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Learned Tolerance to the Corticosterone-Increasing Action of Ethanol in Rats

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SEELEY, R. J., M. H. HAWKINS, D. S. RAMSAY, C. W. WILKINSON AND S. C. WOODS. *Learned tolerance to the corticosterone-increasing action of ethanol in rats.* PHARMACOL BIOCHEM BEHAV **55**(2) 269–273, 1996.—Ethanol administration stimulates the hypothalamic–pituitary–adrenal (HPA) axis, resulting in increased plasma levels of corticosterone. As occurs with many other effects of ethanol, tolerance develops with repeated administration such that plasma corticosterone levels become less effected by subsequent ethanol administration. The present experiment explored the possibility that the environmental cues associated with the administration of ethanol can control the expression of tolerance to ethanol's corticosterone-elevating effects. Male Long–Evans rats received intragastric administrations of ethanol (3.2 g/kg) in association with one set of environmental cues and intragastric saline in association with a different set of environmental cues. Plasma corticosterone levels were elevated after the first ethanol administration, but after the tenth ethanol administration, corticosterone levels failed to increase significantly above control values. After demonstrating tolerance, rats were administered ethanol in the saline-paired environment and plasma corticosterone levels were higher than in the ethanol-paired environment. This environmental specificity suggests that tolerance to the neuroendocrine effects of ethanol is not simply the result of long-term alterations in sensitivity of the HPA axis but is, at least in part, mediated by learned responses to cues that predict the effects of ethanol. **Copyright © 1996 Elsevier Science Inc.**

Learned tolerance Ethanol Rats

ALTHOUGH ethanol is known to reduce anxiety [e.g. (2,10)], it also produces neuroendocrine changes commonly associated with increased stress in both humans and rodents (9,22). Specifically, acute ethanol administration to naive subjects elicits an elevation of corticotropic releasing hormone (CRH) (17). In turn, elevated CRH causes the release of ACTH from the anterior pituitary, which circulates to the adrenal cortex where it is a potent secretagogue for the release of glucocorticoids (cortisol in humans and corticosterone in rats). Activation of this hypothalamic–pituitary–adrenal (HPA) axis also occurs when animals are confronted with a variety of acute stressors. Thus, while ethanol is anxiolytic by several behavioral measures in humans and rodents, the robust increase in glucocorticoids indicates simultaneous activation of neuroendocrine stress responses.

As occurs with many of ethanol's other effects, tolerance develops to the corticosterone-elevating effect of ethanol; for instance, the magnitude of the increased levels of steroid in the blood lessens with repeated administrations of ethanol (5,23). Although the mechanism(s) responsible for this tolerance development are unknown, the issue is important given that alcoholics are at increased risk for a number of disorders associated with HPA activity [e.g., pseudo-Cushings's syndrome (16)]. It is unknown, for example, if this risk occurs as a result of ethanol-induced changes in HPA function or is secondary to the wide range of comorbidities associated with alcoholism that have their own impacts on HPA function (e.g., depression) (26).

Research on the mechanism of tolerance to ethanol-induced increases of HPA activity has been based upon pharmacodynamic models of tolerance whereby repeated exposure of target tissues causes them to become less sensitive to drug effects. Two specific hypotheses have been forwarded to account for the development of tolerance to ethanol's enhancement of

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HPA activity [see (23)]. First, the CRH activation caused by repeated exposure to ethanol may result in a reduction of CRH receptors for transducing CRH signal into secretion of ACTH from the pituitary. In fact, chronic ethanol exposure does result in reduced CRH receptor density in the anterior pituitary (4). Second, it is possible that chronic ethanol exposure alters the sensitivity of the adenyl cyclase system responsible for intracellular CRH signaling and several studies support this possibility (4,23,24).

These correlational observations aside, the actual mechanism(s) responsible for the development of tolerance to the corticosterone-increasing effect of ethanol remains unknown. An alternative explanation, that has yet to be explored, is that tolerance to the corticosterone-elevating effect of ethanol is based upon classical conditioning [see (6,19,20) for reviews of the contribution of conditioning to drug tolerance]. In this model, a drug-induced perturbation of a regulated variable (i.e., a drug effect) elicits a neurally mediated corrective response that counters the perturbation and returns the system to its predrug level. With repeated drug exposures, these corrective responses become learned and can be elicited by cues that reliably predict the drug-induced disturbance. These learned responses can diminish the impact of the drug on the regulated system and, therefore, contribute to the development of tolerance.

