FEATURES OF THE ANTIHYPOXIC ACTION OF MEXIDOL ASSOCIATED WITH ITS SPECIFIC EFFECT ON ENERGY METABOLISM

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Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate, I) is a derivative of the 3-hydroxypyridines (3-OP) which are membrane-active antioxidants and inhibitors of lipid peroxidation. These compounds can alter the physicochemical properties of membranes, lipid-protein interactions, membrane-bound enzyme activity, and can elicit modification of receptor, metabolic, and transport functions of membranes [1, 9]. All 3-OP have a wide spectrum of psychotropic actions which are apparently mediated via a GABA-benzodiazepine-Cl-ionophore complex [2, 4]. Besides, a number of 3-OP have antihypoxic activity which is especially marked in the acute hypobaric hypoxia system which imitates the conditions found at high altitudes [5, 10]. Since many antihypoxics can produce positive effects on energy metabolism, and increase the level of cell energy, we studied the effects of I and some other 3-OP derivatives on the function of the respiratory chain; this is known to be one of the metabolic functions most sensitive to lack of oxygen [8].

MATERIALS AND METHODS

Experiments were carried out on white mongrel male rats (160-200 g). Bioenergetic parameters were measured in liver mitochondria isolated by the standard method of differential centrifugation in medium containing 0.25 M sucrose [11]. The following parameters were measured: (a) the rate of respiration in the three Chance states V_2, V_3, and V_4 (by a polarographic method), the rate and time of phosphorylation V_ϕ and τ_ϕ, the ADP/O ratio; these were evaluated by inhibitor analysis of the oxidative pathways catalyzed by succinate oxidase and NADH oxidase; (b) redox changes of flavoproteins (FP), which are the respiratory carriers of the first complex (by a fluorimetric method) [10] (measurements of the level of pyridine nucleotide reduction were difficult because of the intense intrinsic luminescence of 3-OP in the wavelength range of NAD(P)H); (c) changes in the mitochondrial membrane potential $\Delta\Psi_{mt}$ (using the lipophilic probe TPP^+).

The following compounds were studied: I, 2-ethyl-6-methyl-3-hydroxypyridine (II), and 2-ethyl-6-methyl-3-hydroxypyridine hydrochloride (III).

These substances were added to a measuring cell containing mitochondria, to concentrations of 0.01, 0.1 and 1 mM, and changes in the parameters mentioned above were measured.

RESULTS AND DISCUSSION

Compound I (1 mM) produced a three-fold increase in endogenous respiration which was accompanied by FP reduction and the onset of oxidative phosphorylation in the presence of ADP, i.e., I had the properties of a respiratory chain substrate. Since the ADP/O ratio was close to 2, it seemed possible that respiratory chain oxidation of I was occurring via the succinate oxidase route. In fact, a comparison of its effects with those of exogenous succinate (1 mM) showed a great similarity. Both succinate and I increased the reverse transfer of electrons along the respiratory chain, resulting in an increase in the extent of FP reduction (Table 1).

Compounds II and III differed in that they had no effect on endogenous mitochondrial respiration, the level of FP reduction, or $\Delta\Psi_{mt}$, and oxidative phosphorylation did not occur in their presence (see Table 1), i.e., these compounds did not activate the energy-synthesizing mitochondrial fraction.

The following results provided additional confirmation that I is oxidized by the respiratory chain via the succinate oxidase route. Addition of malonate, a competitive in-

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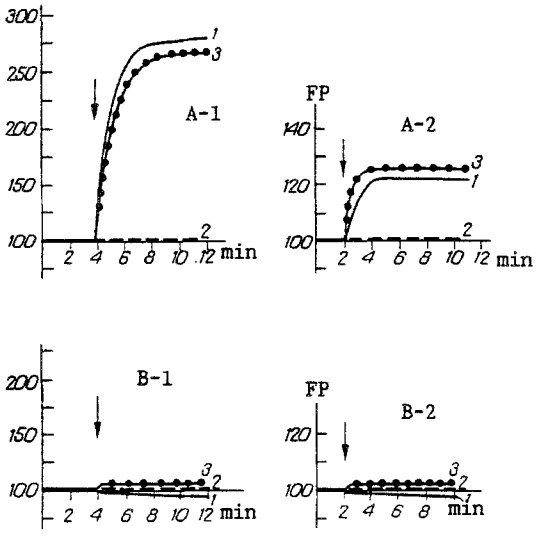

