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BRL 38227 (levcromakalim)-induced hyperpolarization reduces the sensitivity to Ca^{2+} of contractile elements in canine coronary artery

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Summary. Potassium (K^+) channel openers decrease intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$ by hyperpolarizing the membrane and deactivating the Ca^{2+}-channels. To examine whether the hyperpolarization produced by K^+-channel openers has other effects on the mechanical activity of vascular smooth muscle, we investigated the effects of levcromakalim (BRL 38227) on membrane potential, $[Ca^{2+}]_i$, as measured with fura-2, and force of contraction induced by 30 mmol/l KCl-physiological salt solution (PSS), in canine coronary arteries. BRL 38227 hyperpolarized the membrane and reduced increases in $[Ca^{2+}]_i$ and in contractile force induced by 30 mmol/l KCl-PSS. The $[Ca^{2+}]_i$-contractile force curve, determined in the presence of BRL 38227, was located to the right of the control curve determined by decreasing extracellular Ca^{2+} concentrations ($[Ca^{2+}]_o$) in 30 mmol/l KCl-PSS. The $[Ca^{2+}]_i$-contractile force curve, determined by decreasing extracellular K^+ concentrations ($[K^+]_o$), was also located to the right of that determined by decreasing $[Ca^{2+}]_o$ in 30 mmol/l KCl-PSS. The effect of BRL 38227, a reduction in the Ca^{2+}-sensitivity of contractile elements, was antagonized by the ATP-sensitive K^+-channel blocker, glibenclamide (10^{-6} or 10^{-5} mol/l). These results suggest that the membrane hyperpolarization induced by BRL 38227, or the repolarization caused by reducing $[K^+]_o$, decreases the Ca^{2+}-sensitivity of contractile elements of vascular smooth muscle.

Key words: K^+ channel opener – Levcromakalim (BRL 38227) – Hyperpolarization – Repolarization – Ca^{2+}-sensitivity of contractile elements

Introduction

K^+-channel openers are a new class of vasodilating agents. They increase the permeability of the cell membrane to K^+ and induce membrane hyperpolarization in vascular smooth muscle (Hamilton et al. 1986; Quast and Cook 1989). Membrane hyperpolarization produces the deactivation of the voltage-dependent Ca^{2+}-channels. Thus, the K^+-channel openers indirectly act as the Ca^{2+}-channel blockers.

Recent reports have shown that membrane hyperpolarization induced by BRL 38227 (levcromakalim), the active enantiomer of cromakalim (Buckingham et al. 1986; Hof et al. 1988), or cromakalim may inhibit an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and contractile force related to phosphoinositide breakdown induced by noradrenaline (Ito et al. 1991; Quast and Baumlin 1991) or by the thromboxane A_2 analogue U 46619 (Yamagishi et al. 1992a, b). Thus, the vasodilation related to the membrane hyperpolarization induced by K^+-channel openers may be generated not only via the classical mechanism but also through additional mechanisms. We have demonstrated that the K^+-channel opening action of E 4080, (E)-*N*-[3-((*N'*(2-(3,5-dimethoxyphenyl)ethyl)-*N'*-methyl)amino)propyl]-4-(4-(1*H*-imidazol-1-yl)phenyl)-3-butenamide, which is a new bradycardic agent with a coronary vasodilating property (Kawamura et al. 1991), reduces $[Ca^{2+}]_i$ and force of contraction in 30 mmol/l KCl-physiological salt solution (PSS), and we have also suggested that E 4080 reduces the Ca^{2+}-sensitivity of contractile elements (Okada et al. 1992). Thus, our hypothesis is that the membrane hyperpolarization induced by K^+-channel openers, including E 4080, may generate vasodilation by not only deactivation of Ca^{2+}-channels but also by affecting the contractile elements. E 4080, however, has a dual action, K^+-channel opening and Ca^{2+}-channel blocking, that results in vasodilation (Kamouchi et al. 1991; Okada et al. 1992). To test our hypothesis it is, therefore, necessary to use a specific K^+-channel opener as a tool. In the present study, we examined whether the specific K^+-channel opener, BRL 38227 (Hof et al. 1988; Edwards et al. 1991; Noack et al. 1992), affects the Ca^{2+}-sensitivity of contractile elements in 30 mmol/l KCl-induced contraction, by measuring membrane potential, $[Ca^{2+}]_i$ and force of contraction. We also compared the effects of BRL 38227

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with those of membrane repolarization induced by a decrease in $[K^+]_o$ from 30 mmol/l to 5 mmol/l.

