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INFLUENCE OF SOME DIVALENT CATIONS ON HEART SARCOLEMMAL BOUND ENZYMES AND CALCIUM BINDING*

JAMES A. C. HARROW, PRASUN K. DAS and NARANJAN S. DHALLA†

Experimental Cardiology Laboratory, Department of Physiology, University of Mantiboa, Winnipeg, Canada R3E 0W3

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Abstract.—The actions of Ni^{2+}, Co^{2+} and Mn^{2+} on the rabbit heart sarcolemmal ATPases, calcium binding, and adenylate cyclase activities were studied. The ability of sarcolemma to hydrolyze ATP was stimulated by 0.1–4 mM concentrations of Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+}. The sarcolemmal Ca^{2+} ATPase (22.8 μmoles P_i/mg of protein/hr) and Mg^{2+} ATPase (21.6 μmoles P_i/mg of protein/hr) activities were depressed by 0.25–4 mM-Co^{2+}, Ni^{2+} and Mn^{2+}, and the order of their potency was $Ni^{2+} > Co^{2+} > Mn^{2+}$. The sarcolemmal Na^+–K^+ ATPase activity (9.4 μmoles P_i/mg of protein/hr) was decreased by 0.10–4 mM concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}. The sarcolemmal calcium binding in the presence of 0.1 mM Ca^{2+} (98 nmoles/mg of protein/5 min) was depressed by 0.25 mM or higher concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}, whereas that in the presence of 1.25 mM-Ca^{2+} (772 nmoles/mg of protein/5 min) was decreased by 2–4 mM-Co^{2+}, Ni^{2+} and Mn^{2+}. The sarcolemmal adenylate cyclase activities in the absence (124 pmoles cyclic AMP/mg of protein/min) and presence of 2 mM-NaF (517 pmoles cyclic AMP/mg of protein/min) were decreased by 0.1–4 mM-Co^{2+} or Ni^{2+} and stimulated by 0.1–4 mM-Mn^{2+}. The contractile force of the isolated rabbit heart was decreased by varying degrees by 0.1–1 mM of divalent cations ($Ni^{2+} > Co^{2+} > Mn^{2+}$). These results indicate sarcolemma is one of the sites involved in the cardiodepressant actions of Ni^{2+}, Co^{2+} and Mn^{2+}.

Although various divalent cations such as Ni^{2+}, Co^{2+} and Mn^{2+} are known to depress myocardial contractile force [1, 2], the exact mode and site of their actions are not clear at present. In view of the electrophysiological findings that Ni^{2+}, Co^{2+} and Mn^{2+} depress calcium currents into the cardiac muscle cell [3, 4], it can be argued that these cations act at the level of sarcolemma. If this is the case, then it should be possible to demonstrate some effects of these cations on the sarcolemmal bound enzyme and calcium binding activities. It has been shown already that heart sarcolemma contains some important enzymes such as Na^+–K^+ ATPase, adenylate cyclase, Mg^{2+} ATPase and Ca^{2+} ATPase [5–7]. Na^+–K^+ ATPase is thought to maintain the intracellular concentrations of Na^+ and K^+ in myocardium whereas adenylate cyclase is known to catalyze the formation of cyclic AMP. Although the functional roles of Mg^{2+} ATPase and Ca^{2+} ATPase activities in heart sarcolemma cannot be stated with certainty, these enzymes are claimed to be involved in the movements of divalent cations across the cell membrane [5, 7]. Furthermore, heart sarcolemma has been shown to possess a remarkable ability to bind a considerable amount of calcium [8], and it is likely that this may serve as an important source of calcium during the excitation–contraction process [7]. Earlier, we have shown that the cardiodepressant actions of propranolol and quinidine can be explained in terms of changes in the sarcolemmal ATPases, calcium binding and adenylate cyclase activities [9, 10]. In this investigation, we wish to examine the interaction of heart sarcolemma with Ni^{2+}, Co^{2+} and Mn^{2+} in order to gain some information concerning the site of their cardiodepressant effects. The actions of these cations on contractile force development by the isolated rabbit heart were also studied.

