PROTECTIVE ACTION OF PHENAZEPAM AND SODIUM HYDROXYBUTYRATE ON SOMATIC MANIFESTATIONS OF IMMOBILIZATION STRESS

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Many tranquilizers are known to abolish the anxiety and alarm reaction in emotional stress [2]. However, it has not been established whether they are active antistressor agents or whether they affect the dynamics of the stress reaction and modify the somatic manifestations of stress.

In the investigation described above, the tranquilizer phenazepam* and the compound sodium hydroxybutyrate, which in small doses has a tranquilizing action [9], were studied for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180 ± 30 g. The animals were immobolized by fixing them securely in special frames for 30 min and 1, 4, and 24 h. After decapitation of the rat the thymus, spleen, and adrenals were weighed, and the state of the gastric mucosa was studied. Lipids were detected histochemically in the adrenal cortex [5] and the thickness of the lipid zone was subjected to morphometry under the light microscope. The cortical layer of the adrenals also was studied with the electron microscope.

Phenazepam in doses of 0.1 and 1 mg/kg and sodium hydroxybutyrate in doses of 10 and 40 mg/kg were injected intraperitoneally 1 h and immediately before immobilization; the injections were repeated every 4 h during the experiment. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Previous investigations showed [4] that the development of immobilization stress in animals fixed by the method described above goes through three stages: a stage of anxiety (the first 4 h), a stage of adaptation (the next 24 h), and a stage of exhaustion (after 48 h). It will be clear from Fig. 1 that during the first 30-60 min the weight of the lymphoid organs fell to about 60% of its initial level, to which it returned after 4 h; the weight of the adrenals showed a tendency to fall and the thickness of the lipid layer of the adrenal cortex was reduced by about 50% after 1 h of immobilization and was restored after 4 h. In the stage of adaptation the weight of the thymus and thickness of the lipid layer of the adrenals were indistinguishable from normal, moderate hypertrophy of the adrenals and a very small decrease in weight of the spleen were observed, and the gastric mucosa showed edema, hyperemia, and hemorrhages. The results of light and electron microscopy also showed hypertrophy of the adrenal cortex (especially the zona fasciculata) and an increase in the number of lipid drops compared with the stage of anxiety.

To demonstrate the protective effects of phenazepam and sodium hydroxybutyrate against stress the dynamics of the stress reaction was studied at the following time intervals: after 30 min and 1 h — maximal changes in the anxiety stage, after 4 h — the beginning of the adaptation stage, and after 24 h — the end of the adaptation stage. After injection of

*7-Bromo-1,3-dihydro-5-(2'-chlorophenyl)-2H-1,4-benzodiazepin-2-one.

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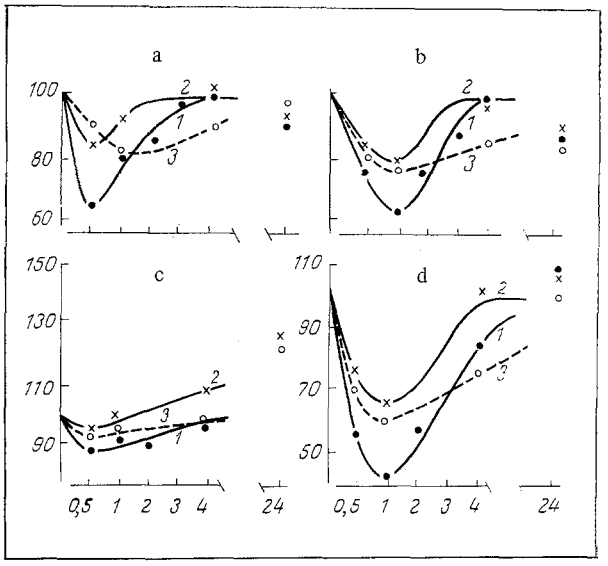

