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Effects of Calcium Channel Blockers on Sodium-Free Contracture in Atrial Muscle of the Rabbit

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=Abstract=**Effects of organic and inorganic Ca-channel blockers on Na-Ca exchange system were investigated in the rabbit atrial muscle. Atrial muscle strips were perfused with K^+-free Tyrode solution in order to depress the sodium pump activity. Removal of external sodium(sodium being replaced by Tris) induced a contracture which reached a maximum after 1 min and effects of Ca-channel blockers on the magnitude of contracture were analysed. The results obtained were as follows: 1. In the concentrations of 30 μM, 100 μM and 300 μM Mn^{2+} increased the magnitude of Na-removal contracture, but decreased it in the concentration above 2 μM. Verapamil(10^{-6} M) pretreatment did not alter the effect of Mn^{2+} on sodium-removal contracture. 2. La^{3+}, as Mn^{2+}, increased the magnitude of contracture in the concentrations of 30-300 μM, and decreased the contracture in higher concentrations(> 1 mM) more prominently than Mn^{2+} did. 3. D-600 also increased the contracture in the concentrations of 5×10^{-8} M, 10^{-7} M and 5×10^{-7} M but had no effect in higher concentrations(10^{-6} – 10^{-5} M), On the other hand diltiazem had no dffect on the contracture in a wide range of concentrations (up to 10^{-4} M). From the above results, it is concluded that Mn^{2+}, La^{3+} and D-600 in lower concentrations stimulate the Na-Ca exchange system, whereas, Mn^{2+}, La^{3+} and D-600 in higher concentrations depress the exchange system and that Na-Ca exchange might be regulated by Ca-channel blockers and this regulation is sensitive to the concentration of Ca-channel blockers.**

Key Words: *Ca-channel blockers, Sodium-calcium exchange, Rabbit atrial muscle, Sodium-removal contracture*

INTRODUCTION

Sodium-calcium (Na-Ca) exchange has received much attention due to the critical role of calcium in cellular regulatory processes. Na-Ca exchange may play a role in maintaining the low intracellular calcium level and in mediating several calcium-dependent responses. In the cardiac muscle, especially, Na-Ca exchange may be essential in regulating contractility (Mullins 1981; Philipson 1985). Three sodium ions are

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exchanged with a calcium ion so that the exchange system generates an electrical current that flows in the direction of the calcium ion exchanges for the sodium ions in cardiac muscle. The exchange, therefore, is electrogenic (Pitts 1979; Reeves and Hale 1984). At present it is believed that Na-Ca exchange is involved in cardiac action potential and the positive inotropic effect of digitalis (Mullins 1981) and that the exchange also contributes to a phenomenon called "calcium-paradox" which occurs when calcium activity is returned to control level after a calcium-free perfusion period (Zimmerman and H Ismann 1966). In the present study we have measured and analysed the magnitude of sodium-free or sodium removal contracture (sodium being replaced by Tris) to investigate the effect of organic and inorganic calcium channel blockers on Na-Ca exchange in the rabbit atrial muscle. We demonstrated that Mn^{2+}, La^{3+} and D–600 in lower concentrations stimulated the Na-Ca exchange system whereas, Mn^{2+} and La^{3+} in higher concentrations depressed the exchange system. We suggest that Na-Ca exchange can be regulated by calcium channel blockers and that this regulation is sensitive to the concentration of calcium channel blockers.

MATERIALS AND METHODS

Preparation

Rabbits of either sex weighing about 1 kg were used. The animals were killed by a blow to the hind neck and exsanguinated by cutting both carotid arteries. Then the heart was extracted quickly and was transfered into a chamber containing oxygenated Tyrode solution. The right atrium was separated from the heart. The atrial wall was incised along between the superior and inferior vena cava, and was opened. Blood remaining in the atrium was washed out with normal Tyrode solution. After the recovery for one hour, the strips of the atrial muscle were prepared in sizes of, 1–1.5 mm in width and 3–5 mm in length. A loop was made with fine cotton thread for connecting to the hook of force transducer (Grass, FTO3) in the one end of the muscle strip. The above preparations were done under a zoom stereomicroscope. After the muscle strips were rested for an hour in the preparation chamber, a muscle strip was transferred into the experimental chamber.

