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A POSSIBLE ACTION OF NICARDIPINE ON THE CARDIAC SARCOLEMMAL Na^+-Ca^{2+} EXCHANGE

SATOSHI TAKEO,* KYOKO ADACHI and MATAO SAKANASHI†

Department of Physiology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Fukuyama 729-02; and † Department of Pharmacology, School of Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa 903-01, Japan

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Abstract—The effects of nicardipine on sodium-calcium exchange activity of cardiac sarcolemma-enriched vesicles isolated from the rat heart were examined. Sodium-loaded, sarcolemma-enriched vesicles, when exposed to a medium containing 40 μM $CaCl_2$, exhibited about 5 nmoles Ca^{2+}/mg protein of the maximal calcium uptake; the initial rate was 21 nmoles Ca^{2+}/mg protein/min. The calcium uptake was dependent on the extravesicular concentration of calcium ion. Nicardipine at concentrations of 0.1 to 10 μM depressed the rate of calcium uptake activity by 60–90%. The isolated membrane vesicles preloaded with Ca^{2+} showed a calcium efflux activity, when exposed to a medium containing sodium ion. The rate of calcium efflux was 2.5 nmoles Ca^{2+}/mg protein/min, when measured in a medium containing 6.5 mM NaCl. The efflux rate was facilitated with increased concentrations of sodium ion in the medium. About 75% of the preloaded calcium in the vesicles was released within 3 min of incubation. The rate of calcium efflux was stimulated in the presence of 0.1 to 10 μM nicardipine (2.5- to 4-fold increase). The present results suggest a possible action of nicardipine on the sodium-calcium exchange mechanism at cardiac sarcolemmal sites.

Calcium influx across the cardiac sarcolemma plays a crucial role in the regulation of contractile mechanisms of the myocardial cell. This is believed to be mediated, in part, through a slow inward current during the plateau phase of the action potential [1, 2], which has been demonstrated to be prevented by organic calcium channel blockers [3–6]. The calcium influx is also believed to be mediated through sodium-calcium (Na^+-Ca^{2+}) exchange at the cardiac sarcolemmal site [7, 8]. Since the Na^+-Ca^{2+} exchange appears to be influenced by calcium antagonists, information concerning the effects of calcium antagonists on this mechanism in myocardial cells has been sought. There are several reports concerning the influence of calcium antagonists on Na^+-Ca^{2+} exchange and passive calcium efflux in mitochondria [9–11] or cardiac membrane vesicles [12]. However, the information is not sufficient to elucidate generalized actions of calcium antagonists on the Na^+-Ca^{2+} exchange mechanism at the sarcolemmal site. Nicardipine, 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5 dicarboxylic acid 3-[2-(*N*-methylamino)] ethyl ester hydrochloride, has been shown to have potent vasodilating actions on the cerebral and coronary arteries [13–15], and to prevent serotonin- or prostaglandin $F_{2\alpha}$-induced spasm of vascular smooth muscle [16]. Such actions have been assumed to be mediated through a calcium antagonistic action [14, 17] or an inhibition of calcium influx into the vascular smooth muscle [18, 19]. Thus, the agent is classified as one of the organic calcium antagonists [20–22]. Because pharmacological influences of nicardipine on the Na^+-Ca^{2+} exchange mechanism have not been understood, we were interested in exploring a possible action of the agent on the Na^+-Ca^{2+} exchange mechanism in the myocardial cell. The present study was undertaken to examine the influence of nicardipine on the cardiac Na^+-Ca^{2+} exchange activity of isolated, cardiac sarcolemma-enriched vesicles in the rat.

* Author to whom all correspondence should be addressed.

