ADAPTATION TO REPEATED RESTRAINT STRESS IN RATS: FAILURE OF ETHANOL-TREATED RATS TO ADAPT IN THE STRESS SCHEDULE

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Abstract — Adaptation to a repeated restraint stress schedule was monitored in ethanol-treated and control rats. A single episode of 2 h restraint decreased food intake in both control and ethanol-treated rats. The decreases in control rats were not observed following the 5th daily restraint of 2 h/day, suggesting that adaptation has occurred. Ethanol-treated rats, however, exhibited decreased food intake even after 5th daily restraint of 2 h/day. Ethanol administration decreased weekly but not daily cumulative food intake in unrestrained rats. Food intakes of ethanol-treated and control restrained rats were comparable following 1st-3rd daily restraints, but were smaller in ethanol-treated rats following the 4th and 5th daily restraints. Open-field ambulatory activities monitored 24 h after the 5th daily restraint on the 6th day were comparable in control restrained and unrestrained rats. Ethanol-treated and control unrestrained rats also exhibited comparable ambulation, but ethanol-treated restrained rats exhibited smaller activity than control restrained or ethanol-treated unrestrained rats. Fluid intakes of ethanol and control rats were comparable during the 2 weeks of ethanol administration, but daily restraint schedule decreased ethanol intake. The findings show adaptation to repeated restraint in control rats and inability of ethanol-treated rats to adapt in the stress schedule. These findings imply that excessive alcohol consumption may impair adaptation to stress and thus conceivably precipitate depression.

INTRODUCTION

The prevalence of depression in alcoholic patients has long been regarded to be greater than in the general population (Woodruff et al., 1973). Symptoms associated with alcohol dependence are often very similar to those of depression (Roy et al., 1987; Virkkunen et al., 1989; Buydens-Branchey et al., 1989), but the relationship between the two disorders is not very clear.

Stress life events precipitate depression (Brown et al., 1987). It is often proposed that alcohol drinking is reinforced as a self-medication because it reduces tension and perception of stress stimuli (Smail et al., 1984). However, depression and alcoholism often co-exist. On the one hand, excessive alcohol consumption can lead indirectly to depression or anxiety. On the other hand, alcohol can be used, especially by males, to cope with depression (Berger and Adesso, 1991).

Studies on experimental animals show that an episode of 2 h restraint, an uncontrollable stress, produces marked anorexia in rats (Kennett et al., 1985; Haleem et al., 1988; Haleem and Parveen, 1994). Open-field ambulation monitored 24 h later was also decreased (Kennett et al., 1985; Haleem et al., 1988). On repeated immobilization, these behavioural deficits were no longer observed. These studies suggested that behavioural adaptation to a stress schedule develops when the same stress is administered repeatedly (Haleem and Parveen, 1994). In order to investigate a possible role of alcohol intake in the precipitation of stress-induced depression, the present study was designed to evaluate behavioural adaptation to repeated restraint in ethanol and control (water-treated) rats. Effects of daily restraints on ethanol intake were also monitored.

MATERIALS AND METHODS

Locally bred male albino Wistar rats weighing 230–250 g, purchased form PCSIR laboratories, Pakistan, were housed individually under a 12 h light/dark cycle (lights on at 06:00) with free access to cubes of standard rodent diet and tap water for at least 3 days before experimentation.

Experimental protocol

Animals were randomly assigned to control and ethanol-treated groups. Ethanol was added in the drinking water for 2 weeks, in concentrations (v/v)
of 5% for the initial 2 days, 7% for the following 2 days and 10% for the remainder of the experimental period as described earlier (Haleem, 1990). Food intake, fluid intake and body weight of rats in both the groups were monitored weekly. Growth rate was calculated as percentage of the preceding week's body weight.

After 2 weeks of ethanol administration, both the ethanol-treated and control groups were further divided into unrestrained and restrained subgroups. Restraint consisted of daily immobilization for 2 h between 09:30 and 11:30 for 5 days. The animals of the two unrestrained sub-groups were left in their home cages during this period. Food intake and fluid (water or ethanol) intake were monitored daily. After 5 daily restraint periods of 2 h each day, ambulatory activities of all the animals in an open-field were monitored on the 6th day between 10:00 and 11:00 h.

