BIOPHYSICS AND BIOCHEMISTRY

Energotropic Effect of Succinate-Containing Derivatives of 3-Hydroxypyridine

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Succinate-containing derivatives of 3-hydroxypyridine, mexidol and proxypin, serve as succinate donors for the respiratory chain and contribute to activation of the succinate oxidase pathway of oxidation. Under conditions of hypoxia, these changes promote recovery of aerobic energy production, normalization of intracellular ATP concentration, and development of the antihypoxic effect. Succinate-free analogues of the test compounds exhibit no such properties. Both agents are considered as energotorpic substances. The specific effect of these compounds is manifested in direct interaction with the respiratory chain and normalization of ATP synthesis under conditions of hypoxia/ischemia. The test compounds can be used for the correction of energy metabolism disorders during acute oxygen deficiency. Moreover, they can be used for the treatment of associated functional disturbances.

Key Words: *hypoxia; energotropic antioxidants; succinate; mitochondrial enzymes; respiratory chain*

According to modern notions, energy metabolism is the target for hypoxia. The decrease in oxygen supply to cells and mitochondria is accompanied by phasic changes in activity of mitochondrial enzymes [3-5,7-9,15]. Since mitochondrial dysfunction accompanies practically all pathologies, impairment of electron transport and coupling function of mitochondria under these conditions leading to a decrease in the intracellular content of macroergic substances and suppression of energy-dependent functional and metabolic systems are of particular importance.

The prophylactic and therapeutic use of succinate-containing drugs is an effective approach to the prevention and treatment of consequences of hypoxia/ischemia and impairment of aerobic energy synthesis [2,3,6,9-12]. Succinate is an energy substrate. Succinate is oxidized by mitochondrial enzyme succinate dehydrogenase (SDH; mitochondrial enzyme complex II, MEC II). Activation of oxidative metabolism of succinate during hypoxia has thermodynamic advantages under conditions of high degree of reduction of mitochondrial enzyme complex I (MEC I) in the main respiratory chain [2,3,6-9]. This process contributes to the maintenance of ATP synthesis by mitochondria (energotropic effect) [3,4,6,8-15].

Previous experiments showed that succinate can be used for the protection of the organism from hypoxia. Some modern drugs containing succinate [3,5-7,13,14] are successfully used in medical practice. However, the search for succinate-containing drugs of high efficacy is in progress. The synthesis of succinate-containing heterocyclic compounds is a special

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field of research. Much progress has been achieved over the last 30 years [6,7,12-14]. These compounds should increase the permeability of the blood-tissue barriers. 3-Hydroxypyridine (3-HP) derivatives are of considerable interest in this respect. These compounds, structural analogues of vitamin B_6, have a wide range of pharmacological properties. Apart from antioxidant and psychotropic properties, they increase organism's resistance to hypoxia, reduce the degree of ATP loss in ischemic brain and myocardium, and normalize the process of oxidative phosphorylation [12-14]. Some 3-HP derivatives (mexidol and emoxipine) are used as antihypoxic and antioxidant drugs in medical practice.

Here we studied the interaction of two succinate-containing derivatives of 3-HP (mexidol and proxypin) with the respiratory chain. We also evaluated the energotropic properties of these compounds (recovery of intracellular aerobic synthesis of ATP during hypoxia and normalization of functional activity in the organism).

MATERIALS AND METHODS

Experiments were performed on outbred albino rats. The brain was removed after decapitation. The neocortex was isolated. The homogenate of the neocortex (25%) was maintained on ice [10]. Respiration of the homogenate was studied polarographically using a Clark electrode. The homogenate (0.1 μl) and aqueous solution of test compounds were put in a closed polarographic cell (1 cm^3) using a micropipette. We studied the effect of succinate-containing derivatives of 3-HP (mexidol and proxypin) and succinate-free structural analogues (emoxipine and GB-212, respectively) on respiration of the homogenate.