MATERIALS AND METHODS

Male, albino Sprague–Dawley rats, weighing 250–300 g, were used in the experiments. Cardiac sarcolemma-enriched vesicles were isolated from the rat heart according to the method of Pitts [23]. The rat ventricles were homogenized in 4 vol. of 0.6 M sucrose–10 mM imidazole, pH 7.0, with an Ultra-turrax (Werke AG) at a speed of 1600 rpm for four 20-sec periods. The homogenate was centrifuged at 12,000 *g* for 30 min. The supernatant fraction was diluted with one-third of the supernatant volume of 160 mM KCl–20 mM morpholinopropane sulfonic acid (MOPS), pH 7.4 (KCl/MOPS solution), and centrifuged at 96,000 *g* for 60 min. The pellet was resuspended in 2 ml of KCl/MOPS, layered over 30% sucrose solution containing 0.3 M KCl, 50 mM sodium pyrophosphate and 0.1 M Tris/HCl, pH 8.3, and centrifuged at 95,000 *g* for 90 min using a swinging type bucket. The band at the sample-sucrose interface was recovered, diluted with 3 vol. of KCl/MOPS, and centrifuged at 100,000 *g* for 30 min. The pellet was resuspended in either KCl/MOPS, pH 7.4 (K^+-vesicles), or in 160 mM NaCl–20 mM MOPS (Na^+-vesicles) for Na^+-Ca^{2+} exchange study at a concentration of about 1.5 mg/ml. For an assay of ATPase activities, the final pellet was suspended in 320 mM sucrose–20 mM MOPS, pH 7.4. Isolation of the membrane was carried out at 0–4°. Protein concentrations were determined according to the method of Lowry *et al.* [24].

In a marker enzyme study, sodium, potassium-stimulated, magnesium-dependent ATPase (Na^+, K^+-ATPase) [25] and potassium-stimulated, *p*-nitrophenylphosphatase [26] activities were measured. Ouabain-sensitive Na^+, K^+-ATPase activity was estimated as the difference in Na^+, K^+-ATPase activities in the absence and presence of 1 mM ouabain. Measurements of NADPH-cytochrome *c* reductase and cytochrome *c* oxidase activities were performed according to the methods of Masters *et al.* [27] and Wharton and Tzagoloff [28] respectively. Oxalate-supported calcium uptake and potassium-stimulated, EDTA-ATPase activities were also measured by methods described elsewhere [29, 30].

Calcium influx studies were carried out using both Na^+-vesicles and K^+-vesicles according to the method of Pitts [23]. The Na^+-vesicles (30–40 μg protein) were incubated at 37° for 60 min and then added to an incubation medium containing 40 μM $^{45}CaCl_2$ (about 200 cpm/pmole) and 160 mM KCl/20 mM MOPS in a final volume of 500 μl. Four hundred microliters of the reaction mixture was withdrawn at desired intervals and filtered through a Millipore filter (pore size, 0.45 μm) under suction. The filter paper was washed three times, each time with 2.5 ml of a washing solution containing 160 mM KCl, 20 mM MOPS and 0.5 mM $LaCl_3$. The filter papers were dried at 60° in an oven for 1 hr and dissolved in 10 ml of a scintillation mixture containing Omnifluor (New England Nuclear) and Triton X-100, and then counted by a scintillation counter. As a blank, the incubation was carried out in the medium in which Na^+-vesicles were substituted for the same amount of K^+-vesicles. Calcium influx activity was estimated as the amount of calcium taken up by the vesicles, which was calculated as the difference in calcium uptake activities between Na^+-vesicles and K^+-vesicles. Nicardipine was added to the incubation medium at a final concentration of 0.1 to 10 μM. The initial rate of calcium influx was calculated from the data of time-course changes in the calcium influx activity and expressed as nmoles Ca^{2+}/mg protein/min.

Calcium efflux studies were carried out using sarcolemma-enriched vesicles preloaded with $^{45}CaCl_2$ as described in the calcium influx study. That is, Na^+-loaded vesicles were incubated at 37° for 3 min, with the incubation mixture containing 40 μM $^{45}CaCl_2$–KCl/MOPS solution to obtain the maximal calcium uptake by the vesicles. Calcium efflux was initiated at 37° by diluting the incubation medium into an equal volume of the solution prewarmed to 37°, containing 5 mM NaCl, 155 mM KCl, 1 mM ethyleneglycol bis (aminoethylbisther)tetra-acetate (EGTA) and 20 mM MOPS, pH 7.4. At desired intervals, 900 μl of the resulting mixture was taken and then filtered under suction. The filter papers were washed as described in the calcium influx study, followed by counting the radioactivity. Concentrations of nicardipine were adjusted in the medium containing NaCl, KCl, EGTA and MOPS so as to obtain the desired concentration in 1 ml of the reaction mixture. K^+-vesicles were also incubated as described above as a blank, and the difference in calcium efflux activities between the Na^+-vesicles and the K^+-vesicles was taken as the net calcium efflux activity, which was expressed as either residual calcium or percentage calcium efflux to the initial calcium contents in the vesicles. The initial rate of calcium efflux was calculated from the data of time-course changes in the calcium efflux activity and expressed as nmoles Ca^{2+}/mg protein/min. Compositions of NaCl and KCl were varied depending upon the desired concentrations of NaCl by keeping a constant osmolarity of the medium when the kinetic study was performed. It is pointed out that 4 mM NaCl was present in the incubation medium for calcium efflux study, when there was no sodium in the diluting medium, since 25 μl of the Na^+-vesicle suspension was employed in the present experiment.

