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Effect of Ki1769, a Novel K^+-channel Opener, on Sensitivity to Ca^{2+} of Contractile Elements and Inositol Phosphate Formation in Porcine Coronary Artery

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Abstract

To determine whether Ki1769, a novel K^+-channel opener, acts intracellularly in vasorelaxation, we compared the effects of Ki1769 on force of contraction, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and inositol phosphate (IP_1) formation with those of Ca^{2+}-channel blockers in isolated porcine coronary artery.

Ki1769 (10 μM) and verapamil (1 μM), which produced submaximal relaxation, reduced the increase in $[Ca^{2+}]_i$ and force of contraction induced by 25 mM KCl. Verapamil reduced $[Ca^{2+}]_i$ and the force of contraction to a similar extent but Ki1769 reduced force of contraction more strongly than it did $[Ca^{2+}]_i$. Ki1769 also inhibited U46619 (9,11-dideoxy-9α,11α-methano-epoxy-$PGF_{2\alpha}$)-induced IP_1 formation and glibenclamide blocked its inhibitory effect.

These results suggest that the opening of K^+ channels induced by Ki1769 reduces the Ca^{2+} sensitivity of contractile elements and inositol phospholipid hydrolysis which is related to the Ca^{2+} release from intracellular storage.

The ultimate mechanism of action of K^+-channel openers was once thought to be similar to those of Ca^{2+}-channel blockers, because K^+-channel openers produced hyperpolarization of the cell membrane and thus decreased Ca^{2+} influx through inhibition of voltage-dependent Ca^{2+} channels. In contrast, it was found that isradipine, a Ca^{2+}-channel blocker, caused less inhibition of the contractile response caused by endogenous vasoactive substances such as angiotensin II and 5-hydroxytryptamine than cromakalim, a K^+-channel opener, suggesting that the mechanism of action of K^+-channel openers partly differs from that of Ca^{2+}-channel blockers (Cook et al 1988). Recently, K^+-channel openers were reported to decrease the release of Ca^{2+} from intracellular stores (Itoh et al 1991, 1992; Yamagishi et al 1992a, b) and the Ca^{2+} sensitivity of contractile elements in vascular smooth muscle (Okada et al 1992, 1993b). These mechanisms of K^+-channel openers are thought to be associated with hyperpolarization of the cell membrane. Therefore, it seems that K^+-channel openers inhibit contractile responses induced by some agonists more effectively than Ca^{2+}-channel blockers.

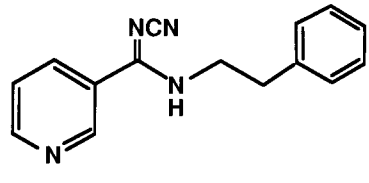

FIG. 1. Chemical structure of Ki1769.

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Recently, we found a novel vasodilator Ki1769 in the course of a screening program (Fig. 1). In vascular smooth muscle, Ki1769 increased the efflux of ^{86}Rb, a marker for K^+ (Kashiwabara et al 1992), and the vasorelaxant effect of Ki1769 was antagonized by glibenclamide (Okada et al 1993a). In addition, Ki1769 had no effect on intracellular guanosine 3′:5′-cyclic monophosphate (cGMP) and adenosine 3′:5′-cyclic monophosphate (cAMP) (Yokoyama et al 1994). From these observations, although Ki1769 structurally differs from cromakalim and pinacidil, its mechanism of action appears to be based on a K^+-channel opening action. However, whether Ki1769 affects the inositol phosphate formation and the Ca^{2+} sensitivity of contractile elements has not yet been examined. Therefore, in the present study, we examined the effect of Ki1769 on force of contraction and intracellular Ca^{2+} concentration using the fura-2 method and inositol phosphate formation in porcine isolated coronary artery.

Materials and Methods

Preparations

Porcine hearts were obtained from a local abattoir. The left circumflex or the left anterior descending coronary arteries (o.d. 2·5–3·0 mm) were removed and rinsed with Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25·0 and glucose 10·0. The arteries were cleaned of adherent tissue and then cut into 2-mm rings or 2-cm helical strips. The endothelium of each preparation was removed by gentle rubbing. The 2-mm ring preparation was used for the simultaneous measurement of muscle tension and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and the helical strip

preparation was used for the determination of inositol phosphate formation.