Methods

Tissue preparation. Hearts were obtained from mongrel dogs (8–15 kg), of either sex, that had been anesthetized with sodim pentobarbital (30 mg/kg, i.v.). Coronary arterial rings (0.8–1.2 mm in diameter, about 1 mm in width) were used. The endothelium was removed by gentle rubbing with forceps. The luminal side of arterial ring was turned to face outwards. We have confirmed that in coronary arterial rings, precontracted with 30 mmol/l KCl-PSS, acetylcholine (10^{-6} mol/l) or bradykinin (10^{-7} mol/l) did not produce any relaxation.

Membrane potential measurement. The endothelium-denuded arterial rings were slit open to form sheets. These were placed in a 1.5 ml organ bath perfused with 5 mmol/l KCl-PSS maintained at 37 °C at a rate of 4 ml/min. Intracellular membrane potentials were measured with glass microelectrodes, filled with 3 mol/l KCl, connected to an amplifier (MEZ-7200, Nihon Kohden, Tokyo, Japan). The resistance of the microelectrodes was 40–70 MΩ. The membrane potentials were displayed on an oscilloscope (COS 5020-ST, Kikusui Electronics, Tokyo, Japan) and also on a pen-writing recorder (FBR-252A, Toa Electronics, Tokyo, Japan).

To examine the effects of BRL 38227 and the decrease in $[K^+]_o$ on the membrane potentials of canine coronary arterial smooth muscles, the preparation was perfused with 30 mmol/l KCl-PSS for 30 min and then impaled with a microelectrode. After stable membrane potentials had been obtained, BRL 38227 (10^{-7}–10^{-5} mol/l) was introduced in a cumulative manner or $[K^+]_o$ was decreased stepwise from 30 to 5 mmol/l. Each concentration of BRL 38227 or $[K^+]_o$ was applied for 5 min.

$[Ca^{2+}]_i$ and contractile force measurements. $[Ca^{2+}]_i$ was measured by a fluorescence method, as described previously (Yanagisawa et al. 1989, Mori et al. 1990). In brief, coronary arterial rings were exposed to 10 μmol/l fura-2 acetoxymethyl ester (fura-2 AM) for about 6 h at room temperature. The noncytotoxic detergent, pluronic F-127 (0.1% W/V), was premixed into PSS to help dissolve the fura-2 AM in PSS. Fluorescence was measured in a fluorimeter equipped with a dual wavelength excitation device (CAM-200 or CAM-220, Japan Spectroscopic, Tokyo, Japan) connected to an inverted microscope (TMD-8, Nikon, Tokyo, Japan). The fluorescence image was obtained by focusing on the smooth muscle cells in the medial layer with a Nikon CF UV (Fluor) 10× objective lens. The muscle ring was placed horizontally in a temperature-controlled, 0.4 ml tissue bath which was mounted on the inverted microscope and perfused with PSS at a rate of 4 ml/min. The muscle ring was stretched to a resting tension of about 5 mN between two tungsten needles, one of which was glued to a transducer (AE 801, AME, Horten, Norway or U-10230, Shinko, Tokyo, Japan). The photosignals and the mechanical activity were measured simultaneously and both were recorded on a chart recorder (Recti-horiz-8K, NEC-San-ei, Tokyo, Japan). They were also digitized by A/D converters and fed into a microcomputer (PC-9801, NEC, Tokyo, Japan) for further calculation and graphical analysis (Himpens and Somlyo 1988). At the end of each experiment, the cell membrane was lysed with a detergent, Triton X-100 (1%), and autofluorescence signals were determined by quenching fura-2 signals with $MnCl_2$. We subtracted the F_{340} and F_{380} values due to autofluorescence from the corresponding values of F_{340} and F_{380}, obtained under conditions of fura-2 loading, to derive a corrected ratio. Since it is difficult to calculate the absolute concentration of $[Ca^{2+}]_i$ (Konishi et al. 1988; Karaki 1989), we used the F_{340}/F_{380} ratio as an indicator of relative concentrations of $[Ca^{2+}]_i$. None of the drugs and chemicals used in the present study, with the exception of $MnCl_2$, affected fluorescence signals at the concentrations used.

To examine the effects of BRL 38227 on $[Ca^{2+}]_i$ and contractile force in the artery, 30 mmol/l KCl-PSS was perfused for 30–40 min and then BRL 38227 (10^{-8}–10^{-5} mol/l) was introduced in a cumulative manner. Each concentration of BRL 38227 was applied for 10 min. In the experiments with glibenclamide (10^{-6} or 10^{-5} mol/l), glibenclamide was introduced 10 min before the start of the application of BRL 38227.

