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European Journal of Pharmacology 333 (1997) 261–268

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Differential effects of Ca^{2+} channel blockers on Ca^{2+} overload induced by lysophosphatidylcholine in cardiomyocytes

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Received 24 April 1997; revised 30 June 1997; accepted 4 July 1997

Abstract

The effects of Ca^{2+} channel blockers (verapamil, diltiazem, nicardipine, bepridil and flunarizine) on Ca^{2+} overload induced by lysophosphatidylcholine were examined in rat isolated cardiomyocytes. Addition of lysophosphatidylcholine (15 μM) produced Ca^{2+} overload as evidenced by a marked increase in the concentration of intracellular Ca^{2+} and hypercontracture of cells. Verapamil, flunarizine and bepridil concentration dependently inhibited the lysophosphatidylcholine-induced Ca^{2+} overload, whereas diltiazem and nicardipine did not. Lysophosphatidylcholine increased the release of creatine kinase, which was significantly attenuated by verapamil, flunarizine or bepridil (5 μM for each), but not by diltiazem or nicardipine (20 μM for each). Verapamil, flunarizine, bepridil (which attenuated the lysophosphatidylcholine-induced Ca^{2+} overload) and nicardipine (which did not) inhibited the veratridine-induced increase in the concentration of intracellular Na^+ (indicated by the increase in fluorescence ratio of Na^+-binding benzofuran isophthalate) and cell contracture, whereas diltiazem did not. These results suggest that verapamil, bepridil and flunarizine attenuate the Ca^{2+} overload induced by lysophosphatidylcholine, and that the Ca^{2+} channel blocking action of these drugs does not contribute substantially to this effect. The Na^+ channel inhibition together with high lipophilicity of these drugs may be important for the attenuation of the lysophosphatidylcholine-induced Ca^{2+} overload. © 1997 Elsevier Science B.V.

Keywords: Cardiomyocyte; Lysophosphatidylcholine; Ca^{2+} overload; Ca^{2+} channel blocker; Creatine kinase

1. Introduction

Ca^{2+} channel blockers have been used for treatment of ischemic heart disease, and the beneficial effects of the drugs are considered to be due to the cardiac depression and vasodilation that result from specific inhibition of the voltage-dependent Ca^{2+} channel. Nisoldipine, however, protects the heart from ischemic injury without inducing cardiac depression (Watts et al., 1987), and L-*cis*-diltiazem, an optical isomer of D-*cis*-diltiazem with minimum Ca^{2+} channel-blocking action, has an anti-ischemic action similar to that of D-*cis*-diltiazem (Nasa et al., 1990). These findings suggest that the cardioprotective effect of Ca^{2+} channel blockers against ischemia-reperfusion damage is not only due to their Ca^{2+} channel blocking action but is also due to other actions.

Lysophosphatidylcholine is an amphipathic metabolite of membrane phospholipids that accumulates in the myocardium during ischemia (Kinnaird et al., 1988) and exerts deleterious effects on biological membranes, causing electrophysiological alterations and contractile dysfunction (Corr et al., 1982; Hoque et al., 1995). In addition, exogenous lysophosphatidylcholine produces Ca^{2+} overload resulting from the physico–chemical alteration of membranes (Liu et al., 1991; Woodley et al., 1991). Therefore, lysophosphatidylcholine is regarded as one of the substances responsible for ischemia-reperfusion damage.

If lysophosphatidylcholine is one of the major substances responsible for ischemia-reperfusion damage, Ca^{2+} channel blockers may attenuate the Ca^{2+} overload induced by lysophosphatidylcholine. To test this hypothesis, we examined the effects of Ca^{2+} channel blockers on the lysophosphatidylcholine-induced Ca^{2+} overload in rat isolated cardiomyocytes. We used five Ca^{2+} channel blockers belonging to different classes: verapamil, bepridil, nicardipine, diltiazem and flunarizine from phenylalkylamines, diarylaminopropyl amines, dihydropyridines, benzodiazepines, and diphenylpiperazines, respectively. In additional experiments, veratridine was used instead of lysophosphatidylcholine in order to examine whether Na^+

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channel inhibition is involved in attenuation of the lysophosphatidylcholine-induced Ca^{2+} overload.