Restrained procedure

Ethical conditions were maintained throughout the experiment. The animals were restrained by an approved procedure (Kennett et al., 1985; Haleem et al., 1988; Haleem and Parveen, 1994). Wire grids of 10" x 9" fitted with a perspex plate of 9" x 6.5" as described earlier (Haleem et al., 1988; Haleem and Parveen, 1994) were used. Immobilization was effected by pressing the forelegs of the rat through the gaps in the metal grid and taping them together with zinc oxide plaster. Hind limbs were also taped and the head of the animal rested on the perspex plate. At the end of the daily restraint period the animals were released and returned to their home cages.

Open-field activity

The open-field apparatus used in the present investigation consisted of a square area 76 cm x 76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity, a rat was placed in the centre square of the open field and the numbers of squares crossed with all four paws were scored for 5 min as described earlier (Haleem et al., 1988). Activities of control and ethanol-treated unrestrained and restrained animals were monitored in a balanced design to avoid order effect.

Statistical analysis

Data on the effects of ethanol administration on cumulative weekly food intake, fluid intake and growth rates were statistically tested by two-way ANOVA (repeated measure design). Effects of repeated restraint on daily changes of food intake and fluid intake in ethanol-treated and control rats were analysed by three-way ANOVA. Differences in the open-field activity of ethanol-treated and control restrained and unrestrained rats were analysed by two-way ANOVA. Post-hoc comparisons were done by Newman–Keuls test. P values >0.05 were considered insignificant.

RESULTS

Figure 1 shows the effects of 2 weeks of ethanol administration on cumulative weekly food intake (Fig. 1A), fluid intake (Fig. 1B) and growth rates (Fig. 1C). Two-way ANOVA (repeated measure design) performed on weekly cumulative food intake values (Fig. 1A) showed significant treatment (F = 45, d.f. 1, 22; P < 0.01) and insignificant weekly measure (F = 3.7, d.f. 1, 22; P > 0.05) effect. Interaction between the two factors (F = 0.13, d.f. 1, 22; P > 0.05) was also insignificant. Post-hoc analysis showed that ethanol administration decreased weekly food intakes. Two-way ANOVA (repeated measure design) performed on weekly fluid intake values (Fig. 1B) showed insignificant treatment (F = 0.01, d.f. 1, 22; P > 0.05), weekly measure (F = 0.14, d.f. 1, 22; P > 0.05) effect and insignificant interaction (F = 0.38, d.f. 1, 22; P > 0.05) between the two factors. Two-way ANOVA (repeated measure design) performed on weekly growth rate values also revealed insignificant treatment (F = 1.3, d.f. 1, 22; P > 0.05), weekly measure (F = 2.9, d.f. 1, 22; P > 0.05) effect and insignificant interaction (F = 0.04, d.f. 1, 22; P > 0.05) between the two factors.

Figure 2 shows the effects of repeated restraint stress on daily changes of food intake in control (water-treated) and ethanol-treated rats. Three-way ANOVA showed significant stress (F = 14.6, d.f. 1, 100; P < 0.01), ethanol administration (F = 79, d.f. 1, 100; P < 0.01) effect and an insignificant day factor effect (F = 1.1, d.f. 4, 100; P > 0.05). Interactions between stress*ethanol (F = 4.6, d.f. 1, 100; P < 0.05), day*ethanol (F = 9.7, d.f. 4, 100; P < 0.01) and day*stress (F = 20.3, d.f. 4, 100; P < 0.01) were significant. Ethanol*stress*day interaction was insignificant.
Fig. 1. Effects of 2 week ethanol administration on weekly cumulative food intakes (A), fluid intakes (B) and growth rates (C). Values are means ± SD (n = 12) of control (water-treated; filled column) and ethanol-treated (open column) rats. Significant difference by Newman–Keuls test: *P < 0.05 from control (water-treated) rats following two-way ANOVA (repeated measure design).

