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Effects of Restraint Stress on Voluntary Ethanol Intake and Ulcer Proliferation in Rats

G. E. ROCKMAN,[1] A. HALL AND G. B. GLAVIN[2]

Department of Psychology, University of Winnipeg, Winnipeg, MB., Canada R3B 2E9

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ROCKMAN, G. E., A. HALL AND G. B. GLAVIN. *Effects of restraint stress on voluntary ethanol intake and ulcer proliferation in rats.* PHARMACOL BIOCHEM BEHAV **25**(5) 1083–1087, 1986.—The present study examined the effect of exposure to a schedule of predictable restraint stress on voluntary ethanol consumption and ulcer proliferation in rats. Following ethanol screening rats were divided into high, medium and low ethanol consuming groups on the basis of daily ethanol intake (g/kg/day) and exposed to daily 1 hr restraint stress for 10 consecutive days. Voluntary ethanol consumption was monitored both during the stress period and for an additional 25 days post-stress. Stomach pathology was assessed on days 1, 5 and 10 of the stress period as well as at the conclusion of the post-stress period. Results indicated a differential effect of stress on ethanol intake in that high ethanol preferring rats consumed less ethanol in the first 5 days of the post-stress period as compared to non-stressed controls. In contrast, the medium ethanol preferring group drank more ethanol than controls during days 1–5 of the post-stress period. Ethanol consumption for the low ethanol groups did not change during the entire experiment. Stomach pathology data revealed no ulcer formation in the stressed groups during the stress period. At the end of the post-stress period, however, stressed animals exhibited a significantly greater ulcer severity (mean cumulative ulcer length) and ulcer frequency (mean number of ulcers per rat) than non-stressed groups. Stomach pathology for ethanol consuming groups did not differ from controls, indicating that ethanol did not, by itself, affect ulcer development.

Restraint stress Ethanol Ulcer

CONSIDERABLE research has been conducted examining the interaction between stress and ethanol consumption. Unfortunately, much of the information emanating from these studies is contradictory [11]. 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 level [1], brain monoamine activity [6], and plasma non-esterified fatty acid level [12], other studies have demonstrated either a potentiation or no effect of ethanol on stress-induced gastric mucosal injury [5, 7, 14–16].

Another aspect of the stress-ethanol interaction concerns the effect of various stressors on ethanol consumption. For example, while it was originally demonstrated that stress increased ethanol consumption in cats [8], few subsequent studies have found similar results [2,3]. In two recent studies, contrasting effects of stress on ethanol intake were observed. Rockman and Glavin [15] demonstrated that activity stress induced a decrease in ethanol consumption among high ethanol preferring rats during the stress period. Medium and low ethanol consuming groups did not alter their ethanol intake. In contrast, Nash and Maickel [9] observed an increase in ethanol consumption in rats which were exposed to a schedule of unpredictable immobilization stress. It is interesting to note that this increase in ethanol intake occurred during the post-stress period. Unfortunately, in this latter study, ethanol consuming animals were not grouped according to different levels of ethanol intake. These two studies serve to highlight the contradictory results in this area as well as the importance of assessing ethanol consumption of low, medium and high ethanol preferring rats during both the stress and post-stress periods. The present study was designed to investigate the effect of exposure to chronic restraint stress on long-term voluntary ethanol consumption and ulcer proliferation during stress and post-stress periods.

METHOD

Subjects

Male Wistar rats (Holtzman Co., Madison, WI) weighing 190–210 g on delivery were used. Rats 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).

