EFFECT OF DIAZEPAM ON OXIDATIVE METABOLISM OF MOUSE BRAIN TISSUE

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Diazepam inhibits oxygen absorption by homogenates during oxidation of endogenous respiration substrates. When added to mitochondrial fractions it inhibits oxidation of glutamate but does not effect oxidation of succinate. These results indicate that diazepam inhibits activity of those dehydrogenases in brain tissue whose coenzyme is NAD.

Benzodiazepine derivatives are widely used in clinical medicine and, in particular, during surgical operations [5] when the oxygen supply to the tissue is disturbed. Diazepam has been shown to increase the resistance of animals to hypoxia [1].

The effect of diazepam on oxidative metabolism of the brain tissue of animals was investigated.

EXPERIMENTAL METHOD

Male albino mice weighing 20-25 g were used. Tissue specimens were obtained from the cerebral hemispheres. The tissue was homogenized in a medium of the following composition: mannitol 0.25 M, tris 0.01 M, EDTA 0.2 mM, KCl 0.01 M, pH 7.4. The buffer solution was the homogenization medium together with 0.01 M K_2HPO_4, pH 7.4. In experiments with homogenates the tissue was homogenized in 2 volumes of medium. The incubation medium in these experiments consisted of 0.5 ml homogenate (8-10 mg protein) and 0.5 ml of buffer solution. Mitochondria were isolated by differential centrifugation from 10% homogenates. The composition of the incubation medium was: 0.5 ml mitochondrial suspension (5-7 mg protein) and 0.5 ml buffer solution containing hexokinase, ATP, $MgCl_2$ [2], and respiration substrate (glutamate or succinate), 10 μmoles. The diazepam was dissolved in 0.01 N HCl and added to the samples in buffer solution in a concentration of $4 \cdot 10^{-4}$ M.

The absorption of oxygen by the homogenates and mitochondria of the brain tissue was studied by Warburg's manometric method. The samples were incubated in the small tubes of the Warburg apparatus at 26°C for 15 min. The intensity of respiration was expressed in microatoms oxygen absorbed per minute per gram protein of the sample. The protein content was determined by the biuret reaction. Mean values calculated from the results of five to six analogus experiments are given in Table 1.

EXPERIMENTAL RESULTS AND DISCUSSION

Diazepam very slightly inhibits oxidation of endogenous respiration substrates in mouse brain tissue homogenates (Table 1).

Experiments were carried out with mitochondria isolated from the brain tissue homogenates in order to detect the part of the mitochondrial respiratory chain which is sensitive to the inhibitory effect of diazepam. As the results in Table 1 show, when added to the mitochondrial fraction in a concentration of $4 \cdot 10^{-4}$ M, diazepam had no effect on the oxidation of succinate but considerably inhibited the oxidation of glutamate.

This result demonstrates that diazepam inhibits those dehydrogenases whose coenzyme is NAD.

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TABLE 1. Effect of Diazepam ($4 \cdot 10^{-4}$ M) on Oxygen Absorption By Homogenates and Mitochondria of Mouse Brain Tissue

Tissue sample	Respiration substrates	Absorption of O_2 (in $\mu A/min \cdot g$)		Inhibition of oxygen absorption by diazepam (in percent)
		control	diazepam	
Homogenates	Endogenous	25.0	21.4	14.4
Mitochondria	Succinate	39.5	39.5	0
	Glutamate	30.0	21.0	30

Comparison of the results obtained on homogenates and mitochondria shows that the oxidation of endogenous respiration substrates by homogenates is less sensitive to diazepam than the oxidation of glutamate by mitochondria. The explanation of this difference must be that among the endogenous respiration substrates (in the experiments with homogenates) there are some which are oxidized by both NAD-dependent dehydrogenases, i.e., those sensitive to diazepam, and by NAD-independent dehydrogenases, not sensitive to the inhibitory action of diazepam.

The ability of diazepam to prolong the survival of animals exposed to hypoxia, discovered previously [1], can evidently be attributed both to a reduction in the animal's energy requirements on account of the sedative and hypothermic effect of the drug, and also to the maintenance of NAD-independent tissue respiration.

Chlorpromazine differs from diazepam in this respect. Although chlorpromazine has both hypothermic and sedative effects, it does not significantly prolong the survival of mice during exposure to hypoxia [1]. The reason for this may be that chlorpromazine inhibits succinate dehydrogenase and cytochrome oxidase [4], so that the transport of hydrogen and electrons is blocked at both the beginning and end of the respiratory chain. In the presence of diazepam, on the other hand, succinate can still be oxidized because of the insensitivity of NAD-independent respiration to diazepam. Since the oxidation of succinate during hypoxia evidently plays an essential role [3], this may be an important factor in the general action of diazepam in prolonging the survival of animals in a state of hypoxia.

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