Simultaneous measurement of muscle tension and $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ was measured as reported by Yanagisawa et al (1989). Coronary arterial rings, whose luminal side had been turned outwards, were exposed to 10 μM fura-2 acetoxymethyl ester (fura-2 AM) for about 8 h at room temperature (21 ± 2°C) in Krebs-Henseleit solution. A nontoxic detergent, pluronic F127 (0·1% w/v), was added to increase the solubility of fura-2 AM. After fura-2 AM loading, muscles were rinsed with Krebs-Henseleit solution for 1 h. The muscle ring was placed horizontally in a temperature-controlled 10-mL organ bath and stretched to a resting tension of about 2 g between two stainless-steel needles, one of which was connected to a transducer (TB-611T, Nihon Kohden) to monitor muscle contraction. The ring was illuminated at two excitation wavelengths (340, 380 nm) and the intensity of fluorescence (F_{340}, F_{380}) at 500 nm was measured (CAF100, Japan Spectroscopic Co.). We used the F_{340}/F_{380} ratio as an indicator of $[Ca^{2+}]_i$ instead of absolute $[Ca^{2+}]_i$ because the dissociation constant of fura-2 for Ca^{2+} in cytosol may be different when obtained in-vitro (Karaki 1989). The ratio F_{340}/F_{380} and tension obtained at rest and those obtained in 25 mM KCl were taken as 0 and 100%, respectively.

KCl depolarizing solution, 25 mM, was prepared by replacing NaCl with equimolar KCl in the control solution. Low Ca^{2+} solution was made by decreasing $CaCl_2$ in the 25 mM KCl. These solutions were saturated with 95% O_2–5% CO_2 at 37°C.

Determination of inositol phosphate (IP_1)

IP_1 formation was assayed by the method of Endoh et al (1993). Coronary arterial strips were incubated with Krebs-Henseleit solution for 30 min at 37°C. Subsequently the tissues were labelled with 10 μCi mL^{-1} myo-[^{3}H]inositol (sp. act. 106 Ci mmol^{-1}) in Krebs-Henseleit solution at 37°C for 2 h. After incubation, the tissues were washed twice with Krebs-Henseleit solution and exposed to 100 nM U46619 in the presence of 10 mM LiCl for 1 h. Ki1769 (10 μM) or nifedipine (1 μM) was added 5 min before and throughout the application of U46619. In some experiments, glibenclamide (1 μM) was applied before the addition of Ki1769. The reaction was terminated by freezing the arteries in liquid nitrogen followed by homogenization in 1 mL $CHCl_3$/CH_3OH/HCl (100:200:1, v/v/v) mixture. Samples were centrifuged and the supernatant was loaded onto an anion exchange column (Bio-Rad AG1-X8). The column was washed with 5 mL water, which was followed by 8 mL 60 mM ammonium formate/5 mM sodium tetraborate. IP_1 was eluted with 0·2 M ammonium formate/0·1 M formic acid. The eluate was collected in scintillation vials, and 8 mL scintillation fluid was added. The sample was counted in a liquid scintillation counter and the results were expressed with respect to wet weight of tissue.

Drugs

Fura-2 AM and pluronic F127 were purchased from Dojin, Kumamoto, Japan; myo-[^{3}H]inositol was from Amersham International, UK. U46619 (9,11-dideoxy-9α,11α-methano-epoxy-$PGF_{2\alpha}$) was purchased from Funakoshi, Tokyo, Japan. Glibenclamide and nifedipine were obtained from Sigma, St Louis, MO, USA. Verapamil was from Wako, Osaka, Japan, and Ki1769 was synthesized in our laboratory.

Statistics

Differences were considered significant when $P < 0.05$ by an unpaired Student's t-test. All data are expressed as mean ± s.e.m.

Results

Ki1769 and verapamil produced concentration-dependent relaxation in porcine coronary arteries contracted by 25 mM KCl (Fig. 2). The pD_2 values for Ki1769 and verapamil were 5·78 ± 0·10 and 6·60 ± 0·05, respectively. From these results, we selected concentrations of 10 μM for Ki1769 and 1 μM for verapamil, which produced submaximal relaxations, in the subsequent experiments.

Fig. 3 shows typical recordings of the effects of verapamil and Ki1769 on $[Ca^{2+}]_i$ and force of contraction. KCl (25 mM) induced sustained increases in $[Ca^{2+}]_i$ and force of contraction, and verapamil (1 μM) and Ki1769 (10 μM) produced reductions in $[Ca^{2+}]_i$ and force of contraction. The relationship between $[Ca^{2+}]_i$ and force of contraction, obtained when the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) was decreased, was compared with those in the presence of Ki1769 or verapamil (Fig. 4). The decrease in $[Ca^{2+}]_o$ (30 μM) reduced $[Ca^{2+}]_i$ and force of contraction to similar extents. Verapamil as well as decreasing $[Ca^{2+}]_o$ reduced $[Ca^{2+}]_i$ and force of contraction almost in parallel. However, Ki1769 (10 μM) reduced the force of contraction more strongly than $[Ca^{2+}]_i$.

