 and days 9–12)], appropriate post hoc (Tukey) tests, and simple main effects analysis when interactions were significant. For the statistical analysis, the activity stress period was divided into 3 time periods of 4 days each to produce a total of 4 equivalent (including habituation) time periods

RESULTS

Figures 1 and 2 indicate that both low and medium ethanol-drinking groups in either home cage or wheel cage conditions did not significantly change their ethanol consumption over time. For low and medium ethanol-consuming groups, no main effects of group (wheel-housed vs home-caged housed) or of time period (habituation, days 1–4, days 5–8, days 9–12) or of groups × time period interactions were significant (group × time period: low etoh; % TDF, $F(3,60)=2.18$, $p>0$ 05, g/kg; $F(3,60)=0.06$, $p>0.05$; medium etoh; % TDF, $F(3,54)=1.38$, $p>0.05$, g/kg, $F(3,54)=0$ 11, $p>0.05$). In contrast, high ethanol-preferring animals' % TDF (Fig. 3) yielded a significant effect of group, $F(1,63)=9.48$, $p<0.005$, and of group × time period interaction, $F(3,63)=12$ 12, $p<0$ 01 In addition, for high ethanol-preferring animals' g/kg, only a significant group × time period interaction, $F(3,63)=3$ 10, $p<0.03$, was observed. More specifically, ethanol consumption for the wheel-housed animals significantly decreased during days 1–4 and 5–8 of the activity-stress period both in comparison to their pre-stress habituation period levels as well as to control group levels ($p<0$ 01). During days 9–12 of the activity-stress period, ethanol consumption returned to control group levels ($p>0$ 05) It is important to note that the reduction in ethanol consumption was not the result of a general reduction in fluid intake, since water intake for the high ethanol-preferring animals (Fig. 4) was not affected by activity stress relative to their water intake during habituation, $F(3,33)=0.01$, $p>0$ 05 Furthermore, a comparison of Figs. 3 and 4 further illustrates this point. Specifically, it can be noted that during the early phase of activity stress there was an inverse relationship between the animals' ethanol intake and water intake in that when ethanol intake was low the animals maintained (or increased) water consumption. Hence it is clear the activity stress specifically reduced ethanol consumption rather than

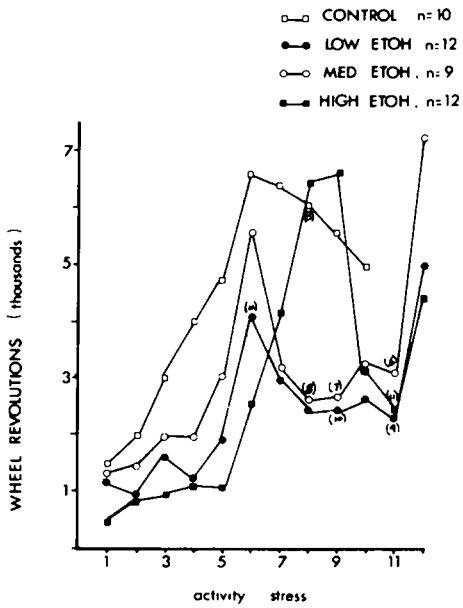

FIG 6 Wheel revolutions (thousands) for all groups during activity stress period

producing a general debilitating effect on the animals In addition, during the entire experiment no overt signs of intoxication were observed in any of the ethanol consuming animals. Food intake data for all groups during the activity stress period (days 1–4, 5–8 and 9–12) are illustrated in Fig 5 No significant differences were detected (group × time period $F(2,78)=1\ 43$, $p>0\ 05$)

Analysis of running wheel activity (days 1–4, 5–8 and 9–12, Fig 6) indicated a significant group × time period interaction, $F(2,78)=2\ 85$, $p<0.05$. Post hoc tests revealed that this interaction was the result of a difference in running-wheel activity during days 5–8 of the activity-stress period All ethanol-drinking groups differed significantly from non-ethanol-exposed wheel-cage controls ($p<0\ 01$). Because of high variability within groups, no other significant differences were detected

Stomach pathology data are summarized in Table 1 All data refer to glandular ulcers No instance of rumenal ulcer (indicative of starvation) was observed. No significant differences were found in either mean number of ulcers per animal or in mean cumulative ulcer length per animal. Ulcer incidence (number of rats per group developing ulcers), however, was affected, in that the incidence of ulcers in all ethanol-exposed groups was approximately 50% of that seen in control (non-ethanol-exposed) animals ($\chi^2=9\ 59$, $p<0.05$)

TABLE 1
SUMMARY OF STOMACH PATHOLOGY FOR EXPERIMENTAL AND CONTROL RATS

Group	Ulcer Incidence	Mean (±SE) Number of Ulcers (Ulcer frequency)	Mean (±SE) Cumulative Ulcer Length (mm) (Ulcer severity)
Wheel-housed High Ethanol	6/12	8 3 (4 7)	14 7 (6 9)
Wheel-housed Medium Ethanol	4/9	6 0 (2 6)	14 7 (8 6)
Wheel-housed Low Ethanol	5/12	7 9 (3 8)	15 2 (8 9)
Wheel-housed Water Only	10/10	10 2 (2 8)	16 4 (3 1)
Home Cage High Ethanol	1/11	0 09 (0 02)	0 27 (0 04)
Home Cage Medium Ethanol	0/9	0 0 (0 0)	0 0 (0 0)
Home Cage Low Ethanol	0/13	0 0 (0 0)	0 0 (0 0)
Home Cage Water Only	0/10	0 0 (0 0)	0 0 (0 0)

