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Adrenalectomy Reduces Alcohol-Stimulated Activity: Blood and Brain Alcohol Content

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WALLIS, C. J , R F ANTON AND C. L. RANDALL *Adrenalectomy reduces alcohol-stimulated activity Blood and brain alcohol content* PHARMACOL BIOCHEM BEHAV **20**(6) 883–886, 1984.—It has been shown that adrenal glucocorticoids have a permissive role in some of the actions of alcohol. To determine if an intact adrenal was necessary for the stimulation of locomotor activity, 24 female C3H mice were tested for open field activity with ethanol or saline Two weeks after adrenalectomy or sham surgery, animals were tested for activity again with ethanol or saline. One week later, alcohol disappearance curves were generated for blood and brain Adrenalectomy reduced but did not abolish the alcohol-stimulated locomotor activity In addition, adrenalectomy significantly reduced estimated peak alcohol levels in blood and brain but significantly reduced the disappearance rate for alcohol only in brain. These data suggest that adrenalectomy significantly changes alcohol distribution, with greater impact on brain alcohol levels than on blood levels, and that this may be responsible, at least in part, for the reduction in stimulated locomotor activity

Adrenalectomy Brain ethanol Blood ethanol Open field activity

THE adrenal gland has been implicated as a possible effector organ through which alcohol may produce some of its effects. The response of the adrenal gland to alcohol treatment is an acute release of corticosterone from the adrenal cortex [10], release of catecholamines from the adrenal medulla [14], and a chronic increase of adrenal weights in mice [17]. While these alcohol-stimulated adrenal responses should have potent direct effects on tissue sensitivity and vascular tone, it has been shown that many of the alcohol effects that depend upon an intact adrenal gland are not the result of the acute release of adrenal hormones.

Alcohol elicits a variety of adrenal dependent biochemical effects. For example, ethanol treatment results in a reduction in plasma insulin levels which can be blocked by adrenalectomy [9]. Additionally, microsomal phosphatidate phosphohydrolase activity is increased by alcohol treatment, and this effect can be blocked by adrenalectomy [19] In this case, glucocorticoids appear to be permissive, not causal, in the action of ethanol.

Behaviorally, a similar permissive role for glucocorticoids has been suggested in the induction of seizures after withdrawal of alcohol. Adrenalectomy significantly reduces the number of animals showing seizures after withdrawal of alcohol and corticosterone replacement reversed the effect, suggesting that it was not the stress-induced increase in corticosterone that was responsible but that the presence of corticosterone was permissive to the development of seizures [20].

Treatment with low doses of alcohol result not only in biochemical and endocrine changes but also in behavioral changes such as increased locomotor activity [6, 15, 18]. It has been shown that ethanol-induced locomotor activity can be modified by dopamine agonists [2], GABA-like drugs [3], and adrenergic blockers [13]. The following work was undertaken to determine if the ethanol-induced increase in locomotor activity is dependent on the integrity of the adrenal gland and if any change in the response to alcohol could be secondary to changes in blood or brain alcohol levels.

METHOD

Female C3H mice [24] (Charles River Breeding Laboratories, Kingston, NY) were housed individually with food and water ad lib. Room temperature, humidity, and lighting were controlled (12:12 light:dark cycle, lights on at 5:30 a.m.). Animals were randomly divided into four treatment groups. ADX-Ethanol, ADX-Saline, SHAM-Ethanol, and SHAM-Saline Prior to surgery, animals were given an initial activity test to assure that there was no sampling difference between groups. Twelve animals were bilaterally adrenalectomized (ADX) by the dorsal approach and the remaining animals were given sham surgery (SHAM). All animals were anaesthetized with sodium pentobarbital (80 mg/kg, 0.02 ml/g body weight, IP, in sterile saline). After surgery, adrenalectomized animals were maintained on normal saline ad lib. Two weeks after surgery, animals were retested for the effect of ethanol on open field activity behavior. One week after the last activity test, animals were tested for the effect of adrenalectomy on blood and brain ethanol clearance rates. The completeness of adrenal removal was verified by gross

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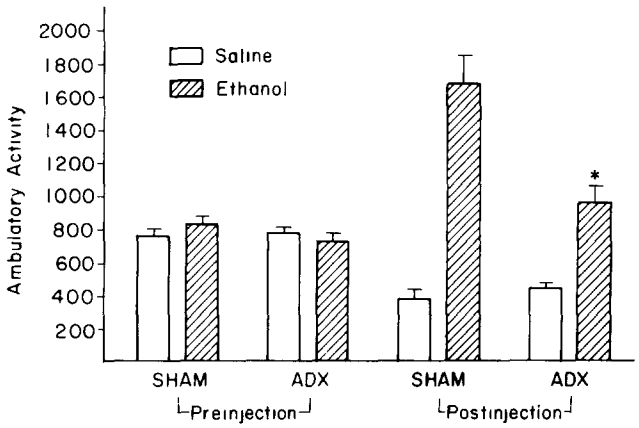

FIG 1 Effect of adrenalectomy (14 days post operation) on open field ambulatory activity before and after injection with 1 5 g/kg ethanol (0 015 ml/g body weight, IP) or saline Values are means±SEM *Denotes significant difference from SHAM operated controls, $p<0$ 01

TABLE 1

THE EFFECT OF ADRENALECTOMY ON ETHANOL STIMULATED STEREOTYPIC BEHAVIOR

Treatment	Group	N	Preinjection Score	Postinjection Score
Saline	SHAM	6	733 ± 47	404 ± 43
	ADX	6	632 ± 74	440 ± 36
Ethanol (1 5 g/kg)	SHAM	6	584 ± 113	1236 ± 119
	ADX	6	658 ± 36	743 ± 48[1]

Each value is the mean ± SEM. Value differs significantly from Sham operated controls, $p<0$ 005

inspection and assay of plasma corticosterone using a competitive protein binding assay [8].

