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DOES ETHANOL INDUCE STRESS IN RATS WITH ESTABLISHED ALCOHOL MOTIVATION?

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A single dose of ethanol of more than 1 g/kg induces considerable release of stressor hormones of pituitary and adrenal origin [3, 5]. In low doses (0.5-1 g/kg), however, ethanol may behave as an antistressor factor, and this can be recorded both in behavioral experiments and as lowering of the plasma corticosterone level in animals previously exposed to stress [1, 4]. Prolonged alcoholization is accompanied by activation of the hypothalamo-hypophyseoadrenal system with disturbance of internal feedback mechanisms, causing changes in the response of this system to classical stress-inducing stimuli [2, 7, 9].

The problem of possible reversal of the antistressor and stress-inducing properties of small and large doses of ethanol in animals with chronic contact with ethanol accordingly arises. The aim of this investigation was to compared the time course of the plasma ACTH level in intact rats and rats with established alcohol motivation.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-250 g were used. To study the effect of ethanol on the plasma ACTH level of intact rats, animals kept in communal cages were divided into three groups. Animals of group 1 received an intraperitoneal injection of physiological saline, those of groups 2 and 3 received a 25% solution of ethanol in doses of 1 and 4 g/kg respectively; the animals were decapitated (5-6 rats in each subgroup) 15, 30, and 60 min later.

Other rats were kept in individual cages with free access to water and 15% ethanol solution, and their liquid consumption was recorded daily for 14 days. The rats were then divided into four groups: animals of group 1, with no access to alcohol, received an intraperitoneal injection of physiological saline (water control); the remaining animals, consuming ethanol by preference, in approximately equal amounts, were given an intraperitoneal injection of physiological saline (group 2 – alcohol control) and a 25% solution of ethanol in doses of 1 and 4 g/kg (groups 3 and 4 respectively), and the animals (5-6 rats in each subgroup) were decapitated 15, 30, and 60 min later.

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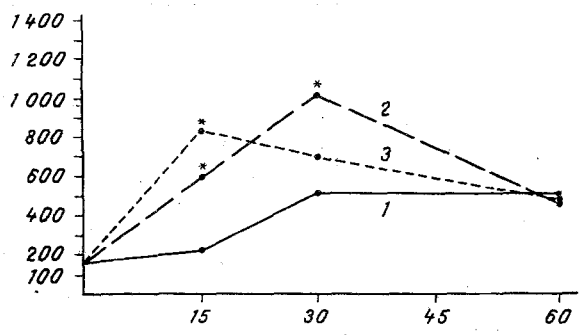

Fig. 1

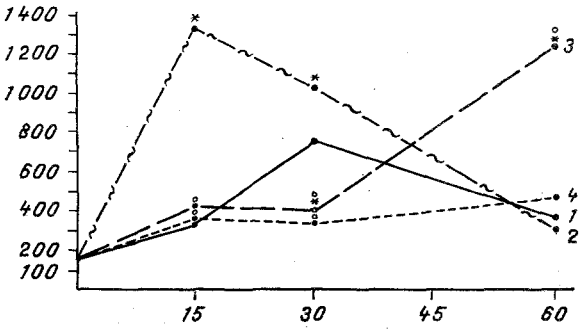

Fig. 2

Fig. 1. Time course of change in plasma ACTH level of rats after a single injection of ethanol in doses of 1 and 4 g/kg. 1) Control; 2) ethanol (1 g/kg); 3) ethanol (4 g/kg). *$p < 0.05$ compared with control at corresponding time interval. Here and in Fig. 2: Abscissa, time (min); ordinate, ACTH level (ng/ml).

Fig. 2. Time course of changes in plasma ACTH level in rats with established alcohol motivation, after a single injection of ethanol in doses of 1 and 4 g/kg. 1) Water control; 2) alcohol control; 3) ethanol (1 g/kg); 4) ethanol (4 g/kg). *$p < 0.05$ compared with water control, **$p < 0.05$ compared with alcohol control at corresponding time interval.