DISCUSSION

The results of the present study indicate that only the high ethanol-preferring rats (4.5–6.0 g/kg/day) reduced their ethanol consumption during activity-stress In addition, no significant differences were observed in ulcer severity (cumulative ulcer length) among all groups. However, it was noted that in all ethanol-consuming groups, ulcer *incidence* was approximately 50% and was markedly lower than the 100% incidence observed in non-ethanol-exposed rats

The reduction in ethanol intake among high ethanol-preferring rats may be related to the well-documented effects of stress on brain monoamine neurotransmitters More specifically, activity-stress has been shown to progressively and significantly accelerate NA turnover in many brain regions [20] After several days of such stress, NA synthesis cannot meet the demands of stress-induced excess NA utilization, thereby resulting in a net deficit of NA in many brain regions [2] Accordingly, it is possible that such a rapid utilization followed by a net reduction in brain NA activity was responsible for the observed decrease in ethanol intake in these animals. This explanation is also consistent with the data implicating NA in the mediation of alcohol self-administration [1,4] In particular, these authors demonstrated that inhibition of dopamine-beta-hydroxylase, which produces a reduction in brain NA level, reduced ethanol consumption. Thus, we suggest that the reduction in ethanol consumption observed in the high-preferring group was a result of the known neurochemical (NA) aberrations induced by the chronic activity-stress procedure

It is not clear, however, why the low and medium ethanol-preferring animals did not show a similar reduction in ethanol drinking during the activity-stress period One explanation concerns the amount of ethanol consumed by the low, medium, and high-preferring groups This may be relevant in light of the evidence indicating a direct effect of ethanol on brain monoamine function Specifically, ethanol has been shown to accelerate brain NA turnover [5,9] and, in some instances, reduce brain NA level using high ethanol doses. We suggest that the combination of high ethanol intake and exposure to chronic stress potentiated alterations

(reductions) in brain NA in these animals, resulting in decreased ethanol consumption. Because of the lower amount of ethanol consumed in the low and medium-preferring groups, such a potentiating effect did not occur in these groups

Another interesting finding of the present study was that stress ulcer incidence was reduced to approximately 50% in all ethanol-consuming groups (relative to the 100% incidence observed in control animals) In addition, ethanol-consuming rats exposed to activity-stress exhibited a very low mortality rate relative to controls. By the end of the study, all control animals had died, while the low, medium and high-preferring groups lost 1, 3, and 3 animals, respectively It thus appears that the presence of ethanol enhances survival probability in the activity-stress paradigm. Furthermore, unlike the effects of ethanol in acutely stressful situation [18], no potentiation of ulcer severity by ethanol was observed with chronic activity-stress Although ethanol is itself a gastric irritant and at high concentrations can induce gastric lesions [17], there is evidence to support a protective effect of ethanol against some stress-induced pathology including protection from accelerated brain monoamine turnover [7,10], and elevated plasma corticosterone [3,15]

Another explanation for the increased survival rats and decreased ulcer incidence observed among ethanol-consuming rats exposed to activity-stress concerns the caloric value of ethanol. Control animals exposed to activity-stress usually die in 5–7 days A clinical evaluation of these rats [14] revealed low blood glucose, high serum urea nitrogen, low serum protein and increased bilirubin, all suggesting that these animals had exhausted their metabolic substrate. Although activity-stressed animals have access to food for 1 hr each day, a typical feature of this model is that after several days, the rats largely ignore the feeding period In the present study, only the high ethanol-preferring rats decreased their ethanol consumption, and did so only for the first part of the stress period. It is possible that the caloric value of the ethanol consumed was sufficient to sustain life in some of these animals and to prevent the development of stomach lesions in this proportion of the animals. Studies are currently underway to investigate this possibility.

An additional result that deserves mention is the observation that in one phase of the activity-stress period (days 5–8), all ethanol consuming animals exhibited less running wheel activity compared to controls. It may be that the central depressant effect of ethanol was responsible for this finding. Less running is associated with increased survival in the activity-stress paradigm, and this may be the reason for the relatively low incidence of both ulcers and mortality among ethanol-preferring rats observed in this study. However, the extreme variability in running wheel activity observed among all groups including the control group, makes it difficult to ascertain whether ulcer incidence and mortality rate were related to running wheel activity Nonetheless, it is of interest to note the difference in the nature of the interaction between ethanol and chronic stress as observed in the present study and that between ethanol and acute stress as seen in our previous study [18]. It appears that ethanol exerts different effects depending upon the chronicity of the stress.

In summary, the present results suggest that (a) exposure to chronic stress reduced ethanol consumption only in high ethanol-preferring animals, and (b) the presence of physiological doses of ethanol was associated with increased survival and lowered ulcer incidence among all ethanol-preferring animals. We are examining these phenomena in animals selectively depleted of NA and 5-HT in order to verify the neurochemical concomitants of the interaction between stress and ethanol consumption

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