Solutions

Normal Tyrode solution contains (mM) NaCl 140, KCl 3, $CaCl_2$ 2, $MgCl_2$ 1, dextrose 5, Tris-HCl 5 (pH was adjusted to 7.4 at 35° C). Sodium-free Tyrode solution was made by replacing NaCl iso-osmotically with tris-HCl. Drugs and chemicals used in the present experiment were verapamil, D-600, diltiazem, $MnCl_2$ and $LaCl_3$.

Experimental apparatus and protocol

Experimental chamber was made of perspex (derivative of leucine) and was of horizontal type. The inflow and outflow of experimental solution were done with hydrostatic pressure changes. Experimental temperature was maintained at about 35° C with a constant temperature circulator (Haake FE). All the muscle strips were allowed to rest in the horizontal chamber for one hour in tris-buffered Tyrode solution at 35° C equilibrated with 100% O_2, before experiments were done. Sodium-free contractures were recorded by using a force transducer (Grass, FTO3) and a recorder (Device).

RESULTS

A contracture, which reached maximum in 1 minute and relaxed spontaneously, was evoked in potassium-free, sodium-free solution (sodium replaced by equimolar Tris). Before the contracture, strip was exposed to potassium-free Tyrode solution in order to increase intracellular sodium concentration. Spontaneous twitch contractions usually appeared during potassium-free perfusion and just after washing out with normal Tyrode solution and tonic tension increased gradually in potassium-free solution. The magnitude of contracture was considered as the result of Na-Ca exchange (Fig.1).

Fig.2. shows the effects of various concentrations of manganese ion on the sodium-free contracture. The magnitude of sodium-free contracture was always increased by relatively lower concentrations (30 μM 100 μM and 300 μM) of Mn^{2+}, and it was decreased in very high concentrations (> 2 mM) of Mn^{2+} compared with control value. In the presence of 8 mM Mn^{2+}, sodium-free contracture was not abolished but decreased to about 50% of control value. Spontaneous contractions were decreased in a concentration-dependent manner in the presence of Mn^{2+} and finally disappeared in the presence of higher concentrations of Mn^{2+} (> 2 mM).

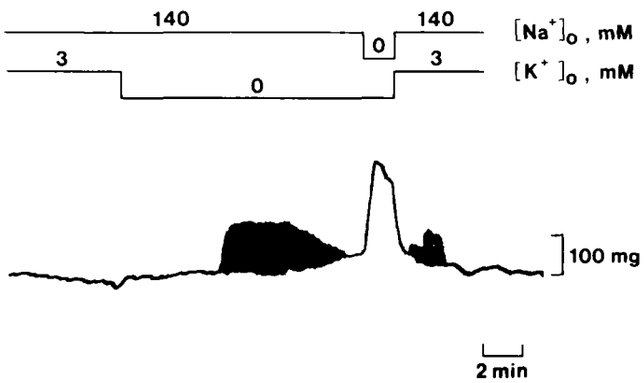

Fig.1. Experimental protocol for the induction of sodium-free contracture in rabbit atrial muscle. The magnitude of contracture was considered as the result of Na-Ca exchange.

