Inhibition of vasoconstriction by potassium channel opener aprikalim in human conduit arteries used as bypass grafts

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Aims Potassium channel openers (KCOs) are of potential therapeutic value. Little is known about the effect of these drugs on human conduit arteries used as coronary bypass grafts. The purpose of this study was to determine the effect of the KCO aprikalim (RP52891) on human arteries used as coronary bypass grafts with emphasis on the possible difference in the inhibitory effect on depolarizing agent-mediated rather than receptor-mediated contraction.

Methods Human internal mammary artery segments (IMA, $n=88$) taken from 28 patients were studied. Concentration-relaxation curves for aprikalim were established in IMA precontracted with three vasoconstrictors (K^+, U46619, and phenylephrine). In IMA rings incubated with aprikalim (1 or 30 μM) for 10 min concentration-contraction curves for the three vasoconstrictors were constructed.

Results Aprikalim-induced relaxation was less in K^+ ($37.3 \pm 6.4\%$) than in U46619 ($80.2 \pm 7.7\%$, $P=0.002$), or phenylephrine ($67.5 \pm 7.0\%$, $P=0.038$) -precontracted IMA. The EC_{50} for K^+-(-5.40 ± 0.12 log M) was significantly higher than that for phenylephrine (-6.43 ± 0.30 log M, $P=0.007$) but not significant compared with that for U46619 (-5.81 ± 0.11, $P>0.05$). Pretreatment with aprikalim depressed the contraction by phenylephrine from $140.6 \pm 27.6\%$ to $49.3 \pm 14.1\%$ ($P=0.002$) and shifted the EC_{50} 11.0-fold higher in rings treated with 1 μM aprikalim ($P=0.007$). Treatment of aprikalim did not significantly reduce the K^+ and U46619-induced contraction ($P>0.05$) but shifted the concentration-contraction curves rightward (2.8-fold higher for K^+, $P<0.05$ and 2.2-fold higher for U46619, $P<0.05$).

Conclusions This study demonstrates that aprikalim has vasorelaxant effects in human conduit arteries used as coronary artery bypass grafts contracted by a variety of vasoconstrictors and this effect is vasoconstrictor-selective with greater potency for α_1-adrenoceptor agonists than for depolarizing agent K^+. These findings provide information on the possible use of this KCO in various clinical settings.

Keywords: human artery, internal mammary artery, potassium channel opener, aprikalim, K_{ATP}

Introduction

ATP-sensitive potassium channels (K_{ATP}) exist in a wide range of cells, particularly in endocrine cells, smooth muscle, skeletal muscle, neurons, and cardiac cells [1]. Potassium (K^+) channel openers (KCOs) are considered to comprise a heterogeneous group of organic compounds [2]. The common features among K_{ATP} openers include a hydrophobic group, an electrodeficient aromatic ring, and a hydrogen bonding site [2]. KCOs repolarize or hyperpolarize the cell membrane. They therefore decrease the opening probability of voltage-dependent L- and T-type Ca^{2+}-channels and restrain agonist-induced Ca^{2+} release from intracellular sources through inhibition of inositol trisphosphate (IP3) formation, and lower the efficiency of calcium as an activator of contractile proteins. In addition, KCOs may accelerate the clearance of intracellular free Ca^{2+} via the Na^+/Ca^{2+} exchange pathway. The effects drive the vascular smooth muscle cells into a relaxed state and reduce membrane excibility and the reactivity of the cell [2, 3]. It has been suggested that vascular smooth muscle is particularly sensitive to KCOs [1]. Considering these characteristics, KCOs are of potential value as therapeutical agents [3].

The use of vasodilators in the treatment of cardiovascular diseases has recently increased. Although many vasodilators such as calcium antagonists, ACE-inhibitors, long-lasting nitrates, and phosphodiesterase (PDE) inhibitors have been extensively investigated, the vasorelaxant action of KCOs on the human conduit arteries used as coronary bypass grafts has not been reported. In fact, little has been known about the effect of KCOs on human blood vessels.

Theoretically, when a vessel is contracted by the depolarizing agent K^+, because of the higher membrane potential (depolarization), KCO-induced repolarization or hyperpolarization may be more difficult, compared with

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other vasoconstrictors that affect the potential less [4, 5]. This may result in a selective inhibitory effect with less potency to the depolarizing agent-mediated vasoconstriction.