The effects of the decrease in $[Ca^{2+}]_o$ or $[K^+]_o$ on $[Ca^{2+}]_i$ and contractile force were examined with artery that had been contracted by 30 mmol/l KCl-PSS for 30 to 40 min. $[Ca^{2+}]_o$ (2.5 to 0.1 mmol/l), or $[K^+]_o$ (30 to 5 mmol/l), was decreased stepwise and each concentration of $[Ca^{2+}]_o$ or $[K^+]_o$ was applied for 8 or 10 min, respectively.

Drugs and solutions. The composition (mmol/l) of normal PSS (5 mmol/l KCl-PSS) was as follows: NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2.5, glucose 11.1, HEPES 3 (pH 7.4). The solution was equilibrated with 100% O_2 at 37 °C. High KCl-PSS was made by substituting an equimolar amount of KCl for NaCl. The decrease in $[Ca^{2+}]_o$ was achieved by decreasing the $CaCl_2$ concentration in PSS. Drugs and chemicals were obtained from the following sources: BRL 38227 ((−)-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2-oxo-1-pyrrolidyl)-2*H*-1-benzopyran-3-ol, SmithKline Beecham, Worthing, U.K.), glibenclamide (Yamanouchi, Tokyo, Japan), HEPES (*N*-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid), fura-2 AM and pluronic F-127 (Dojin, Kumamoto, Japan), acetylcholine chloride, DMSO (dimethyl sulfoxide) and Triton X-100 (Wako, Tokyo, Japan), bradykinin (Peptide Institutes, Osaka, Japan). BRL 38227 was dissolved in 70% ethanol to give a concentration of 10 mmol/l. Glibenclamide was dissolved in DMSO to give a concentration of 10 mmol/l. These were diluted with PSS immediately before use and the pH of the solutions was adjusted to 7.4. Fura-2 AM was dissolved in DMSO at a concentration of 1 mmol/l.

Analysis of concentration-effect curves and statistics. The concentration-effect curves, for BRL 38227 versus the decrease in the force of contraction and against the increased $[Ca^{2+}]_i$ produced by 30 mmol/l KCl-PSS, were expressed as a % reduction form the pre-drug values, and computer-fitted to a logistic equation:

$$E = 100 - M \times A^p/(A^p + K^p) \quad (1)$$

where E is the normalized effect, M is the maximum effect, A is BRL 38227 concentration, K is the EC_{50} value of BRL 38227 and p is the slope parameter. EC_{50} values are given as pD_2 values ($pD_2 = -\log EC_{50}$). Experimental values are given as means ± SEM. Statistical significance of results was evaluated using an analysis of variance (F-test) followed by Student's test or the Aspin-Welch-*t*-test, or by a paired *t*-test. A *P*-value lower than 0.05 was considered to be significant.

Results

Effect of BRL 38227 on the membrane potential of canine coronary arterial muscles

In 30 mmol/l KCl-PSS, the mean membrane potential of canine coronary arterial smooth muscles was −34.3 ± 0.8 mV ($n = 9$). The application of BRL 38227 (10^{-7}–10^{-5} mol/l) produced membrane hyperpolarization in a concentration-dependent manner (Fig. 1). The mean membrane hyperpolarization produced by BRL 38227 (10^{-5} mol/l) was −5.0 ± 0.5 mV ($n = 5$, $P < 0.001$) (Fig. 1B).

Effect of BRL 38227 on $[Ca^{2+}]_i$ and contractile force

In rings of canine coronary artery loaded with fura-2, perfusion with 30 mmol/l KCl-PSS increased $[Ca^{2+}]_i$ and elicited a contraction. BRL 38227 (10^{-8}–10^{-5} mol/l) reduced both $[Ca^{2+}]_i$ and contraction in a concentration-dependent manner (Fig. 2). The effects of BRL 38227 were reversed by washing. Summarized data from 7 muscle preparations are shown in Fig. 3. The max-