Open Field Activity

Open field activity was measured in a Digiscan Activity Monitor (Omnitech, Columbus, OH) in a dark room between 1–4 30 p.m The monitor uses an 8×8 grid of infrared sensors spaced five centimeters apart. Control of this test session and recording of the test data were done with a modular programming system (Coulbourn Instruments, Leigh Valley, PA). Total, ambulatory, and stereotypic activity (total-ambulatory) were recorded. Trials consisted of two tests, a fifteen (15) minute test prior to injection and a fifteen (15) minute test after injection with either 1.5 g/kg ethanol (0.015 ml/g body weight, IP, in sterile saline) or an equivalent volume of sterile saline.

Alcohol Clearance Rates

Animals were injected with 1 5 g/kg ethanol (0 015 ml/g body weight, IP, in sterile saline). At 30, 60, and 90 minutes post-injection, blood samples were collected from the retro-orbital sinus, the animals were killed by decapitation, and the brains were removed quickly. The right cerebral hemisphere was weighed and used for the assay. Blood and brain samples were diluted 50:1 with 3.4% perchloric acid (volume·volume for blood, volume:weight for brain) Brain samples were homogenized by sonication. All samples were vortexed and centrifuged at 2000 rpm. The supernatent was used in the assay The alcohol assay was based on the Calbiochem method (La Jolla, CA) which uses the enzyme alcohol dehydrogenase (Sigma Chemical Co., St. Louis, MO) to convert ethanol to acetaldehyde. The cofactor, NAD, is reduced stoichiometrically during the reaction to NADH which is detectable by UV spectrophotometry. All values are read directly from a standard curve (mg/dl or mg%). Data were analyzed by linear regression to determine the rate of clearance (slope) and estimated peak levels (y intercept). Samples were collected at 30, 60, and 90 minutes, since in preliminary studies we determined that this was the most linear portion of the disappearance curve for ethanol after an intraperitoneal injection.

Data Analysis

Activity data were analyzed by a three factor analysis of variance (drug × surgery × trial) with repeated measures on one factor However, since there was always a significant interaction between test trial and drug treatment, data were analyzed by a two way analysis of variance (drug × surgery) within either the pre- or postinjection condition Simple effects were analyzed where appropriate [21]

Differences between alcohol clearance rates and peak levels for the ADX versus SHAM groups were determined by derivation of a t-statistic [11].

RESULTS

In the preoperative tests of activity, alcohol increased both ambulatory and stereotypic behavior compared to saline (2 6× for ambulatory and 3.0× for stereotypic activity). There was no difference between the arbitrarily assigned operative groups for pre- or postinjection activity scores. Two weeks after surgery there was still no significant difference in activity scores of ADX versus SHAM operated animals on preinjection test scores. Postinjection, alcohol significantly elevated both ambulatory and stereotypic behavior in both surgical groups, $F(1,18)=61$ 93 and $F(1,18)=57.35$, respectively, $p<0.001$. There was a significant interaction between drug and surgical treatments for both ambulatory and stereotypic behavior ($p<0$ 01) Analysis for simple effects showed that adrenalectomy significantly diminished the effect of ethanol on both ambulatory and stereotypic behavior, $F(1,18)=18$ 94 and $F(1,18)=21.58$, respectively, $p<0.005$, while there was no difference between surgical groups for saline-injected animals (Fig. 1, Table 1).

The blood ethanol clearance curves (Fig 2) show a significantly lower estimated peak blood ethanol concentration in the ADX versus SHAM operated animals (ADX=158, SHAM=186 mg/dl, $p<0$ 05), while there is no significant difference in the clearance rate from blood In brain, there is also a significantly lower estimated peak level of alcohol in the ADX group than in SHAM operated controls (ADX=108 versus SHAM=142 mg/dl, $p<0.001$) and there is a significantly slower rate of clearance for ADX than SHAM animals (ADX= −0 89 versus SHAM= −1.11 mg/dl/min, $p<0.05$, Fig. 3).