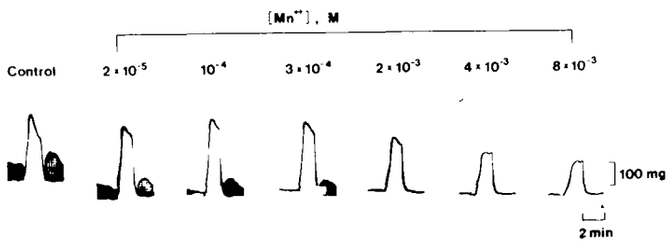

Fig.2. Effects of manganese ion on the sodium-free contracture. While Mn^{2+} increased the magnitude of sodium-free contracture in the concentrations of 2 × 10^{-5}, 10^{-4} and 3 × 10^{-4} m, it decreased the contracture in higher concentrations (> 2 mM), Even in 8mM of Mn^{2+} sodium-free contracture was never abolished but decreased about 50% of control magnitude. Spontaneous contractions were decreased and finally disappeared when the concentrations of Mn^{2+} were increased.

Fig.3. shows the relationship between the concentration of Mn^{2+} and the relative amplitude of contracture. The relative amplitude in various concentrations of Mn^{2+} was obtained with reference to the control value (100%). It shows clearly that in lower concentrations Mn^{2+} increased sodium-free contractures and in higher concentrations (> 2mM) decreased them.

In order to exclude the possible effect of Mn^{2+} on sodium-free contracture via calcium channel, effects of Mn^{2+} on sodium-free contracture were evaluated after pretreatment with 10^{-6} M verapamil for 15 minutes (Fig.4).

In control experiments, 10^{-6} M verapamil induced a small increases in sodium-free contracture and spontaneous contraction disappeared before treatment with Mn^{2+}. The magnitude of sodium-free contracture was also increased by the lower concentrations (30 μM, 100 μM and 300 μM) of Mn^{2+}, and decreased by very high concentrations (> 2 mM) of Mn^{2+} compared to control value. Similar effects were observed in the absence of verapamil-pretreatment. These data indicate that Mn^{2+} acts on sodium-free contracture directly.

Fig.5. shows the effects of lanthanum ion

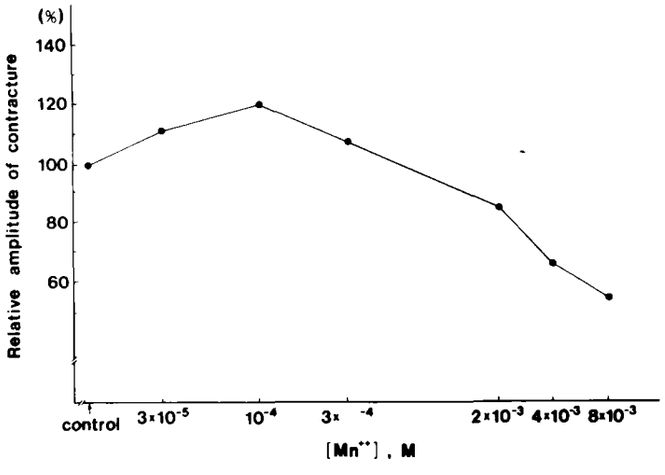

Fig.3. Plot of the relationship between Mn^{2+} concentrations and the relative amplitude of contracture (% of control amplitude). It shows clearly that in lower concentrations Mn^{2+} increased sodium-free contractures and in higher concentration (> 2 mM) decreased them.

with various concentrations

on sodium-free contracture. As in the case of Mn^{2+}, La^{3+} increased sodium-free contracture in lower concentra-tions (30 μM, 100 μM and 300 μM) and decreased the contracture in higher concentrations (> 1 mM). Fig.6. shows the relationship between the concentration of La^{3+} and the relative amplitude of contracture. The relative amplitude in various concentrations of La^{3+} was obtained with reference to the control value (100%). In order to exclude the possible effect of La^{3+} on sodium-free contracture via calcium channel, the effects of La^{3+} on sodium-free contracture were evaluated after pretreatment with 10^{-6} M verapamil for 15 minutes (Fig.7).

