EFFECT OF ETHIMIZOLE ON THE HYPOTHALAMO – HYPOPHYSEO – ADRENAL SYSTEM OF RATS WHEN INHIBITED BY DEXAMETHASONE

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Ethimizole decreased the inhibition of the hypothalamo – hypophyseo – adrenocortical system due to dexamethasone, showing that the effect of the compound takes place through its influence on central (hypothalamic) mechanisms of regulation of the secretion of ACTH-glucocorticoids.

The well-marked excitatory effect of alkylamides of imidazole-dicarboxylic acid (the antiphein group of compounds) on the hypothalamo – hypophyseo – adrenocortical system (HNA) and their ability to weaken the inhibition of this system produced by injections of corticosteroids are well known [2-5].

The object of the present investigation was to study the mechanism of action of one of the most active compounds of this group of neurotropic agents, namely, ethimizole (the bis-methylamide of 1-ethylimidazole-4,5-dicarboxylic acid).

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-200 g. Ethimizole was injected intraperitoneally in doses of 2-20 mg/kg, and into the median eminence of the hypothalamus in a dose of 300 μg. This last injection was given through microcannulas implanted previously with the aid of a stereotaxic apparatus using the coordinates of Szentagothai et al. [7]. The cannulas were glass tubes 425 μ in diameter. The compound or distilled water was injected into the median eminence of the hypothalamus in a volume of 20 μl. The effect of ethimizole was compared with the action of formalin (10% solution), injected subcutaneously in a volume of 0.15-0.1 ml, or with the effect of laparotomy and extirpation of one adrenal. The rats were decapitated 90 min after injections of the substances, and blood was collected. The state of the HHA system was estimated with respect to the concentrations of 11-hydroxycorticosteroids (11-HCS) in the plasma of the circulating and adrenal blood, determined by the fluorometric method of De Moore in the modification of Usvatova and Pankov [6]. Blood flowing from the adrenal was collected in the acute experiment under superficial chloralose anesthesia (55 mg/kg). In some tests the ascorbic acid concentration in the adrenals of the rats was determined [8]. The experiments were carried out on intact animals and also after experimentally induced depression of the HHA system by intraperitoneal (2 h before injection of the tested

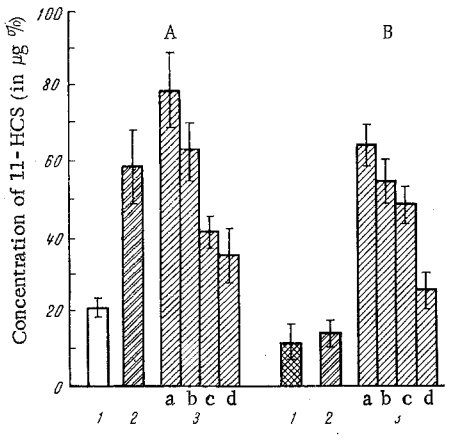

Fig. 1. Concentration of 11-HCS in circulating blood plasma of intact rats (A) and rats receiving dexamethasone (B): 1) control; 2) formalin (0.15 ml); 3) ethimizole (a 20, b 10, c 7.5, d 5 mg/kg).

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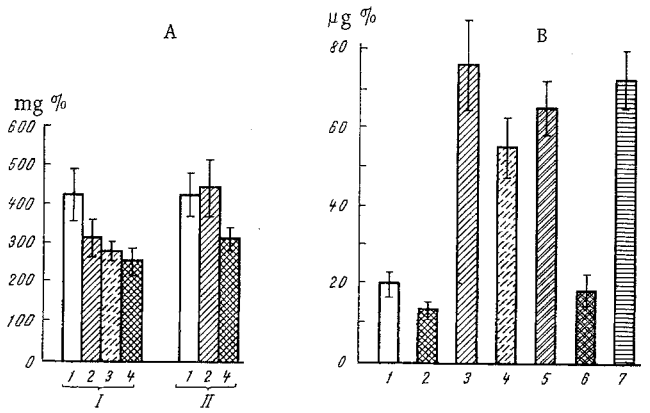

Fig. 2. Effect of ethimizole on adrenal response to laparotomy or to injection of formalin. A: ascorbic acid concentration in adrenals (in mg %); 1) control; 2) laparotomy; 3) ethimizole (300 μg); 4) ethimizole + laparotomy; I) intact rats; II) rats receiving dexamethasone (300 μg/kg); B: 11-HCS concentration (in μg%) in peripheral blood plasma; 1) control; 2) dexamethasone (300 μg/kg); 3) ethimizole (20 mg/kg); 4) dexamethasone + ethimizole; 5) formalin (0.15 ml); 6) dexamethasone + formalin; 7) dexamethasone + ethimizole + formalin.

compound) injection of dexamethasone (16 α-methyl-9-α-fluorohydrocortisone) in a dose of 300 μg/kg. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS AND DISCUSSION

In the experiments on intact rats the 11-HSC concentration in the circulating blood plasma 90 min after injection of ethimizole (7-20 mg/kg) was increased by 2-4 times (Fig. 1A). A similar effect was produced by stimulation as the result of subcutaneous injection of formalin (0.1-15 ml). In response to the surgical operation, the ascorbic acid concentration in the adrenals fell by 25% ($P < 0.05$; Fig. 2A). These results indicate that the severity of the response of the adrenal cortex to injection of ethimizole in these doses is similar to its response to formalin stimulation or to surgical trauma.