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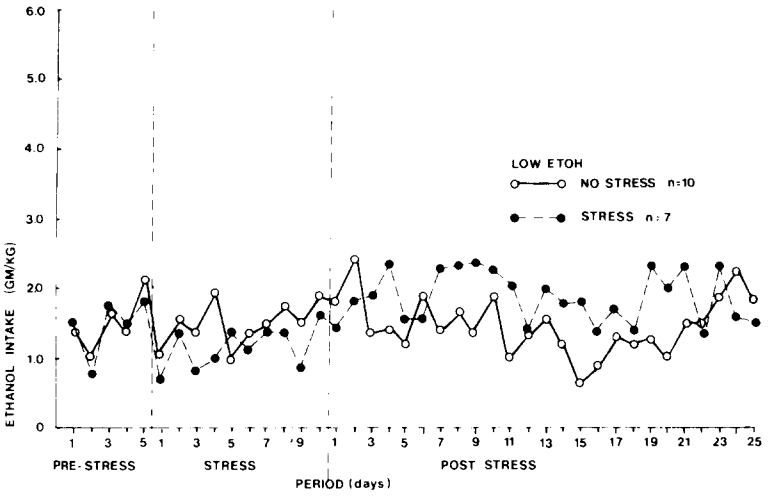

FIG. 1. Ethanol consumption in terms of mean grams per kilogram for low-ethanol consuming rats.

Procedure

Ethanol screening. After 5 days of adaptation, the ethanol screening procedure began. Two calibrated drinking tubes were attached to the left front of each rat's cage. One contained tap water while the other initially contained a 3% (v/v) solution of ethanol. This concentration was presented every other day for 8 days; that is, every other day the rats received two tubes of water and on alternate days, they received one tube of water and one tube of ethanol. The position of the ethanol tube was changed upon each presentation to eliminate the possibility of a position preference by the rats. The same alternate day presentation was continued for ethanol in increasing concentrations of 5%, 7% and finally 9% (v/v). At the end of the screening procedure, all rats received the 9% (v/v) ethanol in an everyday free-choice with water for 20 consecutive days. Therefore, the rats were exposed to ethanol for a total of 36 days prior to restraint. 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 access to water throughout. Low, medium and high ethanol preferring groups were defined by their mean daily ethanol consumption (1.5–2.5; 2.5–4.5; 4.5–6.0 g/kg/day, respectively) throughout the entire 36 day screening procedure.

Restraint stress. Immediately following the last day of the ethanol screening, half of the rats (stressed group) from each of the 4 groups were randomly selected and exposed to 1 hour of restraint (at approximately 1100 hr) in the supine position at room temperature [4] for a total of 10 days. The other half of the rats (non-stressed groups) were moved into an adjoining room and handled at the beginning and at the end of the 1 hour daily stress periods but were not restrained. This procedure ensured that both restraint stressed and control rats received equal amounts of handling *per se*. Both stressed and non-stressed rats were denied access to ethanol, water and food for that hour only. Following daily stress periods, all rats were returned to their home cages with food, water and ethanol (9%) available ad lib. Body weight, water and ethanol consumption were monitored daily, both during the 10 day stress period and during a subsequent 25 day post-stress period.

Stomach pathology. Four rats from each group were sacrificed with chloroform on days 1, 5 and 10 of the stress period. The remaining rats were sacrificed on completion of the post-stress period. Stomachs were excised, preserved in 10% formaldehyde 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 [4].

Statistical Analysis

Ethanol consumption for all groups was calculated in terms of 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 the appropriate control groups. For statistical analysis, the stress and post-stress periods were divided into 7 time periods of 5 days each to produce a total of 8 equivalent (including pre-stress) time periods. All data were analyzed by repeated measures analysis of variance [group (stress vs. no-stress) × time period], appropriate post hoc (Tukey) tests, and simple main effects analysis when interactions were significant.

