Biochemical Pharmacology, Vol. 23, pp. 2887–2893. Pergamon Press. 1974. Printed in Great Britain.

EFFECT OF ISOPROTERENOL ON THE ACTIVITY OF Na^+,K^+-ADENOSINE TRIPHOSPHATASE FROM DOG HEARTS

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(*Received* 5 *November* 1973; *accepted* 6 *March* 1974)

Abstract—The effect of isoproterenol on the activity of Na^+,K^+-stimulated ATPase, a membrane-bound enzyme, from dog hearts was studied. In isoproterenol-treated hearts the yield of the membrane fraction enriched in sarcolemma was increased, and Mg^{2+}- and N^+,K^+-ATPase, 5′-nucleotidase and succinic dehydrogenase activities were decreased. A decrease in the activity of marker enzymes in the fractions of heavy microsomes and mitocondria was also observed. Thus using marker enzymes as a measure of purity, no increase in mutual contamination of the isolated subcellular fractions could be detected. Investigation of special properties of Na^+,K^+-ATPase showed alteration in pH optimum, changed K_m value and increased sensitivity to $HgCl_2$, without any significant increase in the content of protein-bound sulfhydryl groups. These effects of isoproterenol can be attributed to destruction of membrane and/or other cellular proteins allowing more non-enzyme protein to sediment with the isolated fraction or to a more specific influence on the properties of Na^+,K^+-ATPase.

OVERDOSAGE of isoproterenol has been known to produce, in experimental studies, heart enlargement and "infarct-like" myocardial lesions.[1–5] Several hypotheses were proposed regarding the etiology of the lesions. Almost all of these hypotheses presume cell membrane injury associated with altered permeability to mono- and divalent cations, water etc.,[6–8] altered intracellular ATP level, loss of ATP synthesis[9] and disturbance of the Ca^{2+} signal regulating energy flux in heart muscle,[10] all of which may generate, together with a probable lack of oxygen, uncoupled ATPase activities.[11] In view of the fact that the membrane-bound Na^+,K^+-ATPase (ATP phosphohydrolase, EC 3.6.1.3) enzyme complex has been established to be directly related to the energy-dependent translocations of Na^+ and K^+ across the plasma membrane[12–14] we decided to study the effects of isoproterenol on cardiac Na^+,K^+-ATPase activity.

MATERIALS AND METHODS

Adult mongrel male dogs were used. A single subcutaneous injection of 7·5 mg isoproterenol per kg body weight was administered. Twenty-four hours later the hearts were removed, under pentobarbital anaesthesia. Approximately 5 g of heart left ventricles was freed from fat and connective tissue, thoroughly washed in ice-cold medium (0·25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl, pH 7·4) to remove blood, and the subcellular fraction containing Na^+,K^+-stimulated, Mg^{2+}-dependent ATPase was isolated according to the procedure of Matsui and Schwartz.[15] The

amount of protein sulfhydryl groups in this preparation was determined using Ellman's reagent.[16] To assay Na^+,K^+- and Mg^{2+}-ATPase activities the fraction was incubated at 37° for 10 min in a total volume of 1 ml containing 50 mM Tris–HCl, 1 mM EDTA, 5 mM $MgCl_2$, 4 mM Tris–ATP, 100 mM NaCl and 20 mM KCl at pH 7·4, with or without 2 mM ouabain. Both KCl and NaCl were omitted from the reaction mixture for the determination of Mg^{2+}-ATPase activity. The reaction was stopped by addition of 1 ml of 12% trichloroacetic acid. The amount of P_i released into the medium through ATP hydrolysis was estimated in the protein-free filtrate by the method of Taussky and Shorr[17] and values were corrected in respect to the non-enzymatic hydrolysis of ATP. Protein concentration was determined by the procedure of Lowry *et al.*[18] The purity of the membrane fraction was examined electronmicroscopically and also by testing its Mg^{2+}- and Na^+,K^+-ATPase activities in the presence of ouabain, oligomycin and NaN_3.