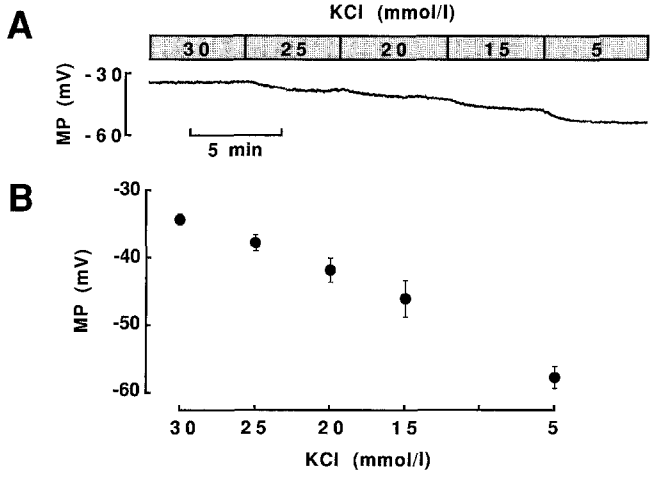

Fig. 1 A, B. Effect of BRL 38227 on membrane potential (MP) of canine coronary arterial muscle in 30 mmol/l KCl-PSS. **A** A typical record of the effect of BRL 38227 on membrane potential of a preparation. **B** The summarized data obtained from five preparations; mean values ± SEM; *PSS*, physiological salt solution. Significance of difference from the control: $*P<0.05$; $***P<0.001$

imum effects of BRL 38227 in reducing $[Ca^{2+}]_i$ and inhibiting contraction were 33.5 ± 1.2% and 57.4 ± 5.0%, respectively. The pD_2 values for BRL 38227 on $[Ca^{2+}]_i$ and contraction were 6.34 ± 0.17 and 6.18 ± 0.09, respectively. The maximum % inhibitory effect on contraction was significantly ($P<0.001$) larger than the % reduction in $[Ca^{2+}]_i$, whereas the pD_2 values were not significantly different. The effects of BRL 38227 on $[Ca^{2+}]_i$ and force of contraction were effectively antagonized by the ATP-sensitive K^+-channel blocker glibenclamide (10^{-6} or 10^{-5} mol/l) (Fig. 3). $[Ca^{2+}]_i$ and force of contraction in 30 mmol/l KCl-PSS were not significantly changed by glibenclamide.

The relation between $[Ca^{2+}]_i$ and force of contraction in the presence of BRL 38227 in 30 mmol/l KCl-PSS

Decreasing $[Ca^{2+}]_o$ from 2.5 to 0.1 mmol/l, stepwise, in 30 mmol/l KCl-PSS reduced the $[Ca^{2+}]_i$ and contractile force in a concentration-dependent manner (Fig. 4). Summarized data obtained from 9 preparations are shown in

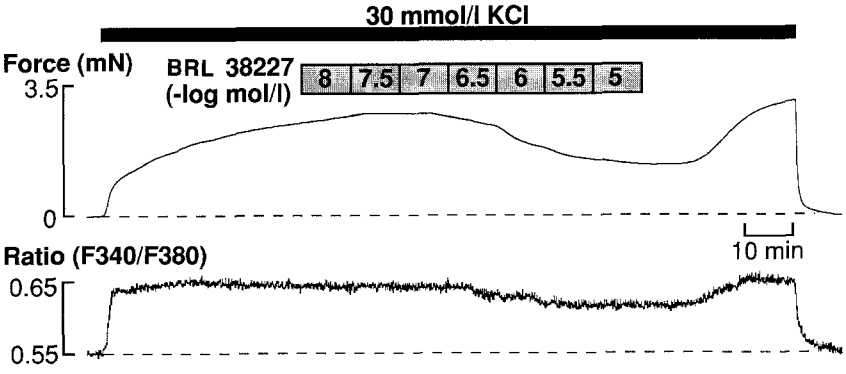

Fig. 2. Typical traces of the effects of BRL 38227 on the force of contraction (mN) and on the increase in $[Ca^{2+}]_i$ produced by 30 mmol/l KCl-PSS in a canine coronary arterial muscle loaded with fura-2. The $[Ca^{2+}]_i$ was expressed as the F_{340}/F_{380} ratio

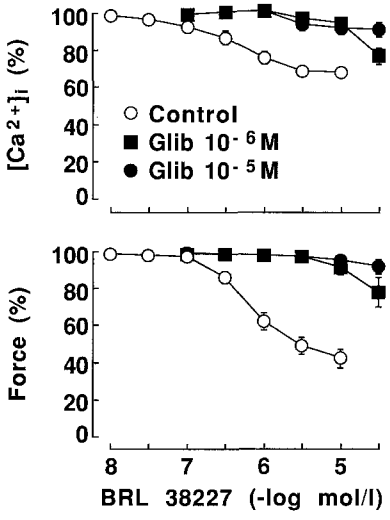

Fig. 3. Effects of BRL 38227 on the increases in $[Ca^{2+}]_i$ and on the force of contraction produced by 30 mmol/l KCl-PSS in the absence (○, $n=7$) and the presence of glibenclamide 10^{-6} (■, $n=4$) or 10^{-5} mol/l (●, $n=6$). Glibenclamide was introduced 20 min after the perfusion with 30 mmol/l KCl-PSS, and BRL 38227 was applied 10 min after the addition of glibenclamide. The $[Ca^{2+}]_i$ or force of contraction is expressed as a percentage of the value of each just before the application of BRL 38227

