THE EFFECT OF CERTAIN PHENOTHIAZINE DERIVATIVES ON RESPIRATORY PHOSPHORYLATION IN THE CARDIAC MUSCLE OF THE RABBIT

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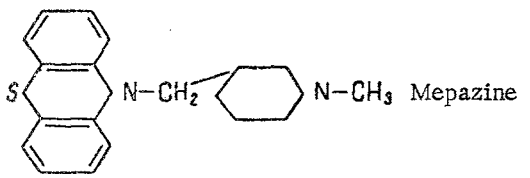
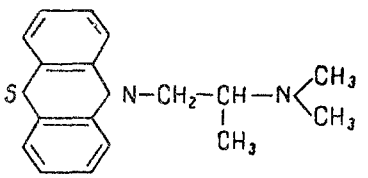
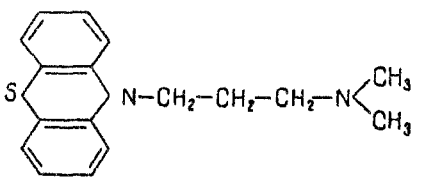
Certain derivatives of the phenothiazine series, possessing a neuroplegic action, are widely employed in clinical practice. In this connection the attention of biochemists has been drawn to the study of the effect of phenothiazine derivatives on a number of enzymic processes. The drug most studied in this respect is aminazin (chlorpromazine). Data are available showing the inhibition by chloropromazine of respiration [7], phoshporylation [3, 5, 9], adenosinetriphosphatase [3, 8, 9] and cytochrome oxidase [7, 6] activity, and phospholipid metabolism [4]. Regarding the analogs of chloropromazine - promethazine (phenergan) and promazine, those are known to depress cytochrome oxidase and adenosinetriphosphatase [8].



Mepazine



Phenergan



Promazine

In the present research we investigated the effect of three phenothiazine derivatives - mepazine [(acetate-10-) N-methylpiperidyl-3-methylphenothiazine], phenergan [(hydrochloride-10-) 2-dimethylaminopropylphenothiazine] and promazine [(hydrochloride-10-) 3-dimethylaminopropylphenothiazine] - on the processes of respiration and the accompanying phosphorylation in homogenates of the cardiac muscle of the rabbit.

METHOD

Male rabbits weighing 2.0-2.5 kg were decapitated, the heart was rapidly extracted and placed in cold physiological saline and, through a cannula introduced into the aorta, it was perfused in order to free it from the greater part of its blood. The cardiac muscle was then separated from connective tissue and fat, cut into small pieces with scissors and homogenized for one minute in the cold in a glass homogenizor in two volumes (in proportion to the weight of tissue) of a saline medium. The homogenate used in the experiments was filtered through several layers of gauze. The concentrations of the components of the saline medium for homogenization were as follows: NaCl - $1.4 \cdot 10^{-1}$ M, $MgSO_4$ - $2 \cdot 10^{-3}$ M, KCl - $5 \cdot 10^{-3}$ M. The reaction of the medium was adjusted to pH = 7.8-8.0 with caustic soda (0.5 N). The experiments on oxidative phosphorylation were conducted in Warburg flasks. Each flask contained 1 ml of incubation medium, consisting of 0.5 ml of homogenate and 0.5 ml of buffer solution (pH = 8.0). The concentrations of the various components of the buffer solution were: Na_2HPO_4 - $4.6 \cdot 10^{-2}$ M, $MgSO_4$ - $2 \cdot 10^{-3}$ M, KCl - $5 \cdot 10^{-3}$ M, NaCl - $1 \cdot 10^{-1}$ M. Creatine, which was used as the final phosphate acceptor, was added in an amount of 5 mg of a sample volume of 1 ml. The phenothiazine derivatives were introduced into the composition of the buffer solution.

The experiments were performed in an atmosphere of oxygen, the absorption of which was measured in the Warburg apparatus. Incubation was at 260° for 18 minutes. The intensity of phosphorylation was judged by the formation of phosphocreatine and by the decrease in inorganic phosphorus in the medium after incubation. At the end of incubation the proteins were precipitated by an equal volume of 5% trichloroacetic acid, and separated by centrifugation. The phosphocreatine of the supernatant fluid was determined by A. M. Alekseeva's method [1] and the inorganic phosphate by the Fiske-Subbarow method [2]. The experiments on the study of the oreatine kinase activity were carried out on muscle extracts [2]. Minced

muscle from the washed heart was extracted in the cold with two volumes of distilled water for 20 minutes. The tissue was then squeezed between gauze, the extract centrifuged and the supernatant fluid was used in the experiments as an enzyme solution. The experimental samples consisted of 0.5 ml of extract and 0.5 ml of buffer solution, to which had previously been added adenosinetriphosphate (10 mg), creatine (5 mg) and NaF (1 mg). In the experiments on the study of the adenosinetriphosphatase activity, a homogenate of cardiac muscle was used as enzyme solution. The experimental samples consisted of 0.5 ml of homogenate and 0.5 ml of buffer solution, to which had been previously added adenosinetriphosphate (10 mg) and monoiodoacetate (0.2 mg).