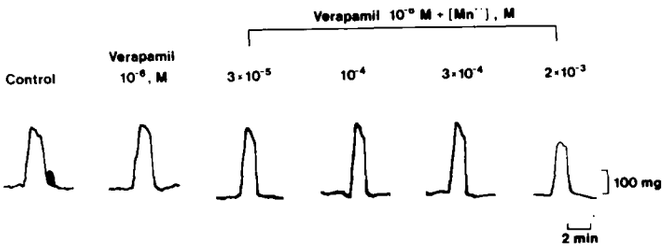

Fig.4. Effects of pretreatment of 10^{-6} m verapamil on the Na-free contractures. In order to exclude the possible effects of Mn^{2+} through calcium channel on sodium-free contractures, 10^{-6} M verapamil was treated before the exposure to potassium-free solution. Verapamil alone increased the magnitude of contracture and almost abolished spontaneous contractions. Effects of Mn^{2+} on contractures were not altered by verapamil pretreatment.

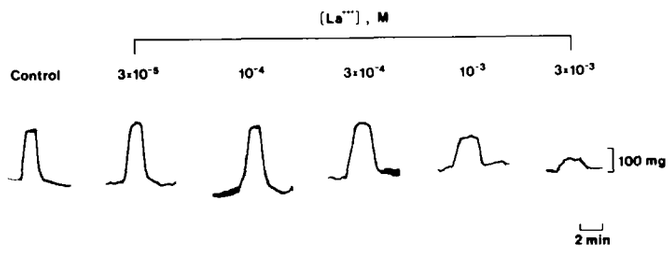

Fig.5. Effects of lanthanum ion on sodium-free contracture. La^{3+} increased sodium-free contracture in the lower concentrations (3×10^{-5}, 10^{-4} and 3×10^{-4} M) but decreased the contracture in higher concentrations (> 1 mM). La^{3+} has almost similar action on contracture to Mn^{2+} but decreasing effect in higher concentration was more prominent than Mn^{2+}.

In control experiments, 10^{-6} M verapamil induced a small increases in Na-free contracture. The magnitude of sodium-free contracture was also increased by the lower concentrations (30 μM, 100 μM and 300 μM) of La^{3+}, and decreased in very high concentrations (> 1 mM) of La^{3+} compared with control value. Similar effects were observed in the absence of verapamil-pretreatment. These data indicate that La^{3+} act on sodium-free contracture directly. La^{3+} has almost the same action on sodium-free contracture to Mn^{2+} but the reducing effect of

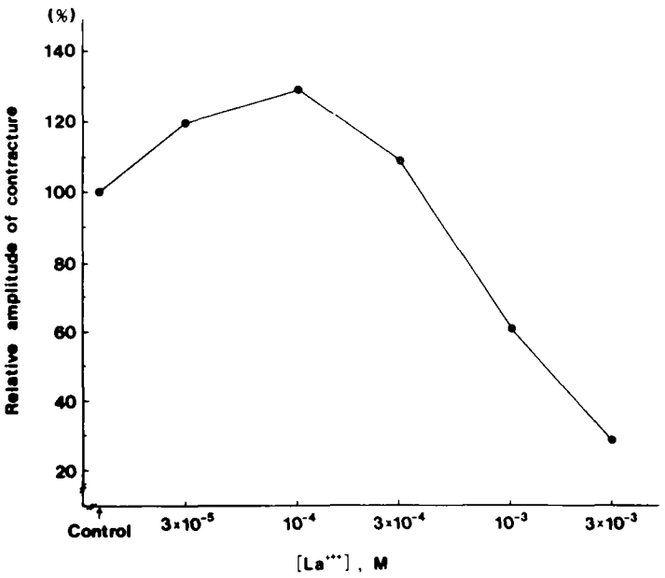

Fig.6. The dose-response relationship between La^{3+} concentrations and the amplitude of contracture (% of control magnitude). La^{3+} inhibits the development of sodium-free contracture in high concentrations and decreased the contracture to 20% of control magnitude.

