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Stimulative and protective action of Sr^{2+} and Ba^{2+} on (Na^+–K^+)-ATPase from cultured heart cells

The use of monolayers of cultured cells, containing only one cell type, provides an ideal system for evaluating the activity of a variety of pharmacological agents in the absence of complicating tissue and neural effects. Such a system of cultured myocardial cells was recently used by SPERELAKIS AND LEHMKUHL[1] in an attempt to separate the electrophysiological effects of cardiac glycosides and local anesthetics at the tissue level from those at the cellular level. Their results indicated that ouabain, cocaine and tetracaine produced depolarization and a loss of electrical excitability in individual cells. This inhibition of electrical activity could rapidly be reversed, concomitant with a marked hyperpolarization, by the addition of either Sr^{2+} or Ba^{2+} (5–10 mM) to the culture bath. Among several possible explanations, the authors favored the view that their results could best be explained by assuming these drugs inhibited the (Na^+–K^+)-ATPase, and that Sr^{2+} or Ba^{2+} had a protective and stimulative role on Na^+ pump activity. In addition, this view is consistent with the observations that Sr^{2+} usually hyperpolarizes cultured heart cells bathed in normal media within 1–2 min, even in the presence of high external K^+ (partial Na^+ replacement); however, Sr^{2+} is not effective in Na^+-free Li^+–Ringer, presumably because Li^+ can not be actively extruded. The present communication is an investigation of the direct effects of Ba^{2+} and Sr^{2+} on (Na^+–K^+)-dependent ATPase activity of cultured heart cells. The data support the mechanism of Ba^{2+} and Sr^{2+} action suggested by the earlier work, and represents a further localization of the effects of local anesthetics from the cellular to the molecular level.

Monolayers of cultured heart cells were prepared and grown as previously described[2–4]. Routinely, four culture flasks containing approx. 250 mg of cells were used to prepare "a membrane preparation" of myocardial cells. The cells were lysed using osmotic shock, washed twice in distilled water, and sonicated for 5 min. The sonicate was centrifuged at 100000 $\times$ g for 3 h at 4°, and the pellet redissolved in 4 ml of 0.25 M sucrose. This membrane preparation was prepared daily and immediately used as the source of ATPase activity. After incubation in the appropriate medium (see Table I) for 30–60 min, inorganic phosphate was estimated by the method of FISKE AND SUBBAROW[5].

The results are summarized in Table I. It is clear that Ba^{2+} and Sr^{2+} both further activate the ATPase from cultured heart cells. The degree of activation of the enzyme as a function of either Sr^{2+} or Ba^{2+} concentration is shown in Fig. 1. The results are similar to those reported by WINS AND SCHOFFENIELS[6] for Sr^{2+} and Ba^{2+} activation of the ATPase of red cell ghosts, though their maximum activity was obtained at lower cation concentrations. The protection of the enzyme from the effect of ouabain by Ba^{2+} or Sr^{2+} is entirely consistent with the electrophysiological data of SPERELAKIS AND LEHMKUHL[1]. A greater activation of the ATPase by Sr^{2+} is consistent with the greater hyperpolarization produced by Sr^{2+} compared to Ba^{2+}. The ability of Sr^{2+} and Ba^{2+} to rapidly hyperpolarize these cells in the presence of ouabain could be due either to the re-establishment of the ionic gradients or to an electrogenic Na^+ pump potential. The present studies do not differentiate between

TABLE I

SUMMARY OF THE DATA SHOWING STIMULATION OF THE $(Na^+–K^+)$-ATPase FROM CULTURED HEART CELLS BY Sr^{2+} AND Ba^{2+}, AND THE PROTECTIVE ACTION OF THESE DIVALENT CATIONS ON THE ACTIONS OF CARDIAC GLYCOSIDES AND LOCAL ANESTHETICS

The control medium contained 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4), 3 mM ATP and 0.4 ml enzyme in a final volume of 2 ml. The solution was incubated at 37° for 5 min, and the reaction started by the addition of ATP and stopped after the appropriate time by the addition of 0.2 ml 35% trichloroacetic acid. In all cases two controls, one without protein and one without ATP, were included.