RESULTS

Figures 1, 2 and 3 illustrate ethanol consumption for low, medium and high ethanol drinking groups respectively. Figure 1 indicates that low ethanol consuming groups (stressed and non-stressed) did not change their ethanol intake throughout the entire experiment. No main effects of group, time period or group × time period interactions were significant (group × time period, $F(7,105)=1.91$, $p>0.05$). In contrast, ethanol consumption for the medium ethanol groups

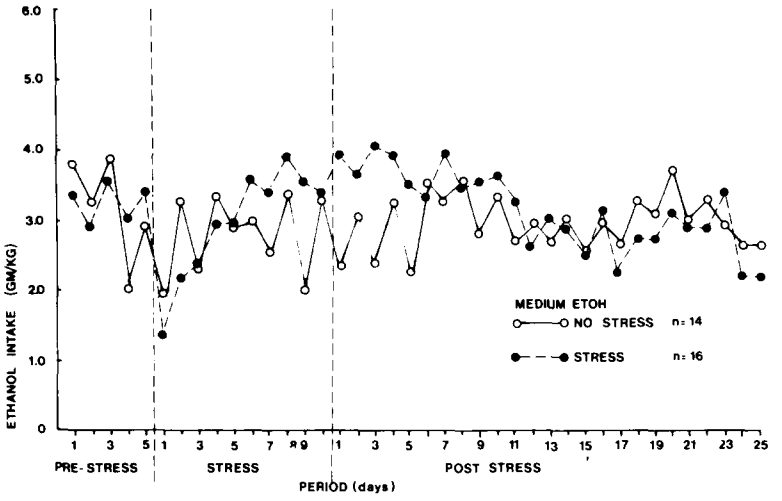

FIG. 2. Ethanol consumption in terms of mean grams per kilogram for medium-ethanol consuming rats.

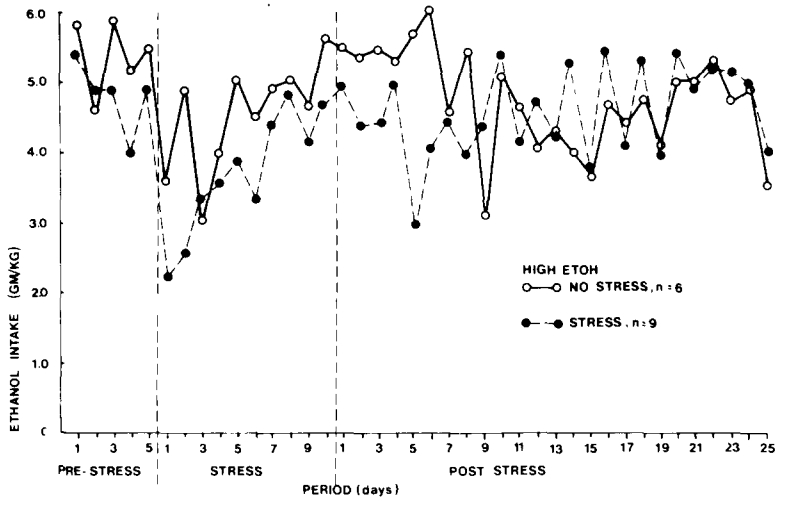

FIG. 3. Ethanol consumption in terms of mean grams per kilogram for high-ethanol consuming rats.

(Fig. 2) yielded a significant effect of period, $F(7,196)=3.97$, $p<0.001$, and of group × time period interaction, $F(7,196)=3.28$, $p<0.005$. More specifically, post-hoc Tukey tests revealed that ethanol consumption for the stressed rats significantly increased during days 1–5 of the post-stress period as compared to the non-stressed rats. No other 5 day drinking periods were significantly different between stressed and non-stressed groups. Analysis of ethanol intake for the high ethanol consuming groups (Fig. 3) indicated a significant effect of time period, $F(7,91)=5.04$, $p<0.001$, and of group × time period interaction, $F(7,91)=2.20$, $p<0.05$. Specifically, post-hoc Tukey tests indicated that during days 1–5 of the post-stress period ethanol consumption of the stressed rats was significantly lower as compared to the non-stressed group. No other significant differences in ethanol intake during any other 5 day periods were found.

It is important to note that body weight and water intake for all groups during the entire experiment did not significantly differ. In addition, no behavioral signs of overt intoxication were observed at any time in this experiment, suggesting that blood alcohol levels were below the maximal oxidizing capacity of these rats.