As a further test for the purity of the membrane fraction the activities of 5′-nucleotidase (5′-ribonucleotide phosphohydrolase EC 3.1.3.5) and succinic dehydrogenase [succinate (acceptor) oxidoreductase EC 1.3.99.1] were estimated in the crude fraction, before NaI treatment, in the NaI-treated fraction as well as in the fractions of heavy microsomes and mitochondria, the last being isolated as described elsewhere.[19] 5′-Nucleotidase activity was measured according to Muir *et al.*[20] using a protein concentration of 250–300 μg/ml and 10 mM $NiCl_2$ as inhibitor of unspecific AMP-splitting enzyme activities. Succinic dehydrogenase activity was determined by the method of Price and Thimann[21] modified by Wrogemann and Blanchaer.[22] The reagents used were the purest obtainable, purchased from Merck, Darmstadt and Sigma Chemical Co., St. Louis.

RESULTS

Changes in enzyme activities and yield of the sarcolemma-enriched membrane fraction. The yield of the subcellular fraction from control hearts was 0·65 ± 0·07 mg protein/g muscle whereas that from isoproterenol-treated hearts was increased, to 0·84 ± 0·03 mg protein/g muscle ($P < 0·05$). On the other hand, the activities of Mg^{2+}-ATPase and Na^+,K^+-ATPase in the above preparation decreased following isoproterenol treatment from 7·85 ± 0·60 to 3·06 ± 0·80 and from 12·70 ± 1·50 to 4·36 ± 0·80 μmoles of ATP hydrolysed/mg of protein per hr, respectively. All changes were significant with $P < 0·001$ ($n = 7$ in all groups).

The depression of Mg^{2+}-dependent, Na^+,K^+-stimulated ATPase(s) activity after isoproterenol administration was accompanied by a decrease in the activities of 5′-nucleotidase and succinic dehydrogenase in crude (before NaI treatment) and in purified (after NaI treatment) membrane fractions enriched in sarcolemma as well as in fractions of heavy microsomes and mitochondria (Table 1), which were isolated to check the validity of the fractionation procedure. In the control heart both the crude and the NaI-treated fractions enriched in sarcolemma were contaminated by mitochondria, containing 4·74 and 2·63 per cent respectively of the succinate dehydrogenase (SDH) activity of the mitochondrial fraction. The contamination by mitochondria of the same fractions from isoproterenol-treated hearts established by the presence of SDH activity represented 4·47 and 2·63 per cent of the lowered mitochondrial SDH activity observed after isoproterenol treatment. However, no measurable

TABLE 1. ACTIVITIES OF MARKER ENZYMES IN FRACTIONS CONTAINING SARCOLEMMA, HEAVY MICROSOMES AND MITOCHONDRIA ISOLATED FROM CONTROL AND ISOPROTERENOL TREATED HEARTS

	Crude sarcolemma fraction before NaI treatment		Sarcolemma after NaI treatment		Heavy microsomes		Mitochondria	
	Control	Isoprot.	Control	Isoprot.	Control	Isoprot.	Control	Isoprot.
5′-Nucleotidase	2·83 ± 0·25	0·67 ± 0·07	1·10 ± 0·09	0·06 ± 0·01	7·20 ± 0·60	4·50 ± 0·54	0·11 ± 0·02	0·06 ± 0·01
Succinic dehydrogenase	0·27 ± 0·03	0·17 ± 0·02	0·15 ± 0·01	0·10 ± 0·01	—	—	5·70 ± 0·60	3·80 ± 0·32

The results are expressed as mean ± S.E. of three separate experiments and are expressed in μmoles $P_i\ mg^{-1}\ hr^{-1}$. Isoproterenol against control $P < 0{\cdot}01$.

