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Metyrapone-Induced Suppression of Corticosterone Synthesis Reduces Ethanol Consumption in High-Preferring Rats

CLAUDIA FAHLKE,* ERNEST HÅRD,* ROSITA THOMASSON,* JÖRGEN A ENGEL† AND STEFAN HANSEN*[1]

*Department of Psychology, University of Göteborg, P.O. Box 14158, S-400 20 Göteborg, Sweden
†Department of Pharmacology, University of Göteborg, Medicinaregatan 7, S-413 90 Göteborg, Sweden

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FAHKLE, C., E. HÅRD, R. THOMASSON, J. A. ENGEL AND S. HANSEN. *Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats.* PHARMACOL BIOCHEM BEHAV **48**(4) 977–981, 1994.—The fluid intake of male Wistar rats with simultaneous access to water and 6% ethanol was determined between 0900 and 1500 h. In high-preferring males (normally covering > 60% of their daily fluid consumption in the form of ethanol), two injections with the corticosterone synthesis inhibitor metyrapone (50 mg/kg) at 0900 h and 1200 h for 4 consecutive days significantly reduced ethanol preference such that they preferred water over alcohol. Treatment with corticosterone (0.6 mg/kg) 2 h before each metyrapone injection partially cancelled this effect of the synthesis inhibitor. By contrast, there was no significant effect of metyrapone treatment on the drinking of ethanol in low-preferring rats (normally covering < 30% of their daily fluid consumption in the form of ethanol). These results suggest that the adrenal secretion of corticosterone directly or indirectly modulates the intake of alcohol in high-preferring rats.

Ethanol preference Metyrapone Corticosterone Adrenal gland Drug abuse Hormone Chemical adrenalectomy

IT has recently become clear that steroid hormones can modulate several behavioral actions of drugs of abuse. For example, Robinson (23) showed that testicular secretions exert suppressive effects on amphetamine sensitization, and ovarian sex steroids modulate the self-administration of cocaine (22). Sex steroids also influence ethanol intake in the rat strain used in our laboratory (8), but their impact seems relatively minor compared with the large changes observed following endocrine manipulations involving the adrenal gland. Thus, Fahlke et al. [(9); see also (19)] recently found a dramatic and persistent decrease in alcohol drinking following adrenalectomy in the rat. Administration of corticosterone, the major corticosteroid in this species, restored ethanol intake to preoperative levels in adrenalectomized animals, whereas treatment with aldosterone was without significant effect (9). These findings, and the fact that ethanol activates adrenal hormone secretion (25,26), suggest that glucocorticoids, through an as yet unspecified mechanism, are involved in the regulation of alcohol intake in the rat.

The present experiment was concerned with the effect on alcohol drinking of the 11β-hydroxylase inhibitor, metyrapone, which blocks the formation of corticosterone (4,10, 12,15). It was predicted, on the basis of our previous study (9), that blockade of corticosterone synthesis would result in a lowered intake of ethanol. The results offer further support for the hypothesis that adrenal glucocorticoids regulate the consumption of alcohol in the rat.

METHOD

Subjects

Animals were 50 male Wistar rats purchased from Möllegaard Breeding Laboratories (Denmark). They were about 90 days of age at the beginning of the experiment, and were

[1] To whom requests for reprints should be addressed.

housed in an air-conditioned colony room at a temperature of 23°C, a humidity of 50–60%, under a reversed artificial light : dark cycle (lights off 1000–2200 h). The rats had free access to food pellets (Ewos AB, Södertälje, Sweden) and tap water throughout the experiment.

Procedure

Upon arrival in the laboratory, the animals were housed in groups of four per cage for 2 weeks to adapt to the novel laboratory conditions. During the ensuing 2-week period, the rats had continuous access to a second bottle containing an ethanol solution in addition to the water bottle. The ethanol concentration was gradually increased (2–4–6% w/v) over this period.