The effect of Ki1769 on U46619 (100 nM)-stimulated IP_1 accumulation was examined in the absence or presence of glibenclamide (1 μM) (Table 1). U46619-stimulated IP_1 accumulation was inhibited by Ki1749 but was not affected in the presence of Ki1769 plus glibenclamide. Nifedipine had no significant inhibitory effect on U46619-stimulated IP_1 accumulation at a concentration that produced submaximal inhibition of U46619-induced contraction (Kasai et al 1993).

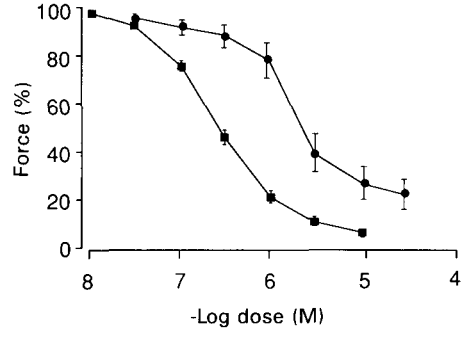

FIG. 2. Concentration-response curves for Ki1769 (●) and verapamil (■) in porcine coronary arteries contracted by 25 mM KCl. Each point represents the mean ± s.e.m. of four experiments.

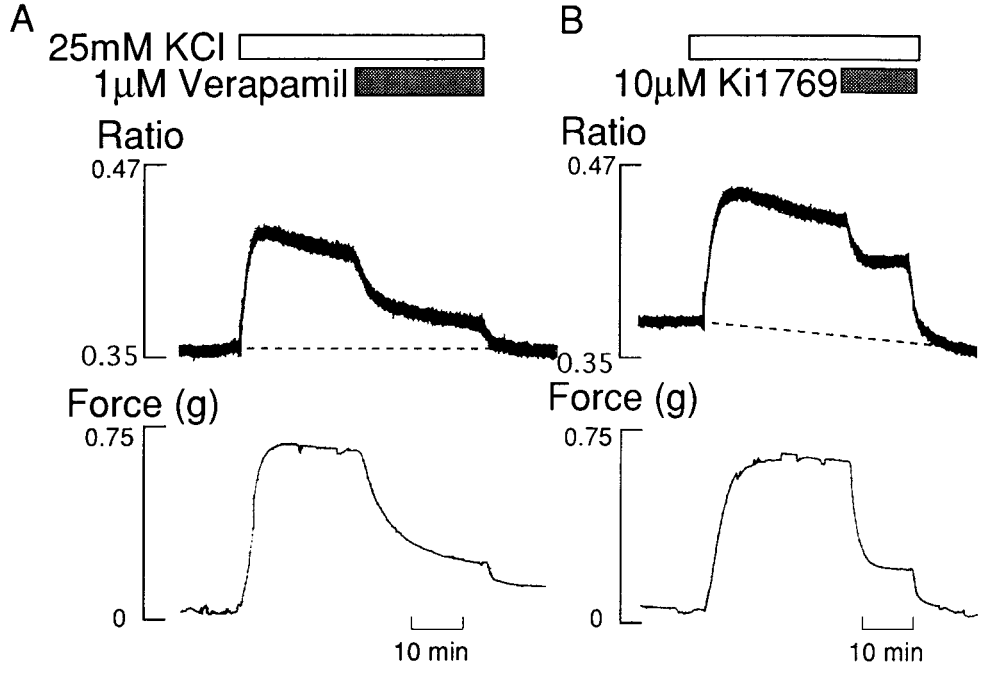

FIG. 3. Effects of verapamil (A) and Ki1769 (B) on $[Ca^{2+}]_i$ (indicated by the F_{340}/F_{380} ratio) and force of contraction induced by 25 mM KCl. Traces are from typical results of four experiments. After 25 mM KCl-stimulated $[Ca^{2+}]_i$ and force of contraction reached steady states, 10 μM Ki1769 or 1 μM verapamil was added.

Discussion

Ki1769 and verapamil produced reductions in force of contraction and $[Ca^{2+}]_i$ in porcine coronary arteries contracted with 25 mM KCl. However, the relationship between force of contraction and $[Ca^{2+}]_i$ with Ki1769 differed from that with verapamil. Verapamil reduced force of contraction and $[Ca^{2+}]_i$ to almost the same extent, which is in good agreement with the previous reports (Takizawa et al 1993). In contrast, Ki1769 reduced force of contraction more strongly than $[Ca^{2+}]_i$. These results suggest that Ki1769 partly shows a relaxing mechanism of action which is not dependent on $[Ca^{2+}]_i$. Such a different characteristic is thought to be based on the difference in mechanisms of action between Ki1769 and verapamil. It is known that the mechanism of verapamil is a Ca^{2+}-channel-blocking action, i.e. inhibition of Ca^{2+} influx through Ca^{2+} channels. In contrast, Ki1769 is thought to be a K^+-channel opener, because Ki1769 produced ^{86}Rb efflux (Kashiwabara et al 1992) and its relaxant effect was antagonized by glibenclamide (Okada et al 1993a). Recently, it has been reported that levcromakalim and E4080, K^+-channel openers which differ structurally from Ki1769, reduce the Ca^{2+} sensitivity of the contractile elements (Okada et al 1992, 1993b). This reduction in Ca^{2+} sensitivity is thought to be associated with membrane hyperpolarization through the opening of K^+ channels. Therefore, it is considered that the characteristics of Ki1769 observed in the present study, are due to a reduction in the Ca^{2+} sensitivity of the contractile elements in addition to the inhibition of voltage-dependent Ca^{2+} channels as a result of membrane hyperpolarization.