MATERIALS AND METHODS

New Zealand white male rabbits, each weighing about 2 kg, were killed by cervical dislocation and the hearts removed quickly. The ventricles were washed in ice-cold 10 mM-Tris–HCl, pH 7.4, and homogenized with 10 vol. of 10 mM-Tris–HCl, 1 mM-EDTA and 1 mM-dithiothreitol (DTT), pH 7.4. The sarcolemmal fraction, free from cytoplasmic contamination, was prepared by methods described elsewhere [6, 9]. The sarcolemmal Na^+–K^+ ATPase activity was determined in 1 ml of a medium containing 50 mM-Tris–HCl, pH 7.4, 4 mM-$MgCl_2$, 100 mM-NaCl, 10 mM-KCl and 4 mM-ATP in the absence or presence of 2 mM-ouabain. The difference of the activities in the absence and presence of ouabain is referred to as Na^+–K^+-stimulated, Mg^{2+}-dependent, ouabain-sensitive ATPase (Na^+–K^+ ATPase). On the other hand, the Ca^{2+} ATPase and Mg^{2+} ATPase activities were assayed in a total volume of 1 ml containing 50 mM-Tris–HCl, pH 7.4, 4 mM-$CaCl_2$ or $MgCl_2$ and 4 mM-ATP. The ATP hydrolysis that occurred in the absence of Ca^{2+} or Mg^{2+} was subtracted in order to calculate the activity due to Ca^{2+}-stimulated or Mg^{2+}-stimulated ATPase. In all these cases, the sarcolemmal fraction (0.02–0.05 mg protein/ml) was preincubated for 3 min in the absence or presence of different concentrations of

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† To whom reprint requests should be sent.

Co^{2+}, Ni^{2+} and Mn^{2+} and the reactions were started by the addition of 4 mM-ATP. After 10 minutes of incubation at 37°, the reactions were stopped by the addition of 12% cold trichloroacetic acid and centrifugation. The inorganic phosphate released in the clear supernatant fraction was measured by the method of Taussky and Shorr [11]. The protein concentration was determined by the method of Lowry *et al.* [12]. The adenylate cyclase activity was assayed by the method of Drummond and Duncan [13] for which the sarcolemmal fraction (50 μg protein/0.15 ml) was preincubated in a medium containing 50 mM-Tris–HCl, pH 8.5, 8 mM-caffeine, 5 mM-KCl, 20-mM phosphoenol pyruvate, 15 mM-$MgCl_2$, 2 mM-cyclic AMP and 130 μg/ml of pyruvate kinase for 3 min at 37° followed by the addition of 0.4 mM-[^{14}C]ATP. The sarcolemmal calcium binding was determined in a medium containing 50 mM-Tris–HCl, pH 7.0, at 37° with a protein concentration of 0.15–0.2 mg/ml. Different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+} were added during the preincubation period. The reaction was initiated by the addition of either 0.1 or 1.25-mM $^{45}CaCl_2$ and terminated by millipore filtration [8].

The rabbit hearts were also arranged for coronary perfusion by the Langendorff technique and the contractile force was monitored according to the procedure described previously [14]. Modified Krebs–Henseleit solution containing 1.25 mM-Ca^{2+} was continuously gassed with 95% O_2 and 5% CO_2 and used as a perfusion medium. The hearts were stimulated at 240 beats/min with a square-wave pulse just above the threshold and the coronary flow rate was maintained at 25 ml/min. For studying the effects of different divalent cations, the hearts were switched to the perfusion medium containing different concentrations of Co^{2+}, Ni^{2+} or Mn^{2+}. The results were analyzed by Student's paired *t*-test.

RESULTS

The effects of different concentrations of Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} on the ability of the sarcolemmal fraction to hydrolyze ATP were compared, and the results are shown in Table 1. All these cations were capable of stimulating ATP hydrolysis by heart sarcolemma; Ni^{2+} was found to be the least effective. The maximal Ca^{2+} ATPase and Mg^{2+} ATPase activities were found to be 22.8 and 21.6 μmoles P_i/mg of protein/hr respectively. It is likely that the sarcolemmal ATP hydrolysis in the presence of Co^{2+}, Ni^{2+} and Mn^{2+} is due to stimulation of the Ca^{2+}/Mg^{2+} ATPase enzyme complex, which has been suggested to participate in opening calcium channels in the heart membrane [7].