Fig. 1. Changes in weight of lymphoid organs and adrenals and thickness of lipid layer in adrenal cortex of rats after immobilization. a) Weight of thymus; b) weight of spleen; c) weight of adrenals; d) thickness of lipid layer in adrenal cortex. 1) After injection of physiological saline; 2) after injection of phenazepam, 1 mg/kg; 3) after injection of sodium hydroxybutyrate, 40 mg/kg. Abscissa, duration of stress (in h); ordinate, changes (in %).

phenazepam in a dose of 1 mg/kg (Fig. 1: 2) changes in the weight of the lymphoid organs and in the lipid content in the adrenal cortex were much less marked in the anxiety stage. For instance, the weight of the thymus (Fig. 1a) after 30 min of stress had fallen only to 83% (compared with 64% in the absence of the drug). The weight of the spleen (Fig. 1b) and the lipid content in the adrenal cortex (Fig. 1d) after 1 h had fallen to 78 and 64% (without the drug to 61 and 42%) respectively. The decrease in weight of the adrenals (Fig. 1c) after exposure to stress for 30 min was not statistically significant.

All the parameters studied, except the thickness of the lipid layer, returned to their initial levels after immobilization for 2.5-3 h and during treatment with phenazepam. After exposure to stress for 4 h, hypertrophy of the adrenals, signifying the beginning of the stage of resistance, was observed after administration of phenazepam. Further evidence of this stage was given by the weight of the thymus and spleen and the lipid content in the adrenal cortex. The stage of adaptation followed a smoother course after phenazepam: Hypertrophy of the adrenals was less marked after 24 h of immobilization.

Phenazepam in a dose of 0.1 mg/kg had no protective action against stress.

The antistressor effect of sodium hydroxybutyrate (10 and 40 mg/kg) was exhibited only with the larger dose (Fig. 1:3), and after a dose of 10 mg/kg the results did not differ significantly from the control. After treatment with this compound the weight of the thymus after immobilization for 30 min was reduced to 91% compared with 64% in the control (Fig. 1a). The maximal decrease in weight of the thymus (to 81%) was observed only after exposure of 1 h, but not of 30 min, just as in the control animals and after treatment with phenazepam. The weight of the spleen and the lipid content in the adrenal cortex after exposure to stress for 1 h were 78 and 61% (without treatment 61 and 42%) respectively. Changes in weight of the adrenals did not differ significantly from the control. After immobilization for 4 h the weight of the lymphoid organs and adrenals and the lipid content in the adrenal cortex had not yet regained their initial values, which they did not reach until after 6 h, evidence of lengthening of the anxiety stage during treatment with sodium hydroxybutyrate. The adaptation stage, like the anxiety stage, was smoother: The weight of the adrenals after stress for 24 h was 124% (155% in the control).