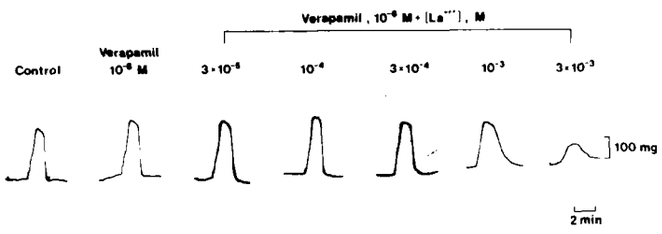

Fig.7. Influence of verapamil pretreatment on the La^{3+} effect on sodium-free contracture. No clear effect was noticed by 10^{-6} M verapamil on the increase or decrease in sodium-free contractures induced by various concentrations of La^{3+}.

La^{3+} on sodium-free contracture in higher concentrations was more prominent than that of Mn^{2+}

Fig.8. shows the effects of D-600 on Na-free contractures. D-600 increased the magnitude of Na-free contracture in the concentrations of 5 × 10^{-8}, 10^{-7} and 5x10^{-7} M. In higher concentrations (> 10^{-6} M) of D-600 we did not observe further decrease in the magnitude of the contracture in contrast to Mn^{2+} and La^{3+}. Fig.9. shows the relationship between the concentration of D-600 and the relative amplitude of sodium-free contracture.

The relative amplitude in various concentrations of D-600 was obtained with reference to the control value (100%). The effect of another organic calcium channel blocker, diltiazem, on sodium-free contracture was observed (Fig.10). Fig.11. shows the relationship between the concentration of diltiazem and the relative amplitude of sodium-free contracture. In all the concentrations of diltiazem tried, the magnitude of sodium-free contracture was unaffected.

DISCUSSION

It has been known that either increasing $[Ca]_o$ or decreasing $[Na]_o$ would result in tension development in cardiac muscle. This effect was first analysed quantitatively by L ttgauüand Niedergerke (1958). Further direct measurements of calcium flux using isotope demonstrated that calcium influx was increased by increasing $[Ca]_o$ (Winegard and Shanlo 1962;

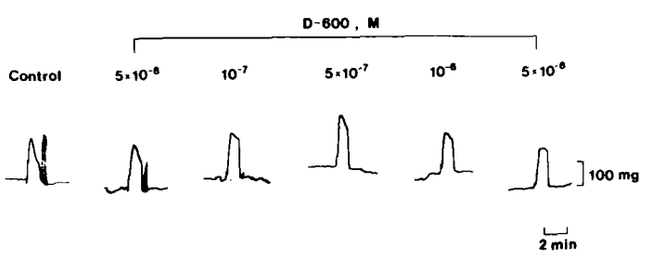

Fig.8. Effects of D-600 on sodium-free contractures. D-600 also has stimulatory effect on the development of sodium-free contracture in the concentrations of 5 × 10^{-8}, 10^{-7} and 5 × 10^{-7} M, but has no inhibitory effect in concentration higher than 10^{-6} M.

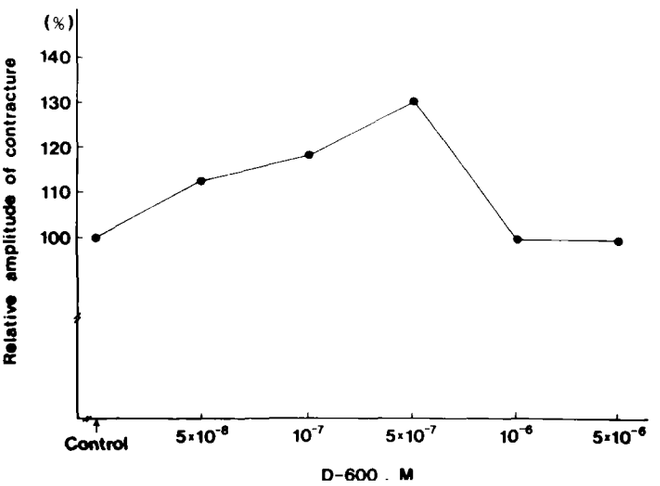

Fig.9. The dose-response relationship between D-600 concentration and relative magnitude of sodium-free contracture (% of controm value). Magnitude of contracture increase in 5 × 10^{-8}, 10^{-7}, 5 × 10^{-7} M of D-600 and shows maximum in 5 + 10^{-7} M.