The phenothiazine derivatives were introduced into the composition of the buffer solution, consisting in this case of $1.5 \cdot 10^{-1}$ M $NaHCO_3$ solution in a 1% solution of $MgSO_4$. After the additives had been dissolved the reaction of the medium was adjusted to pH = 8.0.

Incubation was at 26° for 18 minutes. At the end of incubation, the phosphocreatine content of protein-free centrifugates was determined (in the experiments to study creatine kinase activity) and the increase in mineral phosphorus (in the experiments to study adenosinetriphosphatase activity).

RESULTS

The effect of all three phenothiazine derivatives on respiration and phosphorylation is shown in Fig. 1.

phosphorylation (at a concentration of $0.8 \cdot 10^{-3}$ M) and of the less sharp lowering of phosphorylation than of respiration (at a concentration of $1.2 \cdot 10^{-3}$ M), the P : O coefficient was increased with low concentrations of promazine.

Mepazine, in a concentration of $0.8 \cdot 10^{-3}$ M, slightly depressed respiration and stimulated phosphocreatine formation. Concentrations exceeding $0.8 \cdot 10^{-3}$ M caused considerable depression of phosphocreatine formation. Particularly marked depression of phosphocreatine formation was caused by mepazine in a concentration of $1.6 \cdot 10^{-3}$ M; at this concentration dissociation of respiration and phosphorylation took place, expressed as a fall in the P : O coefficient.

In contrast to promazine and mepazine, phenergan in a concentration of $0.8 \cdot 10^{-3}$ M depressed respiration and phosphocreatine formation equally. In concentrations higher than $0.8 \cdot 10^{-3}$ M, and especially above $1.2 \cdot 10^{-3}$ M, phenergan depressed phosphorylation more strongly than respiration, as a result of which the P : O coefficient fell considerably.

In order to discover whether the depression of phosphocreatine formation was the result of inhibition of oxidative phosphorylation or inhibition of creatine kinase, or whether it arose as the result of activation of adenosinetriphosphatase, we carried out experiments in which we studied the effect of the phenothiazine derivatives on the activity of creatine kinase and adenosinetriphosphatase.

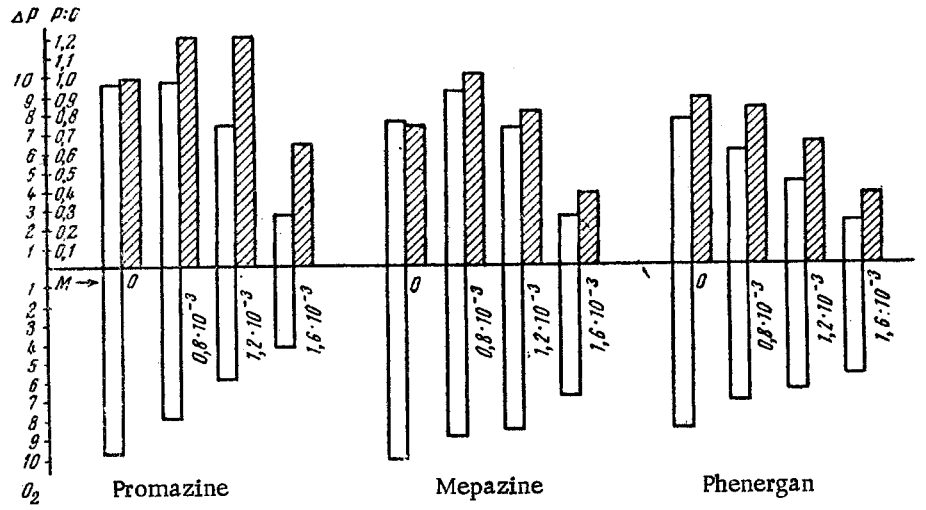


Fig. 1. The effect of different concentrations of phenothiazine derivatives on oxidative phosphorylation. ΔP – Formation of phosphocreatine in microatoms of phosphorus; ΔO_2 – absorption of oxygen in microatoms; shaded columns – P : O coefficient.