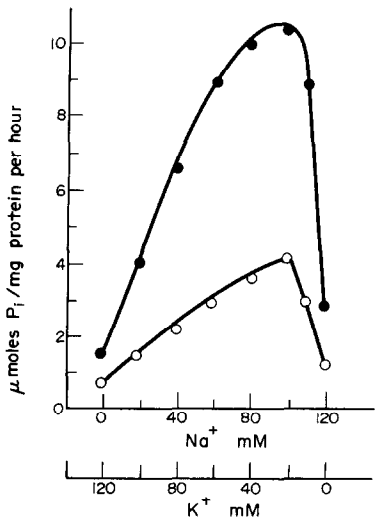

FIG. 1. Na^+,K^+-stimulated ATPase activities of the hearts from control and isoproterenol-treated dogs in the presence of varying concentrations of Na^+ and K^+ in an incubation medium containing 50 mM Tris–HCl, pH 7·4, 1 mM EDTA, 5 mM $MgCl_2$, 4 mM Tris–ATP with and without ouabain (0·2 mM) for 10 min at 37°. Na^+,K^+-stimulated ATPase activity was calculated by subtracting the values in the presence of ouabain from those in the absence of ouabain (n = 5). (●) Control; (○) isoproterenol-treated.

Na^+,K^+-ATPase activity could be detected in fractions of heavy microsomes and mitochondria.

The content of protein-bound sulfhydryl groups in the fraction containing Na^+,K^+ stimulated Mg^{2+}-ATPase increased in the isoproterenol treated hearts from 8·60 ± 0·85 to 9·55 ± 1·05 μmoles/mg protein, but the difference was not significant (n = 5; P > 0·05).

Changes in some special properties of Na^+,K^+-ATPase. The extent of isoproterenol-induced diminution of Na^+,K^+-ATPase activity was found to be characteristic

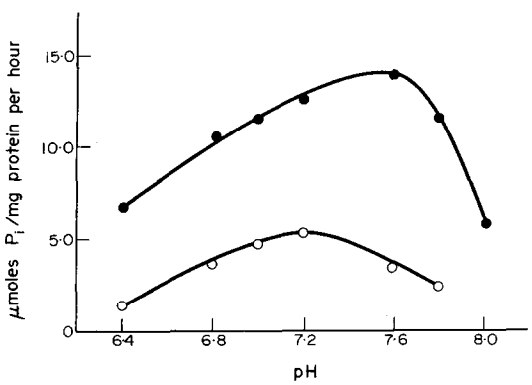

FIG. 2. Na^+,K^+-stimulated ATPase activities of the hearts from control and isoproterenol-treated dogs at different pH of the incubation medium. Membrane fractions (60–70 μg/ml) were incubated in a medium containing 50 mM Tris–HCl, 1 mM EDTA, 5 mM $MgCl_2$, 4 mM Tris–ATP, 100 mM NaCl, 20 mM KCl, at different pH with and without ouabain (0·2 mM) for 10 min at 37°. Na^+,K^+-stimulated ATPase activity was calculated by subtracting the values in the presence of ouabain from those in the absence of ouabain (n = 5). (●) Control; (○) isoproterenol-treated.

TABLE 2. THE INFLUENCE OF SOME INHIBITORS ON Mg^{2+}-ATPASE AND Na^+,K^+-ATPASE ACTIVITIES ISOLATED FROM NORMAL AND ISOPROTERENOL TREATED HEARTS

	Inhibitor	Inhibition in % of original activity Mg^{2+}-ATPase	Na^+,K^+-ATPase
Control hearts	10^{-5} M Ouabain	⩽2	⩾98
	5 μg/ml Oligomycin	⩽2	64
	5 mM NaN_3	19	⩽1
	0·1 mM $HgCl_2$	5	76
Isoproterenol treated hearts	10^{-5} M Ouabain	⩽2	⩾98
	5 μg/ml Oligomycin	⩽1	60
	5 mM NaN_3	20	⩽1
	0·1 mM $HgCl_2$	⩽1	⩾99

The results are expressed as mean ± S.E. of three estimations. Membrane fractions (60–70 μg/ml) were incubated in a medium containing 50 mM Tris–HCl pH 7·4, 1 mM EDTA, 5 mM $MgCl_2$, 4 mM Tris–ATP, 100 mM NaCl, 20 mM KCl.

in a wide range of $Na^+:K^+$ ratios (Fig. 1); maximal activities in both controls and in isoproterenol-treated dogs were observed in the presence of 100 mM Na^+ and 20 mM K^+. The pH optimum for Na^+,K^+-stimulated ATPase was 7·6 for control and 7·2 for isoproterenol-treated hearts (Fig. 2).