Ethanol causes a myriad of effects other than activation of the HPA axis. For example, under normal ambient temperatures, ethanol administration to naive subjects causes a decrease in core body temperature, and this effect declines with subsequent administrations of ethanol, and learning has been compellingly implicated in the development of tolerance to this hypothermic effect. Mansfield and Cunningham (12) found that when ethanol is administered to rats in the presence of one set of environmental cues, and saline is administered to the same rats on alternate days but in the presence of a different set of environmental cues, tolerance develops to the hypothermia caused by ethanol and is evident only in the ethanol-paired environment. Hence, rats are tolerant to ethanol's hypothermic effect in the ethanol-paired environment but not in the saline-paired environment [see also (11)]. Consistent with this interpretation, Hjeresen et al. found that if rats never experience a perturbation of temperature after ethanol is administered, tolerance to the hypothermic effect does not develop (8). This implies that experiencing the drug effect (e.g., hypothermia), rather than drug exposure alone, is critical for tolerance to develop.

The present experiment investigated the possibility that learning is important in the development of tolerance to the corticosterone-increasing effect of ethanol. To do so, one set of environmental cues was paired with the administration of ethanol and a second set of environmental cues was paired with the administration of saline in the same rats. Tail blood samples for measurement of plasma corticosterone were collected after the first and tenth administrations of ethanol and saline. Each rat was then administered ethanol in the saline-paired environment. The tolerance to ethanol that develops in this paradigm is environmentally specific, implying that learning plays a critical role in the development of tolerance to this neuroendocrine effect.

METHOD

Subjects

Thirty naive male Long–Evans rats were obtained from the colony maintained by the Department of Psychology at the University of Washington and were housed in individual wire mesh cages in a temperature-controlled (22°C) vivarium with ad lib access to pelleted food and water except where noted. The room was on a 12 L:12 D schedule. Testing was conducted between 2 and 5 h into the light cycle.

Procedures

Gastric Catheterization. Gastric catheters were constructed from 15 cm of silastic tubing. A ring of silastic bonding was applied approximately 2 cm from the gastric end of the catheter. Next, a dacron and silastic mesh disc about 1 cm in diameter was threaded down the tubing. The disc and bonding together served as an anchor for the catheter once it was placed in the stomach. Rats were food deprived for 24 h prior to surgery, and anesthetized with 3.7 mg/kg equithesin. A 1-cm incision was made on the skull and a 2-cm incision was made along the ventral abdominal midline just posterior to the rib cage. The abdominal muscle wall was opened and the stomach exteriorized. A short incision was made in the greater curvature of the stomach, the disc end of the catheter was inserted into the stomach, and the incision closed with two small stitches. The opposite end of the catheter was subcutaneously routed to the incision at the top of the skull. A short length of 23-gauge stainless steel tubing was force fit into the cranial tip of the silastic tubing. Four screws were placed in the skull and the catheter was anchored to the screws with dental acrylic such that the tip was exteriorized through the skin.

Plasma Corticosterone Measurements

Blood samples were obtained from the tip of the tail into 250 μl heparinized capillary tubes. The tubes were centrifuged for 5 min at 4000 rpm. The plasma was separated and stored at −75°C. Corticosterone was measured by radioimmunoassay in unextracted plasma (1). Samples were assayed in triplicate in a volume of 1 μl plasma diluted with phosphate buffer and heated for 20 min at 80°C to denature corticosteriod-binding globulin. Corticosterone antibody was obtained from ICN Biomedicals (Costa Mesa, CA). The detection limit for corticosterone is 5 pg/tube (or 5 ng/ml with a 1 μl sample). Intra- and interassay coefficients of variation were 7.7% and 17%, respectively.

Experimental Protocol

Training Phase. At the same time each day rats were administered a 10-ml intragastric bolus of either ethanol (3.2 g/kg) diluted in saline or isotonic saline in one of two distinct environments. One environment was the home cage in the vivarium. The alternate environment consisted of a smaller mouse-type cage in an adjacent room. The two environments were made more distinct by placing sand paper on the cage floor and adding a maple smell, low ambient lighting, and the presence of white noise to the alternate environment. The two environments, therefore, differed in visual, tactile, auditory and olfactory cues. The drug treatment (ethanol or saline) paired with each environment was counterbalanced across subjects.

Prior to a trial in the alternate environment, a rat was removed from its home cage and placed in a transport cage, carried to the adjacent room, and placed into the mouse cage. After 5 min it was removed briefly and received the injection appropriate for that environment and returned to the mouse cage. For a trial in the vivarium environment, a rat was removed from its home cage, injected and returned to its home

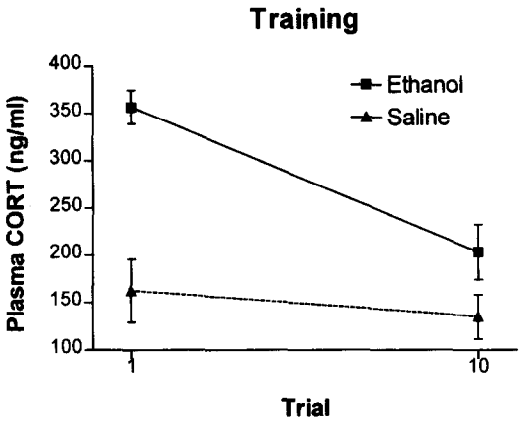

FIG. 1. Mean (± SEM) plasma corticosterone (CORT) levels after the first and 10th administrations of ethanol and saline.