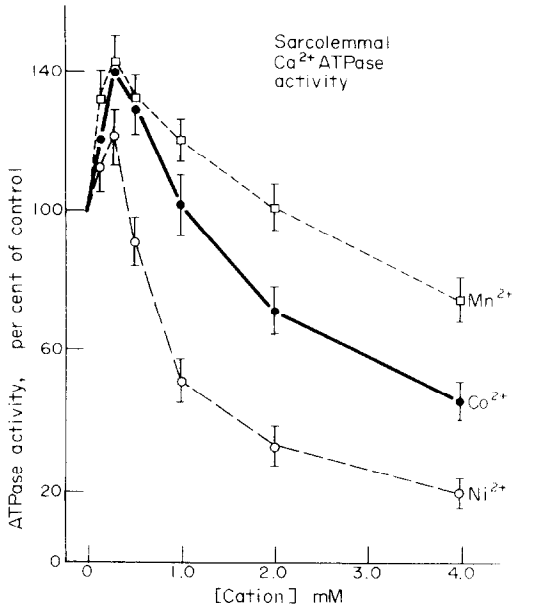

Fig. 1. Sarcolemmal Ca^{2+} ATPase activity in the presence of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}. The incubation medium was the same as that described in Materials and Methods except that 1.25 mM Ca ATP was used. The control value for Ca^{2+} ATPase was 12.1 ± 0.8 μmoles P_i/mg of protein/hr. Each value is the mean ± S. E. of four experiments.

In one series of experiments, the effects of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+} on the ability of sarcolemma to hydrolyze ATP in the presence of 1.25 and 4 mM-Ca ATP were studied. The sarcolemmal Ca^{2+} ATPase activity in the presence of 1.25 mM-Ca^{2+} was stimulated by 0.10–0.25 mM-Ni^{2+}, 0.10–0.50 mM-Co^{2+} and 0.10–1 mM-

Table 1. Sarcolemmal ATP hydrolysis in the presence of different concentrations of divalent cations*

Concn of cation (mM)	ATP hydrolysis (μmoles P_i/mg protein/hr)				
	Ca^{2+}	Mg^{2+}	Co^{2+}	Ni^{2+}	Mn^{2+}
0.10	2.96 ± 0.56	4.43 ± 0.41	3.97 ± 0.58	3.09 ± 0.58	2.78 ± 0.29
0.25	3.52 ± 0.20	5.68 ± 0.47	5.79 ± 0.35	3.60 ± 0.28	3.06 ± 0.25
0.50	5.74 ± 0.26	6.33 ± 0.39	8.88 ± 0.34	5.00 ± 0.51	7.38 ± 0.61
1.0	9.76 ± 0.40	9.01 ± 0.44	14.9 ± 1.3	5.48 ± 0.52	12.7 ± 0.40
2.0	15.1 ± 1.8	12.8 ± 0.81	20.5 ± 1.0	5.69 ± 0.41	21.1 ± 1.3
4.0	22.8 ± 1.4	21.6 ± 1.2	18.9 ± 0.95	9.14 ± 0.39	17.6 ± 1.1

* The incubation medium was the same as that described for sarcolemmal ATPase activity in Materials and Methods. The value for sarcolemmal ATP hydrolysis in the absence of any added cation was 1.95 ± 0.31 μmoles P_i/mg of protein/hr. The concentration of ATP was 4 mM. Each value is the mean ± S. E. of four experiments.

Mn^{2+} but depressed by 1–4 mM-Ni^{2+}, 2–4 mM-Co^{2+} and 4 mM-Mn^{2+} (Fig. 1). It should be pointed out that the effects of these cations on Ca^{2+} ATPase activity by employing low concentrations of Ca^{2+} could not be appreciated because these cations were capable of stimulating ATP hydrolysis by themselves. On the other hand, the Ca^{2+} ATPase activity, when assayed under optimal conditions in the presence of 4 mM-Ca^{2+}, was depressed significantly ($P < 0.05$) by 0.25–4 mM-Ni^{2+} or Co^{2+} and 1–4 mM-Mn^{2+} (data not shown). These divalent cations also produced similar effects on the sarcolemmal Mg^{2+} ATPase activity. The Ca^{2+} ATPase activity (23.3 ± 1.6 μmoles P_i/mg of protein/hr) was depressed by 76, 45 and 38 per cent whereas the Mg^{2+} ATPase activity (22.0 ± 2.5 μmoles P_i/mg of protein/hr) was depressed by 78, 50 and 40 per cent by 4 mM-Ni^{2+}, Co^{2+} and Mn^{2+} respectively. The order of potency in decreasing Ca^{2+}/Mg^{2+} ATPase activities was $Ni^{2+} > Co^{2+} > Mn^{2+}$. The depressant effects of Co^{2+}, Ni^{2+} or Mn^{2+} on Ca^{2+}/Mg^{2+} ATPase were not prevented by the addition of 1–4 mM excess ATP in the incubation medium. Thus, the observed actions of these divalent cations are unlikely to be due to a decrease in the concentrations of Ca ATP and Mg ATP, which serve as substrates for the ATPase reaction.