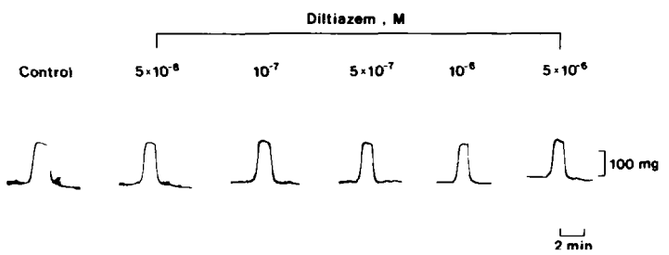

Fig.10. Effects of diltiazem on sodium-free contractures. In the whole range of Concentrations (5 × 10^{-8} – 10^{-4} M) diltiazem has no stimulatory or inhibitory action on the contracture.

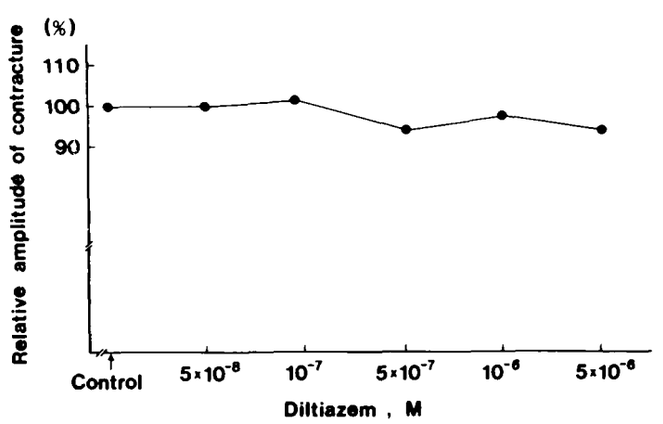

Fig.11. Plot of the dose-response relation between diltiazem concentrations and the relative amplitude of the contracture.