Fig. 1

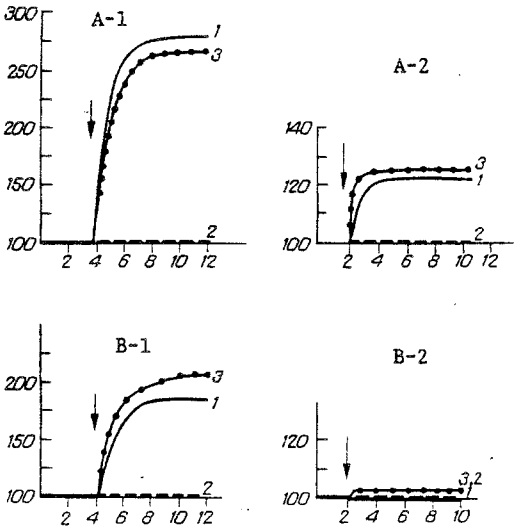

Fig. 2

Fig. 1. The effects of I, base (II), and succinate on the rate of oxygen consumption and the level of FP luminescence in isolated liver mitochondria oxidizing endogenous substrates (A), and in conditions of malonate (20 mM) inhibition of the succinate oxidation route (B). Arrows show the times of addition of compounds: (1) mexidol, 1 mM; (2) base II, 1 mM; (3) succinate, 1 mM. The abscissa shows time in minutes, and the ordinate shows V, the rate of oxygen consumption (% of initial) in A-1 and B-1, and reduced FP (% of initial) in A-2 and B-2.

Fig. 2. The effects of I, base (II), and succinate on the rate of oxygen consumption and the level of FP luminescence in isolated liver mitochondria oxidizing endogenous substrates (A), and in conditions of amytal (4 mM) inhibition of the NAD-dependent oxidation route (B). For explanations, see the caption for Fig. 1.

TABLE 1. The Effect of Mexidol (I), Emoxypine (III), and the Base (II) on the Functional Properties of the Respiratory Chain of Isolated Liver Mitochondria Oxidizing Endogenous Substrates

Property studied	Compound	Concentration of compound, M		
		10^{-5}	10^{-4}	10^{-3}
Rate of oxygen utilization, % of initial	I	100	170	278
	III	100	100	100
	II	100	100	100
	Succinate	–	–	269
Reduced FP, % of initial	I	100	117	122
	III	100	100	100
	II	100	100	100
	Succinate	–	–	124
ADP/O	I	2.0	1.78	1.72
	III	No phosphorylation		
	II	Same		
	Succinate	–	–	1.43
Mitochondrial membrane potential ($\Delta\Psi$)	I	100	121	126
	III	100	100	100
	II	100	100	92
	Succinate	–	–	108

hibitor of succinate dehydrogenase, (20 mM) to mitochondria produced a threefold reduction in the rate of endogenous respiration; both I and succinate failed to stimulate respiration in the presence of malonate. FP reduction did not occur, which supports the suggestion of a link between reduction of respiratory carriers (without malonate) and reverse transport of electrons, and showed a decrease in this process on inhibition of succinate dehydrogenase with malonate. Thus, reduction of FP by I was actually caused by activation of the

TABLE 2. The Effects of Mexidol (I), Emoxypine (III), and the Base (II) on Oxidative Phosphorylation of Isolated Liver Mitochondria Oxidizing Succinate and Glutamate (% of control)

Concentration of compound, M	Glutamate (5 mM)					Succinate (5 mM)				
	V_2	V_3	V_4	ADP/O	V_{ad}	V_2	V_3	V_4	ADP/O	V_{ad}
					Mexidol (I)					
10^{-4}	187	138	161	89	123	98	98	102	111	100
10^{-3}	228	172	395	71	121	88	78	105	85	60
					Emoxypine (III)					
10^{-4}	100	85	87	94	78	100	98	130	95	96
10^{-3}	136	96	120	85	72	109	80	85	89	68
					Base (II)					
10^{-4}	102	87	103	105	95	—	—	—	—	—
10^{-3}	136	97	136	94	91	110	82	116	90	74

succinate oxidase oxidation route (Fig. 1). In contrast, blocking the oxidation of NAD-dependent substrates with the NADH oxidase inhibitor amytal (4 mM) had no effect on the ability of I to activate endogenous mitochondrial respiration. In these conditions the initial (prior to addition of amytal) level of respiration was restored, and was even exceeded by 40-50%. However, FP reduction did not occur in conditions of electron transport block at the NADH-CoQ stage, as would be expected. Qualitatively similar effects were obtained on addition of exogenous succinate, although they were of lesser magnitude. Compounds II and III had no effect on respiration or FP in the presence of malonate or amytal (Fig. 2).