The sarcolemmal Na^+–K^+ ATPase activity (9.4 μmoles P_i/mg of protein/hr) was significantly decreased ($P < 0.05$) by Co^{2+}, Ni^{2+} and Mn^{2+} in 0.1–4 mM concentrations (Fig. 2). Since Mg ATP serves as a substrate for the Na^+–K^+ ATPase, it is likely that Co^{2+}, Ni^{2+} and Mn^{2+} may be reducing the substrate concentration in the reaction medium. However, increasing the concentration of Mg^{2+}, ATP or both in the incubation medium by 2–4 mM did not modify the depressant effects of these divalent cations on the sarcolemmal Na^+–K^+ ATPase activity. The effects of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+} on the sarcolemmal calcium binding activity in the presence of 0.1 and 1.25 mM-calcium were also examined, and the results are given in Table 2. At 0.1 mM-Ca^{2+}, 0.25–4 mM-Co^{2+}, Ni^{2+} and Mn^{2+} decreased ($P < 0.05$) calcium binding whereas at 1.25 mM-Ca^{2+}, 4 mM-Co^{2+} or Ni^{2+} and 2–4 mM-Mn^{2+} were effective in decreasing significantly calcium binding by sarcolemma.

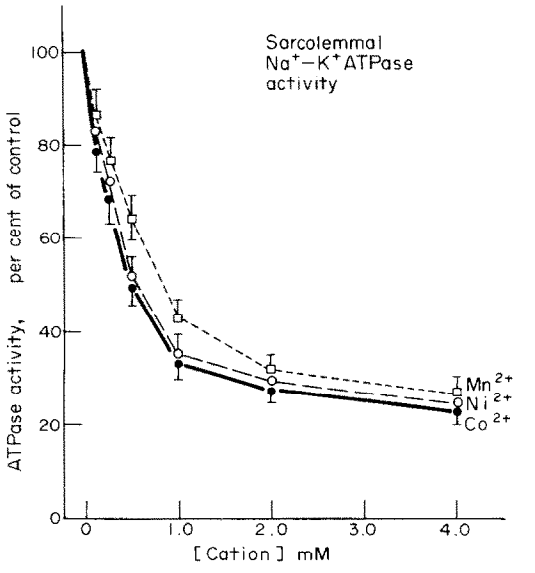

Fig. 2. Sarcolemmal Na^+–K^+ ATPase activity in the presence of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}. The incubation medium was the same as that described in Materials and Methods. The control value for Na^+–K^+ ATPase activity was 9.4 ± 0.46 μmoles P_i/mg of protein/hr. Each value is the mean ±S.E. of four experiments.

The sarcolemmal adenylate cyclase activities in the absence (basal) and presence of 2 mM-NaF were 124 and 517 pmoles cyclic AMP/mg of protein/min respectively. Both Co^{2+} and Ni^{2+} at 0.1–4 mM concentrations were found to decrease ($P < 0.05$) the sarcolemmal basal and NaF-stimulated adenylate cyclase activities, whereas Mn^{2+} at 0.1–4 mM concentrations was observed to increase ($P < 0.05$) these activities (Table 3). Since the effects of divalent

Table 2. Sarcolemmal calcium binding in the absence and presence of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}*