The animals were subsequently housed individually in clear plastic Macrolon 3 cages (45 × 30 × 16 cm), while having continued access to two bottles (plastic 300 ml bottles with ballvalve spouts; ALAB, Sweden) containing tap water or 6% ethanol solution for the remainder of the experiment. This particular ethanol concentration was chosen because in our experience it stimulates peak levels of consumption in the present strain of rats. Fluid intake was measured twice a week (Mondays and Thursdays) when the bottles were cleaned and refilled with fresh beverages. Body weight was recorded once a week. On the basis of their ethanol preference (i.e., proportion of ethanol intake relative to total fluid consumption in percent) during 3 weeks, 14 high-preferring (>60%) and 10 low-preferring (<30%) rats were selected for further study. They were repeatedly familiarized to being injected IP with 0.9% saline for a few days.

The experiment comprised four consecutive 4-day phases, during which time fluid consumption was recorded daily at 0900 and 1500 h. All injections were given at 0900 and 1200 during the three first phases. During the first phase, the subjects were injected twice daily with vehicle (tartaric acid and distilled water; 1 ml/kg) and fluid intake was determined. During the second phase, one group of animals (seven high-preferring and five low-preferring rats) received two daily IP injections of 50 mg/kg metyrapone (2-methyl-1, 2-di-3-pyridyl-1-propanone; Sigma). Another group (seven high-preferring and five low-preferring rats) was given the corresponding volume of vehicle. During the third phase, all animals received two daily vehicle injections. Fourth, the former metyrapone-treated group was started on metyrapone (2 × 50 mg/kg) again for 4 days. Two hours before each metyrapone injection the rats were given SC 0.6 mg/kg corticosterone (Sigma), dissolved in 0.9% saline containing 1% ethanol. Control rats received the appropriate vehicle injections at corresponding times. The subjects weighed 460 ± 10 g (mean ± SE) at the beginning of the experiment.

Statistics

For each individual, the mean intake of ethanol and water during the four phases of the experiments was computed and used in the statistical calculations. Changes in the mean fluid intake relative to the first vehicle phase were calculated for two time segments: between 0900 and 1500 h and between 1500 and 0900 h. Group differences in these difference scores were then assessed separately for the high- and low-preferring subjects with the Mann–Whitney *U*-test. Within-groups comparisons were made with the Wilcoxon test. Two-tailed levels of significance were consistently used (24).

RESULTS

High-Preferring Animals

0900–1500 h. Figure 1 shows the mean 6 h fluid intakes (0900–1500 h) of high-preferring rats during the vehicle, metyrapone, vehicle and corticosterone + metyrapone phases of the experiment. Injections of metyrapone (50 mg/kg), given at 0900 and 1200 h, significantly reduced ethanol preference ($p < 0.001$) and ethanol intake ($p < 0.001$) in comparison to rats receiving vehicle during this period (second panel of Fig. 1). Compared to baseline values, ethanol preference and consumption was reduced by more than 50% in rats receiving metyrapone. Although there was a significant compensatory increase in water intake during metyrapone treatment ($p < 0.01$), total fluid intake was lower in this group of animals compared to controls ($p < 0.05$).

When metyrapone was omitted from the injected solution, there was an immediate increase in ethanol preference and intake back to baseline levels (third panel of Fig. 1). Thus, there were no statistically significant differences between experimental and control vehicle-treated animals during this wash-out period.