It can be seen that promazine, in a concentration of $0.8 \cdot 10^{-3}$ M, depressed respiration while the phosphocreatine formation was increased or remained within normal limits. Inhibition of phosphocreatine formation by promazine began when its concentration was increased further (above $0.8 \cdot 10^{-3}$ M). As a result of the more pronounced lowering of respiration and the slight increase in

The results of these experiments, shown in Figs. 2 and 3, demonstrate that mepazine, phenergan and promazine (in concentrations depressing oxidative phosphorylation) neither depressed creatine kinase nor activated adenosinetriphosphatase. It was characteristic that, in the concentrations used, mepazine and promazine showed a depressing action on adenosinetriphosphatase activity.

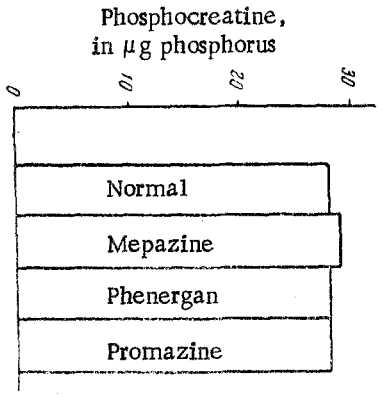


Fig. 2. Effect of mepazine, phenergan and promazine (in concentrations of $1.6 \cdot 10^{-3}$ M) on creatine kinase activity.

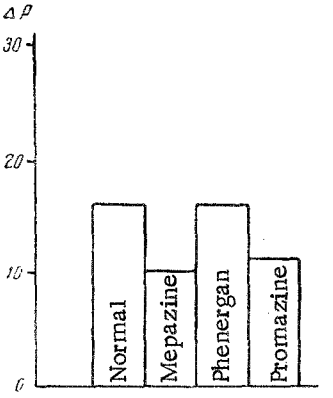


Fig. 3. Effect of mepazine, phenergan and promazine(in concentration of $1.6 \cdot 10^{-3}$ M on adenosinetriphosphatase activity. P – increase in mineral) phosphate (in microatoms).

It may be concluded from the results obtained that the mechanism of action of the tested phenothiazine derivatives on respiratory phosphorylation differed slightly. In the presence of mepazine, respiration was depressed to a lesser degree than in the presence of the two isomers investigated – phenergan and promazine, and promazine had a more pronounced effect than phenergan.

The action of mepazine was also characterized by stimulation of phosphorylation at low concentrations of the drug, with subsequent depression of the process as the concentrations were increased. Phenergan depressed phosphorylation in low concentrations, whereas promazine had no effect on this process in these concentrations. A special feature of promazine was the sharp depression of respiration, preceding the depression of phosphorylation.

By their effect on phosphorylation, the drugs tested could be placed in order: Mepazine in a concentration of $0.8 \cdot 10^{-3}$ M increased phosphorylation; promazine increased it only slightly, and phenergan depressed it. In concentration of over $0.8 \cdot 10^{-3}$, phenergan depressed phosphorylation to a greater degree than promazine and mepazine.

The difference between the effect of the tested phenothiazine derivatives on respiration and phosphorylation is accounted for by differences in the chemical structure of these substances. It may be postulated that the presence of an N-methylpiperidyl-3-methyl side-chain in the structure of mepazine explains the dissociating effect of this preparation on respiration and the associated phosphorylation. On replacement of the mepazine side-chain by a 2-dimethylaminopropyl or a 3-dimethylaminopropyl chain, the capacity of the preparations to dissociate respiration and phosphorylation is reduced.

SUMMARY

Phenothiazine derivatives, mepazine and promazine, when added to homogenates of cardiac muscle of the rabbit in a concentration of $0.8 \cdot 10^{-3}$ M, cause a rise in the efficacy of oxidative phosphorylation, increasing the ΔP and the P : O coefficient. In a $1.2 \cdot 10^{-3}$ M concentration of mepazine and promazine ΔP is reduced, though to a lesser degree than ΔO_2, the P : O coefficient remaining slightly above the normal level.

A $0.8 \cdot 10^{-3}$ M phenergan concentration uniformly reduces both respiration and phosphorylation. With a further rise of the phenergan concentration phosphorylation decreases more sharply than respiration. High concentrations of mepazine, phenergan and promazine ($1.6 \cdot 10^{-3}$ M) sharply depress phosphocreatine formation and cause a decrease of P : O. The phenothiazine derivatives neither depress creatine kinase nor activate adenosinetriphosphatase.

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