Concn of cation (mM)	Calcium binding (nmoles/mg protcin)					
	Co^{2+}		Ni^{2+}		Mn^{2+}	
	A	B	A	B	A	B
0	98 ± 3.2	772 ± 31	95 ± 3.7	780 ± 27	101 ± 6.2	776 ± 23
0.10	96 ± 2.4		90 ± 2.6		95 ± 3.4	
0.25	83 ± 3.4†		81 ± 2.0†		80 ± 3.3†	
0.50	73 ± 2.8†	781 ± 22	72 ± 3.1†	783 ± 24	73 ± 2.9†	763 ± 29
1.0	68 ± 2.9†	776 ± 25	67 ± 3.3†	783 ± 18	62 ± 2.5†	756 ± 34
2.0	63 ± 2.0†	763 ± 33	63 ± 2.6†	758 ± 20	53 ± 2.9†	729 ± 23†
4.0	52 ± 1.7†	717 ± 18†	51 ± 2.0†	712 ± 23†	49 ± 2.4†	706 ± 21†

* The incubation medium was the same as that described for sarcolemmal calcium binding in Materials and Methods. Sarcolemmal fractions were incubated in the presence of (A) 0.1 mM $^{45}Ca^{2+}$ or (B) 1.25 mM $^{45}Ca^{2+}$ for 5 min at 37°. Each value is the mean ±S. E. of four experiments.

† Significantly different from control ($P < 0.05$).

Table 3. Sarcolemmal adenylate cyclase activity in the presence of different concentrations of Co^{2+}, Ni^{2+} and Mn^{2+}*

Concn of cation (mM)	Adenylate cyclase activity (% of control)					
	Co^{2+}		Ni^{2+}		Mn^{2+}	
	A	B	A	B	A	B
0.10	85 ± 5.8	85 ± 4.3	77 ± 4.0	81 ± 4.6	148 ± 7.3	115 ± 5.1
0.25	77 ± 4.3	74 ± 4.0	72 ± 3.7	64 ± 3.2	252 ± 12.3	135 ± 6.4
0.50	71 ± 3.6	66 ± 3.2	53 ± 3.0	51 ± 2.7	278 ± 14.6	144 ± 6.8
1.0	61 ± 3.0	52 ± 2.9	48 ± 2.7	46 ± 2.6	283 ± 15.1	178 ± 7.3
2.0	56 ± 2.7	43 ± 2.5	34 ± 2.3	30 ± 1.8	286 ± 17.3	191 ± 8.6
4.0	42 ± 2.2	34 ± 2.0	21 ± 1.7	12 ± 1.0	308 ± 19.2	197 ± 8.3

* The incubation medium was the same as that described for sarcolemmal adenylate cyclase activity in Materials and Methods. (A) Basal activity, (B) activity in the presence of 2 mM NaF. The control values for basal adenylate cyclase activity and activity in the presence of NaF were 124 ± 4.7 and 517 ± 31 pmoles cyclic AMP/mg protein/min respectively. Each value is the mean ±S. E. of three experiments. All values in the presence of divalent cations were significantly different from the control ($P < 0.05$).

cations on adenylate cyclase were similar under basal and optimal (in the presence of NaF) conditions and since Mn^{2+} and Co^{2+} or Ni^{2+} produced opposite actions, it is unlikely that the observed effects are due to changes in the availability of substrate in the incubation medium.

When the isolated rabbit hearts were perfused with medium containing different concentrations of divalent cations, the contractile force started declining within 3–5 sec. With a 1 mM concentration of Ni^{2+}, Co^{2+} and Mn^{2+}, the contractile force decreased by about 90, 80 and 33 per cent, respectively, in 20 sec. When measured at 2 min after starting perfusion with divalent cations, the contractile force was depressed by about 40, 80 and 100 per cent by Ni^{2+}, 25, 45 and 95 per cent by Co^{2+}, and 20, 60 and 80 per cent by Mn^{2+} at 0.1, 0.5 and 1 mM concentrations respectively. The time course and dose–response analysis indicated that the order of potency with respect to depression in contractile force was $Ni^{2+} > Co^{2+} > Mn^{2+}$.