Magnesium-dependent ATPase (Mg^{2+}-ATPase) activity of sarcolemma-enriched vesicles was measured at 37° in a medium containing 50 mM Tris/HCl, pH 7.4, 4 mM $MgCl_2$, 1 mM EDTA, 5 mM NaN_3, 4 mM Tris-ATP and about 25 μg protein. Total ATPase activity was measured at 37° in a medium containing 50 mM Tris/HCl, pH 7.4, 4 mM $MgCl_2$, 1 mM EDTA, 5 mM NaN_3, 4 mM Tris-ATP, 100 mM NaCl, 10 mM KCl and about 25 μg protein. The reaction was initiated by the addition of ATP and, 5 min later, was terminated by the addition of cold 12% trichloroacetic acid. The inorganic phosphate liberated was determined by the method of Taussky and Shorr [31] as described elsewhere [25]. Sodium-potassium ATPase (Na^+, K^+-ATPase) activity was taken as the difference between total ATPase and Mg^{2+}-ATPase activities. Nicardipine was added to the incubation medium at a final concentration of 0.1 to 10 μM.

Student's *t*-test was used for comparison of the control value versus values in the presence of nicardipine. Differences at the 95% confidence level were considered significant. Nicardipine was provided by the Yamanouchi Pharmaceutical Co. Ltd., Japan.

RESULTS

Isolated cardiac membrane vesicles and the homogenate used in the present experiment were characterized by measuring the marker enzyme activities; the results are shown in Table 1. The membrane vesicles show 16-fold enriched Na^+- K^+-ATPase and 5.7-fold enriched potassium-stimulated phenylphosphatase activities, compared with those of the heart homogenate. NADPH-cytochrome *c* reductase, oxalate-supported calcium uptake, and cytochrome *c* oxidase activities of the isolated membrane vesicles were not higher than those of the homogenate. Potassium-stimulated, EDTA-ATPase activity of the isolated membrane was about 10% of the homogenate. Ouabain-sensitive Na^+, K^+-ATPase activity was 14 ± 3% of the total Na^+,K^+-ATPase detected in the native membrane vesicles (N = 4).

Calcium influx activities of the isolated, cardiac membrane vesicles in the absence (control) and the presence of nicardipine are shown in Table 2, in which the activities are expressed as the net calcium uptake as well as the initial rate of calcium uptake. A rapid influx of calcium into sodium-loaded vesicles was seen, which was maximal at about 3 min. Calcium taken up by the control vesicles at 10 and 20 sec of incubation was about 50 and 80% of the maximal

Table 1. Marker enzyme activities of the rat heart homogenate and the sarcolemma-enriched vesicles*

	Homogenate (A)	Sarcolemma-enriched vesicles (B)	Ratio (B/A)
Na^+,K^+-ATPase (μmoles P_i/mg protein/hr)	1.8 ± 0.2	28.8 ± 3.0	16
K^+-stimulated phosphatase (μmoles nitrophenol/mg protein/hr)	0.51 ± 0.02	2.92 ± 0.30	5.7
NADPH-cytochrome *c* reductase (μmoles cytochrome/mg protein/hr)	3.1 ± 0.4	0.9 ± 0.4	0.3
Oxalate-supported calcium uptake (nmoles Ca^{2+}/mg protein/5 min)	14.1 ± 0.7	12.8 ± 2.1	0.9
Cytochrome *c* oxidase (μg cytochrome/mg protein/min)	0.99 ± 0.03	0.95 ± 0.03	1.0
K^+-stimulated, EDTA-ATPase (μmoles P_i/mg protein/hr)	8.52 ± 0.25	0.93 ± 0.51	0.1

* Each value represents a mean ± S.E.M. of four experiments. Ratios represent values of the homogenate/values of the sarcolemma-enriched vesicles, which roughly suggest the degree of the purification.