No response of the adrenals was observed to intraperitoneal injection of ethimizole (2 mg/kg). If, however, the compound was injected into the median eminence of the hypothalamus in a dose of 300 μg (1.5-2 mg/kg), 3 h after its injection a significant decrease was observed in the ascorbic acid concentration in the adrenals. These results indicate that the action of ethimizole is mediated through its effect on nervous, predominantly hypothalamic, centers of regulation of the secretion of ACTH-glucocorticoids.

In the next series of experiments the rats received a preliminary intraperitoneal injection of dexamethasone (300 μg/kg). The corticosteroid level 3.5 h after injection of dexamethasone was reduced in the circulating blood of the animals by approximately half compared with the controls (Fig. 1B). The experimental results show that dexamethasone, in the dose used, completely blocked the response of the rats' adrenals to subcutaneous injection of formalin (Fig. 1B) and also to surgical trauma (Fig. 2A). These facts indicate inhibition of the HHA system by dexamethasone. However, after injection of ethimizole into the median eminence of the hypothalamus, surgical trauma led to a marked ($P < 0.02$) decrease in the ascorbic acid concentration in the adrenals of the rats, despite the preliminary injection of dexamethasone. Similar results were also obtained when the corticosteroid content was determined in the blood plasma of rats receiving dexamethasone and subcutaneous injections of formalin (Fig. 2B); the 11-HCS concentration after intraperitoneal injection of ethimizole (10 mg/kg) and subcutaneous injection of formalin was higher than after injection of formalin alone.

It must be emphasized that dexamethasone only partially reduced the adrenocortical response to injection of ethimizole (Fig. 1B). These results indicate that ethimizole weakens the depression of the HHA system by dexamethasone.

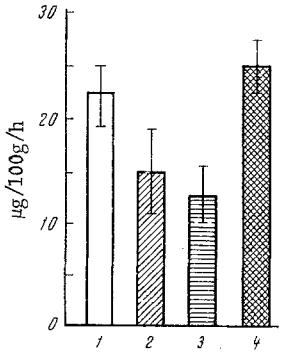

Fig. 3. Effect of ethimizole on corticosterone secretion by the adrenal (in μg/100 g/h): 1) control; 2) dexamethasone (300 μg/kg); 3) dexamethasone + ethimizole (20 mg/kg 1.5 h before collection of blood); 4) dexamethasone + ethimizole (20 μg/kg immediately before collection of blood).

The next series of acute experiments on rats showed that corticosterone secretion into blood flowing from the adrenal was 22.2 μg/100 g/h (Fig. 3). In rats receiving dexamethasone 3-3.5 h before the collection of blood began, the rate of corticosterone secretion was 14.7 μg/100 g/h ($P < 0.05$). This indicates depression the HHA system. Injection of ethimizole (20 mg/kg) immediately before collection of blood draining from the adrenal, in animals receiving dexamethasone, caused an increase in the corticosterone secretion by the adrenal to 24.7 μg/100 g/h, indicating activation of the HHA system by ethimizole.

If ethimizole was injected in the above dose 1.5 h before collection of the blood into animals which had first received dexamethasone, it did not produce an increase in the secretion of glucocorticoids but, on the contrary, a slight decrease in the level of corticosterone secretion (to 12.5 μg/100 g/h). Meanwhile, as mentioned above (Figs. 1B and 2B), the concentration of corticosteroids in the circulating blood plasma of the rats receiving dexamethasone was high 1.5 h after injection of ethimizole. This suggests that the absence of an increase in the intensity of corticosteroid secretion by the adrenal at these times was due to potentiation of the anesthesia by the action of ethimizole. However, another possibility is that when the compound was injected 1.5 h before the collection of blood began, its stimulant effect on the HHA system had already reached its full extent, so that no further increase in the secretion of corticosteroids took place as the result of the additional trauma. These results indicate that in the initial period, ethimizole led to an increase in the secretion of corticosteroids, which disappeared 1.5-2 h after injection of the compound.

The results of the experiments on rats thus confirm those obtained previously in experiments on guinea pigs [3, 5], indicating the ability of ethimizole to decrease the depression of the HHA system by corticosteroids. In additon, the results of this study of the mechanism of this effect of ethimizole indicate that the compound acts through its influence on central (hypothalamic) mechanisms of regulation of the secretion of ACTH-glucocorticoids.

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