The present study was designed to investigate the effect of aprikalim, one of the KCOs, on the most commonly used arterial graft—the human internal mammary artery (IMA) with emphasis on the possible difference in the inhibitory effect on depolarizing agent-mediated compared with receptor-mediated contraction.

Methods

General

Eighty-eight human IMA segments were collected from 28 patients undergoing IMA graft surgery. There were 21 males and 7 females with a mean age of 62.5 ± 4.1 years. The investigation conforms with the principles outline in the Declaration of Helsinki. Approval to use discarded IMA tissue was given by the Institutional Review Board of St. Vincent Hospital and Human Ethics Committee of Grantham Hospital. Any discarded distal IMA segments were collected and put in a container with oxygenated, physiological solution (Krebs') maintained at 4° C, and then transferred to laboratory. The IMA was transferred into a glass dish and dissected out from its surrounding connective tissue. The vessels were cut into 3-mm-long rings and suspended on wires in organ baths [6–7]. The number of rings taken from each patient varied from 2–6. The Krebs' solution had the following composition (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, and glucose 11. The solution was aerated with a gas mixture of 95% O_2^- 5% CO_2 at 37° C.

Organ-bath technique

A normalization technique was used to set the vascular rings at a pressure comparable with that at the *in vivo* situation. The details of the technique were published before [6, 7]. Briefly, the rings were stretched-up in progressive steps to determine the length-tension curve for each ring. A computer iterative fitting program (VESTAND 2.1, Yang-Hui He, Princeton University, NJ.) was used to determine the exponential line, pressure and the internal diameter. When the transmural pressure on the rings reached 100 mmHg, determined from their own length-tension curves, the stretch-up procedure was stopped and the rings were released to 90% of their internal circumference at 100 mmHg. This degree of the passive tension was then maintained throughout the experiment.

The endothelium was intentionally preserved by cautiously dissecting and mounting the rings in our study since endothelium plays a modulatory role in the contractility of the human IMA. We previously found that this technique allowed the experiments carried out with an intact endothelium, as determined by the functional relaxation response to acetylcholine in the canine IMA, canine [6] and porcine [4] coronary artery. We have also showed that the endothelium was functionally preserved in the isolated human IMA rings [8–11].

Protocol

After the normalization procedure, the IMA rings were equilibrated for at least 45 min.

Relaxation Aprikalim-induced relaxation was studied in IMA rings contracted with potassium chloride (K^+, 25 mM, $n=7$), U46619 (10 nM, $n=7$), and phenylephrine (PE, 3 μM, $n=7$). The concentrations of these vasoconstrictor substances were submaximal as determined from the logistic-curve fitting equation [6]. These concentrations are equal to EC_{50}–EC_{80} for the U46619, K^+, and PE-induced contraction in the human IMA from previous studies [7–12]. Cumulative concentration-relaxation curves to aprikalim were then established. Only one concentration-relaxation curve was obtained from each IMA ring. From seven rings (taken from four–six patients), a mean concentration-relaxation curve was constructed. The contraction-relaxation curve to aprikalim was also established in rings treated with glibenclamide, a K_{ATP} channel blocker, for 30 min before the contraction-relaxation curve was induced in either K^+ or PE-precontracted IMA rings ($n=4$ in each group).

Depression of contraction by pretreatment with aprikalim After equilibration, 100 mM K^+ was added into the organ bath and the contraction force was recorded. The ring was frequently washed to restore the baseline. To determine whether pretreatment with aprikalim would alter the contraction response to various vasoconstrictors (K^+, U46619, and PE), cumulative concentration-contraction curves were constructed in IMA ring segments. These rings were equilibrated for 10 min with -6 or -4.5 log M (1 or 30 μM) aprikalim. The time for the pretreatment was decided by the average time to reach a plateau for each dose of aprikalim in the relaxation experiments from the present study. The contraction was expressed as percentage of the contraction force induced by 100 mM K^+.

Data analysis

The effective concentration of the constrictor (or dilator) agent that caused 50% of maximal contraction (or relaxation) was defined as EC_{50}. The EC_{50} was determined from each concentration-contraction (or relaxation) curve by a logistic, curve-fitting equation: $E = MA^P/(A^P + K^P)$ where E is response, M is maximal contraction (or relaxation), A is concentration, K is EC_{50} concentration, and p is the slope parameter [6]. A computerized program was used for the curve-fitting.