The effects of I on the oxidative metabolism of isolated mitochondria were not apparent in the presence of high concentrations of exogenous succinate (5 mM), showing that I and succinate act synergistically and at the same point in energy metabolism. In contrast, in the presence of NAD-dependent substrates (glutamate + malate), I increased mitochondrial respiration 2-3-fold in different metabolic states, and reduced the ADP/O ratio by 30% (Table 2). This could be due to the competitive relationship between the NADH oxidase and succinate oxidase routes of oxidation, and to monopolization of the respiratory chain by succinate with I. Compounds II and III had no such effect.

It is known that in solution I forms a complex which under some conditions can apparently undergo partial dissociation to a base (2-ethyl-6-methyl-3-hydroxypyridine, II) and succinic acid (IV). The base can accumulate in biological membranes, increasing their permeability and producing the psychotropic effects of these substances. As we have shown, the acid compound IV is oxidized by the respiratory chain; the presence of the base within the membrane may increase its availability to respiratory chain enzymes, which would also explain the greater energizing effect of I in comparison with that of exogenous succinate, as evaluated by the magnitude of $\Delta\Psi_{mt}$ (see Table 1). It is also possible that IV itself, within this complex, functions as an intermembrane carrier, i.e., it has properties resulting from a free carboxyl group not bound to the 3-OP molecule. Complexes of IV with a variety of therapeutic compounds are known to have higher pharmacological efficacy, and IV is used in pharmacological practice with this aim [6]. Because of these properties, I has clear advantages over other 3-OP which do not contain IV, but which do have antihypoxic properties (II and III). In conditions of oxygen deficiency, where NAD-dependent oxidation in the respiratory chain is limited, I can increase the activation of the succinate oxidase route, which is compensatory for cell energetics, especially in animal tissues such as the myocardium which are highly resistant to hypoxia, and in which this route is potentially highly activatable [8].

A second conclusion arises from our data, which is that the antihypoxic action of 3-OP derivatives lacking IV in their structure results from other mechanisms, which are not associated with their direct action on the respiratory chain as energy substrates, but depend on other factors, such as their modifying effects on membrane receptor functions [2, 3, 10]. If this is the case, then I must differ in having a stronger antihypoxic effect than II and III, since its mechanism of action will be a combination of the antihypoxic membrane-binding effect of the base and the energizing activity of compound IV, which is utilized by mitochondria as an oxidation substrate.

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ANALYSIS OF DILTIAZEM AND ITS METABOLITES BY HPLC AND MASS SPECTROMETRY

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Diltiazem (I) is a calcium antagonist which is finding ever wider use in cardiological practice for the treatment of various forms of cardiac failure. Contradictory data have appeared in the literature in recent years as to which metabolites are formed during the biotransformation of diltiazem, and in what ratios. Thus, it has been suggested that compound II is the major metabolite [3], accounting for up to 90% of the plasma diltiazem concentration. These authors also observed other metabolites, namely compounds III and IV.

Other authors [4] have observed compound III, desacetyldiltiazem, as a major metabolite.

The aim of the present work was to study diltiazem and its major metabolites in the plasma of patients with coronary failure, using a combination of HPLC and fast atom mass spectrometry.

MATERIALS AND METHODS

The following solvents were used after additional purification: methanol, hexane, iso-propanol, and chloroform (all high-purity grade), and mono- and disubstituted sodium phosphates (chemical grade), HEPES (BDH "Analar" grade, USA), and tablets containing 60 mg of diltiazem produced by "Alkaloid," Yugoslavia.

Six patients with ischemic heart disease received single p.o. doses of diltiazem (90 mg). Blood samples were taken before, and 1, 2, 3, 4, and 24 h after administration. Plasma was taken and, when necessary, was frozen and stored at −20°C.

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