Fig. 4B. The decrease in the force of contraction on reducing $[Ca^{2+}]_o$ was similar to that in $[Ca^{2+}]_i$. Because BRL 38227 inhibited the force of contraction more effectively than it inhibited the fall in $[Ca^{2+}]_i$, the relations between $[Ca^{2+}]_i$ and contractile force in the presence of BRL 38227 were expressed as % changes, in order to make a comparison with the relation obtained by decreasing $[Ca^{2+}]_o$ in 30 mmol/l KCl-PSS (control) (Fig. 5). The curve for BRL 38227 was located to the right of the control curve and the slope of the curve was steeper than that of control. Thus, in the presence of BRL 38227, a decrease in $[Ca^{2+}]_i$ generated a larger relaxation than the same decrease in the control: BRL 38227, in 30 mmol/l KCl-PSS, reduced the sensitivity of contractile elements to Ca^{2+}.

Modification, by glibenclamide, of the effect of reducing the Ca^{2+} sensitivity caused by BRL 38227

Figure 6 shows the influences of glibenclamide (10^{-6} or 10^{-5} mol/l) on the concentration-effect curves for BRL 38227 against the reduction in the Ca^{2+}-sensitivity i.e. the contractile force corresponding to a given $[Ca^{2+}]_i$ of contractile elements of coronary arterial muscle in 30 mmol/l KCl-PSS. To show the extent of the reduction

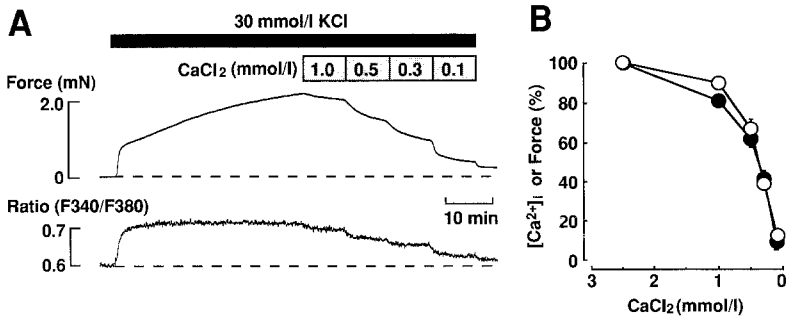

Fig. 4. Effects of decreasing $[Ca^{2+}]_o$ on the increases in $[Ca^{2+}]_i$ and force of contraction produced by 30 mmol/l KCl-PSS in canine coronary arteries. **A** Typical traces of the contractile force and $[Ca^{2+}]_i$ in 30 mmol/l KCl-PSS. The concentrations of $[Ca^{2+}]_o$ were decreased stepwise from 2.5 to 0.1 mmol/l. **B** The summarized data (mean values±SEM) obtained from 9 preparations. The $[Ca^{2+}]_i$ (●) and force of contraction (○) are expressed as a percentage of the value of each when the muscle was exposed to 2.5 mmol/l $CaCl_2$ and 30 mmol/l KCl-PSS

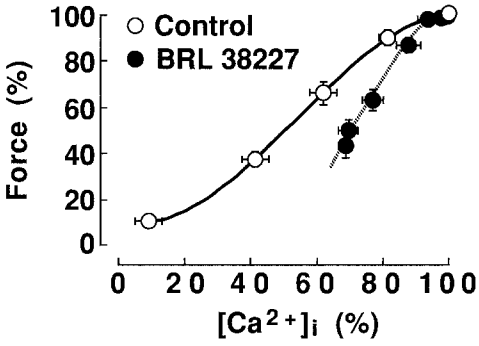

Fig. 5. Relation between $[Ca^{2+}]_i$ and contractile force in the absence (control), and the presence of BRL 38227 in 30 mmol/l KCl. The control curve was obtained by decreasing $[Ca^{2+}]_o$ (2.5, 1.0, 0.5, 0.3 and 0.1 mmol/l) in 30 mmol/l KCl-PSS. (See also Fig. 4) ($n = 9$). The curve in the presence of BRL 38227 (10^{-8}–10^{-5} mol/l) was obtained from the data in Fig. 3 ($n = 7$). The $[Ca^{2+}]_i$ and the contractile force is expressed as a percentage of each pre-drug (2.5 mmol/l $CaCl_2$ and 30 mmol/l KCl-PSS) value

