$6.0\times10^{-5}$ M. Hence, $^3$H-NECA low-affinity binding sites are targets of 6-MP cytostatic action.

The experimental findings indicate that the selective sensitivity of bone marrow lymphoblasts to 6-MP depends on the presence of specific binding sites of the cytostatic on the surface of these cells. At the same time, azathioprine, a chemical analog of 6-MP, metabolized in the body to 6-MP [4], may directly interact with peripheral blood lymphocyte purine receptors and thus regulate their functional activity.

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# Bioenergetic Mechanisms of the Antihypoxic Effect of Mexidol, a Succinate-Containing Derivative of 3-Hydroxypyridine

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One of the priority research trends at present in the creation of antihypoxic agents is an attempt to activate in hypoxia the compensatory metabolic streams supplying energy substrates to the respiratory system and acting as emergency adaptation mechanisms in this pathological process. The succinate oxidase oxidation pathway in the mitochondria is one of these streams [2,3]. Since the direct administration of succinate as an antihypoxic agent is ineffective because of its relatively poor penetration through the tissue-blood barriers, attempts are being made to use for this purpose various organic compounds containing succinic acid and facilitating its transfer across the cell membrane [1]. We have demonstrated the antihypoxic effect of mexidol, a succinate-containing 3-hydroxypyridine derivative whose properties are manifested predominantly in rats liable to hypoxia [5] and in the myocardium of such animals.

The present research was aimed at studying the bioenergetic effects of mexidol in hypoxia and at proving that succinate oxidation underlies the antihypoxic effect of the drug, succinate being a component of hydroxypyridine, which acting as an energy

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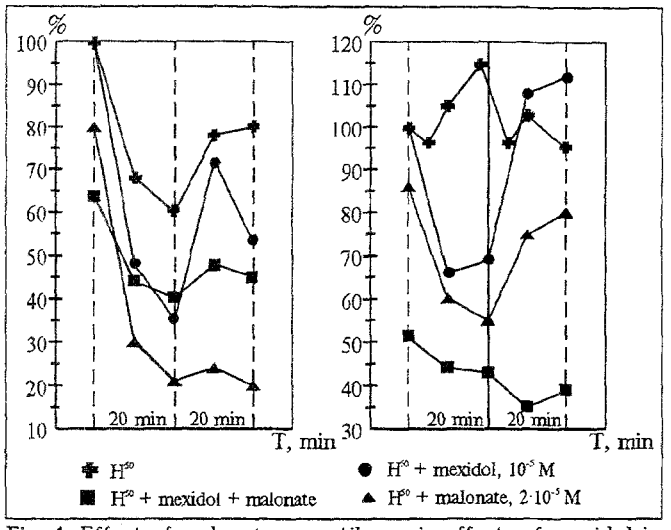


**Fig. 1.** Effect of malonate on antihypoxic effects of mexidol in the myocardium of rats unresistant to hypoxia.

substrate "carrier" delivering the substrate to the myocardial respiratory chain under conditions of $O_2$ insufficiency.

## MATERIALS AND METHODS

Experiments were carried out with albino outbred male rats weighing 180 to 220 g, predivided in a pressure chamber into groups in accordance with their resistance to acute hypoxia. Only rats highly resistant or unresistant to hypoxia were used in the experiments. The antihypoxic effects of emoxipin (not containing succinate) and of the succinate-containing mexidol, both 3-hydroxypyridine derivatives, were studied on a model of Langendorff isolated perfused contracting heart in our modification [4]. For assessment of the agents' effects on the functional and metabolic parameters of the isolated contracting myocardium, the heart beat intensity (HBI), heart rate (HR), the product of these parameters, reflecting the mechanical "work" of the heart (HR × HBI), the myocardial respiration velocity ($V_{resp}$), the coronary flow velocity ($C_{fl}$), and the ATP and creatine phoshate levels were recorded. The myocardium was perfused with carbogen-saturated solution (95% $O_2$ + 5% $CO_2$) ("normoxia") or with a gas mixture (50% $O_2$ + 45% $N_2$ + 5% $CO_2$) to simulate medium-severe hypoxia ($H_{50}$). The temperature of the perfusate (37°C) was maintained automatically, the pH was 7.4. The chemical compounds studied were added to the reservoir with the perfusion solution in a volume under 1% of perfusate volume 10 min before the 20-min hypoxia simulation. The heart rate and heart beat intensity and the myocardial respiration and coronary flow velocities were constantly recorded during the course of the experiment. ATP and creatine phosphate in the isolated heart were measured at the end of the reoxygenation period (at the 20th minute). The data were processed after Student.