From this fitted equation, the mean EC_{50} value $\pm$ s.e. mean was calculated in each group. Unpaired *t*-test or analysis of variance were used to test statistical significance among different constrictors and dilators regarding the maximal response or EC_{50}. Scheffe's *F*-test was used as post-hoc test between groups. $P<0.05$ was considered significant.

Materials

Drugs used in this study and their sources were: U46619 (Cayman Chemical, Ann Arbor, MI); phenylephrine and

other chemicals (Sigma, St Louis, MO). Stock solution of U46619 was held frozen until required. Aprikalim is generously provided by Rhone-Poulenc Rorer Recherche-Developpement, France.

Results

Resting vessel parameters

The mean internal diameter of the 88 rings at an equivalent transmural pressure of 100 mmHg (D100) [6, 7] was 2.7 ± 0.19 mm as determined from the normalization procedure. When the IMA rings were set at a resting diameter of $0.9 \times$D100, the equivalent transmural pressure was 70.1 ± 3.1 mmHg, and the resting force was 4.6 ± 0.7 g.

Relaxation by aprikalim in the IMA precontracted by K^+, U46619, or PE

The precontraction force was 5.3 ± 0.8 g for K^+ (25 mM), 4.2 ± 0.8 g for PE (3 μM), and 3.6 ± 1.0 for U46619 (10 nM) ($P=0.3$, ANOVA). Aprikalim caused less relaxation in IMA precontracted by K^+ ($37.3 \pm 6.4\%$) than by U46619 ($80.2 \pm 7.7\%$, $P=0.002$) or by PE ($67.5 \pm 7.0\%$, $P=0.02$, Scheffe's *F*-test, Figure 1) at the concentration of 100 μM (-4 log M) (Figure 1). There was a significant difference among the relaxations in these three vasoconstrictor-precontracted IMA ($P=0.001$, ANOVA). The EC_{50} for K^+-induced contraction (-5.40 ± 0.12 log M) was significantly higher than that for PE (-6.43 ± 0.30 log M, $P=0.007$, Scheffe's *F*-test) but not significant compared with the U46619-precontracted rings (-5.81 ± 0.11, $P>0.05$).

In glibenclamide-pretreated IMA rings, aprikalim induced significantly less relaxation (Figure 2a, b). In the K^+-induced contraction, the maximal relaxation was $10.0 \pm 10.0\%$ ($n=4$, $P=0.04$ *vs* the control of $37.3 \pm 6.4\%$, Figure 2a). In the PE-induced contraction, it was $31.0 \pm 16.7\%$ ($n=4$, $P=0.04$ *vs* the control of $67.5 \pm 7.0\%$, Figure 2b).

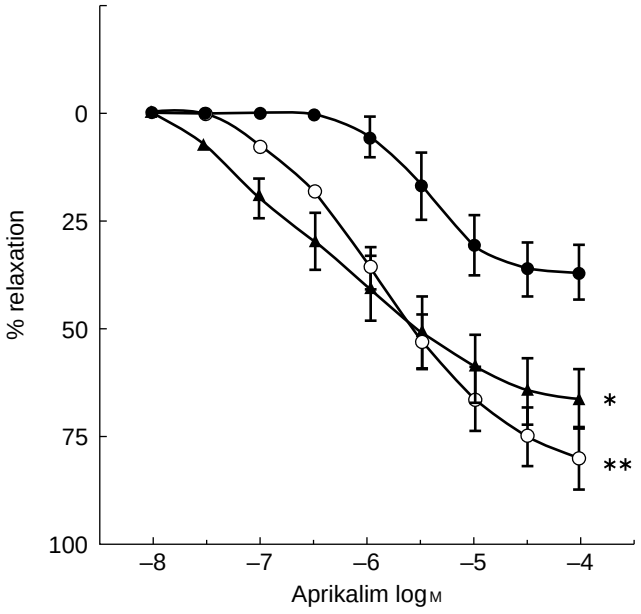

Figure 1 Mean concentration ($-$log M)-response (% relaxation) curves for aprikalim in the human internal mammary artery precontracted by potassium chloride (●, K^+, 25 mM, $n=6$), U46619 (○, 10 nM, $n=6$), and phenylephrine (▲, 3 μM, $n=6$). The rings were taken from 5–6 patients. Vertical error bars are 1 s.e.mean. $P<0.01$, ANOVA among the three groups. ⋆$P<0.05$, ⋆⋆$P<0.01$, Scheffe's *F*-test compared with the relaxation in K^+-precontracted rings.