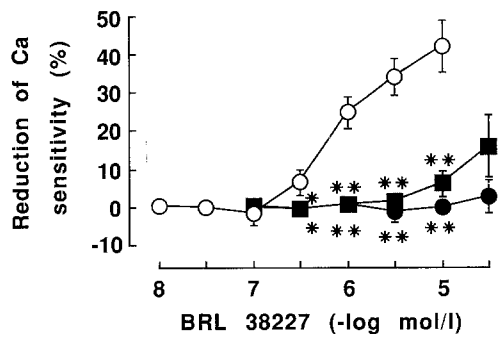

Fig. 6. Effects of glibenclamide (10^{-6} or 10^{-5} mol/l) on the concentration-effect curve for BRL 38227 versus the reduction in Ca^{2+}-sensitivity of contractile elements (in 30 mmol/l KCl-PSS). Reduction of Ca^{2+}-sensitivity is shown as the contractile force in the presence of BRL 38227 expressed as a percentage inhibition of the value at the same $[Ca^{2+}]_i$ obtained from the control curve in Fig. 5. Significance of difference from the control value; $^*P<0.05$; $^{**}P<0.01$; BRL 38227 alone (○), +Glib 10^{-6} M (■), +Glib 10^{-5} M (●)

in the Ca^{2+}-sensitivity, the contractile force, in the presence of BRL 38227, was expressed as a % reduction from that, at the same $[Ca^{2+}]_i$, obtained from the control curve in Fig. 5. The BRL 38227 reduced the Ca^{2+}-sensitivity in a concentration-dependent manner. The concentration-effect curve for BRL 38227 versus the reduction in the Ca^{2+} sensitivity was shifted to the right by 10^{-6} mol/l glibenclamide and the effect was almost abolished by 10^{-5} mol/l glibenclamide. Thus, the ability of BRL 38227 to reduce the Ca^{2+}-sensitivity seems to be due to the opening of K^+-channels that are sensitive to glibenclamide and the membrane hyperpolarization.

Relation between $[Ca^{2+}]_i$ and contractile force obtained by decreasing $[Ca^{2+}]_o$ or $[K^+]_o$ in 30 mmol/l KCl-PSS

Since it had become obvious that the hyperpolarization induced by BRL 38227 had the effect of reducing the Ca^{2+}-sensitivity of contractile elements in coronary arterial muscle, we examined membrane repolarization induced by decreasing $[K^+]_o$ stepwise and its effect on the increases in $[Ca^{2+}]_i$ and force of contraction in canine coronary arteries previously depolarized by 30 mmol/l KCl-PSS. Figure 7A shows the efffect of reducing $[K^+]_o$ on membrane potential in a canine coronary artery. Summarized data, obtained from four muscles, are shown in Fig. 7B. The reduction of $[K^+]_o$ produced concentration-dependent membrane repolarization. The reduction

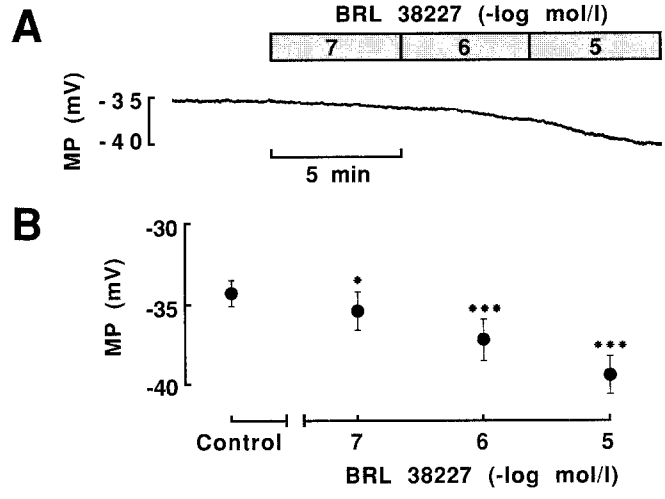

Fig. 7A, B. Membrane repolarization induced by a stepwise decrease in $[K^+]_o$ from 30 mmol/l KCl-PSS. **A** A typical record of the effect of the decrease in $[K^+]_o$ on membrane potential (MP) in an arterial strip. **B** Summarized data (mean values±SEM) obtained from four preparations

of $[K^+]_o$ from 30 to 25 mmol/l resulted in repolarization of -4.0 ± 0.4 mV ($n = 4$, $P<0.01$).