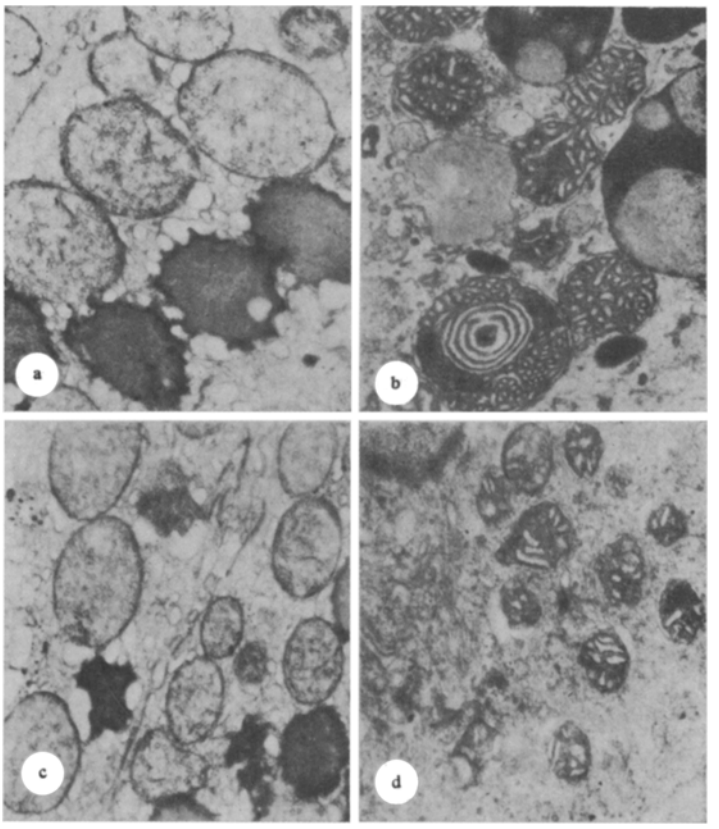

Fig. 2. Ultrastructure of adrenocorticocytes of zona fasciculata and zona glomerulosa of rat adrenals during stage of adaptation to stress. a) Cell of zona fasciculata of adrenal of control rats. b) Cell of zona glomerulosa of adrenal of control rat; c) cell of zona fasciculata after treatment with phenazepam (1 mg/kg); d) cell of zona glomerulosa after treatment with phenazepam (1 mg/kg).

An electron-microscopic study of the adrenal cortex of the control animals in the anxiety stage showed that stress induces mainly ultrastructural changes in the mitochondria and smooth endoplasmic reticulum and in the number of lipid drops. These changes were more marked in the zone fasciculata (Fig. 2a). For instance, after immobilization of the animals for 1 h dilatation of the structures of the endoplasmic reticulum, moderate swelling of the mitochondria with translucency of their matrix and a decrease in the number of vesicular cristae in them, and a decrease in the number of lipid inclusions were observed. These changes were characteristic of "pale" adrenocorticocytes. In the adaptation stage considerable hypertrophy of all zones of the cortex was observed, but this was primarily connected with an increase in volume of the cells of the zona fasciculata. In all zones of the cortex "pale" and "dark" cells could be distinguished; the latter were increased in number. The nuclei of these cells were irregular in shape, with masses of chromatin at the periphery. Hyperplasia of the mitochondria was noted, with an increase in the number of their cristae. The smooth endoplasmic reticulum also was hypertrophied and had dilated tubules. The number of lysosomes was increased in the cells of the zona fasciculata (Fig. 2a). Similar ultrastructural changes were found in the zona reticularis and zona glomerulosa (Fig. 2b).

After treatment with phenazepam and sodium hydroxybutyrate the submicroscopic changes indicated less marked reorganization of the structural and functional apparatus of the adrenocorticocytes. The ultrastructure of the cortical cells was almost indistinguishable from that in intact animals. The only evidence of stress was the appearance of "dark" cells, moderate hypertrophy of the smooth endoplasmic reticulum and Golgi complex, and enlargement and fusion of some of the liposomes (Fig. 2c). Hyperplasia of the mitochondria was observed in cells of the zona glomerulosa, and their matrix had increased electron density (Fig. 2d).

A protective action against stress was thus clearly revealed in the various preparations studied by the methods described above. Both phenazepam and sodium hydroxybutyrate could reduce the severity of the manifestations of stress in the anxiety and adaptation stages. This antistressor effect can evidently be explained by the tranquilizing action of phenazepam and sodium hydroxybutyrate in the doses given [1, 2]. However, the dynamics of the stress reaction in the anxiety stage was changed differently by the two drugs. After administration of phenazepam the anxiety stage was shortened (to 2.5-3 h), whereas after administration of sodium hydroxybutyrate, on the other hand, it was prolonged. Lengthening of the anxiety stage was perhaps connected with the shorter tranquilizing effect [8] of sodium hydroxybutyrate than of phenazepam [3]. However, whereas the antistressor action of phenazepam can be explained mainly by its anxiolytic effect, in the mechanism of the protective action of sodium hydroxybutyrate against stress other factors besides the tranquilizing effect may also be important. It has been shown, for example, that in analogous doses sodium hydroxybutyrate has a marked antihypoxic effect [6], the mechanism of which is explained not only by normalization of oxidative metabolism, but also by prevention of disturbances of nitrogen metabolism [7].

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