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STIMULATION OF HEART SARCOLEMMAL Na^+-Ca^{2+} EXCHANGE BY CONCANAVALIN A

Naoki Makino, Dayuan Zhao and Naranjan S. Dhalla

Division of Cardiovascular Sciences,
St. Boniface General Hospital Research Centre,
and Department of Physiology, University of Manitoba,
Winnipeg, Canada R2H 2A6

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Summary: The effects of Concanavalin A (Con A) on membrane Ca^{2+} translocation activities were examined by employing rat heart sarcolemmal preparations. Con A stimulated Na^+-dependent Ca^{2+} uptake and ATP-dependent Ca^{2+} uptake activities in the sarcolemmal vesicles; maximal stimulation was seen at a concentration of 10 ug/ml. These effects of Con A were blocked by α-methylmannoside. Sarcolemmal Na^+-induced Ca^{2+} release was not affected by Con A. It is suggested that Con A-like substances may play a regulatory role in Ca^{2+}-translocation processes of heart sarcolemma.

The presence of Na^+-Ca^{2+} exchange and Ca^{2+} pump has been demonstrated in cardiac sarcolemmal preparations (1-4). These mechanisms are believed to be involved in the efflux of Ca^{2+} from the cell and thus are considered to play an important role in the metabolism of cellular Ca^{2+} (5-7). Although the regulation of these Ca^{2+} extruding activities is not fully understood, alterations of the Na^+-Ca^{2+} exchange and Ca^{2+} pump activities have been observed under pathological conditions as well as due to some pharmacological interventions (2,6,8-14). Since the cell membrane composition and fluidity are changed under these conditions, it was thought worthwhile to examine whether an agent, which is known to alter the fluidity of the plasma membrane, has any action on Ca^{2+} translocating activities. In this study we report the effects of a mitogenic lectin, Con A, which is commonly used as a probe to alter the fluidity of plasma membrane, on the Na^+-Ca^{2+} exchange and Ca^{2+}-pump activities of rat heart sarcolemma.

Materials and Methods

Isolation and characterization of heart membranes: Sarcolemmal vesicles were prepared from rat ventricles by the method of Pitts (3). The final pellet was suspended in either 160 mM KCl, 20 mM MOPS, pH 7.4 or 160 mM NaCl, 20 mM MOPS, pH 7.4 at a concentration of 1.5 to 2.2 mg protein/ml. The sarcolemmal vesicles employed in this study were characterized with

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respect to marker enzyme activities by methods used previously (15). The activity of Na^+-K^+ ATPase, a well known marker for plasma membrane, was 22.8 ± 1.9 umol Pi/mg/hr; this indicated 14 to 16 fold purification with respect to the heart homogenate enzyme activity. Cytochrome c oxidase and rotenone insensitive NADPH cytochrome c reductase activities in the sarcolemmal fraction indicated minimal (3-5%) contamination with mitochondrial and sarcoplasmic reticular fragments, respectively.

Measurement of Na^+-Ca^{2+} exchange: Na^+-dependent Ca^{2+} uptake and Na^+-induced Ca^{2+} release activities were determined by the methods employed previously (9,11,13). For studies involving Na^+-dependent Ca^{2+} uptake, sarcolemmal vesicles suspended in 160 mM NaCl, 20 mM MOPS, pH 7.4 were incubated at 37°C for 30 min. The NaCl-loaded vesicles were then added (10 ul) to a series of tubes containing an incubation mixture (at 37°C) consisting of 160 mM KCl, 20 mM MOPS, pH 7.4 plus 40 uM of $^{45}CaCl_2$ (50 uCi/nmol of Ca^{2+}) in a final volume of 500 ul in the absence or presence of different concentrations of Con A. The reaction was arrested at desired times by the addition of 100 ul of 160 mM KCl, 5 mM $LaCl_3$, 20 mM MOPS, pH 7.4. Aliquots (100 ul) were withdrawn, filtered through Millipore filters (0.45 uM) and then washed with 2 ml (1 ml aliquots) of 160 mM KCl, 20 mM MOPS, 1 mM $LaCl_3$, pH 7.4 to displace the externally bound Ca^{2+}. The sarcolemmal vesicles were also incubated for 30 min at 37°C in 160 mM KCl, 20 mM MOPS, pH 7.4 to load K^+ and then Ca^{2+} uptake was determined in a manner similar to that described for the Na^+-vesicles. The net Ca^{2+} influx was calculated as the difference between the Ca^{2+} uptake activities of the Na^+-loaded vesicles and the K^+-loaded vesicles. The nonspecific Ca^{2+} uptake in the K^+-loaded vesicles varied between 2 to 4 nmol Ca^{2+}/mg protein under the conditions used in this study and Con A was found to exert no effect on this parameter. For studies on Na^+-induced Ca^{2+} release, Na^+-loaded vesicles were allowed to accumulate $^{45}Ca^{2+}$ for 5 min in KCl/MOPS, following the procedure outlined for the Ca^{2+} uptake study. Ca^{2+} release was initiated by diluting the incubation medium into an equal volume of the solution containing desired concentrations of NaCl or KCl, 20 mM MOPS and 1 mM EGTA, pH 7.4 in the absence or presence of 10 ug/ml Con A. The content of Ca^{2+} within the vesicles after various times of efflux was determined by applying 200 ul of the mixture through 0.45 um Millipore filters under suction. The filters were washed and assayed for radioactivity in 10 ml of the scintillation fluid.