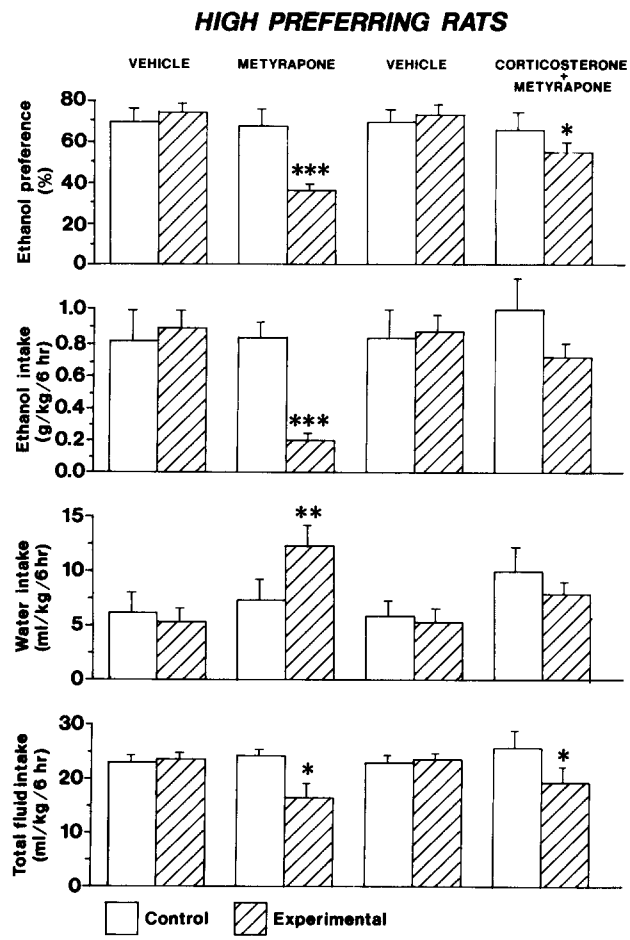

FIG. 1. Ethanol preference, ethanol intake, water intake, and total fluid intake (between 0900 and 1500 h) in high-preferring male rats treated with vehicle, metyrapone, vehicle and metyrapone + corticosterone (mean ± SE). Control rats received vehicle throughout. $p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ (Mann–Whitney *U*-test).

The effect of combined corticosterone (0.6 mg/kg) and metyrapone administration on fluid consumption was examined in the final phase of the experiment (fourth panel of Fig. 1). In comparison to the vehicle group, rats given corticosterone + metyrapone showed reduced ethanol preference ($p < 0.05$) and total fluid intake ($p < 0.05$), whereas ethanol or water intakes were not significantly affected. Examination of Fig. 1 suggests that corticosterone pretreatment attenuated the inhibitory effect of metyrapone. Thus, within-groups comparisons revealed that ethanol preference ($p < 0.05$) and ethanol intake ($p < 0.01$) were higher when the rats were given corticosterone + metyrapone instead of metyrapone alone.

1500–0900 h. Table 1 shows the average 18 h ethanol preference and intake (1500–0900 h) of high-preferring rats during the four phases of the experiment. There were no statistically significant differences between experimental and control animals.

Low-Preferring Animals

0900–1500 h. In contrast to the high-preferring rats, there were no significant alterations in ethanol preference and ethanol intake following treatment with metyrapone in the low-preferring group (Fig. 2). Thus, experimental and control animals in this subgroup did not differ significantly on any measure during any phase of the experiment.

1500–0900 h. There were no statistically significant differences between experimental and control animals in the mean 18 h ethanol preference or intake (Table 1).

DISCUSSION

The present study investigated the effect of metyrapone, a corticosterone synthesis inhibitor, on the voluntary intake of a 6% ethanol solution in rats. The response to the drug was found to vary according to the baseline levels of ethanol consumption. In low-preferring animals, alcohol drinking remained entirely unaffected by the multiple metyrapone injections. Administration of the identical treatment to high-preferring rats, on the other hand, switched their fluid preference such that they favored water rather than ethanol. Thus, metyrapone significantly depressed ethanol preference and ethanol intake by more than 50% in these high-preferring rats. Although there was a concomitant increase in water consumption, total fluid intake was slightly but significantly lowered during the treatment period.