2. Materials and methods

2.1. Isolation of myocytes

Calcium-tolerant cardiomyocytes were isolated from male Sprague–Dawley rats (about 250 g body weight) according to the method described in a previous study (Hashizume et al., 1994). The isolated cardiomyocytes were suspended in Krebs–Ringer bicarbonate (KRB) buffer containing NaCl 119 mM, $NaHCO_3$ 15 mM, KCl 2.6 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, glucose 11 mM and $CaCl_2$ 1.0 mM. All experiments were performed at 37°C.

2.2. Loading the cells with fura-2 or Na^+-binding benzofuran isophthalate

To measure the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), myocytes were loaded with fura-2 by incubating the cells with 5 μM fura-2/acetoxymethyl ester (AM) in KRB constantly bubbled with $5\%CO_2/95\%O_2$ for more than 1 h at room temperature. Thereafter, 1 ml of cell suspension was taken and washed twice with fresh KRB buffer before use. The fluorescence intensities were measured using a fluorescence spectrometer (model CAF-110, Japan Spectroscopic, Tokyo, Japan). The excitation wavelengths for fura-2 were 340 and 380 nm, and the emission wavelength was 510 nm. Calibration of the fluorescence intensity of fura-2 was done at the end of each experiment by the addition of digitonin (100 μM) and ethyleneglycol-bis-(β-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (20 mM) to the cell suspension to obtain the maximum and minimum fluorescence levels, respectively. The $[Ca^{2+}]_i$ was estimated according to the method described by Grynkiewicz et al. (1985).

Because lysophosphatidylcholine inhibits the inactivation process of the Na^+ channel (Burnashev et al., 1991) to produce Ca^{2+} overload and because lysophosphatidylcholine interferes with fluorescence of Na^+-binding benzofuran isophthalate (SBFI), a Na^+-sensitive probe, an additional experiment with veratridine, which increases intracellular Na^+ concentration ($[Na^+]_i$) and hence $[Ca^{2+}]_i$ without interfering with SBFI fluorescence, was carried out to examine whether or not Ca^{2+} channel blockers attenuate the lysophosphatidylcholine-induced Ca^{2+} overload through their inhibitory action on the Na^+ channel. The cells were loaded with 5 μM SBFI/AM, and the loading process and the measurement of fluorescence intensities of SBFI were carried out under the same conditions as those for fura-2. Since calibration of $[Na^+]_i$ has many problems, there is no accepted method by which the fluorescence SBFI signal can be converted exactly to the $[Na^+]_i$ (Levi et al., 1994). In the present study, therefore the change in $[Na^+]_i$ was simply expressed by the change in fluorescence ratio of SBFI at 340/380 nm, which was converted into a percentage of the maximum change in fluorescence ratio of SBFI obtained after the addition of gramicidin (10 μM), a Na^+ ionophore, at the end of each experiment.

2.3. Observation of morphological change

The myocyte samples were fixed with 2.5% glutaraldehyde. About 150 myocytes were counted under a light microscope, and the number of rod-shaped cells as a percentage of the total of cells was calculated and used as an indicator of morphological change.

2.4. Measurement of creatine kinase

Creatine kinase activity was measured by means of a kit purchased from Sigma (St. Louis, MO, USA). The cell samples were centrifuged, and creatine kinase was extracted from pellets (cells) with 1% Triton X-100. The creatine kinase activity in both the supernatant and the cellular extract was measured. The activity of creatine kinase released into supernatant was expressed as a percentage of the total creatine kinase activity (supernatant plus pellets).

2.5. Materials

Lysophosphatidylcholine (L-α-lysophosphatidylchline, palmitoyl), veratridine and gramicidin were purchased from Sigma, fura-2/AM and SBFI/AM from Dojindo Laboratory (Kumamoto, Japan). Verapamil was obtained from Nacalai Tesque (Kyoto, Japan), bepridil and nicardipine from Sigma, diltiazem from Tanabe Seiyaku (Osaka, Japan) and flunarizine from Kyowa Hakko Kogyo (Tokyo, Japan). Solutions of lysophosphatidylcholine and all the Ca^{2+} channel blockers were prepared immediately before use with distilled water. Veratridine was dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO was about 0.07%, which did not induce any change in SBFI ratio or cell shape.

