TYPE B MONOAMINE OXIDASE AND FUNCTIONS OF Ca^{2+},Mg^{2+}-DEPENDENT ADENOSINETRIPHOSPHATASE IN PREPARATIONS FROM SARCOPLASMIC RETICULUM VESICLES

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Oxidative deamination of β-phenylethylamine or benzylamine by type B monoamine oxidases (MAO) in preparations of sarcoplasmic reticulum vesicles from rabbit skeletal muscles is accompanied by inhibition both of active Ca^{2+} transport into the vesicles and of the activity of Ca^{2+},Mg^{2+}-dependent ATPase, which is preventable by deprenil, a specific inhibitor of type B MAO. Aldehydes formed during enzymatic deamination of substrates of type B MAO may perhaps participate in the regulation of Ca^{2+},Mg^{2+}-dependent ATPase, activity.

KEY WORDS: *ATPase; active Ca^{2+} transport; type B monoamine oxidases; biogenic aldehydes and amines.*

A connection has been postulated between the activity of monoamine oxidases (MAO) and the permeability of biological membranes [4, 10, 11]. Vesicles of the sarcoplasmic reticulum (SR) from skeletal muscles, the major protein component of which is Ca^{2+},Mg^{2+}-dependent ATPase [9], are used to study active Ca^{2+} transport as an indicator of biological membrane function.

The object of this investigation was to detect MAO activity in SR vesicles and to ascertain if a functional connection exists between MAO activity and the activity of Ca^{2+},Mg^{2+}-dependent ATPase.

EXPERIMENTAL METHOD

Preparations of SR vesicles were isolated from homogenates of rabbit hind limb muscles by differential centrifugation [5], in the course of which fractions sedimented at 15,000g in 20 min were removed twice and fractions sedimented at 40,000g in 90 min were collected. The ATPase activity was measured with a pH meter [2] or from the liberation of inorganic phosphate [1]. When determining MAO activity, oxygen uptake was measured polarographically (pH 7.0, 37°C) and the liberation of ammonia was measured by an isothermic diffusion method followed by nesslerization [12]. The protein concentration in the samples was determined by Lowry's colorimetric method with bovine serum albumin as the standard. The sources of the amines and MAO inhibitors used in the work and their characteristics were described previously [12].

EXPERIMENTAL RESULTS AND DISCUSSION

Incubation of samples containing 0.4 mg protein of SR vesicles in 2 ml of 0.02 M imidazole–HCl buffer, pH 7.0; and benzylamine, dopamine, or β-phenylethylamine (5 mM), but not serotonin; led to excessive (compared with the control without amines) uptake of oxygen and liberation of ammonia. Preincubation at pH 7.0 of the SR visicles for 30 min at 20°C with benzyl-

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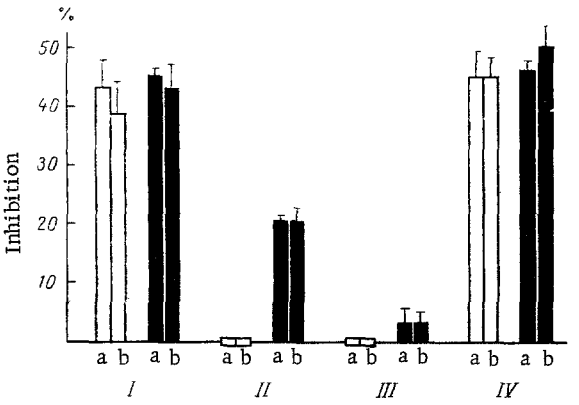

Fig. 1. Inhibition of Ca^{2+},Mg^{2+}-dependent ATPase activity (a) and of active Ca^{2+} transport (b) during preincubation of SR vesicles with benzylamine (unshaded columns) or with β-phenylethylamine (black columns) without preliminary treatment with MAO inhibitors (I), and also after preliminary treatment with iproniazid (0.5 mM) (II). Deprenil (0.05 mM) (III) or chlorgyline (0.05 mM) (IV). To each sample (5 ml) were added 0.4 mg protein of SR vesicle preparations; 0.02 M imidazole–HCl buffer, pH 7.0; and one of the MAO inhibitors. After treatment with inhibitors for 30 min at 20°C, benzylamine or β-phenylethylamine was added to the samples (final concentrations 0.2 mM), which were then preincubated for 30 min at 20°C and treated with ATP to determine Ca^{2+},Mg^{2+}-ATPase activity and active Ca^{2+} transport by a pH-metric method. MAO inhibitors in the above concentrations did not affect these indices. Mean values of results of three independent experiments (with two parallel determinations in each) are shown.

amine, β-phentlethylamine (even 0.01 mM), or dopamine, but not with noradrenalin or serotonin, led to inhibition of Ca^{2+},Mg^{2+}-dependent ATPase activity and of active Ca^{2+} transport (Table 1). Without preincubation, these amines had no such action. Deprenil [8], a specific inhibitor of type B MAO, but not chlorgyline, an inhibitor of type A MAO, in low concentrations prevented the inhibitory action of substrates of type B MAO (benzylamine or β-phenylethylamine) on Ca^{2+},Mg^{2+}-ATPase activity and on active Ca^{2+} transport. Iproniazid, without the ability to selectively inhibit the different types of MAO, had an action intermediate between that of deprenil and chlorgyline (Fig. 1). The products of deamination of benzylamine catalyzed by type B MAO (benzaldehyde), in low concentrations (10^{-6} M), inhibited Ca^{2+},Mg^{2+}-ATPase activity and active Ca^{2+} transport sharply (by 40-50%) and virtually independently of the duration of preincubation, and these effects were not prevented by Deprenil. Purified SR preparations thus contained MAO activity which, in view of the nature of its substrate and inhibitor specificity, was evidently activity of a type B MAO specifically oxidizing β-phenylethylamine or benzylamine (but not serotonin or noradrenalin), and blocked by low concentrations of Deprenil (but not chlorgyline). Oxidative deamination of β-phenylethylamine or benzylamine by these enzymes in preparations of SR vesicles was accompanied by inhibition of Ca^{2+},-Mg^{2+}-dependent ATPase activity and active Ca^{2+} transport, evidently due to the action of

TABLE 1. Effect of Preincubation with Amines on Ca^{2+},Mg^{2+}-Dependent ATPase Activity and Active Ca^{2+} Transport in Preparations of SR Vesicles from Rabbit Skeletal Muscles

Amine (0.1 mM)	Inhibition, % of control (without amines)	
	ATPase activity	active Ca^{2+} transport
Serotonin	0	0
Noradrenalin	0	0
Dopamine	34	32
Benzylamine	43	37
β-phenylethylamine	45	43

Legend. In control samples (preincubation without amines) mean Ca^{2+},Mg^{2+}-ATPase activity was 300-500 nmoles P_i/mg protein/min of incubation. Mean rate of active Ca^{2+} transport through SR membrane was 600-1000 nmoles Ca absorbed/mg protein/min of incubation [2].

aldehydes produced in the course of these reactions. Other examples of effects produced apparently by amines on biochemical reactions but in fact due to aldehydes, as products of the enzymatic deamination of amines, have been described in the literature [3, 6, 7].

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