to the efferent component [10]. With this in mind, and also considering that it is in the anterior cortical zones that information from the limbic system is compared with the possibilities of efferent action, and in this way the cortical control of the emotions is brought about [6], it will be clear that the results now obtained showing the marked effect of benzodiazepine on the anterior cortical zones are of great importance for the assessment of the mechanism of action of these substances.

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EFFECT OF ETHIMIZOLE ON ENERGY METABOLISM IN THE RAT BRAIN

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In a dose of 25 mg/kg, 20 min after intraperitoneal injection, ethimizole stimulates oxidative phosphorylation, increases the creatine phosphate content and reduces the concentration of inorganic phosphorus in the brain tissue of rats. It is postulated that ethimizole stimulates energy metabolism through its activating effect on adenyl cyclase.

KEY WORDS: *high-energy compounds*; *oxidative phosphorylation*; *brain*; *ethimizole*.

A previous investigation showed that the molecular mechanism of the action of ethimizole is connected with its activating effect on adenyl cyclase [1, 3]. Ethimizole has also been shown to stimulate glycolysis in the brain [4].

In the investigation now described oxidative phosphorylation and the concentration of high-energy phosphorus compounds in brain tissue were investigated after administration of ethimizole.

EXPERIMENTAL METHOD

Male albino mice weighing 180-200 g were used. Ethimizole was injected intraperitoneally in doses of 2.5 and 25 mg/kg 20 min before sacrifice. To investigate oxidative phosphorylation the rats were decapitate., and to determine the concentrations of ATP, creatine phosphate,

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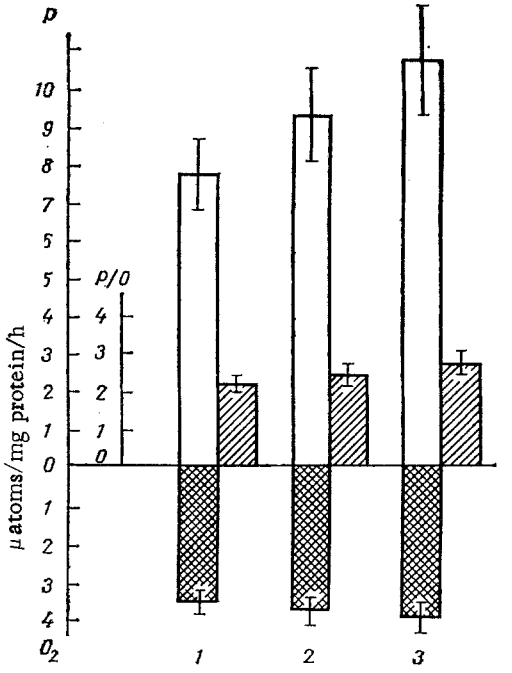

Fig. 1. Effect of ethimizole on oxidative phosphorylation in rat brain: 1) control; 2, 3) experiments with ethimizole in doses of 2.5 and 25 mg/kg respectively. Unshaded columns) transfer of inorganic phosphorus; cross-hatched) oxygen uptake; obliquely shaded) P:O ratio. Values of M ± 2.5 m given.

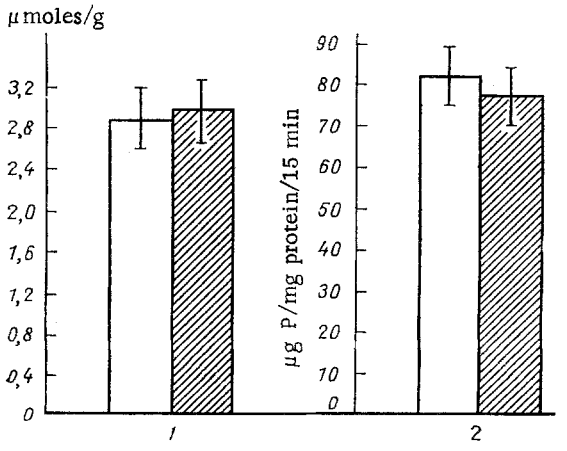

Fig. 2. Effect of ethimizole (25 mg/kg) on ATP content and ATPase activity in rat brain: 1) ATP content; 2) ATPase activity; unshaded columns) control; shaded columns) experiments with ethimizole.

and inorganic phosphorus, they were immersed whole in liquid oxygen. Oxidative phosphorylation in mitochondria isolated from the brain [11] was measured in a Warburg apparatus at 26°C for 20 min. The incubation medium contained: 0.02 M potassium phosphate buffer, 0.005 M $MgCl_2$, 0.001 M NaF, 0.01 M KCl, 0.05 M glucose, 0.02 M α-ketoglutarate, 200 μg ATP, and 300 μg hexokinase. The decrease in the phosphate concentration in the incubated samples [6], the content of protein [13] and ATP [12], ATPase activity [5], and the levels of creatine phosphate [10] and inorganic phosphorus (by the method of Fiske and Subbarow) also were determined.