2.6. Statistical analysis

Results are given as means ± S.E.M. In all experiments, *n* indicates the number of experiments done with cell samples obtained from different rats. Significance of difference was determined with an analysis of variance followed by Dunnett's multiple comparisons test with a significance level of $P < 0.05$.

3. Results

3.1. The effects of Ca^{2+} channel blockers on lysophosphatidylcholine-induced increase in $[Ca^{2+}]_i$

To examine the effects of Ca^{2+} channel blockers on the lysophosphatidylcholine-induced increase in $[Ca^{2+}]_i$, myo-

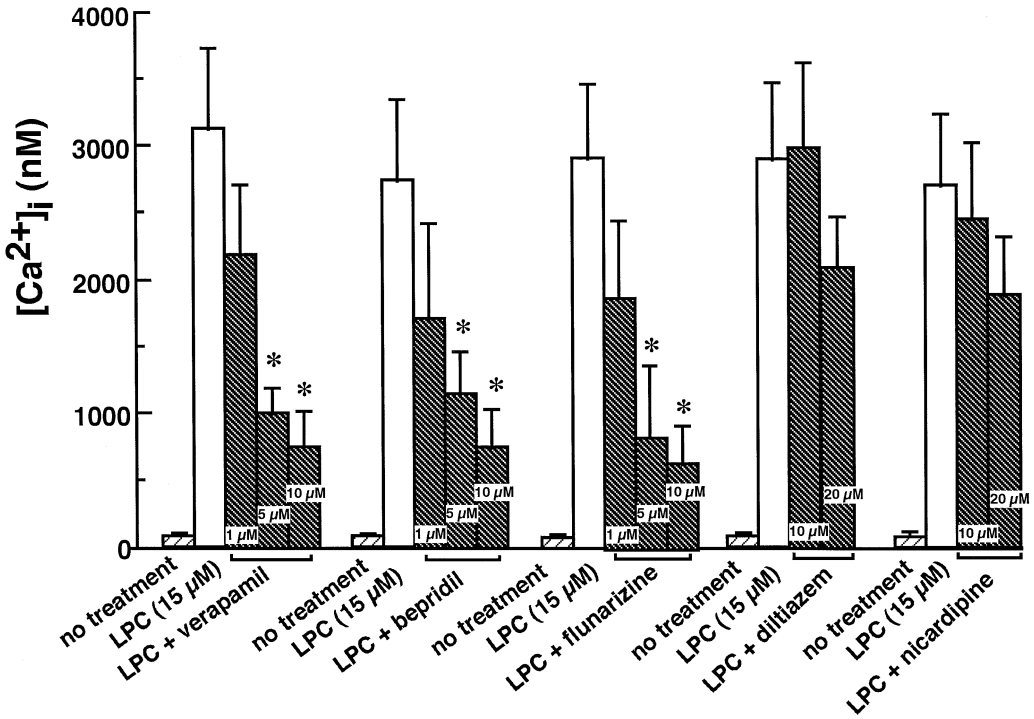

Fig. 1. Effects of Ca^{2+} channel blockers on the increase in $[Ca^{2+}]_i$ induced by lysophosphatidylcholine (LPC) (15 μM) in rat isolated cardiomyocytes. Cells were pretreated with each of the Ca^{2+} channel blockers for 5 min, and then exposed to lysophosphatidylcholine for 5 min. Values are means ± S.E.M., $n = 5–7$. $^{*}P < 0.05$ vs. the value of LPC (15 μM).

cytes were preincubated with one of the drugs for 5 min, and then lysophosphatidylcholine was added. Addition of lysophosphatidylcholine (15 μM) to the cell suspension produced a rapid and marked increase in $[Ca^{2+}]_i$. The full response was obtained about 2 min after the addition of lysophosphatidylcholine. The value of $[Ca^{2+}]_i$ 5 min after

