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BRIEF COMMUNICATION

Glucocorticoids Antagonize the Sedative Action of Ethanol in Mice

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SZE, P. Y. *Glucocorticoids antagonize the sedative action of ethanol in mice.* PHARMACOL BIOCHEM BEHAV **45**(4) 991–993, 1993.—The effect of corticosterone on sleep time in mice following a hypnotic dose of ethanol (3 g/kg) was determined. An acute dose of the steroid (10 mg/kg) administered 15 min prior to ethanol injection significantly shortened the sleep time (by 55%). Brain levels of ethanol were not affected by the steroid treatment. The effect was specific to glucocorticoids because steroids without glucocorticoid activity including testosterone and 17β-estradiol were ineffective. These results indicate that glucocorticoids have an antagonistic effect to the acute action of ethanol in the brain. The rapid onset of the corticosterone action in antagonizing ethanol-induced sedation suggests that the action is mediated by a membrane mechanism rather than the classical steroid mechanism involving an intracellular receptor and gene expression.

Ethanol Glucocorticoids Sedative action Steroid actions on membranes

THE central depressant action of ethanol has been attributed to a variety of neurochemical effects in the brain, including changes in neurotransmitter receptors (2,9), adenylate cyclase (14), and calcium channels (4). In recent studies, we found that ethanol inhibits the binding of calmodulin to synaptic plasma membrane (3,12). Because calmodulin is a regulatory factor for a number of membrane-mediated processes in pre- and postsynaptic sites, the inhibition of membrane binding of calmodulin may lead to cascading alterations in synaptic events. Opposite to the inhibition by ethanol, the binding of calmodulin to synaptic plasma membrane is enhanced by glucocorticoids (3,13). At the behavioral level, it has long been known that glucocorticoids have central stimulant effects, although the underlying mechanism is not understood. For example, hypersecretion of cortisol in Cushing's syndrome is known to produce psychosis (6); among the side effects of glucocorticoid therapy are euphoria and increased seizure susceptibility (17). Thus, glucocorticoids and ethanol appear to have opposite behavioral effects, one stimulant and the other depressant, consistent with their opposite biochemical effects on calmodulin binding. In the present study, we found that the sedative action of ethanol is indeed antagonized by glucocorticoids.

METHOD

Male C57/BL6J mice (Jackson Laboratory, Bar Harbor, ME), 6–8 weeks old and weighing 24–26 g, were maintained in groups of five in standard laboratory cages. The sedative effect of ethanol was assessed by determining the sleep time after a hypnotic dose of ethanol (3 g/kg), as previously described by us (11). The animal was treated with corticosterone (IP, in 0.1 ml saline containing 0.2% polyvinylpyrrolidone) 15 min before administration of ethanol (IP, in 0.2 ml saline). Upon the loss of righting reflex, the ethanol-injected mouse was placed on its back in a V-shaped trough. Sleep time was considered the time between the loss of righting reflex and the time the animal regained the ability to right four times within 1 min. All experiments were performed at the same hour of the day (between 8:00–9:00 a.m.), beginning with corticosterone (or vehicle) treatment.

In animals sampled at random, the whole brain was removed 30 min following injection of 3 g/kg ethanol. The brain was homogenized in 4 vol ice-cold distilled water and the homogenate centrifuged at $10{,}000 \times g$ for 10 min. The supernatant was used for the determination of ethanol concentration. Ethanol was assayed enzymatically by measuring the conversion of NAD to NADH after the addition of alcohol dehydrogenase (8).

Sample sizes are identified in the tables. Analysis of variance (19) was applied to the data.

RESULTS

Control mice without corticosterone treatment had a sleep time of 33 min following a hypnotic dose of 3 g/kg ethanol

TABLE 1

DOSE RESPONSE OF CORTICOSTERONE EFFECTS ON ETHANOL-INDUCED SLEEP

Corticosterone Dose (mg/kg)	Sleep Time (min)	Brain Ethanol Level (mg/g tissue)
0	33 ± 4 (18)	4.20 ± 0.12 (6)
1	31 ± 5 (16)	4.26 ± 0.10 (6)
5	23 ± 4* (18)	4.15 ± 0.13 (6)
10	15 ± 6* (16)	4.08 ± 0.12 (6)
20	14 ± 6* (18)	4.21 ± 0.16 (6)

Sleep time was determined following an injection of ethanol (3 g/kg, IP) 15 min after corticosterone or vehicle administration. The value is mean ± SEM. Sample size is shown in parentheses.

*$p < 0.01$ for the difference from the control (vehicle only).

(Table 1). In mice receiving 1 mg/kg corticosterone prior to ethanol administration, no effect of the steroid on sleep time was found. However, as the steroid dose increased to 5 mg/kg the sleep time was reduced to 70% of the control level. At the steroid dose of 10 mg/kg, the sleep time was shortened to 45% of control. No further reduction of sleep time was found as the steroid dose increased to 20 mg/kg (Table 1) and higher (data not shown).