The K_m value of Na^+,K^+-stimulated ATPase was $6{\cdot}6 \times 10^{-4}$ M and $1{\cdot}20 \times 10^{-4}$ M. For control and isoproterenol-treated heart respectively.

Influence of some inhibitors on Na^+,K^+-ATPase activity. No essential differences in ouabain, sodium azide and oligomycin sensitivity could be found in Na^+,K^+-ATPase and Mg^{++}-ATPase preparations obtained from control and isoproterenol-treated hearts. An increase in the inhibition of Na^+,K^+-ATPase by $HgCl_2$ was found in isoproterenol-treated heart (Table 2).

DISCUSSION

In trying to explain the observed decrease of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities and the increased yield of the respective subcellular fraction at least two

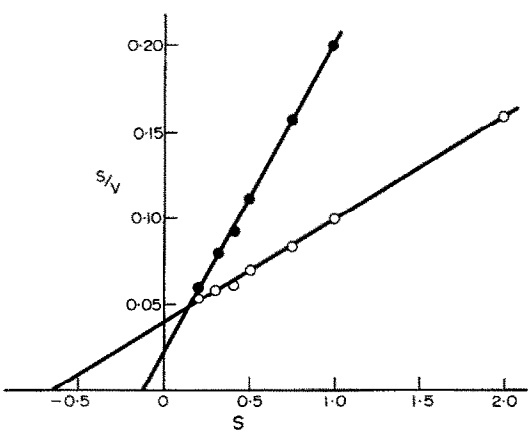

FIG. 3. Hanes plot of the effect of ATP concentrations on Na^+,K^+-ATPase activity. Concentrations of ATP ([S]) are expressed in mM and the velocity (V) in μmoles P_i liberated/mg protein per hr. The straight line was plotted by linear regression and extrapolated to esimate K_m. The incubation medium was the same as described in Figs. 1 and 2. (○) Control; (●) isoproterenol-treated.

interrelated influences should be considered: (a) unspecific overall tissue destruction by isoproterenol accompanied by elevated intracellular Ca^{2+} concentration resulting in an overall decrease in the activities of structurally bound enzymes such as actomyosin ATPase,[23] Na^+,K^+-ATPase, Mg^{2+}-ATPase, SDH and 5′-nucleotidase in various membrane fractions (for the latter see Table 1). (b) Changes of some specific properties of enzymes particularly those of Na^+,K^+-ATPase, such as pH optimum, some kinetic properties (K_m value) and increased sensitivity to $HgCl_2$ (see Figs. 2 and 3 and Table 2).

The above conception is supported by the fact, that the observed decrease by 65·6 per cent of Na^+,K^+-ATPase by 61·0 per cent of Mg^{2+}-ATPase, by 74·6 per cent of 5′-nucleotidase and by 33·4 per cent of SDH activities cannot simply be explained by a 29·0 per cent increase in the yield of the NaI-treated fraction after isoproterenol administration. Neither can these results be explained by contamination of the sarcolemma-enriched membrane fraction by other membranes nor by altered validity of the fractionation procedure for isoproterenol-treated plasma membranes, since an approximately similar depression in the activities of marked enzymes could be observed in plasma membrane fractions, as well as in microsomal and mitochondrial fractions (see Table 1) thus not showing any increase in their mutual contamination. The increased yield of the NaI-treated membrane fraction can therefore be considered to be due to more non-enzyme protein being separated by the same procedure. This protein may originate from destroyed membranes and other destroyed cellular compartments[6,10] which then may have altered properties in regard to isolation.

As regards changes of specific properties of Na^+,K^+-stimulated Mg^{2+}-dependent ATPase it should be pointed out that our control values were similar to those reported by other authors as to pH optimum,[14] influence of Na^+ and K^+,[24] K_m and V_{max}[25] and influence of certain inhibitors.[14,15,26]

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