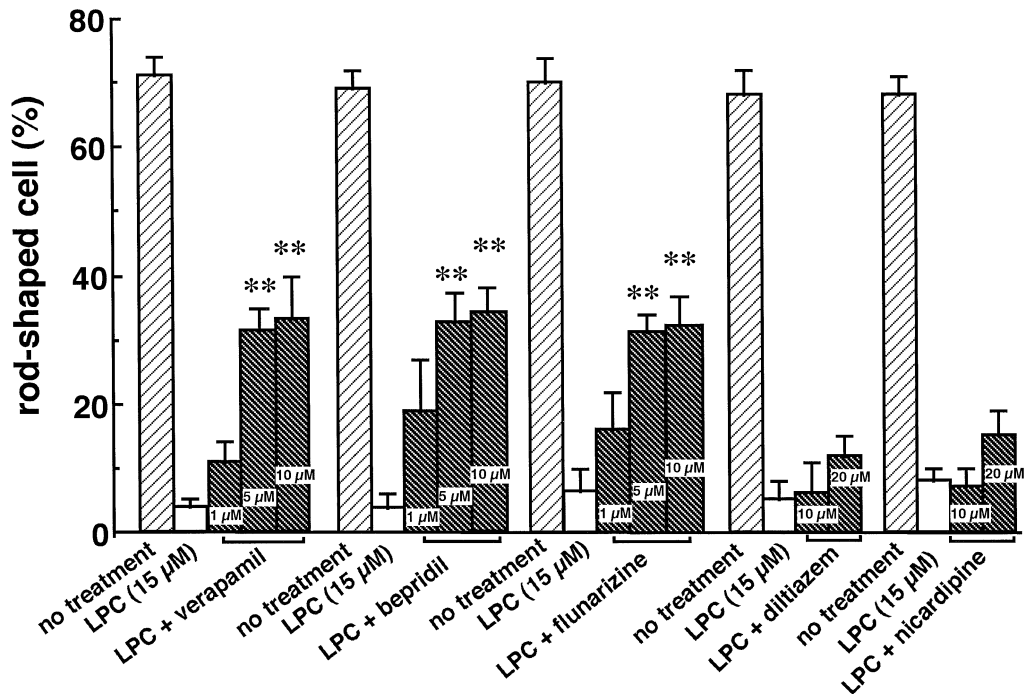

Fig. 2. Effects of Ca^{2+} channel blockers on the decrease in the percentage of rod-shaped cells induced by lysophosphatidylcholine (LPC) (15 μM) in rat isolated cardiomyocytes. Cells were pretreated with each of the Ca^{2+} channel blockers for 5 min, and then exposed to lysophosphatidylcholine for 5 min. Values are means ± S.E.M., $n = 5–7$. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the value of LPC (15 μM).

the addition of lysophosphatidylcholine was used to evaluate the effects of drugs. Fig. 1 shows that 5 min after the addition of lysophosphatidylcholine the $[Ca^{2+}]_i$ increased from about 70 nM to about 3000 nM. Pretreatment with verapamil, bepridil or flunarizine attenuated the lysophosphatidylcholine-induced increase in $[Ca^{2+}]_i$ in a concentration-dependent manner, with P values less than 0.05 at the concentration of 5 μM. However, diltiazem and nicardipine did not attenuate the lysophosphatidylcholine-induced $[Ca^{2+}]_i$ rise significantly even when a high concentration (20 μM) was used.

3.2. The effects of Ca^{2+} channel blockers on lysophosphatidylcholine-induced change in cell shape

The sample for observation of the change in cell shape was part of the sample used for measurement of $[Ca^{2+}]_i$. Addition of lysophosphatidylcholine (15 μM) to the cell suspension produced hypercontracture of cells, leading to a decrease in the percentage of rod-shaped cells. Fig. 2 shows that 5 min after the addition of lysophosphatidylcholine the percentage of rod-shaped cells decreased from about 70% (before addition) to 5% (after addition). In harmony with the results with $[Ca^{2+}]_i$, only verapamil, bepridil and flunarizine attenuated the change in cell-shape induced by lysophosphatidylcholine in a concentration-dependent manner, whereas diltiazem and nicardipine did not.

3.3. The effects of Ca^{2+} channel blockers on lysophosphatidylcholine-induced release of creatine kinase

Fig. 3 shows the effects of Ca^{2+} channel blockers on the release of creatine kinase induced by lysophosphatidylcholine. Without lysophosphatidylcholine and drugs, the amount of released creatine kinase as a percentage of the total creatine kinase was $9.7 \pm 1.4\%$ in the cells after 5 min of incubation. Significant release of creatine kinase ($57.6 \pm 4.2\%$) occurred 5 min after the addition of lysophosphatidylcholine (15 μM). Verapamil, bepridil or flunarizine (5 μM for each) significantly attenuated the creatine kinase release induced by lysophosphatidylcholine ($P < 0.05$), whereas neither diltiazem nor nicardipine (20 μM) did.