Table 2. Effects of nicardipine on calcium influx activity of cardiac sarcolemma-enriched vesicles*

Nicardipine (μM)	Initial rate (nmoles Ca^{2+}/mg protein/min)	Calcium uptake (nmoles Ca^{2+}/mg protein)		
		10 sec	20 sec	3 min
0 (control)	21.5 ± 1.0	2.49 ± 0.11	3.26 ± 0.40	4.91 ± 0.55
0.1	8.7 ± 1.0†	1.14 ± 0.13†	1.65 ± 0.30†	2.72 ± 0.58†
1	6.6 ± 1.3†	0.83 ± 0.16†	1.23 ± 0.49†	2.10 ± 0.67†
10	2.0 ± 0.7†	0.37 ± 0.13†	1.12 ± 0.25†	1.90 ± 0.48†

* Calcium influx activity is expressed as calcium uptake by the vesicles as well as the initial rate of calcium uptake. Each value represents a mean ± S.E.M. of four to five experiments.
† Significantly different from control ($P < 0.05$).

uptake (about 5 nmoles Ca^{2+}/mg protein at 3 min) respectively. Nicardipine at concentrations of more than 0.1 μM elicited a significant depression in the calcium influx activity at all the incubation intervals monitored, in a dose-dependent manner. Nicardipine at concentrations of more than 0.1 μM decreased the calcium uptake by the vesicles (45–54%), whereas a 10 μM concentration of the agent depressed the calcium influx activity by 61–85%. Nicardipine-induced depression in the activity was most obvious at 10 sec after the onset of incubation. Nicardipine at concentrations of 0.1, 1 and 10 μM depressed the initial rate of calcium influx by 60, 70 and 90% respectively. Since Na^+-Ca^{2+} exchange has been demonstrated to be a rapid process [32, 33], we thought it important to examine the calcium influx activity at 10 sec of incubation. Calcium influx activities in the presence and absence (control) of 1 μM nicardipine were measured at different concentrations of calcium (10–100 μM) in the incubation medium (Fig. 1). Calcium uptake by the vesicles was augmented with increased concentrations of calcium, and the apparent V_{max} for control vesicles was found to be about 6.5 nmoles Ca^{2+}/mg protein, whereas that for the vesicles in the presence of 1 μM nicardipine was 1.5 nmoles Ca^{2+}/mg protein. Apparent *n* for the calcium uptake of the control vesicles, analyzed according to Hill's plots, was 1. Nicardipine at a concentration of 1 μM shifted the apparent *n* to about 1.5, indicating a positive cooperativity.

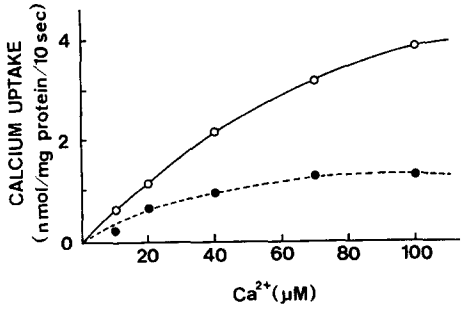

Fig. 1. Calcium influx activities of rat heart sarcolemma-enriched vesicles at different concentrations of $CaCl_2$ in the assay medium. The influx activity was estimated as the amount of calcium taken up by the vesicles and is expressed as nmoles Ca^{2+}/mg protein/10 sec. The calcium uptake was measured at 37° for 10 sec in medium containing 160 mM KCl–20 mM MOPS, Na^+-preloaded vesicles and different concentrations of $^{45}CaCl_2$ according to the method of Millipore filtration in the absence (○; control) and presence (●) of 1 μM nicardipine.