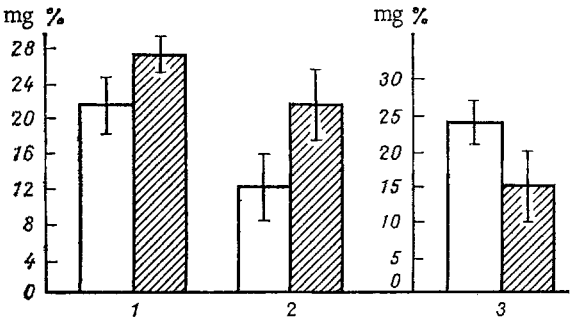

Fig. 3. Effect of ethimizole on levels of creatine phosphate and inorganic phosphorus in rat brain: 1, 2) creatine phosphate in experiments with ethimizole in doses of 2.5 and 25 mg/kg respectively; 3) inorganic phosphorus in experiments with ethimizole in a dose of 25 mg/kg; unshaded columns) control; shaded columns) experiments with ethimizole.

EXPERIMENTAL RESULTS AND DISCUSSION

Ethimizole, in a dose of 25 mg/kg, increased the phosphorylating activity of the brain mitochondria and did not change their respiratory activity (Fig. 1). Esterification of inorganic phosphorus was increased by 36%, the uptake of oxygen was unchanged, and the P:O ratio was increased by 23%, indicating the stronger coupling of oxidation with phosphorylation in the experimental animals than in the controls.

Similar but less marked changes were observed after administration of ethimizole in a dose of 2.5 mg/kg. The tendency observed in these experiments for the P:O ratio and the transfer of inorganic phosphorus to rise did not reach the level of statistical significance ($P > 0.05$).

Oxidative phosphorylation is known to be the principal process supplying ATP for the needs of the cell [2, 8, 9]. In the present experiments no significant changes were found in the ATP level or ATPase activity in the brain tissue of the rats following administration of ethimizole (Fig. 2); meanwhile in doses of 25 and 2.5 mg/kg this substance significantly ($P < 0.05$) increased the creatine phosphate concentration (by 75 and 20% respectively) and lowered the inorganic phosphorus concentration (by 36%) in the brain (Fig. 3). The effect of ethimizole was independent of the season of the year although the creatine phosphate concentration in the brain of intact rats is higher in winter than in spring.

The results indicate that ethimizole causes considerable changes in the energy metabolism of the rat brain, and these changes increase with an increase in the dose of drug. The changes consist of stimulation of oxidative phosphorylation on account of an increase in the phosphorylating activity of the mitochondrial respiratory chain, an increase in the concentration of creatine phosphate, the source of the additional reserves of energy, and a decrease in the concentration of inorganic phosphorus.

The absence of changes in the ATP content in the brain tissue of the rats receiving ethimizole can be explained on the grounds that the ATP content in the cells is maintained at a constant level through special intracellular systems [7]. Since the rate of breakdown of ATP in ATPase reactions was unchanged by ethimizole, it can be postulated that the level of this adenine nucleotide is maintained by the creatine–creatine phosphate system.

The accumulation of cyclic AMP and activation of glycolysis under the influence of ethimizole demonstrated previously [1, 3, 4], combined with the results of the present investigation, indicate that ethimizole increases energy metabolism as a whole, including both its anaerobic and aerobic phases.

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EFFECT OF ACETYLCHOLINE AND ATROPINE ON THE SECRETION OF BLOOD CLOTTING COMPOUNDS INTO THE BLOOD STREAM BY THE KIDNEYS

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Experiments with perfusion of the kidneys of cats in situ showed that the secretion of clotting factors and fibrinolytic substances by the kidneys into the blood stream is a controlled process. Acetylcholine reduces the supply of blood clotting substances and of antiheparin compounds into the blood stream but increases the liberation of plasminogen activators from the kidneys to some extent. Atropine stimulates the liberation of thromboplastic substances and antiheparin components from the kidneys but reduces the secretion of antithrombin compounds. Atropine slightly increases the fibrinolytic activity of the perfusion fluid.

KEY WORDS: *kidney*; *blood clotting*; *fibrinolysis*; *acetylcholine*; *atropine*.

Adrenalin and choline chloride have been shown to stimulate the discharge of thromboplastin and fibrinolysis activators into the blood stream [4, 6-10]. It has also been shown that the kidney is one of the organs which participates actively in the regulation of blood clotting and fibrinolysis [3, 5, 11, 13-15]. However, the role of the kidneys in the modifications of blood clotting observed during changes in the functional state of the autonomic nervous system has received insufficient study.

An attempt was accordingly made to determine whether the kidneys secrete blood clotting compounds into the blood stream and also to examine the effect of acetylcholine and atropine on this process.

EXPERIMENTAL METHOD

Experiments were carried out on 27 cats. Under thiopental anesthesia (50 mg/kg) the renal vessels of the animals were cannulated and the kidney was perfused through the artery with warm Ringer–Locke solution. For a period of 2 h samples of perfusion fluid were collected every 20 min. In special series of experiments, after the first two samples of perfusion fluid had been taken, acetylcholine (0.1 mg/kg) or atropine (0.1 mg/kg) was added to the perfusion fluid, and samples were then again taken in accordance with the scheme above. The

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