## RESULTS

The succinate oxidase pathway of substrate oxidation in the respiratory chain is known to be characterized by thermodynamic advantages over the NAD-dependent oxidation at low $pO_2$ values. This fact seems to explain the higher myocardial resistance of highly resistant rats in comparison with unresistant animals, because in the former the myocardium plays a more significant role in oxidative metabolism [2,3]. Hence, it may be assumed that activation of the succinate oxidase oxidation pathway in hypoxia is conducive to the improvement of myocardial resistance to oxygen deficiency. It is quite possible that such a mechanism underlies the antihypoxic effect of succinate-containing hydroxypyridine derivatives [5].

If the antihypoxic properties of mexidol are related to succinate oxidation mediated by the succinate oxidase pathway of the respiratory chain, then suppression of this pathway may lead to a reduction or even abolishment of the protective effect of the substance.

**TABLE 1.** Effects of Succinate – Containing Hydroxypyridine Derivatives on Macroerg Content (μ/g) in Myocardium of Rats Highly Resistant (HR) or Unresistant (UR) to Hypoxia at 20th min of Reoxygenation for Malonate ($2\times10^{-3}$) inhibition of Succinate Oxidase Oxidation Pathway

| Experimental conditions | ATP | | | | Creatine phosphate | | | |
|---|---|---|---|---|---|---|---|---|
| | HR | | UR | | HR | | UR | |
| | abs. | % | abs. | % | abs. | % | abs. | % |
| $H_{50}$ | 10.88±0.65 | 100 | 10.32±0.52 | 100 | 15.06±0.73 | 100 | 14.34±0.69 | 100 |
| Mexidol (10 min) + $H_{50}$ | 11.55±0.67 | 106 | 11.85±0.70 | 115* | 16.56±1.1 | 110* | 15.96±0.3 | 111* |
| Malonate (10 min) + $H_{50}$ | 7.55±0.42 | 69* | 7.65±0.45 | 74* | 12.12±1.01 | 80* | 12.53±0.95 | 87 |
| Malonate (10 min) + mexidol (10 min) + $H_{50}$ | 5.23±0.35 | 48** | 5.82±0.41 | 56** | 7.30±0.53 | 41*** | 6.80±0.56 | 40*** |

Note. one asterisk – $p<0.1$, two asterisks – $p<0.05$, three asterisks – $p<0.01$.

To verify this hypothesis, we inhibited the succinate oxidase pathway of myocardial oxidation by malonate, a concurrent succinate dehydrogenase inhibitor ($2\times10^{-3}$ M). The addition of malonate to the perfusate under conditions of simulated hypoxia resulted in grave disorders of the measured myocardial functional and metabolic parameters, primarily of the heart beat intensity and myocardial respiration velocity, which were depressed 65-70% in contrast to the 30-40% depression that was characteristic of malonate inhibition in a carbogen-containing medium (Fig. 1). Hence, the succinate oxidase oxidation suppressed by malonate plays an appreciable role in the aerobic process of energy formation in the myocardium under conditions of hypoxia. The restriction of this oxidation pathway affects mainly such oxygen-dependent processes as respiration and heart beat intensity.

Mexidol in a concentration $10^{-5}$ M, when its antihypoxic effect was highest, exerted no protective effect in the presence of malonate either during or after hypoxia (Fig. 1). Therefore the antihypoxic effect of mexidol on the isolated myocardium was thought to be really due to succinate oxidation via the succinate oxidase pathway.

The observed effects of the drugs fully correlated with the myocardial macroerg content as compared with hypoxia alone (Table 1). Malonate, for instance, was conducive to a drastic reduction of myocardial macroerg content in comparison with hypoxia alone. In the presence of malonate mexidol promoted a higher macroerg level in the posthypoxic period as against $H_{50}$ alone. These facts may be regarded as evidence that mexidol succinate is oxidized via the succinate oxidase pathway of the myocardial respiratory chain. The maintenance of its activity and capacity for oxidative phosphorylation at the second and third conjugation sites during $H_{50}$ with limited NAD-dependent oxidation seems to be due to this, promoting the maintenance of a higher macroerg level in the heart under such conditions in comparison with hypoxia alone.

Thus, mexidol is an antihypoxant characterized by a direct energizing action whose effects, appearing to be due to substance hydrolysis and release into the extracellular space of succinate, which is subsequently oxidized in the respiratory chain, are aimed at the recovery under conditions of acute oxygen insufficiency of disturbed oxidative phosphorylation because of limitation of the $NADH_2$-oxidase oxidation pathway.

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