Calcium efflux activities of isolated, cardiac sarcolemma-enriched vesicles in the absence (control) and presence of nicardipine were also examined, the values of which were expressed as residual calcium in the vesicles (Table 3). A significant increase in the

Table 3. Effects of nicardipine on calcium efflux activity of cardiac sarcolemma-enriched vesicles*

Nicardipine (μM)	Initial rate (nmoles Ca^{2+}/mg protein/min)	Residual calcium (nmoles Ca^{2+}/mg protein)		
		30 sec	1 min	3 min
0 (control)	2.52 ± 0.26	3.64 ± 0.38	2.48 ± 0.22	1.30 ± 0.25
0.1	6.32 ± 1.21†	2.41 ± 0.46†	1.94 ± 0.43	1.12 ± 0.26
1	8.18 ± 1.69†	1.99 ± 0.41†	1.56 ± 0.35†	1.31 ± 0.50
10	10.68 ± 1.42†	1.73 ± 0.23†	1.51 ± 0.33†	0.83 ± 0.45

* Calcium efflux activity is expressed as the residual calcium in the vesicles as well as the initial rate of the efflux. Each value represents a mean ± S.E.M. of four to five experiments.
† Significantly different from control ($P < 0.05$).

calcium efflux activity of the vesicles exposed to 0.1 to 10 μM nicardipine was seen at the early stage of the incubation (at 30 sec). At 1 min of incubation, the residual calcium in the vesicles in the presence of 1 and 10 μM nicardipine was significantly lower than controls, whereas there were no significant differences in the residual calcium between the control vesicles and the vesicles in the presence of 0.1 to 10 μM nicardipine at 3 min of incubation. The initial rates of calcium efflux in the presence of 0.1, 1 and 10 μM were 2.5-, 3.2- and 4.2-fold higher than control values respectively.

To examine further the facilitating action of nicardipine on the rate of calcium efflux, percent calcium efflux (ratio of residual calcium to the initial calcium content in the vesicles) from the vesicles preloaded with 40 μM calcium was measured at different concentrations of sodium (6.5 to 60 mM) (Fig. 2). Nicardipine-induced changes in calcium efflux at low concentrations of sodium were larger than those at high concentrations of sodium. The control value for apparent n, calculated from Hill's plots, was about 1.2. The value was similar to that in the presence of nicardipine.

The effects of nicardipine on Mg^{2+}-ATPase and Na^+,K^+-ATPase activities of cardiac sarcolemma-enriched vesicles were examined (Table 4). Control values for the Mg^{2+}-ATPase and the Na^+,K^+-ATPase activities were about 122 and 28 μmoles P_i/mg protein/hr respectively. There were no significant differences in the Mg^{2+}-ATPase activities between the control vesicles and the vesicles exposed to nicardipine. A slight decrease in the Mg^{2+}-ATPase activity was observed, when the ATPase activity was measured in the presence of 1 and 10 μM nicardipine, but it was not significant ($P > 0.05$). Nicardipine induced an increment in the Na^+,K^+-ATPase activity of the vesicles at only the 1 μM concentration.

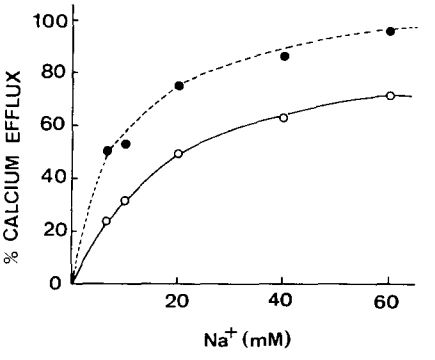

Fig. 2. Calcium efflux activities of rat heart sarcolemma-enriched vesicles at different concentrations of NaCl in the assay medium. The efflux activity was determined by estimating residual calcium in the vesicles after the onset of reaction, and the activities were evaluated as percent calcium efflux of the vesicles. The residual calcium was measured at 37° for 30 sec in medium containing 20 mM MOPS, 1 mM EGTA, Ca^{2+}-preloaded vesicles and different concentrations of NaCl and KCl with a constant osmolarity (160 mM) in the absence (○; control) and presence (●) of 1 μM nicardipine.