Depression of contraction by pretreatment with aprikalim

In the control rings, the maximal contraction force was 6.5 ± 1.0 g for K^+ ($n=8$), 4.1 ± 0.5 g for PE ($n=9$), and 8.7 ± 0.9 g for U46619 ($n=6$). Pretreatment of IMA with aprikalim for 10 min significantly depressed the magnitude of the PE-induced contraction (Figure 3). In comparison with $140.9 \pm 17.9\%$ in the control, the maximal contraction was $74.6 \pm 12.6\%$ ($P=0.02$) and $49.3 \pm 14.1\%$ ($P=0.002$, Scheffe's *F*-test) with the pretreatment of aprikalim at the concentration of 1 and 30 μM. The pretreatment of aprikalim also decreased the sensitivity of IMA to PE. The EC_{50} was 11-fold higher in the aprikalim (1 μM)-pretreated rings ($P<0.01$, Table 1). In the IMA treated with 30 μM aprikalim, the EC_{50} was so high that it could not be determined by the logistic fitting program (Table 1).

In contrast, there was no difference in the maximal contraction with regard to the pretreatment of aprikalim on either K^+- or U46619-induced contraction (Table 1, Figures 4 and 5); although there was an inhibition by aprikalim. The inhibition was shown by the observation that the EC_{50} was significantly increased by the pretreatment with aprikalim (Table 1). The EC_{50} was 2.8-fold higher following pretreatment with aprikalim (30 μM, $P<0.05$) on K^+-induced contraction and 2.2-fold higher ($P<0.05$) in U46619-induced contraction.

Discussion

In this study, we have found in the human IMA, the major arterial graft for coronary artery bypass surgery, that 1) aprikalim relaxes the IMA to a different extent in contractions induced by various vasoconstrictors and that 2) aprikalim may have a selectivity with greater potency to inhibit the contraction mediated by adrenoceptor-agonists than by depolarizing agent K^+ or thromboxane A_2.

The relaxing effect of KCOs on blood vessels has been reported as species independent [3]. Those vessels include rat and rabbit aortas [15] and veins [16, 17], rabbit mesenteric arteries [15], guinea pig and rabbit pulmonary arteries, and porcine or canine coronary arteries [16, 17]. There have been studies on the effect of KCOs on human blood vessels such as cerebral [18, 19], mesenteric [19], and coronary arteries [20]. However, little has been known about the vasorelaxant effect of KCOs on human conduit arteries used as coronary bypass grafts. In the present study, the effect of aprikalim in the human IMA, the major arterial conduit for coronary artery bypass grafting (CABG), was examined.

Although the long-term patency rates of arterial grafts are superior to venous grafts, the use of arterial grafts such as IMA, gastroepiploic artery, inferior epigastric artery, and radial artery has raised the question of whether hypoperfusion may occur due to the small diameter of the arterial grafts. In fact, recent studies have demonstrated that blood flow through arterial grafts, under certain circumstances, may be

