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Ethanol-induced hypothermia in rats is antagonized by dexamethasone

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Abstract

The effect of dexamethasone on ethanol-induced hypothermia was investigated in 3.5-month old male Wistar rats (N = 10 animals per group). The animals were pretreated with dexamethasone (2.0 mg/kg, *ip*; volume of injection = 1 ml/kg) 15 min before ethanol administration (2.0, 3.0 and 4.0 g/kg, *ip*; 20% w/v) and the colon temperature was monitored with a digital thermometer 30, 60 and 90 min after ethanol administration. Ethanol treatment produced dose-dependent hypothermia throughout the experiment (-1.84 ± 0.10, -2.79 ± 0.09 and -3.79 ± 0.15°C for 2.0, 3.0 and 4.0 g/kg ethanol, respectively, 30 min after ethanol) but only the effects of 2.0 and 3.0 g/kg ethanol were significantly antagonized (-0.57 ± 0.09 and -1.25 ± 0.10, respectively, 30 min after ethanol) by pretreatment with dexamethasone (ANOVA, $P<0.05$). These results are in agreement with data from the literature on the rapid antagonism by glucocorticoids of other effects of ethanol. The antagonism was obtained after a short period of time, suggesting that the effect of dexamethasone is different from the classical actions of corticosteroids.

Key words
- Ethanol
- Dexamethasone
- Hypothermia
- Glucocorticoids
- Rats

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It is well known that ethanol produces a wide range of effects in the body as a consequence of its action on the central nervous system (CNS) or peripheral organs (1,2). In humans, ethanol tends to lower body temperature, an effect that depends on different factors such as the dose, clothing or physical activity (3). In general, ethanol administered by the peripheral route (4) or intracerebroventricularly (*icv*) (5) induces a rapid fall in body temperature in laboratory animals, monitored at normal ambient or at lower temperatures (3,6).

The mechanisms by which ethanol affects body temperature are susceptible to a variety of internal and external influences which contribute to its complexity. There are several mechanisms by which body temperature can be regulated, and ethanol can affect most of them by different actions including the suppression of shivering, production of hypoglycemia, and production of cutaneous vasodilatation which leads to increased loss of heat to the environment (for a review, see Ref. 3). It was shown that rats treated with ethanol present a decrease in their body temperature setpoint (7,8). Thus, the effect of ethanol on body temperature is considered to be poikilothermic and depends on the ambient temperature at which the animals are tested (7,9).

Ethanol affects CNS function by several different mechanisms such as the induction of primary perturbations of neuronal membranes (10), reduction of calcium influx through voltage-sensitive calcium channels (11), alteration in release of the neurotrans-

mitters dopamine (12), acetylcholine (13), norepinephrine (14), and serotonin (15), and alteration in neurotransmission mediated by inhibitory and excitatory amino acids (16). Hypothermia induced by ethanol has been related, at least partially, to neurotransmission mediated by monoamines (3,17,18) and to cellular Ca^{2+} (9,19), and may be modulated by other substances such as prostaglandins (20).

Interactions between ethanol and glucocorticoids have been described. For example, corticosterone increased the severity of symptoms exhibited by mice after withdrawal from ethanol and other depressant drugs (21), and adrenalectomy reduced ethanol consumption of rats (22). Recently, it has been reported that dexamethasone suppresses the hypertension induced by ethanol in humans (23). Moreover, it was observed that the hypnotic effect of ethanol on mice was consistently antagonized by the previous injection of large doses of glucocorticoids (20 mg/kg dexamethasone) probably because a high dose of ethanol was needed in this animal model (24). Regarding the control of body temperature, other studies have shown that, although dexamethasone could antagonize the hyperthermia induced by lipopolysaccharide (25), at a higher dose it significantly reduced the hypothermia induced by a high dose of lipopolysaccharide (26). Considering the interactions between glucocorticoids and the effects of ethanol, and that the sensitivity of animals to ethanol-induced CNS hypnosis can be influenced by body temperature (27,28), the present study was undertaken to investigate whether ethanol-induced hypothermia could be antagonized by pretreatment with the glucocorticoid dexamethasone.

A total of 120 male Wistar rats from our own colony, 3.5 months old, weighing approximately 350 g, were used. After weaning at 25 days of age, the animals were housed in groups of 6-8 in polyethylene cages (38 x 36 x 15 cm) in a room maintained at constant temperature (23 ± 1°C) and on a 12-h dark-light cycle (lights on from 6:00 to 18:00 h), with free access to tap water and standard laboratory chow. On the day of the experiment, the animals were transferred to the laboratory 2 h before the beginning of the test in order to habituate to the experimental environment. Room temperature was maintained at 23.0 ± 0.5°C throughout the experiment. Thirty minutes before the test, the animals were individually placed in wire cages in order to minimize the anticipatory anxiety which could interfere with temperature measurements. Each animal was then removed from its cage and gently restrained on the table with a cloth. The basal temperature was monitored by inserting a Vaseline-lubricated thermistor probe 5 cm into the rectum of the animal. Then, the rats received intraperitoneally (*ip*) the injectable form of dexamethasone (Merck, Sharp and Dohme; 2.0 mg/kg) or saline (0.15 M NaCl). After 15 min the animals were injected *ip* with ethanol, 20% w/v, in the appropriate volume to provide doses of 2.0, 3.0 or 4.0 g/kg, or saline. Thus, each ethanol dose group had a respective saline or dexamethasone control group. The doses of ethanol and dexamethasone were obtained from the literature and from previous experiments (20,24). Colon temperature was measured again 30, 60 and 90 min after ethanol administration. Statistical analysis was performed using the two-way ANOVA for repeated measures for each dose of ethanol.