(F = 1.9, d.f. 4, 100; P > 0.05). Post-hoc analysis showed that an episode of 2 h restraint stress given on the 1st day decreased 24 h cumulative food intakes of water-treated rats. The decreases were attenuated following 2nd and 3rd day restraints and were not significant after the 4th day restraint. The deficits of food intake were not observed following the 5th day restraint. The episode of 2 h restraint given on the first day also decreased 24 h cumulative food intake in ethanol-treated rats. The decrease was slightly attenuated following the 2nd day restraint, but there was no further decrease following 3rd, 4th and 5th day restraints. Mean values of daily food intake were smaller in ethanol-treated unrestrained rats. The differences were insignificant by Newman–Keuls test. Mean values of daily food intakes were also smaller in ethanol-treated restrained than in water-treated restrained rats. A post-hoc test showed that the differences were significant following 4th and 5th day restraints.

Figure 3 shows the effects of daily restraints on water and ethanol intake of rats. Three-way ANOVA showed significant stress (F = 83.1, d.f. 1, 100; P < 0.01), ethanol administration (F = 47.4, d.f. 1, 100; P < 0.01) and day factor (F = 2.6, d.f. 4, 100; P < 0.05) effect. Interaction between stress*ethanol (F = 47.4, d.f. 1, 100; P < 0.01) was significant. Day*ethanol (F = 0.59, d.f. 4, 100; P > 0.05) and day*stress
Fig. 2. Effects of restraint on daily food intake in control (A) and ethanol-treated (B) rats. Values are means ± SD (n = 6) of unrestrained (continuous line) or restrained (broken line) rats. Significant differences by Newman-Keuls test: $P < 0.01$ from respective unrestrained rats; $^{*}P < 0.01$ from control (water control treated) restrained rats following three-way ANOVA.

(F = 0.65, d.f. 4, 100; P > 0.05) interactions were insignificant. Stress*ethanol*day interaction ($F = 3.18$, d.f. 4, 100; $P < 0.05$) was significant. Mean values of daily water intake following 2nd–5th day immobilizations were greater in restrained than unrestrained rats. Post-hoc analysis showed that the increases were not significant. On the other hand, mean values of ethanol intakes following 1st and 4th immobilization days were smaller in restrained than unrestrained rats. The decreases were significant following 2nd and 5th day immobilizations. Ethanol and water intakes of unrestrained rats, following 1st to 5th day immobilizations, were not significantly different. Fluid intakes of restrained rats following 2nd–5th day restraints were significantly smaller in ethanol treated than water treated rats.

Figure 4 shows open-field activity of ethanol-treated and control unrestrained and restrained rats monitored 24 h after the 5th day restraint of 2 h/day. Two-way ANOVA showed significant effect of ethanol administration ($F = 10$, d.f. 1, 20; $P < 0.01$) and 5 day restraint ($F = 12.2$, d.f. 1, 20; $P < 0.01$). Interaction between the two factors was insignificant ($F = 2.3$, d.f. 1, 20; $P > 0.05$). A post-hoc test showed that open-field ambulatory activities of ethanol-treated and control unrestrained rats were not different. Control rats restrained 2 h/day for 5 days and control unrestrained rats also exhibited comparable ambulatory activities. Ethanol-treated rats restrained 2 h/day for 5 days exhibited smaller ambulatory activity than control restrained rats or ethanol-treated unrestrained rats.

**DISCUSSION**

The effects of daily restraints on food intake in control (non-ethanol treated) rats (Fig. 2A) largely agreed with those of previous studies (Kennett *et al.*, 1985; Haleem *et al.*, 1988; Haleem and Parveen, 1994). Thus a single daily episode of
2 h restraint decreased food intake. The decreases were attenuated following the 2nd and 3rd day restraints and were not observed following 4th and 5th day restraints, suggesting that adaptation has occurred. In addition, the present study shows that following a single episode of 2 h restraint, food intake is also decreased in ethanol-treated rats. The deficits of food intake in ethanol-treated rats were, however, not normalized after the 5th day restraint (Fig. 2B), suggesting an inability of ethanol-treated rats to adapt in the stress schedule.