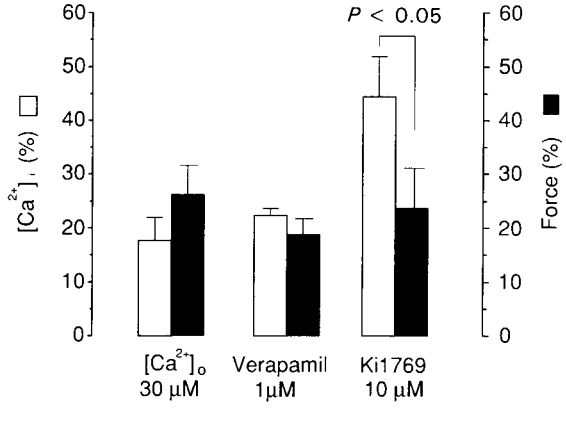

FIG. 4. Effect of Ki1769 on $[Ca^{2+}]_i$ (indicated by the F_{340}/F_{380} ratio) and force of contraction stimulated by 25 mM KCl. After 25 mM KCl-stimulated $[Ca^{2+}]_i$ and force of contraction reached steady states, 10 μM Ki1769 or 1 μM verapamil was added, or $[Ca^{2+}]_o$ was decreased to 30 μM. $[Ca^{2+}]_i$ and tension are expressed as a percentage of each pre-drug value. Each column represents the mean of 4–6 experiments and the s.e.m. is shown by a vertical bar.

Table 1. Effect of Ki1769 (10 μM) on U46619 (100 nM)-induced [^{3}H]inositol phosphate (IP_1) accumulation in porcine coronary artery. Basal IP_1 accumulation in the absence of drug administration was 13·4 ± 1·3 d min^{-1} (mg wet weight)$^{-1}$. Results are presented as percentage of the basal value (basal value = 100%).

	Inositol phosphate accumulation (% basal)
Control	234 ± 15
Ki1769 (10 μM)	159 ± 12**
+ glibenclamide (1 μM)	219 ± 30
Nifedipine (1 μM)	229 ± 35

Mean ± s.e.m., n = 8–12. ** $P < 0.01$ compared with the response to U46619 alone.

Recent studies have shown that the membrane hyperpolarization induced by levcromakalim or cromakalim inhibits agonist-induced inositol phosphate formation in addition to the characteristic mentioned above (Itoh et al 1991; Yamagishi et al 1992a, b). In the present study, U46619-stimulated IP_1 accumulation was inhibited by Ki1769, but not by nifedipine. This finding for nifedipine agrees with previous reports using diltiazem (Eskinder et al 1989) and nicardipine (Araki et al 1989). Furthermore, the inhibitory effect of Ki1769 on IP_1 accumulation was antagonized by glibenclamide, suggesting that the reduction of IP_1 accumulation is based on its K^+-channel opening action as with levcromakalim and cromakalim. Agonist-induced inositol phosphate formation is also reported to be inhibited by increases in intracellular cAMP or cGMP levels (Rapoport 1986; Lang & Lewis 1989; Ahn et al 1992). However, cAMP or cGMP is not thought to be related to the inhibitory effect of Ki1769 on inositol phosphate formation, as we observed that Ki1769 had no effect on intracellular cAMP and cGMP levels in the same preparations as used in the present experiment (Yokoyama et al 1994).

Although Ki1769 is structurally different from levcromakalim and cromakalim, the characteristics of Ki1769 are similar to those of other K^+-channel openers. It has also been reported that Ki4032, in which the phenyl moiety of Ki1769 is replaced by an acetoxyl group, had a K^+-channel opening action, reduced Ca^{2+} sensitivity (Okada et al 1993c) and inhibited Ca^{2+} release from storage sites (Yamagishi et al 1992a). Thus, these observations show that K^+-channel openers of the pyridinecarboximidamide type as well as structurally different K^+-channel openers, reduce the influx of extracellular Ca^{2+}, the Ca^{2+} sensitivity of the contractile elements and agonist-induced inositol phosphate formation.

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