Examination of stomach pathology data from rats sacrificed on days 1, 5, and 10 of the stress period indicated no ulcer development in any of the stressed groups. Ulcer data from the remaining rats at the end of the post-stress period are illustrated in Table 1. All data refer to glandular ulcers; no instance of rumenal ulcer was observed. Results of the

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)
High Ethanol Stress	5/9	3.3 (2.02)	18.9 (9.68)
Medium Ethanol Stress	6/16	1.3 (0.53)	8.4 (4.35)
Low Ethanol Stress	4/7	1.6 (0.78)	9.7 (0.25)
Water Only Stress	1/6	0.8 (0.83)	8.5 (8.5)
High Ethanol No Stress	0/6	0.0 (0.0)	0.0 (0.0)
Medium Ethanol No Stress	1/14	0.07 (0.07)	0.4 (0.43)
Low Ethanol No Stress	1/10	0.2 (0.20)	0.6 (0.60)
Water Only No Stress	2/6	0.3 (0.21)	4.3 (3.59)

analysis of variance of mean ulcer length (ulcer severity) for stressed groups and non-stressed groups indicated a significant effect of stress, $F(1,66)=6.50$, $p<0.02$, but no significant effects of ethanol on ulcer severity. Similarly, stressed groups had significantly more ulcers (ulcer frequency) than non-stressed groups, $F(1,30)=9.41$, $p<0.005$, with ethanol exposure not affecting mean number of ulcers per rat. Finally, ulcer incidence (number of rats within a given group which developed ulcers) did not significantly differ between stressed groups.

DISCUSSION

The results of the present study reveal several interesting observations. First, with regard to ethanol consumption, it was observed that the stressed medium ethanol-preferring group demonstrated a temporary increase in ethanol consumption during the first 5 days of the post-stress period as compared to the non-stressed control group. This increase in ethanol consumption is consistent with the recent report of Nash and Maickel [9]. In contrast, ethanol consumption for the stressed high ethanol-preferring group was observed to be lower than controls during the same post-stress period (days 1–5 post-stress). This observation, while discrepant from the medium ethanol group and other reports [3, 8, 9], is consistent with recent data from our laboratory [15] demonstrating a decrease in ethanol consumption among high ethanol-consuming rats as a consequence of chronic stress. These data considered together suggest that the effect of chronic stress on ethanol consumption may differ depending on the level of ethanol intake during baseline periods. This result is also consistent with the observation that the extent of ethanol exposure seems to interact with stress responsivity [5,14]. In addition, these results serve to highlight the importance of assessing the effects of stress on various levels of ethanol intake (that is low, medium and high), since differential effects of stress on ethanol drinking may be obscured by combining data from rats consuming different levels of ethanol. A mechanism for this observation is not clear at the present time, but research in this laboratory is being conducted to investigate possible neurochemical alterations underlying the effects of stress on ethanol consumption.

Another interesting aspect of the present results concerns the relatively small change in ethanol drinking as compared to other reports [9,15]. The most likely explanation for this observation seems to be a consequence of the stress paradigm employed in this experiment. Restraint stress in the present study was delivered on a predictable schedule which has been demonstrated to be less "stressful" than an unpredictable schedule of stress presentation [10,13]. The fact that no ulcers were observed in the stress groups during the stress period supports this view. A replication of the present study employing an unpredictable stress schedule is underway.

The ulcer data from the present study indicating that ethanol did not affect ulcer number or ulcer severity, are consistent with other reports which suggest that ethanol, in certain stress paradigms, may not affect ulcer proliferation [5, 7, 15]. That we observed ulcer formation only at the conclusion of this study, suggests that a schedule of predictable stress is not ulcerogenic, whereas the initial days of the post-stress period may actually be perceived by the rats as an unpredictable stressor thereby inducing ulcers.

In summary, the present results suggest that (1) exposure to predictable restraint stress produces differential effects on ethanol consumption depending upon the rat's pre-stress level of ethanol intake levels and (2) that ethanol exposure does not alter stress responsivity to predictable stress.

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