Figure 8A shows the concentration-response curves for $[K^+]_o$ versus $[Ca^{2+}]_i$, and versus contractile force, when $[K^+]_o$ was reduced from 30 to 15 mmol/l in 2.5 mmol/l $CaCl_2$-PSS. The reduction of $[K^+]_o$ produced a more prominent inhibition of the force of con-

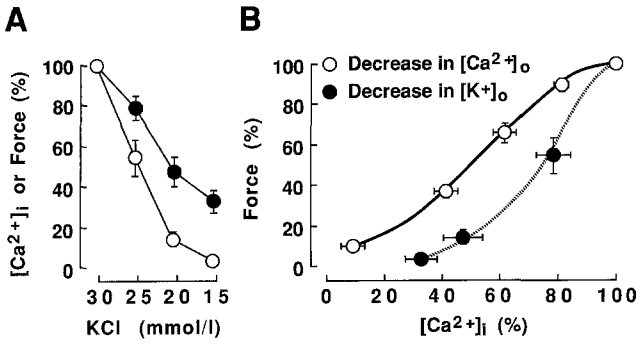

Fig. 8. A Effects of decreasing $[K^+]_o$ on the increases in $[Ca^{2+}]_i$ and force of contraction produced by 2.5 mmol/l $CaCl_2$ and 30 mmol/l KCl-PSS. The concentrations of $[K^+]_o$ were decreased from 30 to 15 mmol/l, stepwise. The $[Ca^{2+}]_i$ (●) or the force of contraction (○) are expressed as percentages of the values obtained with 2.5 mmol/l $CaCl_2$ and 30 mmol/l KCl-PSS. ($n = 6$). **B** Relation between $[Ca^{2+}]_i$ and contractile force in arterial muscle preparations depolarized with 2.5 mmol/l $CaCl_2$ and 30 mmol/l KCl-PSS when $[Ca^{2+}]_o$ (2.5 – 0.1 mmol/l), or $[K^+]_o$ (30 – 15 mmol/l), was decreased in a stepwise manner

traction than of $[Ca^{2+}]_i$. Figure 8B shows the relation between $[Ca^{2+}]_i$ and contractile force which was obtained either by decreasing $[Ca^{2+}]_o$ in 30 mmol/l KCl-PSS or by decreasing $[K^+]_o$ in 2.5 mmol/l $CaCl_2$-PSS. The curve obtained by decreasing $[K^+]_o$ was located to the right of that obtained by decreasing $[Ca^{2+}]_o$. Thus, a decrease in $[Ca^{2+}]_i$ induced by reducing $[K^+]_o$ produced a larger relaxation than did the same decrease in $[Ca^{2+}]_i$ produced by a decrease in $[Ca^{2+}]_o$.

Discussion

In the present study, the membrane hyperpolarization induced by the K^+-channel opener, BRL 38227, and the membrane repolarization induced by a reduction in $[K^+]_o$ have been shown to reduce the Ca^{2+}-sensitivity of contractile elements in canine coronary arteries contracted with 30 mmol/l KCl-PSS.

BRL 38227 caused membrane hyperpolarization and reduced the increase in $[Ca^{2+}]_i$, and the contractile force, produced by 30 mmol/l KCl-PSS. BRL 38227 has been shown to open glibenclamide-sensitive K^+-channels and to relax various smooth muscles (Edwards et al. 1991; Noack et al. 1992). The K^+-channel openers inhibit activation of the voltage-dependent Ca^{2+}-channels as a result of the membrane hyperpolarization (Hamilton et al. 1986; Quast and Cook 1989). The deactivation of the Ca^{2+}-channels by the K^+-channel openers decreases the $[Ca^{2+}]_i$ and the force of contraction (Yanagisawa et al. 1990; Okada et al. 1991).