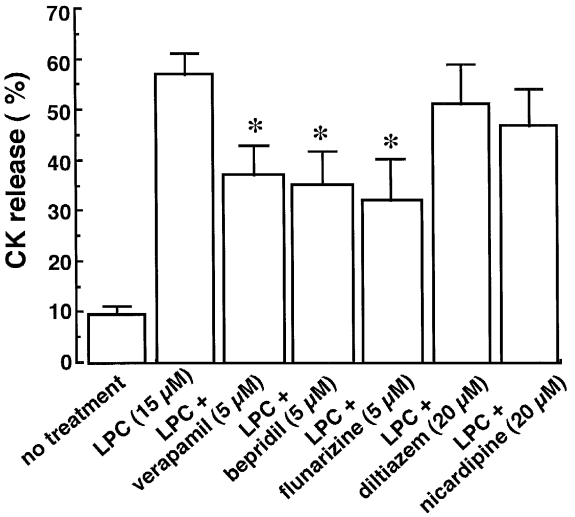

Fig. 3. Effects of Ca^{2+} channel blockers on the creatine kinase release induced by lysophosphatidylcholine (LPC) in rat isolated cardiomyocytes. Cells were pretreated with each of the Ca^{2+} channel blockers for 5 min, and then exposed to lysophosphatidylcholine for 5 min. Values are means ± S.E.M., $n = 5–7$. * $P < 0.05$ vs. the value of LPC (15 μM).

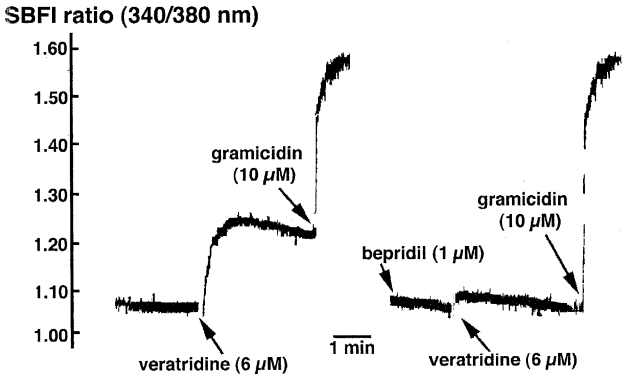

Fig. 4. Representative recordings of the fluorescence ratio of SBFI at 340 and 348 nm (reflecting increase in $[Na^+]_i$) in rat isolated cardiomyocytes. Veratridine (6 μM) increased the SBFI ratio and gramicidin (10 μM) increased it further (left). Bepridil (1 μM) inhibited the veratridine-induced increase in fluorescence ratio of SBFI (right). Maximum value of fluorescence ratio of SBFI was obtained after the addition of gramicidin (10 μM) at the end of experiments.

3.4. The effects of Ca^{2+} channel blockers on veratridine-induced change in SBFI ratio and cell contracture

Fig. 4 displays representative recordings of changes in fluorescence ratio of SBFI (340/380 nm) induced by

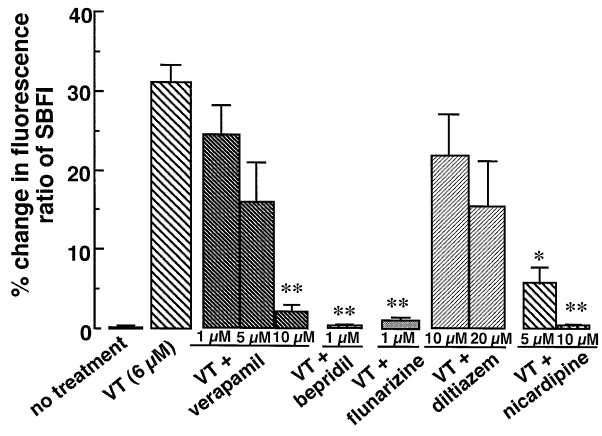

Fig. 5. Effects of Ca^{2+} channel blockers on the increase in fluorescence ratio of SBFI induced by veratridine (VT) (6 μM) in rat isolated cardiomyocytes. The change in fluorescence ratio of SBFI was converted into percentage of the maximum increase in fluorescence ratio of SBFI measured after by the addition of gramicidin (10 μM). Cells were pretreated with each of the Ca^{2+} channel blockers, and then exposed to VT for 3 min. Values are means ± S.E.M., $n = 5$. * $P < 0.05$; * * $P < 0.01$ vs. the value of VT (6 μM).