Plasma containing the sodium salt EDTA in a final concentration of 1 mg/ml was centrifuged at 2000 rpm for 20 min at 4°C. Immediately before determination, the plasma was diluted with the buffer used in the reaction in the ratio of 1:2. The hormone concentration was determined by radioimmunoassay, using commercial kits from "Sorin," (France).

EXPERIMENTAL RESULTS

Since the creation of a model of experimental alcoholism in animals, in most of the methods currently used, involves recording individual ethanol consumption [1], with the corresponding need to keep the animals in isolation, in the present investigation a comparative study was made of the effect of ethanol on the blood ACTH level in animals kept in communal and individual cages.

Two test doses of ethanol were used: 1 g/kg – a dose which, in behavioral experiments, has an anxiolytic action, and 4 g/kg – a subnarcotic dose, inducing depression in animals [1].

The time course of the plasma ACTH level of intact rats, kept in communal cages after a single injection of physiological saline and of ethanol in doses of 1 and 4 g/kg is illustrated in Fig. 1. The point corresponding to the ACTH concentration in pooled intact rat plasma is plotted along the ordinate. Handling animals in any experimental procedure is known to induce a typical stress response – the "handling" effect [6] – and, in conjection with the intraperitoneal injection of physiological saline, this can be regarded as a stress situation. Injection of physiological saline into rats kept in communal cages caused the ACTH level to rise 30 min later, and this effect lasted for the next 30 min. Intraperitoneal injection of ethanol in a dose of 1 g/kg was accompanied by a considerable increase in the plasma ACTH concentration, to reach a peak 30 min after injection. When ethanol was injected in a dose of 4 g/kg the maximal increase in the hormone level was observed only 15 min after injection. The plasma ACTH level in rats at the time of maximal elevation, due to injection of ethanol, whatever the dose, was significantly higher than plasma level of the hormone in animals receiving physiological saline. The plasma ACTH concentration in animals of the experimental and control groups was virtually identical 60 min after injection. Thus irrespective of its dose, ethanol is a stress-inducing factor for animals with no previous contact with ethanol, and kept under comfortable conditions in groups.

The data obtained on animals kept in individual cages are illustrated in Fig. 2. The ACTH level in the isolated rats receiving only water (water control) reached a peak after the same time interval as in intact rats (30 min after injection of physiological saline), but the absolute hormone level in this case was much higher. It can accordingly be concluded

that isolation potentiates the specific response of the pituitary gland to a stress-inducing situation. In rats kept in individual cages with free access to water and 15% ethanol solution (alcohol control) a sharp increase in the ACTH concentration was observed only 15 min after injection of physiological saline. Normalization of the ACTH concentration in this group was observed 60 min after the injection. Thus the formation of alcohol motivation in rats under conditions of isolation caused a marked change in the time course and intensity of the response to the stress situation. Ethanol in a dose of 1 g/kg abolished the response to stress in rats with established alcohol motivation in the course of 30 min after its injection. Elevation of the hormone level compared with that in animals of the control groups was not observed until 60 min after injection of ethanol. Correspondingly, after injection of ethanol in low doses, the rats of this group exhibited a picture of "delayed stress." Increasing the dose of ethanol to 4 g/kg demonstrated the complete reversal of the effect of ethanol on ACTH: the hormone level in rats with established alcohol motivation, 15 and 30 min after injection, was considerably lower than in the "alcohol" control group. The plasma ACTH level of the rats 60 min after injection of ethanol in a dose of 4 g/kg was the same as that in the control groups (alcohol and water controls). The absence of a stress-inducing action of high doses of ethanol in rats with established alcohol motivation cannot be entirely regarded as a manifestation of the development of tolerance to the ACTH-inducing action of ethanol [8], for no such phenomenon was observed 60 min after injection of lower doses (1 g/kg) of ethanol.

If its effect on the ACTH level is used as the criterion, ethanol when given as a single injection thus plays a dual role: it acts as a stress-inducing factor in intact rats but as an antistressor agent in rats with established alcohol motivation.

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