The test solutions of proxypin and mexidol contained 5 mM succinate (similarly to sodium succinate that serves as a respiratory substrate in polarographic studies). The effect of energy substrates on various pathways of oxidation in the respiratory chain was evaluated by inhibitory assay. Experiments were conducted with amytal (MEC I inhibitor, 3 mM) and malonate (MEC II inhibitor, 10 mM). The interaction of the test preparations with the mitochondrial respiratory chain was estimated from changes in energy consumption by the homogenate under conditions of respiratory chain dysfunction caused by inhibitors. The content of ATP and creatine phosphate in samples of rat brain cortex was measured by the luciferin-luciferase method with modifications [1]. Chronic global ischemia was induced by bilateral ligation of the carotid arteries. The rats received intraperitoneal injection of the test preparations 15 min before the "ascent" to a critical altitude of 11,500 m in pressure chamber. Antihypoxic activity of drugs was estimated from their effect on the lifetime of animals under these conditions (second agonal inspiration) [11]. The results were analyzed by Student's *t* test.

RESULTS

Endogenous respiration of homogenates in the absence of exogenous substrates was more sensitive to malonate (competitive inhibitor of SDH, MEC II) than to amytal (inhibitor of the NAD-dependent oxidation pathway, MEC I). Respiratory activity was reduced by 63 and 30%, respectively (Table 1).

Addition of exogenous NAD-dependent substrates (pyruvate+malate, 4:1 ratio) to the cell was followed by stimulation of respiration. The respiratory control was 240%. These changes were accompanied by an increase in the respiratory sensitivity to MEC I inhibitor amytal. The degree of amytal-sensitive respiration was 42% (*vs*. 30%). The sensitivity to malonate decreased slightly under these conditions (Table 1). Therefore, the electron flow through MEC I increases in the presence of exogenous NAD-dependent substrates.

Sodium succinate (5 mM) was more potent than NAD-dependent substrates in increasing the rate of oxygen consumption by homogenates of the cerebral cortex. The respiratory control was 350 and 240%, respectively. Sodium succinate-stimulated respiration was suppressed by malonate by 84%, but was low sensitive to a MEC I inhibitor amytal (the contribution of amytal-sensitive respiration was 16%).

Hence, exogenous succinate contributes to redistribution of substrate flow and monopolization of the respiratory chain (relative increase in electron flux through MEC II).

Succinate-containing compounds proxypin and mexidol stimulated respiration of brain samples. However, they were less potent than exogenous succinate. The respiratory control for proxypin and mexidol was 220 and 260%, respectively. Respiration was highly sensitive to malonate (inhibition by 86 and 80%, respectively), but low sensitive to amytal (15 and 20%, respectively). These features are typical of succinate oxidation (Table 1).

Decreasing the concentration of proxypin and mexidol (*i.e.*, content of succinate, 0.5 mM) was followed by disappearance of the stimulatory effect of the test compounds on respiration, reduction of the contribution of malonate-sensitive respiration, and increase in amytal-sensitive respiration (similar to endogenous respiration). Succinate-free analogues of mexidol and proxypin (emoxipine and GB-212) had no effect on respiration. The action of respiratory chain inhibitors in the presence of these compounds was similar to that observed under conditions of endogenous respiration (Table 1).

TABLE 1. Effects of 3-HP Derivatives (Succinate-Containing Proxypin and Mexidol; and Succinate-Free GB-212 and Emoxipine) on Respiratory Parameters of Homogenates from Rat Brain Cortex

Substances added to a polarographic well	RC, %	Malonate-sensitive respiration, %	Amytal-sensitive respiration, %
None (endogenous respiration)	100	63	30
Pyruvate+malate	241	58	42
Succinate (5 mM)	346	84	16
Proxypin (5 mM succinate)	223	86	14
Proxypin (0.05 mM succinate)	137	62	32
Mexidol (5 mM succinate)	260	80	20
Mexidol (0.05 mM succinate)	119	60	27
GB-212	103	57	38
Emoxipine	105	67	33

Note. RC, respiratory control (ratio of respiration in the presence of oxidation substrates to endogenous respiration, %). Amytal-sensitive respiration, in the presence of amytal (MEC I inhibitor, 3 mM). Malonate-sensitive respiration, in the presence of malonate (MEC II inhibitor, 10 mM).

Our results indicate that succinate entering the structure of 3-HP derivatives proxypin and mexidol serves as the energy substrate for the respiratory chain by a classical for succinate scheme. This is accompanied by an increase in respiration and change in the electron flow from MEC I to MEC II (monopolization of the respiratory chain by succinate confirmed by increased sensitivity of respiration to malonate and decreased amytal-sensitive respiration due to oxidation of NAD-dependent substrates.