Measurement of Ca^{2+} pump activities: The experimental conditions were the same as reported elsewhere (13,16). The concentration of free Ca^{2+} was adjusted by using the EGTA buffer system (17). For ATP-dependent Ca^{2+} uptake assay, sarcolemmal vesicles (50 to 100 ug of protein) were preincubated at 37°C for 5 min in 0.5 ml of medium containing KCl/MOPS, 2 mM $MgCl_2$ in the absence or presence of Con A, and required concentrations of $^{45}CaCl_2$-EGTA to produce 10 uM of free Ca^{2+}. Total Ca^{2+} uptake was initiated by adding 2 mM Tris-ATP (pH 7.4). After 5 min of incubation at 37°C, the contents of each tube were immediately filtered through Millipore filters (0.45 um), and the filters were washed with 2 ml of ice cold KCl/MOPS and 1 mM $LaCl_3$ (pH 7.4), transferred to scintillation vials, dried, and then the radioactivity was determined. The ATP-dependent Ca^{2+}-uptake was calculated by subtracting nonspecific Ca^{2+} uptake (in the absence of ATP) from the total Ca^{2+} uptake. The values for the nonspecific Ca^{2+} uptake varied from 1.5 to 2.5 nmol Ca^{2+}/mg protein and Con A was found to have no effect on the nonspecific Ca^{2+} uptake.

Results

The effects of Con A on Na^+-Ca^{2+} exchange activity were examined and the data are given in Fig 1. It can be seen that Na^+-dependent Ca^{2+}