veratridine (6 μM). The fluorescence ratio of SBFI increased immediately after the addition of veratridine, and the maximum increase was obtained by the addition of gramicidin (10 μM). The veratridine-induced increase in fluorescence ratio of SBFI was about 35% of that induced by gramicidin, and was markedly inhibited by 1 μM bepridil. The effects of Ca^{2+} channel blockers on the veratridine-induced increase in fluorescence ratio of SBFI are summarized in Fig. 5. Veratridine also induced cell contracture, resulting in a decrease in the percentage of rod-shaped cells from about 70% to 0%. The effects of Ca^{2+} channel blockers on the veratridine-induced decrease in the percentage of rod-shaped cells are shown in Fig. 6. Verapamil, bepridil and flunarizine, which inhibited the lysophosphatidylcholine-induced Ca^{2+} overload, attenuated the veratridine-induced increase in fluorescence ratio of SBFI as well as the veratridine-induced cell contracture. The concentrations of verapamil, bepridil and flunarizine needed to exert significant effects on the veratridine-induced changes were 10, 1 and 1 μM, respectively. Diltiazem (10 and 20 μM), which did not inhibit the lysophosphatidylcholine-induced Ca^{2+} overload, had no effect on the veratridine-induced changes in fluorescence ratio of SBFI and cell shape either. Nicardipine (10 μM), which did not attenuate the lysophosphatidylcholine-induced Ca^{2+} overload, however, significantly attenuated the veratridine-induced increase in fluorescence ratio of SBFI and cell contracture.

Table 1
The IC_{50} values for inhibition of Ca^{2+} current in cardiac myocytes and the partition coefficients of Ca^{2+} channel blockers

Drug	Inhibition of Ca^{2+} current IC_{50} (μM)		Partition coefficient (log P)
Verapamil	4.0	(rat) [a]	1.83 [g]
	0.6	(guinea pig) [b]	
Bepridil	0.5	(guinea pig) [c]	2.01 [g]
Flunarizine	5.0	(guinea pig) [d]	5.78 [h]
Nicardipine	0.16	(rabbit) [e]	
Diltiazem	40.0	(rat) [a]	−1.70 [g]
	3.0	(guinea pig) [f]	

[a] Diochot et al. (1995).
[b] Matsuoka et al. (1991).
[c] Yatani et al. (1986).
[d] Tytgat et al. (1990).
[e] Gotoh et al. (1991).
[f] Okuyama et al. (1994).
[g] Pang and Sperelakis (1984) (octanol/Ringer; at room temperature).
[h] Pauwels et al. (1986) (octanol/water).

4. Discussion

The results confirmed the fact that lysophosphatidylcholine produces both Ca^{2+} overload and hypercontracture in rat isolated cardiomyocytes (Chen et al., 1996; Hashizume and Abiko, 1996). Interestingly, however, the effects of Ca^{2+} channel blockers on the lysophosphatidylcholine-induced changes were not the same: verapamil, bepridil and flunarizine attenuated the lysophosphatidylcholine-induced changes in both $[Ca^{2+}]_i$ and cell shape, whereas diltiazem and nicardipine did not. We cannot interpret the results of the present study in terms of Ca^{2+} channel-blocking action, because nicardipine and diltiazem, which have a potent Ca^{2+} channel-blocking action, did not attenuate the lysophosphatidylcholine-induced changes (Table 1). This view is consistent with the findings that the Ca^{2+} overload induced by lysophosphatidylcholine is not due to an increase in Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Liu et al., 1991; Woodley et al., 1991).