The results presented in Figure 1 show that ethanol treatment resulted in a dose-dependent reduction in body temperature, in agreement with previous results (20,29). ANOVA revealed that dexamethasone produced a significant antagonism of the hypothermic effect of 2.0 g/kg ethanol [pretreatment: $F(1,36) = 42.29$, $P<0.00001$; treatment: $F(1,36) = 174.71$, $P<0.00001$; time: $F(2,72) = 35.52$, $P<0.000001$; pretreatment x treatment x time interaction: $F(2,72) = 6.42$, $P<0.002$] (Figure 1, left panel) and of 3.0 g/kg ethanol [pretreatment: $F(1,36) = 46.76$, $P<0.00001$; treatment: $F(1,36) =$

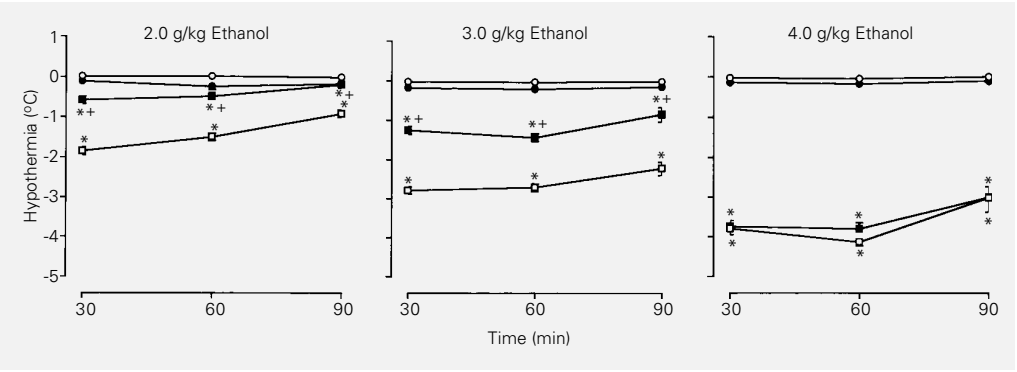

Figure 1 - Effect of dexamethasone pretreatment on ethanol-induced hypothermia in rats. Rats received dexamethasone (2.0 mg/kg, *ip*) or saline administered 15 min before ethanol 20% w/v (2.0, 3.0 or 4.0 g/kg, *ip*) or saline. Colon temperature was measured 30, 60 and 90 min after ethanol administration. The animals were divided into 4 groups: saline + saline (○); dexamethasone + saline (●); saline + ethanol (□), and dexamethasone + ethanol (■). Data are reported as the mean ± SEM of the fall in temperature in degrees Celsius obtained for 10 animals compared to basal values. *P<0.05 compared to saline + saline group; $^{+}$P<0.05 compared to saline + ethanol group (ANOVA).

334.05, P<0.000001; time: F(2,72) = 9.46, P<0.0002; treatment x time interaction: F(2,72) = 8.07, P<0.001] at all time intervals (Figure 1, middle panel). However, the hypothermic effect of the highest dose of ethanol was not significantly antagonized by dexamethasone (Figure 1, right panel).

The results of the present study show that the glucocorticoid dexamethasone antagonized the hypothermia induced by ethanol in rats.These findings are in agreement with the data obtained on the antagonism of ethanol-induced hypnosis by dexamethasone and cortisol in mice (24) and the blockade of ethanol-induced hypertension by dexamethasone in healthy humans (23). It is important to note that, although the dose of dexamethasone we used was higher than that used in studies on hyperpyrexia (30), it was ten times lower than that used in studies on the antagonism of ethanol-induced hypnosis by corticoids (24), and within the same dose range used to counteract lipopolysaccharide-induced hypothermia (26). Moreover, in our study the antagonism of the hypothermic effect was obtained with a dose of dexamethasone which did not alter the body temperature of control animals. This effect does not seem to be a consequence of increased ethanol metabolism since in another study the pretreatment with dexamethasone did not affect blood ethanol concentrations (24). Furthermore, in a previous study we demonstrated a very rapid antagonism of the hypothermic effect of ethanol by *icv* administration of dexamethasone to rats (data not shown). Therefore, our data suggest that a pharmacodynamic rather than a pharmacokinetic interaction between ethanol and dexamethasone seems to be responsible for the antagonism obtained in the present study. Pretreatment with dexamethasone did not block the hypothermic effect produced by the highest dose of ethanol (4 g/kg), perhaps as a consequence of the high degree of hypothermia obtained.

As mentioned before, several mechanisms modulate ethanol-induced hypothermia and some include the influence of Ca^{2+} ions. Chelation of Ca^{2+} ions in the hypothalamus could restore the decrease of the set-point produced by ethanol (7). Since dexamethasone antagonized the cytokine-induced Ca^{2+} increase in the brain accompanying the pyretic response in rabbits (31), we speculate that a reduction in Ca^{2+} ions produced by dexamethasone may be involved in the antagonism of ethanol-induced hypothermia in the present study. Since indomethacin effectively antagonized ethanol-induced hypothermia in rats (20), another possibility is that the inhibition of prostaglandin synthesis by dexamethasone could interfere with this effect. Our findings do not permit a conclusion about the molecular mechanism by which dexamethasone blocked the hypothermic effect of ethanol. Nevertheless, since this antagonism occurred after a short period of time, we suggest that this effect may be mediated by a mechanism involving cell

membrane receptors rather than the typical mechanism of action of corticoids (32). The use of protein synthesis inhibitors or selective glucocorticoid receptor blockers may help identify the mechanisms underlying this antagonism.

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