DISCUSSION

The cardiac membrane isolated in the present experiment showed a high Na^+,K^+-ATPase activity and low activities of cytochrome c oxidase, NADPH-cytochrome c reductase, oxalate-supported calcium uptake and potassium-stimulated EDTA-ATPase. The results suggest that the membrane is enriched primarily with cardiac sarcolemma, and is substantially devoid of other major subcellular organelles such as mitochondria [28], sarcoplasmic reticulum [29] and myofibrils [30]. The membrane also

Table 4. Effects of nicardipine on Mg^{2+}-ATPase and Na^+,K^+-ATPase activities of cardiac sarcolemma-enriched vesicles*

Nicardipine (μM)	ATPase (μmoles P_i/mg protein/hr)	
	Mg^{2+}-ATPase	Na^+,K^+-ATPase
0 (control)	121.8 ± 6.5	27.8 ± 1.1
0.1	119.9 ± 7.5	27.4 ± 2.3
0.3	120.1 ± 6.2	28.5 ± 2.1
1	116.7 ± 5.5	33.3 ± 1.3†
10	116.8 ± 7.4	25.8 ± 2.4

* Each value represents a mean ± S.E.M. of seven experiments.
† Significantly different from control ($P < 0.05$).

exhibited calcium influx activity under the present experimental conditions where non-specific calcium binding was eliminated by washing the membrane with buffer containing La^{3+}. Furthermore, the membrane showed about 14% of ouabain sensitivity to the total Na^+,K^+-ATPase. This suggests that more than 86% of the membrane is practically sealed, because ouabain sensitivity is considered to represent the activity of leaky and/or rightside-out vesicles [33–36].

Pitts [23] demonstrated about 20 nmoles Ca^{2+}/mg protein of the maximal calcium uptake through Na^+-Ca^{2+} exchange by the sarcolemma-enriched fraction from the dog heart. In the present experiment, we confirmed that calcium uptake activity responded in a similar manner, but the maximal activity was less. Calcium uptake activities through Na^+-Ca^{2+} exchange of cardiac membrane vesicles from various animals ranged from 5 to 80 nmoles Ca^{2+}/mg protein. Lamers and Stinis [35] and Kupriyanov *et al.* [12] have shown 5–6 nmoles Ca^{2+}/mg protein of the maximal value for sodium-dependent calcium influx activities of the rat heart sarcolemma-enriched membrane isolated using methods different from ours. Cardiac membrane fraction isolated from the rabbit exhibited maximal calcium influx activity ranging from 8 to 17 nmoles Ca^{2+}/mg protein [37, 38], whereas that for the dog heart was 20–80 nmoles Ca^{2+}/mg protein [23, 32, 39, 40], and that for the bovine heart, 57 nmoles Ca^{2+}/mg protein [33]. Thus, it is likely that differences in the maximal calcium uptake activity of sarcolemma-enriched fractions between the rat heart in the present study and the dog heart demonstrated by Pitts [23] may be attributed to those in animal species.

Nicardipine at concentrations ranging from 0.1 to 10 μM significantly depressed the calcium influx activity of the vesicles. It has also been observed that 0.1 to 10 μM diltiazem, a calcium antagonist, depresses the calcium uptake activity of cardiac sarcolemma-enriched vesicles in a manner similar to that seen in the present experiment, but 0.1 to 10 μM verapamil does not depress the calcium influx activity [41]. These results indicate the divergent action of some calcium antagonists on the calcium influx activity of cardiac membrane vesicles through the Na^+-Ca^{2+} exchange mechanism. Divergent actions of several calcium antagonists on the calcium channel function have also been confirmed by biochemical, pharmacologic and therapeutic studies [42]. Furthermore, nicardipine facilitated the rate of calcium efflux of calcium-preloaded vesicles. The facilitating action was seen most markedly at 30 sec of incubation and was less potent at 1 min of incubation. Since Na^+-Ca^{2+} exchange in the mammalian myocardium has been demonstrated to be a rapid process [32, 33], the facilitating action of the agent at 30 sec of incubation seems to be reasonable, if we accept the notion that calcium efflux in the present experiment was primarily regulated by a Na^+-Ca^{2+} exchange mechanism.

We have not yet determined the sidedness of membrane vesicles employed in the present experiment. However, a sidedness of membrane vesicles appears to be substantially independent of the influence of nicardipine on Na^+-Ca^{2+} exchange activity with respect to its site of action, because recent evidence has shown quite similar affinities at the cytoplasmic and external sites for the Na^+-Ca^{2+} exchanger [43].