Niedergerke 1963) or decreasing $[Na]_o$ (Langer 1964; Reuter and Seitz 1968). These results made it possible to consider the presence of a single exchange mechanism that would move calcium both inward or outward via cell membrane. In 1968, Reuter and Seitz introduced, for the first time, the existence of a Na-Ca exchange mechanism in guinea-pig atria. Na-Ca exchange may be essential in mediating several calcium-dependent responses, with the most prominent example being in regulation of the cardiac contractile state. Contraction of a cardiac muscle fiber depends on the introduction into the myoplasm of sufficient calcium to react with the contractile proteins present in the fiber. Three possible sources of calcium may be involved in this process: (1) the calcium stored in sarcoplasmic reticulum(SR), (2) calcium entry via gated calcium channels, and (3) calcium entry via the Na-Ca exchange mechanism running in a reverse direction. After a perfusion with sodium-free solution, intracellular calcium ion concentration was increased up to several hundred nanomoles (Lee *et al.* 1980; Marhan *et al.* 1980) and the magnitude of external sodium removal-induced contraction was not smaller than normal twitch tension (Chapman 1983). Chapman (1979) also presented evidence that when external sodium was removed, increase in intracellular calcium concentration was not induced by the release of calcium from SR or gated calcium channels. This result would suggest that sodium-free contracture could represent calcium transient induced by the Na-Ca exchange system shown to operate in cardiac muscle when sodium equilibrium potential (E_{Na}) was made negative (when the $[Na]_o$ was made lower than $[Na]_i$). In the present study, therefore, we have measured and analysed the magnitude of sodium-free contracture to investigate the effect of organic and inorganic calcium channel blockers on Na-Ca exchange in the rabbit atrial muscle. On the other hand, some have reported that Na-Ca exchange was insensitive to a wide variety of substances, such as calcium and sodium channel blockers (Gill *et al.* 1981; Reeves and Hale 1984), ouabain (Gill *et al.* 1981), and mitochondrial poisons (Gill *et al.* 1981; Schellenberg and Swanson 1981). Others have reported that Na-Ca exchange was inhibited by polymixin B (Philpson and Nishimoto 1982), dibucaine, tetracaine (Michaelis and Michaelis 1983), chlorpromazine (Caroni *et al.* 1980), amiloride (Schellenberg *et al.* 1983) and also inhibited by calcium channel blockers non-specifically (Baker *et al.* 1969; Coraboeuf *et al.* 1981; Deitmer and Ellis 1978; Horackova and Vassort 1979). Mn^{2+} of high concentr-tions inhibited Na-Ca exchange in nerve (Baker 1972) and squid axons (Blaustein 1977). It was demonstrated that Mn^{2+} inhibited Na transport via cell membrane in cardiac Purkinje fibres (Deitmer and Ellis 1978) and also reduced the magnitude of sodium-free contracture in frog's hearts (Chapman and Ellis 1977). However, Coraboeuf *et al* (1981) observed that 4 mM Mn^{2+} did not have an influence on sodium-free contracture and that very high concentrations (20 mM) of Mn^{2+} inhibited sodium-free contracture in dog Purkinje fibres. It was reported that Na-Ca exchange was inhibited by 0.1 mM La^{3+} in squid giant axon (Baker *et al.* 1969) and by 3 mM La^{3+} in atrial trabeculae of the frog (Horackova and Vassort 1979). Katzung *et al* (1973) reported that Na-Ca exchange was not affected by 0.2–0.4 mM La^{3+} in mammalian heart. There is, therefore, still considerable controversy regarding the effects of calcium channel blockers on Na-Ca exchange. In the present study we examined the effects of calcium channel blockers, such as Mn^{2+}, La^{3+}, D-600 and diltiazem, on sodium-free contracture. It has been reported that low concentrations (0.4 mM) of La^{3+} and Mn^{2+} increased the magnitude of sodium-free contracture (Coraboeuf *et al.* 1981). However, there have been few observations whether D–600 affect the magnitude of sodium-free contracture. We observed that D-600($< 5 \times 10^{-7}$ M) increased the magnitude of sodium-free contracture. Since this effect was also observed after pretreatment with diltiazem, we could exclude the possibility that increase in sodium-free contracture by D-600 was induced by the

calcium channel. On the other hand, diltiazem may not affect Na-Ca exchange. Our data do not necessarily suggest a precise mechanism for the different effects of Mn^{2+}, La^{3+} and D-600 on sodium-free contracture in the presence of different concentrations. In order to elucidate the mechanisms of the regulation of Na-Ca exchange by calcium channel blockers, further studies are, therefore, needed to estimate the effect of calcium channel blockers on Na-Ca exchange at single cell level by using patch clamp techniques. From the above results, it can be concluded that, in cardiac muscle, Na-Ca exchange can be regulated by calcium channel blockers, and that this regulation is sensitive to the concentration of calcium channel blockers.