Similar results were obtained in studying the effect of mexidol on the respiratory chain in isolated mitochondria [11-13].

Due to the ability of mexidol and proxypin to supply the respiratory chain with succinate, they can be used as energotropic drugs. The specific effect of these compounds is manifested in a direct interaction with the respiratory chain and normalization of ATP synthesis during the inhibition of MEC I (hypoxia/ischemia).

In vivo energotropic effect of mexidol were observed in mammals [13,14]. Proxypin had the same effect under experimental conditions of acute hypobaric hypoxia and total cerebral ischemia.

Pretreatment with proxypin prevented the decrease in the content of ATP and creatine phosphate in the neocortex (hypoxia-sensitive rats), which was observed after short-term acute hypobaric hypoxia. Otherwise, proxypin increased the content of these substances compared to controls (highly resistant rats; Fig. 1). There-

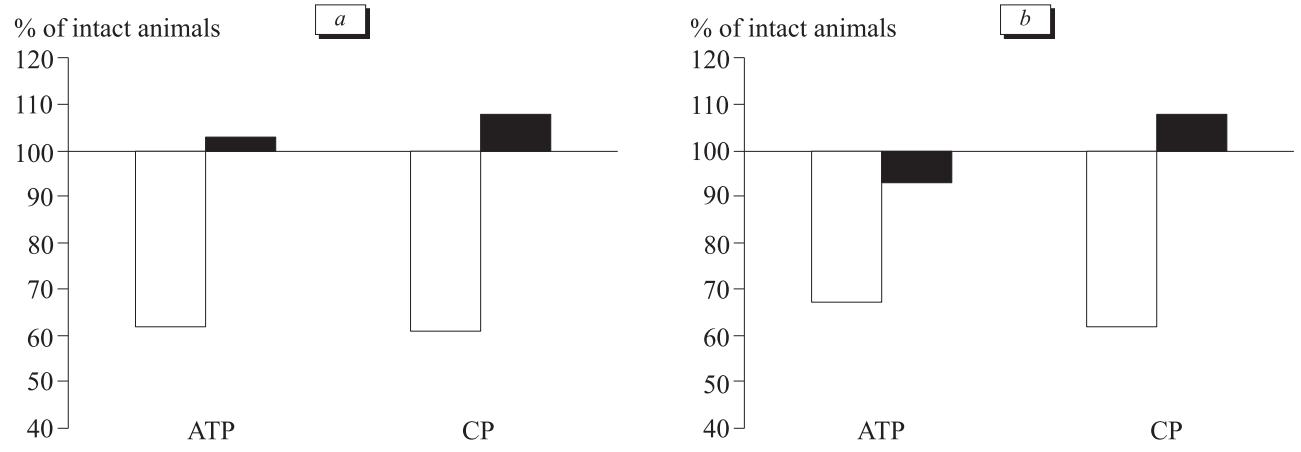

Fig. 1. Effect of proxypin (40 mg/kg intraperitoneally) on the content of ATP and creatine phosphate (CP) in the neocortex of rats with low (*a*) and high resistance to hypoxia (*b*) after "descent" from the critical altitude. Light bars, acute hypobaric hypoxia (AHBH); dark bars, AHBH+proxypin.