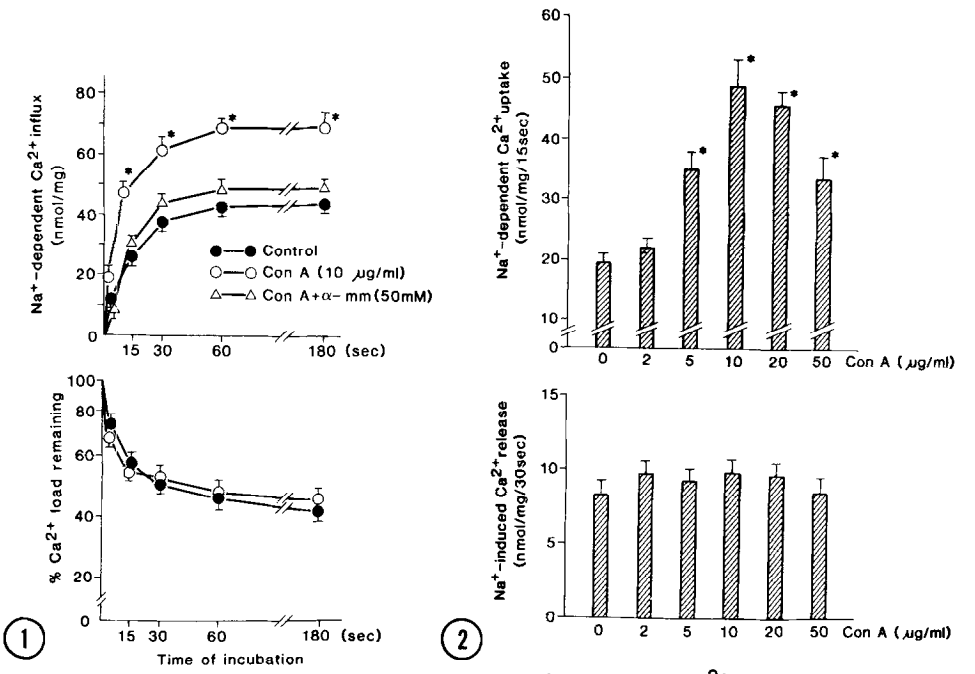

FIGURE 1. Effect of 10 ug/ml Con A on Na^+-dependent Ca^{2+} uptake and Na^+-induced Ca^{2+} release in rat heart sarcolemmal vesicles. Na^+-dependent Ca^{2+} uptake (upper panel) was measured in the presence of 40 uM Ca^{2+} at different times of incubation; the effect of Con A was also tested in the presence of 50 mM α-methylmannoside (α-mm). Lower panel shows the time-course of Na^+-induced Ca^{2+} release by 20 mM Na^+. Values are means $\pm$ S.E. of 6 experiments. * = significantly different ($P<0.05$) from control.

FIGURE 2. Influence of different concentrations of Con A on Na^+-dependent Ca^{2+} uptake (upper panel) and Na^+-induced Ca^{2+} release (lower panel) in rat heart sarcolemmal vesicles. Ca^{2+} uptake was measured in the presence of 40 uM Ca^{2+} whereas Ca^{2+} release was monitored by using 20 mM Na^+. Each value is a mean $\pm$ S.E. of 6 experiments. * = significantly ($P<0.05$) different from control.

uptake in sarcolemmal vesicles was significantly ($P<0.05$) increased by 10 ug/ml Con A and this effect was prevented by α-methylmannoside. However, the Na^+-induced Ca^{2+} release was not altered in the presence of Con A. It should be mentioned that 20 mM Na^+ is capable of releasing about 60% of the accumulated Ca^{2+} in the sarcolemmal vesicles within 3 min. On the other hand, 40 and 80 mM Na^+ released about 80 and 95% of the Ca^{2+} in the sarcolemmal vesicles within 90 sec; Con A also did not affect the Ca^{2+} release when 40 or 80 mM Na^+ was used. Likewise, no effect of Con A on the Na^+-induced Ca^{2+} release was seen when the vesicles were loaded with Ca^{2+} via the ATP-dependent Ca^{2+} uptake process (9,13). Unlike Na^+-induced Ca^{2+} release, Na^+-dependent Ca^{2+} uptake in the sarcolemmal vesicles was significantly increased by 5 to 50 ug/ml concentrations of Con A and the maximal effect was seen with 10 ug/ml Con A (Fig 2). The effect of 10 ug/ml Con A on Na^+-Ca^{2+} exchange in

TABLE 1. Effect of 10 ug/ml Con A on Na^+-dependent Ca^{2+} uptake in rat heart sarcolemmal vesicles in the presence of different concentrations of Ca^{2+}

Concentration of Ca^{2+}	Na^+-dependent Ca^{2+}-uptake (nmol/mg/15 sec)	
	Control	Con A (10 ug/ml)
5 uM	3.1 ± 0.25	6.5 ± 0.43*
10 uM	6.4 ± 0.32	13.8 ± 0.72*
20 uM	11.9 ± 0.64	24.5 ± 0.96*
40 uM	21.6 ± 0.82	46.4 ± 2.32*
80 uM	24.2 ± 1.07	51.7 ± 1.78*

Each value is a mean ± S.E. of 5 experiments. * = significantly different from control values ($P<0.05$).

heart sarcolemma was also examined in the presence of various concentrations of Ca^{2+} (Table 1). The stimulation of Na^+-dependent Ca^{2+} uptake by Con A was evident at all concentrations of Ca^{2+} tested.

The action of different concentrations of Con A was also studied on the sarcolemmal ATP-dependent Ca^{2+} uptake activity and the results are shown in Table 2. ATP-dependent Ca^{2+} uptake was increased significantly ($P<0.05$) by 5 to 50 ug/ml concentrations of Con A. Maximal activation of ATP-dependent Ca^{2+} uptake in the sarcolemmal vesicles was seen at 10 ug/ml Con A. Although α-methylmannoside had no effect on ATP-dependent Ca^{2+} uptake in sarcolemmal vesicles, this agent prevented the stimulatory action of Con A on Ca^{2+} uptake (Table 2). It should be noted that both sarcolemmal and sarcoplasmic reticular fractions exhibited Ca^{2+}-pump activities; however, the sarcoplasmic reticular fraction, unlike sarcolemma, did not show any Na^+-dependent Ca^{2+} uptake activity.