Conditions	*Number of experiments*	*% Activity (± S.E.)*
Control medium	27	100
$-Na^+$, $-K^+$	2	59 ± 7
+ouabain (2 mM)	2	36 ± 11
+ Sr^{2+} (2 mM)	6	163 ± 21
+ Ba^{2+} (2 mM)	2	143 ± 7
+ouabain (2 mM) + Sr^{2+} (2 mM)	4	170 ± 16
+ouabain (2 mM) + Ba^{2+} (2 mM)	2	140 ± 13
+tetracaine (10 mM)	2	22 ± 1
+tetracaine (10 mM) + Sr^{2+} (2 mM)	2	76 ± 1
+tetracaine (10 mM) + Ba^{2+} (2 mM)	2	67 ± 3
+ Li^+ (100 mM) $-Na^+$	3	142 ± 18
+ Li^+ (100 mM) $-Na^+$ +ouabain (2 mM)	2	135 ± 7

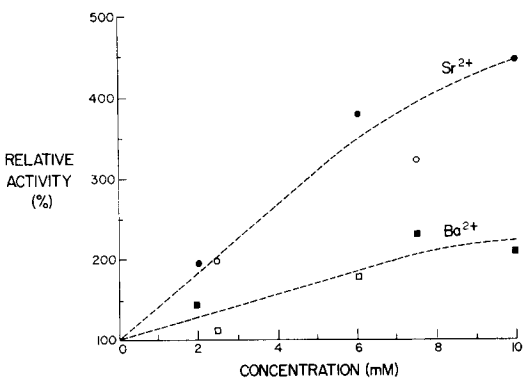

Fig. 1. Stimulation of the transport ATPase of cultured heart cells by Sr^{2+} and Ba^{2+}. The relative enzyme activity relative to the initial control activity of 100% is plotted as a function of the Sr^{2+} or Ba^{2+} concentration. The incubation medium contained 100 mM NaCl, 20 mM KCl, and 5 mM $MgCl_2$. The circles represent Sr^{2+} data and the squares Ba^{2+} data; filled symbols and open symbols are from separate experiments.

these two possibilities, *i.e.* stimulation of an electrogenic *versus* a non-electrogenic pump. The reversal of ouabain inhibition by Ba^{2+} or Sr^{2+} may be due to competition with ouabain for the same binding site. This suggestion appears reasonable since ouabain and K^+ are known to compete for the same site on the $(Na^+–K^+)$-activated ATPase[7], and Ba^{2+} has the apparent ability to bind or plug K^+ sites or channels in the myocardial cell membrane due to their similarity in sizes[8]. However, recently MATSUI AND SCHWARTZ[9] have reported that the reversal of ouabain inhibition by K^+ may not be due to competition for the same binding site on the enzyme. In our

system, the mechanistic interpretation of Ba^{2+} and Sr^{2+} reversal of ouabain inhibition awaits more detailed kinetic studies.

The effect of local anesthetics on cultured heart cells was to depress the electrical activity of these cells and cause a gradual depolarization[1]. This could be due either to an inhibition of the Na^+ pump or a decrease in potassium conductance of the cell membrane. Since the membrane resistance remained nearly constant during the application of the anesthetic, this suggests that pump inhibition might be the cause of the depolarization. The data in Table I indicate that tetracaine does indeed inhibit the cultured heart cell ATPase, and this supports the above suggestion. The rapid hyperpolarization produced by Ba^{2+} and Sr^{2+} of myocardial cells depolarized by cocaine or tetracaine is consistent with the ability of these cations to partially restore the activity of the ATPase as shown in Table I. These results indicate that the loss of electrical excitability and depolarization caused by ouabain and local anesthetics in cultured heart cells could be due to inhibition of the Na^+–K^+ pump, and that the reversal of these effects by Ba^{2+} and Sr^{2+} may be due to their stimulation of the (Na^+–K^+)-ATPase.

The effect of replacing Na^+ in the incubation medium with Li^+ is interesting because electrophysiological studies by PAPPANO AND SPERELAKIS[10] indicate that the cultured heart cell is unable to pump Li^+. However, the activity of the ATPase is increased when Li^+ is substituted for Na^+ (Table I) and this activity is essentially ouabain insensitive. This suggests that Li^+ uncouples the hydrolytic activity of the enzyme from the transport activity. The validity of this suggestion depends on a demonstration that it is actually the activity of the (Na^+–K^+)-dependent enzyme which is stimulated and not the non-specific enzyme. This would be most clearly accomplished by purification of the (Na^+–K^+)-dependent ATPase activity and subsequent kinetic analysis in a Li^+ medium. SKOU[11,12] has shown that in the presence of Na^+, Li^+ partially replaces K^+ as an activator of the enzyme.

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