Mg^{2+}-ATPase activity of the vesicles was not affected by 0.1 to 10 μM nicardipine, whereas about a 20% increase in the Na^+,K^+-ATPase activity was seen at 1 μM nicardipine. As described at the beginning of the paper, nicardipine is known to be one of the organic calcium antagonists. The agent is also subclassified to be a dihydropyridine derivative on the basis of its chemical structure, like nifedipine, nimoldipine and nitrendipine [20–22]. Pan and Janis [44] have demonstrated recently a significant stimulation of Na^+,K^+-ATPase activity of vascular smooth muscle microsomal fraction by several organic antagonists such as nimoldipine, nitrendipine and diltiazem. In the present experiment, nicardipine elicited a significant stimulation of Na^+,K^+-ATPase only at a 1 μM concentration. This appears to be compatible with the findings described above. However, the increment seems to be due, in part, to a slight decrease in the Mg^{2+}-ATPase activity, though the decrement was not significant. Furthermore, the increment in the Na^+,K^+-ATPase was not observed in a dose-dependent manner; rather, a higher concentration of the agent (10 μM) tended to decrease the activity. Thus, an observed increase in the Na^+,K^+-ATPase activity must be emphasized, with some caution in terms of its pharmacological significance.

Effective doses of nicardipine with respect to its pharmacological and biochemical actions on smooth and cardiac muscles have been demonstrated to range from 3 nM to 3 μM: generally, a sensitivity of smooth muscle to the agent was greater than that of cardiac muscles [17–19, 45, 46]. Furthermore, plasma levels of nicardipine of various animals and humans, after administration of its pharmacological dose, have been found to be about 0.2 μM [47, 48]. Although extrapolation of the *in vitro* results to a physiological state should be made with caution, it is noteworthy that nicardipine at a concentration as low as 0.1 μM elicited significant effects on the Na^+-Ca^{2+} exchange activities in the present experiment.

Calcium antagonists are known to inhibit the calcium influx across the cardiac cell membrane through the slow inward current [3–6]. On the contrary, there is little evidence for the direct effect of various organic calcium antagonists on the Na^+-Ca^{2+} exchange system at the sarcolemmal site. Nicardipine showed an inhibitory action on the calcium influx as well as a facilitating action on the calcium efflux of cardiac sarcolemma-enriched vesicles in the present study. This action of nicardipine may be related to its ability to reduce the intravesicular calcium concentration. At present, we do not have any clear explanation for the dual effect of nicardipine on the calcium influx and calcium efflux activities which occur by the concentration gradient of sodium and calcium ions across the isolated vesicle membrane. However, it should be pointed out that verapamil at a 1 μM concentration augmented the calcium efflux activity of rat heart sarcolemma-enriched vesicles without any alterations in the calcium influx activity [41]. Furthermore, as described above, diltiazem at a concentration of 1 μM depresses the calcium influx

activity of the same vesicles, but has little effect on the calcium efflux activity [41]. Moreover, cardiac membrane fraction, isolated from hypertrophied heart induced by pressure-overload, showed a reduction in the calcium efflux activity without any alterations in the calcium influx activity, which was examined under the same conditions as in the present experiment (H. Clayton, S. Takeo and N. S. Dhalla, unpublished observations). Thus, it is likely that mechanisms controlling calcium efflux in the present experiment may be different from those involved in the calcium influx which is considered to be regulated by a Na^+-Ca^{2+} exchanger.

Another problem in evaluating the results on the Na^+-Ca^{2+} exchange in isolated vesicles is a lack of evidence that calcium influx and efflux activities, as observed in isolated membrane vesicles, reflect physiological calcium fluxes across the intact mammalian myocardial cells. Further information on the relationship of Na^+-Ca^{2+} exchange between *in vitro* experiments and the physiological meaning *in vivo* must be awaited. Nonetheless, the present studies strongly suggest a possible action of nicardipine on the cardiac sarcolemmal Na^+-Ca^{2+} exchange mechanism, which may provide another basis for the action of nicardipine on cardiac muscles.

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