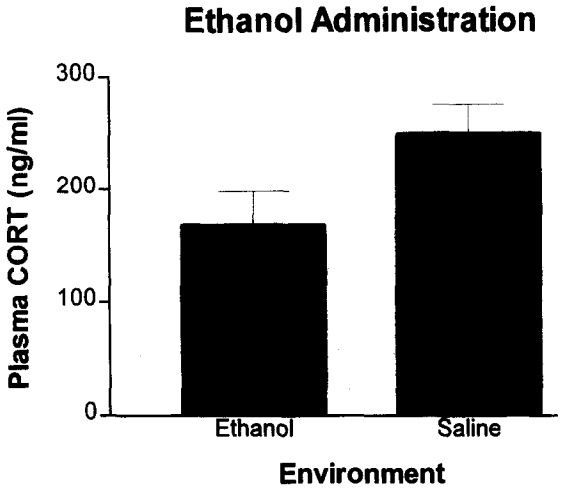

FIG. 2. Mean (± SEM) plasma corticosterone (CORT) levels following ethanol administration in the ethanol-paired environment and in the saline-paired environment. The samples were taken after the 10th and 11th ethanol administrations, respectively. Rats exhibiting a higher CORT level after the 10th ethanol administration than after the 1st ethanol administration were not included in this analysis.

cage. Each animal was given 10 ethanol sessions and 10 saline sessions in the respective environments with the order of ethanol and saline randomized for each pair of days. Twenty minutes after ethanol or saline administration, blood samples were obtained after the initial ethanol and saline administrations (trials 1 and 2) as well as after the tenth ethanol and saline administrations (trials 19 and 20).

Environmental Specificity Test. On the following day (trial 21), each rat received ethanol in the environment that had previously always been paired with saline administration. For half of these animals, ethanol had been administered in the ethanol-paired environment on the previous day while for the other half saline had been administered in the saline-paired environment. Blood samples were taken 20 min after the injection.

Effect of Cues on a Novel Stressor. On trials 22 and 23 each rat was placed in each of the two environments and administered saline with the order counterbalanced across subjects. Ten minutes after the saline was administered each rat was given a series of three intraperitoneal penetrations with a 19 gauge needle as a novel, nonpharmacological stressor. The procedure took approximately 45 s, after which the rat was returned to its environment. Ten minutes later a blood sample was taken. This procedure was included to assess the ability of ethanol-paired cues to suppress activation of the HPA axis by a novel, nonpharmacological stressor.

RESULTS

Training

Data were analyzed using a repeated measures one-way ANOVA, and post hoc analyses utilized the Tukey-Kramer multiple comparisons test. Of the 30 animals that began the experiment, 27 completed the training phase and 25 completed the remainder of the test conditions. Animals were removed either because of failed gastric catheters or abdominal infections. The ANOVA revealed a significant main effect for drug, $F(3, 24) = 14.593$. $p < 0.01$. As depicted in Fig. 1, following the initial administration of ethanol, corticosterone levels were significantly elevated above those observed following saline, $t(26) = 7.5, p < 0.01$. Additionally, tolerance developed for the elevation of corticosterone level over the 10 administrations of ethanol; for instance, the corticosterone levels after the 10th administration of ethanol was significantly less than after the first administration, $t(26) = 5.9, p < 0.01$, and not significantly different than following the tenth administration of saline, $t(26) = 2.65, p > 0.05$, NS.

Environmental Specificity Test

Of the 25 rats that completed the first 20 trials, 20 had a lower corticosterone level following the 10th ethanol administration than following the initial ethanol administration. The values in the other five were higher on the 10th trial. These 20 rats with lower corticosterone levels on the 10th ethanol trial were considered to have developed tolerance and were included in the analysis of the other conditions. Data from the other five rats were not included because the hypothesis under evaluation was that any apparent tolerance would be found to be environmentally specific. Of the remaining 20 rats, 9 had received ethanol in the home cage while 11 had received ethanol in the alternative environment. It should be noted that this slightly unbalanced grouping works against finding a significant elevation of corticosterone in the saline-paired environment because corticosterone levels tended to be higher in the alternative environment. Figure 2 depicts corticosterone levels following ethanol administration in the two distinct environments for rats demonstrating tolerance during training. Corticosterone levels were significantly elevated in the saline-paired environment compared to the ethanol-paired environment [paired samples, one-tailed t-test: $t(19) = 1.8, p < 0.05$].