TABLE 2. Effect of Con A on rat heart sarcolemmal ATP-dependent Ca^{2+} uptake activities

Concentration of Con A (ug/ml)	ATP-dependent Ca^{2+}-uptake (umol/mg/5 min)	
	Without α-MM	With α-MM
Control	15.1 ± 0.78	12.8 ± 0.71
2	15.9 ± 0.81	13.9 ± 0.82
5	19.2 ± 1.0*	11.9 ± 0.92
10	23.4 ± 1.4*	14.5 ± 1.10
20	21.6 ± 0.9*	13.8 ± 1.02
50	20.1 ± 1.1*	13.0 ± 0.96

Each value is a mean ± S.E. of 6 experiments. The concentration of α-MM (α-methylmannoside) was 50 mM. ATP-dependent Ca^{2+} uptake was measured by using 10 uM Ca^{2+}. * = significantly ($P<0.05$) different from control.

Discussion

In this study we have shown that Na^+-dependent Ca^{2+} uptake and ATP-dependent Ca^{2+} uptake in the heart sarcolemmal vesicles were stimulated by Con A. Such an effect of Con A on Ca^{2+}-transport activities cannot be attributed to any interference due to the binding of Ca^{2+} with Con A (18) because the values for both Na^+-dependent Ca^{2+} uptake and ATP-dependent Ca^{2+} uptake were obtained by subtracting the nonspecific Ca^{2+} uptake from the total Ca^{2+} uptake under their respective experimental conditions. Furthermore, the observed augmentation of Ca^{2+}-transport activities were not due to alterations in the permeability of sarcolemmal vesicles because Na^+-induced Ca^{2+} release and nonspecific Ca^{2+} uptake were not changed by Con A. It should be pointed out that Con A has also been reported to increase the ATP-dependent Ca^{2+} uptake activity in lymphocytes (18,19). Furthermore, the activation of Na^+-dependent Ca^{2+} uptake by Con A (100%) was greater than that of ATP-dependent Ca^{2+} uptake (50%) in the heart sarcolemmal vesicles and this may be due to some differences in the sensitivities of these two Ca^{2+}-translocation systems to Con A.

It should be noted that Con A is a well known lectin which interacts with membrane proteins and modifies membrane functions. In this regard Con A has been reported to increase phospholipid unsaturation (20) and amino acid amidation (21), which processes have been shown to influence the membrane fluidity. Since Con A is also known to alter the fluidity of plasma membrane (22-24), it is likely that the stimulation of Na^+-dependent Ca^{2+} uptake and ATP-dependent Ca^{2+} uptake by Con A may be due to its action on the fluidity of heart sarcolemma. This view is supported by the fact that α-methylmannoside, which is known to depress the Con A-induced changes in membrane fluidity by blocking the carbohydrate binding sites on Con A, was found to prevent the stimulation of Na^+-dependent Ca^{2+} uptake as well as ATP-dependent Ca^{2+} uptake in sarcolemmal vesicles. Besides the carbohydrate binding sites, Con A has also been reported to possess an additional site that can be occupied by a hydrophobic ligand (25-27). In fact, the action of Con A on phospholipids has been demonstrated in the vesicles containing neither glycoproteins nor glycolipids (28). Thus, it is possible that Con A may also bind to the hydrophobic sites in order to induce changes in the lipid microenvironment of the Na^+-Ca^{2+} exchanger and Ca^{2+} pump in heart sarcolemma and this may then result in the stimulation of their activities. While a great deal of work needs to be done to settle the question regarding the mechanism of action of Con A, the augmentation of Ca^{2+} transport activities in heart sarcolemma

by Con A suggests that some lectin-like substances present in the myocardium may play a regulatory role in the movement of Ca^{2+} across the cell membrane. The presence of lectin-like substances has been reported in skeletal muscle (29) and these lectins have been suggested to regulate the ATPase activity in the transverse tubular membranes (30).

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