Administration of ethanol increases brain 5-hydroxytryptamine (5-HT) metabolism (Badawy, 1988; Haleem, 1990). Enhancing 5-HT function decreases food intake in experimental animals (Curzon, 1990; Haleem, 1993). In the present study, administration of ethanol for 2 weeks produced a significant decrease in cumulative weekly food intakes (Fig. 1A). Daily intakes were, however, not decreased by ethanol administration in unrestrained rats (Fig. 2A). Weekly decreases of food intake were only marginal and may reflect the caloric contribution of ethanol. Indeed, weekly changes of body weights (growth rates) were highly comparable in ethanol and control rats (Fig. 1C).

On the other hand, brain 5-HT metabolism is also decreased following 2 h restraint (Curzon et al., 1972; Joseph and Kennett, 1983; Haleem et al., 1988; Haleem and Parveen, 1994). Although increased metabolism or synthesis rate may not necessarily imply enhanced function, decreases of food intake following restraint stress are often explainable by increased brain 5-HT metabolism (Donohoe et al., 1987; Haleem et al., 1995). Thus a single episode of 2 h restraint increased 5-HT synthesis rate in the hypothalamus and decreased food intake of previously unrestrained rats (Haleem and Parveen, 1994). Conversely, anorectic effects of restraint did not occur in rats restrained 2 h/day for 5 days and an episode of 2 h restraint did not increase 5-HT synthesis rate in
Failure of ethanol-treated rats to adapt in the stress schedule is also evident from a comparison of food intake of ethanol-treated and control restrained rats (Fig. 2A and B). Restraint-induced decreases of food intakes following 1st–3rd day immobilizations were comparable in ethanol-treated and control rats. However, following 4th and 5th day immobilizations, food intake was smaller in ethanol-treated than control rats.

Administration of ethanol has been shown to modulate locomotor activity. Moderate to high doses of ethanol depress motor activity in rodents while low to moderate doses may have a stimulatory effect, particularly in mice (Carlsson et al., 1972; Lister, 1987; Smoothy and Berry, 1985). In the present study, ethanol administration for 2 weeks in drinking water was found to have no effect on open field ambulatory activity of rats.

Previously, it has been shown that restraint-induced deficits of open-field ambulatory activity do not occur in rats restrained 2 h/day for 5 days (Kennett et al., 1985; Haleem et al., 1988). In the present study, open-field ambulation of control unrestrained and restrained rats monitored 24 h after the termination of the 5th daily 2 h restraint were also comparable (Fig. 4). On the other hand, ethanol-treated restrained rats exhibited smaller ambulatory activity than ethanol-treated unrestrained rats or control restrained rats. These results also show an inability of ethanol-treated rats to adapt in the stress schedule.

Unlike food intake, that of water was not decreased following the episode of 2 h restraint. On the other hand, normalization of food intake in rats restrained 2 h/day for 5 days was associated with 29%, although insignificant, increase of water intake (Fig. 3A). Although free choice consumption of ethanol was decreased in rats exposed to immobilization stress (Suchitra et al., 1991), in the present study, a single episode of 2 h restraint produced only a small decrease of ethanol consumption, possibly because another choice was not available. The decreases were, however, potentiated and became significant particularly following 2nd and 5th day restraints (Fig. 3B). It is important to note that fluid intakes of ethanol-treated and control rats during the 2 weeks of ethanol administration were not different (Fig. 1B). Moreover, daily intakes of water and ethanol in the two groups of unrestrained rats were also comparable (Fig. 3A and B). These results show that restraint stress decreases ethanol consumption.

Pharmacological manipulations which tend to increase 5-HT function in the synaptic cleft have been shown to decrease ethanol consumption, whereas inhibition of 5-HT neurotransmission increased ethanol consumption in rats (Hill, 1974; Rockman et al., 1979; Daoust et al., 1984). Decreases of ethanol consumption following restraint as observed in the present study (Fig. 3A and B) may also occur due to an increase in 5-HT neurotransmission reported to occur following restraint in rats (Curzon et al., 1972; Joseph and Kennett 1983; Haleem and Parveen, 1994).

In conclusion, the present study shows that adaptation to a repeated restraint schedule does not occur in ethanol-treated rats, thereby suggesting that excessive alcohol consumption may impair adaptation to stress and thus conceivably precipitate depression. However, the present model does not appear to be relevant to the human use of alcohol to relieve stress, because intake of ethanol, but not water, was decreased following restraint stress.
REFERENCES


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