Effect of Cues on a Novel Stressor

The novel, nonpharmacological stressor produced a significant elevation of plasma corticosterone levels [10th saline administration vs. stressor in saline environment; $t(24) = 3.49$, $p < 0.01$]. This elevation was not altered by placing the rat in the ethanol-paired environment [ethanol environment: 274 ± 25.6 ng/ml, saline environment 247.0 ± 22.7 ng/ml; $t(24) = 1.08, p > 0.15$, NS].

DISCUSSION

The present study confirms the results of others that ethanol causes a robust increase in plasma corticosterone level when delivered directly into the stomach of naive rats (15). Furthermore, tolerance develops to this endocrine effect of ethanol, for instance, with repeated ethanol administrations the increase in corticosterone levels is reduced. Both of these findings replicate those seen in other ethanol-administration paradigms (5).

The present study assessed the hypothesis that environmental cues that predict ethanol administration influence tolerance. Corticosterone levels following the identical dose of ethanol were greater in the presence of saline-paired environmental cues than in the presence of cues that had been paired with ethanol (see Fig. 2). This environmental specificity of tolerance has several important implications. First, learning would appear to have an important role in the development of tolerance to the corticosterone-increasing effect of ethanol. The environmental cues present when ethanol was administered modified the corticosterone levels as a consequence of their prior association with a drug effect. These observations are consistent with the findings that tolerance to other effects of ethanol, including hypothermia and motor impairment, include learning as an important component (11,12,25). Analogous to the current results with ethanol, the corticosterone response to nicotine is also altered by the presence of nicotine-paired cues in the environment (3). Consequently, the present results are consistent with results from other physiological systems (e.g., temperature regulation) and with similar neuroendocrine measures to other pharmacological agents (e.g., nicotine) with similar effects on the HPA axis. The results are also consistent with theories suggesting that diverse physiological systems are regulated at least in part through learned mechanisms (14,18).

Second, the present results cannot be easily explained by pharmacodynamic theories of tolerance. Because each rat was used as its own control, changes of receptor number, cell membrane properties, or other cellular signaling events caused by repeated exposure to ethanol cannot explain the difference between corticosterone levels to ethanol in the saline or ethanol-paired environments. Consequently, the current results imply that any changes in CRH receptor number caused by repeated ethanol administration are not the basis for the reduced corticosterone response to ethanol after long-term exposure. After all, when the animals were placed in a different environment, much of the corticosterone elevation in response to ethanol returned. Thus, at least part of the lowered sensitivity of the HPA axis to ethanol can be reversed by changing the environment in which ethanol is administered. Tolerance to ethanol in the ethanol-paired environment appears to result from suppression of the HPA axis by ethanol-predictive cues rather than being a consequence of long-term changes to the HPA axis alone.

It could be argued that since the corticosterone elevation to ethanol in the saline-paired environment (trial 21) was not as great as the original corticosterone response (trials 1 or 2), part of the tolerance may be mediated by pharmacodynamic factors such as changes in receptor number or sensitivity. While nothing in the current data eliminates this possibility, caution should be exercised in drawing such a conclusion. It is possible that the difference in the response in the saline-paired environment and the original response to ethanol is a product of learning to associate ethanol with cues that are common to the two environments. For example, both the time of day and the general infusion procedure were the same for both the ethanol and saline administrations. Additionally, some of the cues the animal may use to predict the oncoming effects of ethanol are interoceptive cues caused by ethanol itself (7). Consequently, the difference between the original response to ethanol and the response in the saline-paired environment could be the result of generalization between environmental and interoceptive cues shared by the two environments.

Several groups have shown that repeated ethanol administration can reduce the corticosterone elevation associated with other stimuli that typically activate the HPA axis (13,17,21,23). The present findings suggest that when ethanol exposure is held constant, cues that have been paired with ethanol administration do not reduce the corticosterone increases caused by a nonpharmacological stressor. Therefore, while learned tolerance may be an important determinant of the reduced ability of ethanol to increase corticosterone, changes of the HPA axis observed after chronic ethanol exposure [e.g.,(4)] may mediate the reduced sensitivity of the HPA to other stress stimuli.

While caveats always apply to interpreting such negative data, the data from the nonpharmacological stressor are more consistent with cues acting in an indirect manner to reduce the ethanol-induced activation of the HPA axis rather than suppressing HPA activity directly. Two such models can be applied to the current data. First, ethanol-paired cues may act to suppress activity in central nervous system circuits that are activated by ethanol and produce activation of the HPA axis. Second, HPA activation in response to ethanol may not result from ethanol's direct actions within the central nervous system, but rather may be a secondary effect of peripheral changes (such as hypothermia). In this case, ethanol-paired cues would act to reduce the hypothermia and thereby reduce the peripheral stimulus for HPA activation. Further research is necessary to understand the interaction between ethanol-paired cues and neuroendocrine stress systems.

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