REFERENCES

Baker PF. Transport and metabolism of calciumion in nerve. Prog Biophys Molec Biol 1972; 24:177-223

Baker PF, Blaustein MP, Hodgikin AL, Steinhardt RA. The influence of calcium ion on sodium efflux in squid axons. J Physiol 1969; 200:431-58

Blaustein MP. Effect of internal and external cations and of ATP on sodium-calcium and calcium-calcium exchange in squid axons. Biophys J 1977; 20:79-111

Caroni P, Reinlib L, Carafoli E. Charge movements during the Na-Ca exchange in heart sarcolemmal vesicles. Proc Natl Acad Sci USA 1980; 77:6354-8

Chapman RA. Excitation-contraction coupling in cardiac muscle. Prog Biophys Molec Biol 1979; 35:1-52

Chapman RA. Control of cardiac contractility at the cellular level. Am J Physiol 1983; 245:H535-52

Chapman RA, Ellis DE. The effects of manganese ions on the contraction of the frog's heart. J Physiol 1977; 272:331-54

Coraboeuf E, Gantier P, Guiraudou P. Potential and tension changes induced by sodium removal in dog Purkinje fibres:Role of an electrogenic Na-Ca exchange. J Physiol 1981; 311:605-22

Deitmer JW, Ellis D. The intracellular sodium activity of cardiac Purkinje fibres during inhibition and reactivation of the Na-K pump. J Physiol 1978; 284:241-59

Gill DL, Grollman EF, Kohn LD. Calcium transport mechanisms in membrane vesicles from guinea-pig brain synaptosomes. J Biol Chem 1981; 256:184-92

Horackova M, Vasoort G. Sodium-calcium exchange in regulation of cardiac contractility. Evidence for an electrogenic voltage-dependent mechanism. J Gen Physiol 1979; 73:403-24

Katzung BG, Reuter H, Porzing H. Lanthanum inhibits Ca inward current but not Na-Ca exchange in cardiac muscle. Experimentia 1973; 29:1073-5

Langer GA. Kinetics of calcium distribution in ventricular muscle of the dog. Circ Res 1964; 15:393-405

Lee CO, Uhm DY, Dresdner K. Sodium-calcium exchange in the rabbit heart muscle cells: direct measurement of sarcoplasmic Ca^{2+} activity. Science 1980; 209:699-701

Lüttgau HC, Niedergerke R. The antagonism between Ca and Na ions on the frog heart. J Physiol 1958; 143:486-505

Marhan E, Rink TJ, Tsien RW, Tsien RY. Free calcium in heart muscle at rest and during contraction measured with Ca2+-sensitive microelectrodes. Nature 1980; 286:845-50

Michaelis ML, Michaelis EK. Alcohol and local anesthetic effect on Na^{+}-dependent Ca^{2+} fluxes in brain synaptic membrane vesicles. Biochem Pharmacol 1983; 32:963-9

Mullins LJ. Ion transport in the heart. Raven Press,New York, 1981

Neidergerke R. Movement of Ca in frog heart ventricles at rest and contracture. J Physiol 1963; 167:515-50

Philipson KD. Sodium-calcium exchange in plasma membrane vesicles. Ann Rev Physiol 1985; 47:561-71

Philipson KD, Nishimoto AY. Efflux of Na^{+}-Ca^{2+} exchange in cardiac sarcolemmal vesicles by proteinase pretreatment. Am J Physiol 1982; 243:C191-5

Pitts BJR. Stochiometry of sodium-calcium exchange in cardiac sarcoplasmic vesicles. Coupling to the sodium pump. J Biol Chem 1979; 254:6232-5

Reeves JP. Hale CC. The Stochiometry of the cardiac sodium-calcium exchange system. J Biol Chem 1984; 7733-9

Reuter H, Seits N. the dependence of calcium efflux from cardiac muscle on temperature and external ion composition. J Physiol 1968; 195:451-70

Schellenberg GD, Anderson L, Swanson PD. Inhibition

of Na+-Ca^{2+} exchange in rat brain by amiloride. Molec Pharmacol 1983; 24:251-8

Schellenberg GD, Swanson PD. Sodium-dependent and calcium-dependent calcium transport by rat brain microsomes. Biochem Biophys Acta 1981; 648:13-27

Winegard S, Shanes AM. Calcium flux and contractility in guinea-pig atria. J Gen Physiol 1962; 45:371-94

Zimmerman ANE, H Ismann WE. Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature 1966; 211: 646-7