DISCUSSION

In this study, Co^{2+}, Ni^{2+} and Mn^{2+} at 0.1–1 mM concentrations produced varying degrees of depression in contractile force of the isolated hearts. Furthermore, we have demonstrated that Ni^{2+}, Co^{2+} and Mn^{2+} decreased Ca^{2+} ATPase and Mg^{2+} ATPase activities, when these enzymes were assayed under optimal conditions. The minimum concentrations required for the cations to produce a significant decrease in the enzyme activities were 0.25–0.5 mM for Ni^{2+} and Co^{2+} and 0.5–1 mM for Mn^{2+}. It was also interesting to observe that calcium binding by sarcolemma, when studied in the presence of 0.1 mM-Ca^{2+}, was decreased significantly by 0.25 mM-Mn^{2+}, Co^{2+} or Ni^{2+}. These results indicate that these divalent cations interfere with certain calcium binding sites and thus may decrease the sarcolemmal calcium stores. A reduction in the sarcolemmal calcium has been suggested to decrease the amount of calcium entering the myocardial cell upon excitation and is subsequently associated with a depression in the contractile force [7]. Furthermore, the observed depression in the sarcolemmal Ca^{2+}/Mg^{2+} ATPase, which has been suggested to participate in opening the calcium channels [7], may also contribute to decreasing calcium influx in the presence of these divalent cations. In this regard, it should be noted that these divalent cations have been demonstrated to decrease calcium currents and, therefore, are considered to depress myocardial contractility due to an impairment in the excitation–contraction coupling process [3, 4].

Although Ni^{2+}, Co^{2+} and Mn^{2+} stimulated the sarcolemmal ATP hydrolysis like calcium, it should be noted that both Ni^{2+} and Co^{2+}, unlike calcium, failed to restore the ability of the heart to develop contractile force after perfusion with Ca^{2+}-free medium [1]. Furthermore, these divalent cations did not release intracellularly bound Ca^{2+} from the cardiac cells, but instead were shown to displace and/or release calcium from the superficial sites located on the sarcolemma [1, 15]. Although the depressant effects of Co^{2+}, Ni^{2+} and Mn^{2+} have been explained on the basis of their interference with the release of calcium from the storage sites [16], Ni^{2+} has been suggested to compete for some intracellular sites after the release of calcium from the pool required for the maintenance of contractile force [17]. Thus, the mechanisms by which these divalent cations impair the excitation–contraction coupling appear to be of a complex nature. At present it is tempting to suggest that these divalent cations may displace calcium from the sites on sarcolemma and may enter the myocardial cell upon depolarization, thereby acting as 'false couplers' in the place of calcium in the excitation–contraction coupling mechanism. In this regard it should be noted that a Mn^{2+} action potential has been reported to occur in cardiac muscle [18] and this cation has been shown to accumulate within the cardiac cell [19]. Furthermore, increasing the concentration of extracellular calcium has been shown to overcome the cardiodepressant effect of Mn^{2+} [20].

Inhibition of the sarcolemmal Na^+ K^+ ATPase

activity by Co^{2+}, Ni^{2+} and Mn^{2+} would tend to increase myocardial contractility by making more calcium available, through direct or indirect mechanisms similar to those proposed for cardiac glycosides [21]. On the contrary, concentrations of these divalent cations, which inhibited Na^+–K^+ ATPase activity, were found to depress myocardial contractility. If it is assumed that these divalent cations displace membrane bound calcium, then less calcium would be available from sources associated with Na^+–K^+ ATPase inhibition. This mechanism is similar to that proposed for the cardiodepressant action of propranolol [9]. At any rate, Mn^{2+} has been shown to antagonize the positive inotropic effect of ouabain and its ability to increase calcium exchange [20, 22]. Furthermore, ouabain has also been reported to partially antagonize the effect of Ni^{2+} [20]. While it is difficult to explain the cardiodepressant actions of these divalent cations on the basis of their effects on Na^+–K^+ ATPase, it should be noted that the basal and NaF-stimulated activities of sarcolemmal adenylate cyclase were inhibited by Co^{2+} and Ni^{2+}. Depression in adenylate cyclase activity by Co^{2+} and Ni^{2+} would lead to less formation of cyclic AMP and decreased calcium movements into the myocardium [7]. In this regard, the action of Co^{2+} and Ni^{2+} appears to be similar to that for the cardiodepressant effect of quinidine [10]. On the other hand, stimulation of adenylate cyclase by Mn^{2+} suggests some differences in the mechanism of action of these divalent cations. It is noteworthy, however, that the order of potency of different divalent cations with respect to their depressant effects on contractile force, Ca^{2+}/Mg^{2+} ATPase and adenylate cyclase was $Ni^{2+} > Co^{2+} > Mn^{2+}$ except that Mn^{2+} had a stimulatory effect on adenylate cyclase activity. This is consistent with the well known order of their divalent metal ion–ligand complex association constant [23].

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