The effect of metyrapone on ethanol consumption of high-preferring subjects was transient in that significant effects were detected only on the 6 h observations (0900–1500 h), when metyrapone injections were given just before and halfway through testing. By contrast, ethanol drinking during the remainder of the day (1500–0900 h) seemed unaffected by the drug. Moreover, when metyrapone-treatment was replaced with vehicle injections, the drinking of ethanol reverted to the high baseline levels. The relatively short-lived behavioral effects are probably due to the fact that metyrapone at the dose level used here inhibits corticosterone synthesis for a few hours only (4,15).

It was also found that pretreatment of high-preferring rats with corticosterone, in a dose that produce plasma hormone concentrations within a physiological range 0.5 h after injection (14), partially offset the inhibitory action of the synthesis inhibitor on alcohol drinking. For example, there was only a slight (though significant) lowering of ethanol preference following the combined treatment, the decrease being considerably greater following treatment with metyrapone only. Furthermore, while metyrapone alone depressed ethanol intake dramatically in comparison with vehicle-treated controls, the groups did not differ significantly when corticosterone preceded the metyrapone injections. Considering the rapid clearance of corticosterone from the circulation following injection (14), it is probable that a more sustained corticosterone administration (e.g., hormone implants) would have counteracted the metyrapone effect more completely.

It is difficult to exclude conclusively that the suppressive effect of metyrapone on ethanol drinking was due to unwanted side effects of the drug, such as general malaise. If so, one would expect the development of a conditioned taste aversion (11) because ethanol drinking was paired with the presumed state of

TABLE 1

ETHANOL PREFERENCE AND ETHANOL INTAKE IN HIGH- AND LOW-PREFERRING ANIMALS DURING THE FOUR PHASES OF THE EXPERIMENT (1500–2100 h)

	Vehicle	Metyrapone	Vehicle	Corticosterone + Metyrapone
		High-Preferring Rats		
Ethanol preference (%)				
Control ($n = 7$)	67.15 ± 6.2	68.94 ± 8.0	70.13 ± 6.9	71.67 ± 11.8
Experimental ($n = 7$)	70.52 ± 9.6	70.18 ± 4.2	70.39 ± 8.6	73.16 ± 9.0
Ethanol intake (g/kg/18 h)				
Control	1.98 ± 0.37	1.81 ± 0.39	1.98 ± 0.37	1.68 ± 0.45
Experimental	1.99 ± 0.44	1.81 ± 0.19	1.99 ± 0.41	1.82 ± 0.30
		Low-Preferring Rats		
Ethanol preference (%)				
Control ($n = 5$)	18.00 ± 5.4	22.73 ± 6.9	19.00 ± 2.9	12.65 ± 3.9
Experimental ($n = 5$)	20.20 ± 5.4	19.32 ± 5.5	19.80 ± 3.2	16.69 ± 4.0
Ethanol intake (g/kg/18 h)				
Control	0.43 ± 0.15	0.51 ± 0.20	0.44 ± 0.09	0.32 ± 0.15
Experimental	0.50 ± 0.16	0.45 ± 0.17	0.46 ± 0.10	0.37 ± 0.14

Control animals received vehicle throughout. Data are expressed as group mean ± SE.