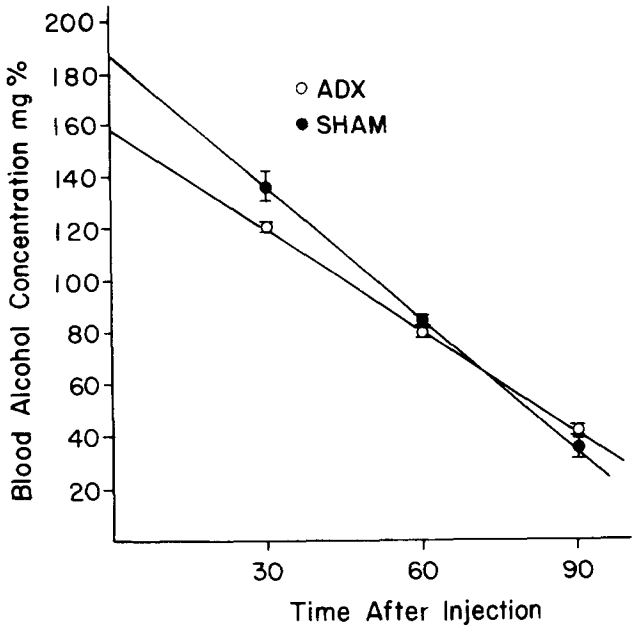

FIG 2 Effect of adrenalectomy (21 days post operation) on blood ethanol clearance rate and estimated peak ethanol concentrations after injection with 1 5 g/kg ethanol (0 015 ml/g body weight, IP) Values are means±SD Each point represents duplicate determinations from four animals

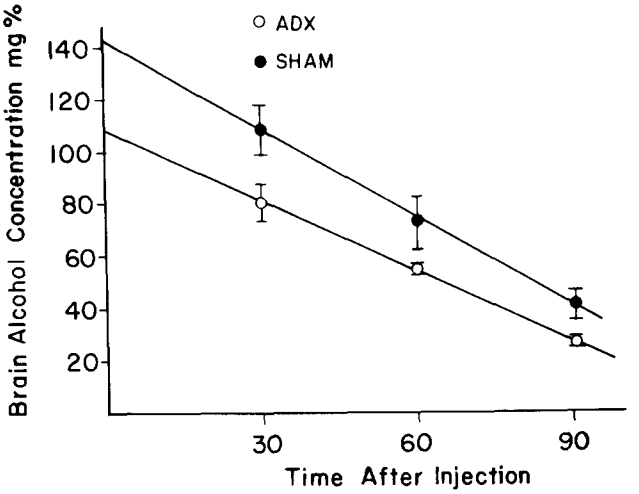

FIG 3 Effect of adrenalectomy (21 days post operation) on brain ethanol clearance and estimated peak ethanol concentrations after injection with 1 5 g/kg ethanol (0.015 ml/g body weight, IP) Values are means±SD Each point represents duplicate determinations from four animals

DISCUSSION

Our results show that adrenalectomy reduces the effect of ethanol on open field activity. In addition, adrenalectomy significantly reduces estimated peak levels of brain ethanol. While we did not measure actual peak values of brain ethanol during the initial 15 minute period, the data from our clearance curves certainly demonstrate a change in ethanol kinetics which may be involved in the effect of adrenalectomy on open field activity. This suggestion would be consistent with the fact that there is a dose-related increase in activity at low doses of ethanol [18]. Typically, a single point comparison of blood ethanol concentrations is done to determine if an effect of a particular treatment is due to changes in blood ethanol. As can be seen from our blood kinetic curves, a single time point analysis could well result in misleading conclusions. In the case of assessing behavioral changes, blood ethanol may not provide an adequate indication of what is occurring in brain

The effects of adrenalectomy on blood ethanol kinetics have been studied in the rat. It has been shown that ADX alters renal function, changes cellular permeability, and decreases hepatic metabolism [12]. Since adrenalectomy reduces body water [16] and ethanol distributes uniformly throughout body water [11], it would follow that ADX would result in increased peak levels of blood ethanol [16]. The same authors report that there is no effect of ADX on the metabolic rate of ethanol However, there are at least two reports that ADX reduces the rate of ethanol metabolism in rats [4,5]. Additionally, there is the suggestion that ADX should modify ethanol absorption due to changes in blood flow as a result of the absence of adrenal epinephrine [7]. Generally, it must be concluded that agreement about the effects of ADX on ethanol kinetics is lacking. Perhaps some of the conflicting results are due to differences in the timing of postadrenalectomy kinetic experiments and the differences in postoperative treatments employed to physiologically maintain the animals (saline versus dexamethasone treatment). Certainly, postoperative treatment may significantly alter total body water and liver metabolism rates.

It is worthwhile to consider the permissive action of glucocorticoids on the development of withdrawal seizures in the mouse [20] in light of our brain ethanol results. If ADX significantly reduces the level of ethanol in the brain, then it may well be that the failure of many ADX animals to develop withdrawal seizures may be in part due to an effectively lower ethanol dose, while corticosterone replacement may reverse peripheral factors influencing ethanol distribution and metabolism. While we do not discount possible direct effects of glucocorticoids on brain, it becomes very important to assure that comparable tissue levels of a drug are being used to assess adrenal modulation of central nervous system sensitivity to that drug. In future studies, we will investigate the effect of adrenalectomy on several alcohol behaviors under conditions that result in comparable brain levels of alcohol, with and without glucocorticoid replacement therapy. The aim of these studies will be to better define the role of the adrenal gland in the actions of alcohol.

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