It has been reported that lysophosphatidylcholine depresses the Na^+ current and that the reduction of the Na^+ current is followed by cell contracture or cell death (Sato et al., 1992). Although the peak of the Na^+ current is decreased, the inactivation process of the Na^+ channel is markedly prolonged (Burnashev et al., 1991). It is possible that there is a massive Na^+ influx through the open channel, and hence Ca^{2+} overload through Na^+/Ca^{2+} exchange. We found that omission of Na^+ from the extracellular solution markedly attenuated the lysophosphatidylcholine-induced $[Ca^{2+}]_i$ rise (unpublished data). Interestingly, Ca^{2+} channel blockers, such as verapamil and bepridil, inhibit the Na^+ channel in addition to the Ca^{2+} channel (Bayer et al., 1975; Yatani et al., 1986), and therefore it is possible that they attenuate the lysophosphatidylcholine-induced Ca^{2+} overload by inhibition of the Na^+ channel. We therefore tried to measure the fluores-

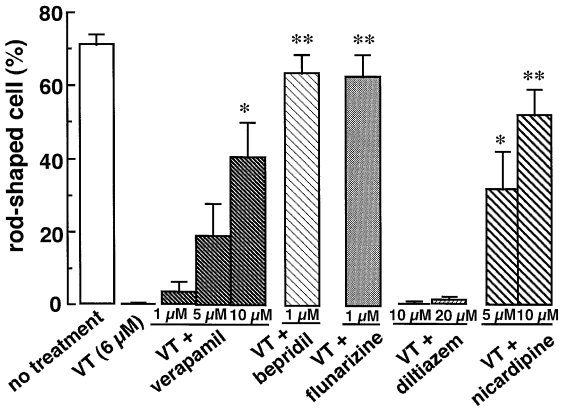

Fig. 6. Effects of Ca^{2+} channel blockers on the decrease in the percentage of rod-shaped cells induced by veratridine (VT) (6 μM) in rat isolated cardiomyocytes. Cells were pretreated with each of the different Ca^{2+} channel blockers, then exposed to VT for 3 min. Values are means ± S.E.M., $n = 5$. * $P < 0.05$; ** $P < 0.01$ vs. the value of VT (6 μM).

cence of SBFI in the presence of lysophosphatidylcholine, but it was not possible because lysophosphatidylcholine interferes with the fluorescence of SBFI. Accordingly, we used veratridine, which elicits effects on the Na^+ channel very similar to those of lysophosphatidylcholine. As reported by Kusaka and Sperelakis (1994), veratridine decreased the peak of the Na^+ current but greatly increased the steady-state inactivating Na^+ current, increasing Na^+ influx. A number of studies showed that veratridine induces Ca^{2+} overload that is secondary to excessive Na^+ influx (Donck et al., 1986; Hashizume et al., 1994). The results demonstrated that verapamil, bepridil and flunarizine, which significantly attenuated the lysophosphatidylcholine-induced Ca^{2+} overload, attenuated both the increase in $[Na^+]_i$ (as evidenced by the increase in fluorescence ratio of SBFI) and the cell contracture induced by veratridine, and that diltiazem, which failed to attenuate the lysophosphatidylcholine-induced changes, did not attenuate the veratridine-induced changes. Our results for veratridine are consistent with those of Donck et al. (1986), and it seems that Na^+ channel inhibition plays a role in the attenuation of the lysophosphatidylcholine-induced Ca^{2+} overload. There is an exception, however, to this view. Nicardipine had a very weak effect on the lysophosphatidylcholine-induced Ca^{2+} overload even at the high concentration of 20 μM, but it attenuated the veratridine-induced changes. In addition, 1 μM was sufficient for bepridil and flunarizine to abolish the veratridine-induced increase in $[Na^+]_i$ and cell contracture, but at this concentration these two drugs exerted only very weak effects on the lysophosphatidylcholine-induced Ca^{2+} overload (Figs. 1 and 5). In contrast, verapamil, which markedly attenuated the lysophosphatidylcholine-induced Ca^{2+} overload at 5 μM, exhibited only a weak effect on the veratridine-induced changes at 10 μM. Moreover, in one of our previous studies (Hashizume and Abiko, 1996) tetrodotoxin, which prevents veratridine-induced depolarization (Sperelakis and Pappano, 1969), did not inhibit lysophosphatidylcholine-induced Ca^{2+} overload. Taken together, these results indicate that inhibition of the Na^+ channel alone cannot explain the protective effects of verapamil, bepridil and flunarizine against the lysophosphatidylcholine-induced Ca^{2+} overload.