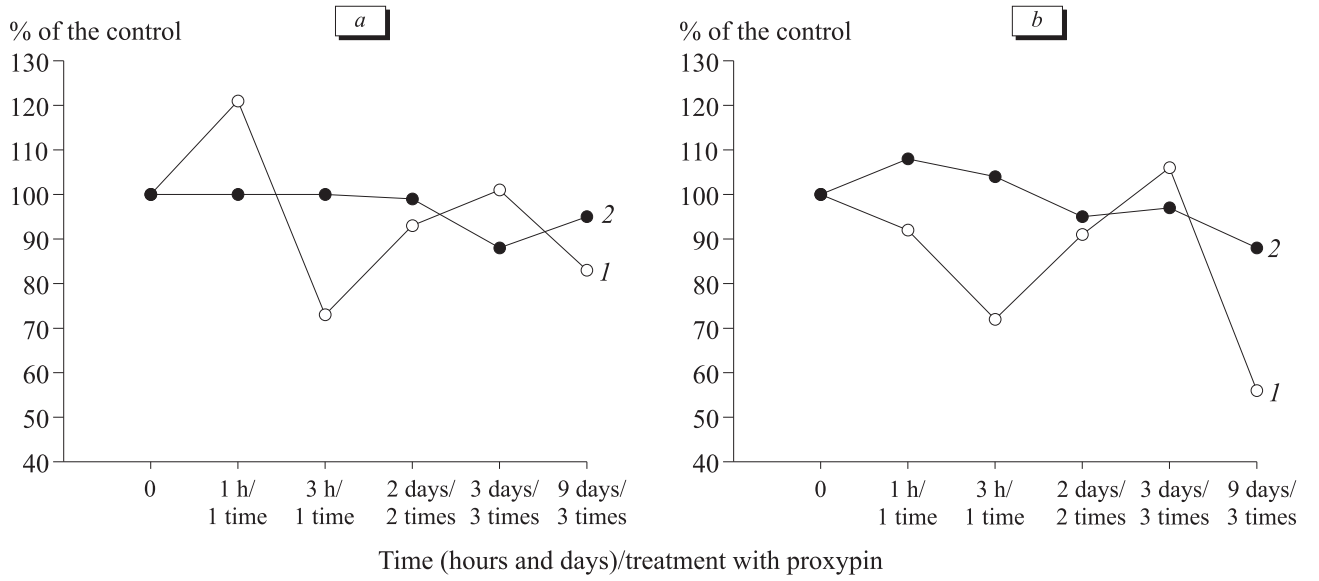

Fig. 2. ATP content in the neocortex of low resistant and highly resistant rats during chronic global ischemia of the brain. Control, sham-operated rats. Ischemia (*1*); ischemia after treatment with proxypin (*2*).

fore, single pretreatment with proxypin has a protective effect on energy metabolism of the brain in rats under conditions of acute hypobaric hypoxia.

Proxypin had a normalizing effect on ATP synthesis during chronic ischemia (Fig. 2). The protective effect of proxypin (prevention of the decrease in energy metabolism in the cerebral cortex) was most pronounced during severe energy deficiency.

The energotropic effect of proxypin and mexidol during hypoxia correlates with antihypoxic activity of these compounds (proxypin was more potent than mexidol and the dose of proxipin producing the antihypoxic effect was lower than that of mexidol and reference preparation γ-hydroxybutyric acid, GHB; Table 2). The antihypoxic effect of GHB in the maximum tolerated dose (300 mg/kg) was probably related to its narcotic activity. Succinate-free analogues of proxipin and mexidol (emoxipine and GB-212) exhibited extremely low antihypoxic activity.

TABLE 2. Antihypoxic Effect of Test Compounds in the Optimal Effective Dose (Succinate-Containing Mexidol and Proxypin; Succinate-Free Analogues Emoxipine and GB-212, Respectively; and Reference Preparation GHB, GHB in a Dose of 300 mg/kg Exhibits the Narcotic Activity)

Test compound	Dose, mg/kg	Lifetime (treatment/control), %	
		low resistant rats	highly resistant rats
Proxypin	40	387*	410*
Mexidol	100	186*	190*
Emoxipine	100	110	120
GB-212	40	115	118
GHB	100	138*	113
	300	230*	105

Note. *$p<0.05$ compared to the control (100%).

Apart from the energotropic and antihypoxic effects, proxipin and mexidol improved various vital functions during hypoxia and ischemia. They decrease the mortality rate, have a normalizing effect on body weight gain, reduce the severity of neurological disorders and aggressiveness (observed in hypoxia), exhibit antistress activity, and recover locomotor and exploratory behavior and emotionality of animals.

We conclude that succinate-containing compounds proxypin and mexidol serve as succinate donors for the respiratory chain and contribute to activation of the succinate oxidase pathway of oxidation during hypoxia. These changes probably determine the recovery of aerobic energy synthesis and antihypoxic effect of test compounds. Therefore, they hold much promise for the correction of energy metabolism disorders under conditions of acute oxygen deficiency. Moreover, they can be used for the treatment of associated functional disturbances. Proxypin and mexidol can be used for the therapy of circulatory disturbances in the brain, encephalopathies of various etiologies, and neurocirculatory dystonia, for prevention of withdrawal syndrome and acute intoxication with neuroleptics and narcotic drugs, and for correction of hyperlipidemic and hemostatic disorders in the early period after ischemic stroke.

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