To ascertain that the alteration of sleep time found after the corticosterone treatment was not due to differences in ethanol metabolism, brain levels of ethanol were determined in sample animals 30 min following injection of 3 g/kg ethanol. The levels of ethanol did not differ among groups (Table 1).

To determine hormone specificity of the glucocorticoid effect, several steroids with different hormonal properties were examined for their effects on sleep time under identical conditions as corticosterone (Table 2). At the high dose used (20 mg/kg), cortisol, the principal glucocorticoid in humans, and dexamethasone, a synthetic glucocorticoid, were as effective as corticosterone in reducing the sleep time. In both cases, the sleep time was shortened to 40–45% of the control level. In contrast, 11-deoxycortisol, a cortisol derivative without glucocorticoid activity, was ineffective, even at the high dose used. Similarly, no reduction of sleep time was found in the two gonadal steroids tested, testosterone and 17β-estradiol.

TABLE 2

EFFECTS OF VAROIUS STERIODS ON ETHANOL-INDUCED SLEEP

Steriod	Sleep Time (min)
Control	34 ± 3 (20)
Cortisol	16 ± 4* (18)
Dexamethasone	14 ± 7* (18)
11-Deoxycortisol	31 ± 4 (12)
Testosterone	32 ± 5 (10)
17β-Estradiol	35 ± 4 (10)

All steriods were 20 mg/kg (IP). Controls received vehicle only. Sleep time was determined following an ethanol injection (3 g/kg, IP) 15 min after steriod administration. The value is mean ± SEM. Sample size is shown in parentheses.

*$p < 0.01$ for the difference from the control.

DISCUSSION

The results of our study indicate that the sedative action of ethanol is antagonized by corticosterone in a dose-dependent manner. The antagonistic effect of the steroid is not due to an alteration of ethanol metabolism because the brain levels of ethanol were not changed following various doses of the steroid. The antagonistic effect appears to be specific to glucocorticoids because steroids without glucocorticoid activity were found to be ineffective to reduce the sleep time. Under our experimental conditions using 3 g/kg ethanol as the hypnotic dose, the maximal antagonistic effect from corticosterone was a reduction of sleep time by about 55%, which was produced whether the steroid dose was 10 mg/kg or higher. It appears that the antagonistic effect to ethanol is saturable at a particular steroid dose. Sedation from a large dose of ethanol may involve multiple neuronal mechanisms; it is possible that corticosterone antagonizes some, but not all, of the underlying events produced by ethanol.

Sleep time following a hypnotic dose of ethanol provides a convenient and accurate approach as an initial step to assess the response of the brain to ethanol. The large doses of glucocorticoids that are required to produce the antagonistic effect may well represent an experimental necessity in dealing with the exaggerated dose of ethanol in such an animal model. The physiological significance of the steroid effect must await future studies using anxiolytic and mild sedative responses to low doses of ethanol. Moreover, it would also be important to gather information from isolated neuronal preparations where neurochemical and electrophysiological analysis can be made at low concentrations of both ethanol and the steroid.

The mechanism of the glucocorticoid action in antagonizing ethanol sedation can only be speculated from the present data. It is important to note that corticosterone was administered only 15 min prior to ethanol induction of sleep. From the rapidity of the steroid action, it is unlikely that the effect is mediated by the classical steroid hormone mechanism, which involves gene expression and protein synthesis following steroid binding with an intracellular receptor. There is substantial evidence that steroid hormones also act on the neuronal membrane (5,18). Two other cases are known where a steroid rapidly elicits or modifies a behavioral response by a membrane-mediated, nongenomic action. Progesterone induces lordosis in female rats within 5–10 min; this specific effect of the steroid is independent of de novo protein synthesis (7). Glucocorticoids suppress sexual behavior in the amphibian newt 5 min after steroid injection; this behavioral response is correlated with steroid binding to the neuronal membrane (5). It appears that the rapid glucocorticoid effect in ethanol sedation is mediated by a similar membrane mechanism. Thus, the fact that ethanol inhibits, whereas corticosterone enhances, the binding of calmodulin to synaptic plasma membrane, as demonstrated in vitro (3), is particularly interesting.

The effect of glucocorticoids found here is not the same as the "permissive" effect of these steroids in ethanol tolerance and dependence described in our earlier studies (10,11). In the permissive role, the steroids are involved as a required factor for the development of neuronal tolerance and physical dependence under chronic ethanol exposure. Ethanol tolerance and dependence are the results of complex adaptive neuronal re-

sponses developed over time; it is likely that alterations of gene expression are involved (15). The participation of glucocorticoids in the development of these adaptive processes may well be mediated by the intracellular receptor (16), which acts as a ligand-dependent transcription factor in the regulation of gene expression (1). In the present study on acute ethanol sedation, the steroids are shown to have a rapid action as an antagonist to ethanol, apparently by a membrane-mediated mechanism. It seems that the overall role of glucocorticoids is to defend normal brain function from sedation, whether by blocking the depressant action of acute ethanol or by promoting the development of adaptive processes under chronic ethanol.

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