The present study also revealed that BRL 38227 reduced the Ca^{2+} sensitivity of contractile elements. This effect was antagonized by the ATP-sensitive K^+-channel blocker glibenclamide. Glibenclamide has been shown to block the electrical, $[Ca^{2+}]_i$, and mechanical changes induced by K^+-channel openers in vascular smooth muscles (Buckingham et al. 1989; Eltze, 1989; Winquist et al. 1989; Yanagisawa et al. 1990), suggesting a role for ATP-sensitive K^+-channels (Standen et al. 1989). These results support our previous suggestion that the K^+-channel opening action of E4080 reduces the Ca^{2+}-sensitivity of contractile elements (Okada et al. 1992). The Ca^{2+}-channel blockers, nifedipine and verapamil, do not affect the Ca^{2+}-sensitivity of contractile elements (Kanmura et al. 1983; Yanagisawa et al. 1989). Thus, although both BRL 38227 and the Ca^{2+}-channel blockers decrease $[Ca^{2+}]_i$ by reducing a Ca^{2+}-influx through Ca^{2+}-channels, BRL 38227, but not the Ca^{2+}-channel blockers, reduces the Ca^{2+}-sensitivity of contractile elements. This difference between BRL 38227 and Ca^{2+}-channel blockers is due to their different effects on membrane potential: BRL 38227 hyperpolarizes the membrane, whereas the Ca^{2+}-channel blockers do not change the resting membrane potential or high KCl-induced depolarization (Ito et al. 1978; Kanmura et al. 1983). Therefore, the reduction of the Ca^{2+}-sensitivity by the BRL 38227 may be due to the membrane hyperpolarization. In line with this consideration, the relations between $[Ca^{2+}]_i$ and contractile force obtained by decreasing $[Ca^{2+}]_o$ or $[K^+]_o$ were different (Fig. 8B). A decrease in $[Ca^{2+}]_i$, induced by reducing $[K^+]_o$, produced a larger reduction in force than that induced by the same decrease in $[Ca^{2+}]_i$ induced by a reduction in $[Ca^{2+}]_o$. Reduction of $[K^+]_o$ in high KCl-PSS caused membrane repolarization in canine coronary arteries. The $[K^+]_o$-membrane potential relation in this study was very similar to that already reported (Harder and Coulson 1979). The decrease in $[Ca^{2+}]_o$ may tend to depolarize the membrane (Casteels et al. 1977). The extent of repolarization caused by the decrease in $[K^+]_o$ from 30 to 25 mmol/l (−4.0 mV) was similar to that of hyperpolarization induced by 10^{-5} mol/l BRL 38227 (−5.0 mV) when there was 30 mmol/l KCl in the bath. The $[Ca^{2+}]_i$-contractile force relation in the presence of BRL 38227 in 30 mmol/l KCl-PSS was very similar to that obtained by reducing $[K^+]_o$ (Figs. 5, 8B). Thus, membrane hyperpolarization by K^+-channel openers and repolarization by reducing $[K^+]_o$ had similar effects on the sensitivity of the contractile elements to Ca^{2+}.

In the present experiments, the decrease in $[K^+]_o$ was produced concomitantly with an increase in extracellular Na^+ concentration ($[Na^+]_o$). We have to consider the influences of an increase in $[Na^+]_o$ on vascular contractility. First of all, the increase in $[Na^+]_o$ was a rather small change. When $[K^+]_o$ was changed from 30 to 25 mmol/l, $[Na^+]_o$ changed only from 110 to 115 mmol/l. Even an increase in $[Na^+]_o$ to such an extent might stimulate Na^+/Ca^{2+} exchange to bring about a Ca^{2+}-efflux. However, $[Ca^{2+}]_i$ was measured in this study. We compared forces of contraction occurring at the same concentration of $[Ca^{2+}]_i$, therefore it is unnecessary to discuss Na^+/Ca^{2+} exchange. An increase in $[Na^+]_o$ might stimulate Na^+/H^+ exchange, resulting in an increase in intracellular pH (pH_i). However, the reported influences of an increase in pH_i on force of contraction in skinned smooth muscle are not consistent; a decrease in force (Gardner and Diecke 1988), no change (Iino 1981), and an increase (Mrwa et al. 1974) have been reported. Thus, it is unlikely that the small increase in $[Na^+]_o$ could

cause the reduced Ca^{2+}-sensitivity of contractile elements induced by the decrease in $[K^+]_o$.

Recent studies have shown that membrane hyperpolarization, induced by BRL 38227 or cromakalim, may inhibit the increase in $[Ca^{2+}]_i$, or in contractile force related to phosphoinositide breakdown induced by noradrenaline or U46619, in vascular smooth muscles (Ito et al. 1991; Quast and Baumlin 1991; Yamagishi et al. 1992a, b). In airway smooth muscle, membrane hyperpolarization also inhibits agonist-stimulated phosphoinositide metabolism (Challiss et al. 1992). These findings suggest that the membrane potential may regulate the activity of phospholipase C. Thus, the reduction of the Ca^{2+}-sensitivity by membrane hyperpolarization may involve an inhibition of phospholipase C, and, consequently, bring about a decrease in protein kinase C activity. We have reported that the activation of protein kinase C by phorbol, 12,13-dibutyrate apparently increases the Ca^{2+}-sensitivity of contractile elements (Mori et al. 1990; Kageyama et al. 1991). Interestingly, it has been reported that a hyperpolarization-activated K^+ efflux appears to regulate directly, adenylyl cyclase activity in *Paramecium* (Schultz et al. 1992; Maelicke 1992). Membrane hyperpolarization is an important mechanism in the effects of some endogenous and exogenous vasodilators (Nelson et al. 1990). Further studies are needed to establish the precise mechanism of actions related to membrane hyperpolarization.

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