We reported in our previous study that both D- and L-propranolol attenuate lysophosphatidylcholine-induced Ca^{2+} overload, and that a high lipophilicity was necessary for these two drugs to exert protective effects against lysophosphatidylcholine-induced Ca^{2+} overload (Chen et al., 1996). Verapamil, bepridil and flunarizine possess a higher lipophilicity than diltiazem (Table 1) (Pang and Sperelakis, 1984). The lipophilicity of nicardipine was not measured in their study, but it is known that nicardipine is more soluble in water than is nifedipine (Diez et al., 1991). The partition coefficient (log P) of nifedipine is 0.43, and therefore the partition coefficient of nicardipine should be lower than 0.43, which is much lower than that of verapamil, bepridil and flunarizine. Thus, verapamil, bepridil and flunarizine, which possess both Na^+ inhibitory effects and high lipophilicity, attenuated the lysophosphatidylcholine-induced Ca^{2+} overload, whereas nicardipine, which has Na^+ inhibitory effects without high lipophilicity, and diltiazem, which has neither Na^+ inhibitory effects nor high lipophilicity, did not. These findings lead to a view that Na^+ inhibitory effects and a high lipophilicity are important to verapamil, bepridil and flunarizine for attenuation of lysophosphatidylcholine-induced Ca^{2+} overload.

In the present study, it was found that lysophosphatidylcholine increased the release of creatine kinase, which reflects membrane damage. Verapamil, bepridil and flunarizine, which attenuated the Ca^{2+} overload induced by lysophosphatidylcholine, reduced the creatine kinase release. Diltiazem and nicardipine, which did not attenuate the lysophosphatidylcholine-induced changes, did not attenuate creatine kinase release either. It was also reported (Pauwels et al., 1990) that veratridine induced release of lactate dehydrogenase in cultured neurons, and the release of lactate dehydrogenase was inhibited not only by flunarizine, bepridil and verapamil but also by nicardipine and diltiazem. These findings suggest that the nature of the membrane damage induced by lysophosphatidylcholine is different from that induced by veratridine. Since the release of lactate dehydrogenase induced by veratridine could be totally prevented by removing extracellular Ca^{2+} (Pauwels et al., 1990), the membrane damage induced by veratridine is dependent on Ca^{2+}. In contrast, we reported recently (Chen et al., 1997) that lysophosphatidylcholine induced release of creatine kinase even in the absence of extracellular Ca^{2+}, suggesting that lysophosphatidylcholine-induced membrane damage does not depend on Ca^{2+}. It is known that lysophosphatidylcholine, which is amphiphilic, is incorporated into cell membrane and elicits profound effects on the biophysical properties of the membrane (Lundbæk and Andersen, 1994), such as an increase in membrane fluidity (Fink and Gross, 1984) and in the formation of ion pores (Katz and Messineo, 1981). It is possible, therefore, that lysophosphatidylcholine binds to the cytoplasmic membrane and associates with it (Man et al., 1990), and that the Ca^{2+} channel blockers with high lipophilicity can readily diffuse into the lipid bilayers of the cell membrane to protect the cell from lysophosphatidylcholine-induced damage.

The antiperoxidant action may also be related to the nonspecific action of Ca^{2+} channel blockers. This suggestion is supported by the findings that structurally unrelated Ca^{2+} blockers, including verapamil, bepridil and flunarizine, depress lipid peroxidation (Janero et al., 1989; Mak and Weglicki, 1990), and that the mechanisms responsible for the antiperoxidant activity of Ca^{2+} channel blockers are not related to their specific Ca^{2+} channel blocking action but are associated with interactions with the phospholipid and protein constituents of the cell membrane (Kauder and Watts, 1996; Mason and Trumbore, 1996).

Nevertheless, the relationship between lipophilicity and antiperoxidant action was not studied in the present study.

In summary, verapamil, bepridil and flunarizine protected cardiomyocytes from lysophosphatidylcholine-induced Ca^{2+} overload. Their protective effects may be unrelated to their Ca^{2+} channel-blocking action. The Na^{+} inhibitory effect plus high lipophilicity are probably important for the protective effects of these drugs against lysophosphatidylcholine-induced Ca^{2+} overload.

Acknowledgements

The authors wish to thank Mr. T. Yokoyama for his technical assistance, Mrs. M. Tajima for her secretarial work and all the other members of the department of pharmacology for their help in carrying out the present study.

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