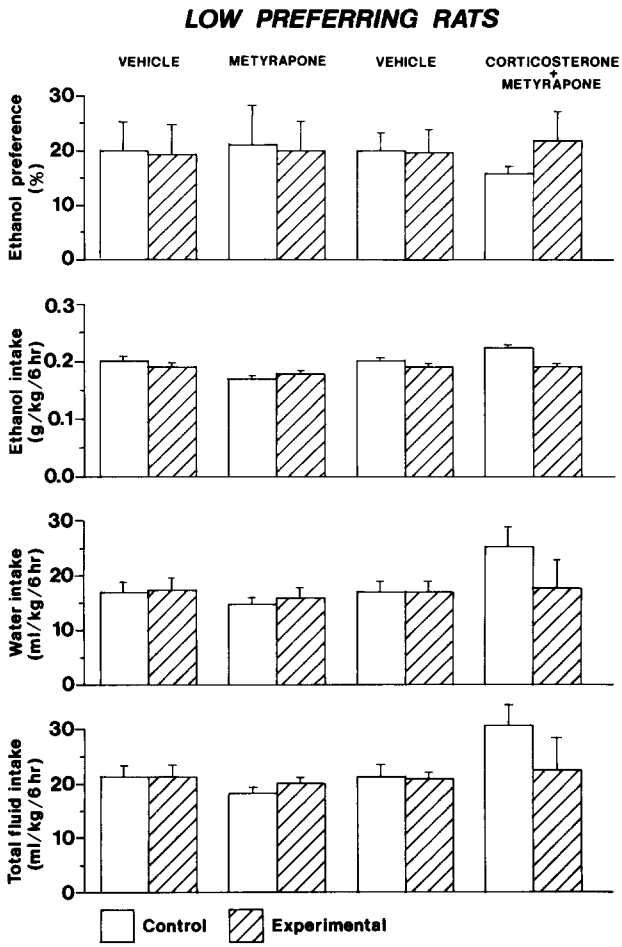

FIG. 2. Ethanol preference, ethanol intake, water intake, and total fluid intake (between 0900 and 1500 h) in low-preferring male rats treated with vehicle, metyrapone, vehicle and metyrapone + corticosterone (mean ± SE). Control rats received vehicle throughout. The differences between groups are not statistically significant.

malaise on 4 consecutive testing days (phase two). There was, however, no difference in ethanol intake during the drug-free periods after metyrapone treatment (1500–0900 h) between the group treated with metyrapone and vehicle group. Nor was there any difference during the third phase, when both groups were treated with vehicle. The absence of persistent aftereffects during these periods of observation speaks against the notion that metyrapone depresses ethanol intake by inducing nausea in the animals.

The behavioral effect could also be due to the accumulation of corticosterone precursors (5) rather than to the shortage of corticosterone per se. However, Fahlke et al. (9) found that adrenalectomy, which abolishes corticosterone levels but gives no accumulation of corticosterone precursors, alters ethanol consumption in a way very similar to metyrapone. It seems reasonable to suggest, therefore, that the suppressive effect of metyrapone on ethanol consumption was due to the reduced availability of corticosterone in the plasma. It may even be the case that elevated corticosterone levels facilitate ethanol intake. Speculatively, it is quite possible that the large individual differences in alcohol preference observed in groups of unselected rats (e.g., present study) is determined, in part, by individual differences in corticosterone secretion. In this context it is interesting to note that the responsiveness to amphetamine, another drug of abuse, is facilitated by corticosterone in the rat (20,21).

The reason why abnormally low corticosterone blood titers attenuate alcohol drinking in high-preferring rats remains to be determined. Chemical or surgical adrenalectomy elicit a number of changes in the hypothalamus and pituitary, including increased vasopressin and corticotrophin releasing factor levels and enhanced adrenocorticotrophic hormone (ACTH) secretion (6). Hypothalamo-hypophyseal alterations of this nature may, hence, mediate the effect of metyrapone or adrenalectomy on alcohol consumption. Support for this hypothesis is offered by the observation that exogenous administration of $ACTH_{4-10}$ decrease ethanol intake in rats (17). It is also becoming increasingly clear that corticosterone affects many other brain regions by interacting with Type I and Type II glucocorticoid receptors, both systems being widely distributed in the brain (1,2,13). For example, glucocorticoid receptors are present in areas rich in serotonin and dopamine (13). Previous research has implicated both of these neurotransmitter systems in the control of ethanol consumption (7), and serotonergic and dopaminergic parameters are altered by adrenalectomy (3,16,18). Thus, shortage of corticosteroids may lower alcohol intake by interfering with mechanisms mediating reward-related processes in the brain.

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