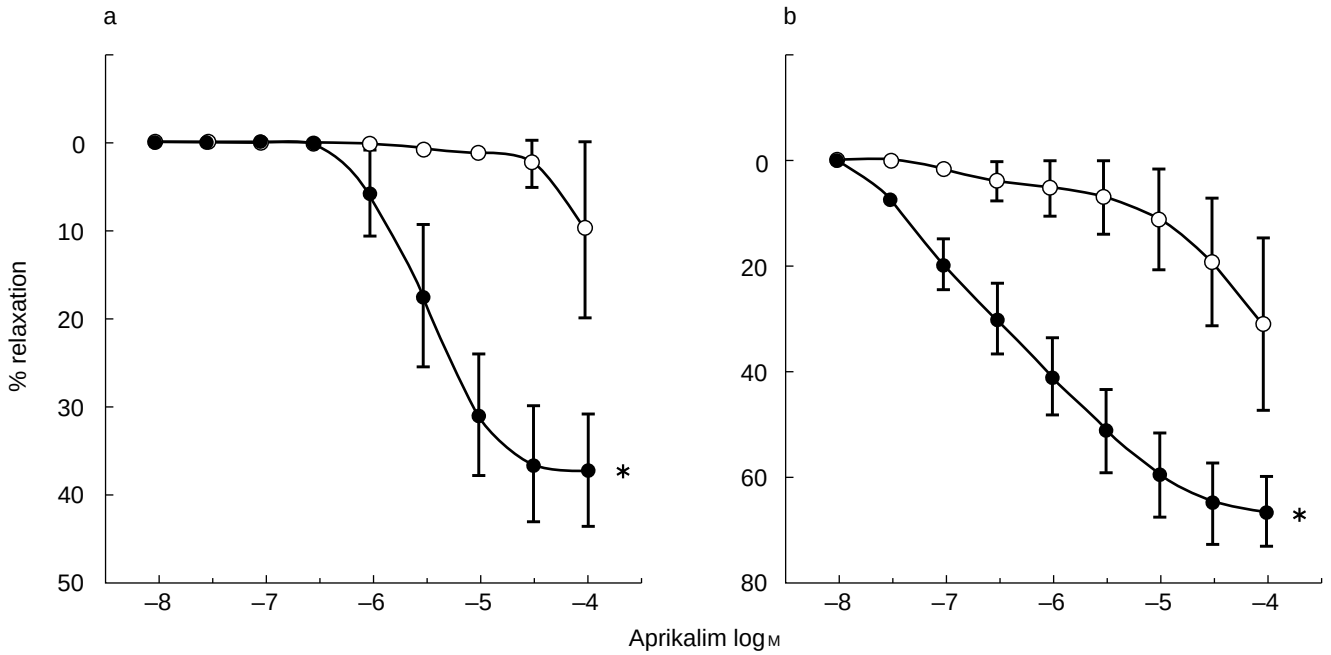

Figure 2 Mean concentration (−log M)-response (% relaxation) curves for aprikalim in the human internal mammary artery precontracted by potassium chloride (K^+, 25 mM, $n=4$, a) or phenylephrine (PE, 3 μM, $n=4$, b) with (○, $n=4$) or without (●, $n=6$) pretreatment by glibenclamide (GBC, 3 μM). Vertical error bars are 1 s.e.mean. ⋆$P<0.05$, unpaired t-test.

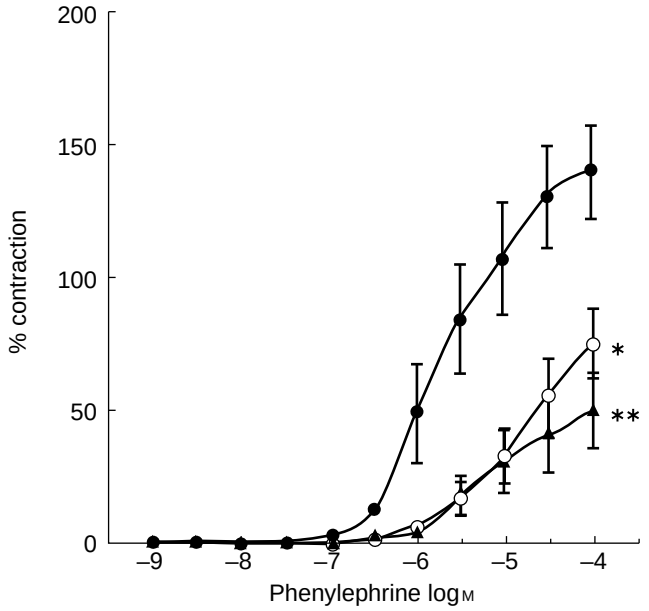

Figure 3 Mean concentration (−log M)-contraction (percentage of 100 mM K^+-induced contraction) curves for phenylephrine. Control (●, $n=9$): without pretreatment of aprikalim. APK −6 (○, $n=7$) or APK −4.5 (▲, $n=7$): aprikalim (−6 or −4.5 log M) was added to the organ bath 10 min before phenylephrine. Vertical error bars are 1 S.E.mean. $P<0.001$ among the three groups at the maximal contraction (ANOVA). ⋆$P<0.05$; ⋆⋆$P<0.01$, Scheffe's F-test compared with the control at the maximal contraction.

Table 1 Inhibition of aprikalim on the contraction induced by various vasoconstrictors in the human internal mammary artery.

	Maximal contraction (% of 100 mM K^+-induced contraction)	*EC_{50} (−log M)*†
K^+		
Control	100	19.53±2.83
Aprikalim (1 μM)	115.2±28.9	37.89±11.82
Aprikalim (30 μM)	105.5±23.8	54.99±10.34⋆
U46619		
Control	315.9±28.9	−8.22±0.07
Aprikalim (1 μM)	316.4±31.7	−8.40±0.10
Aprikalim (30 μM)	385.2±71.7	−7.87±0.06⋆
Phenylephrine		
Control	140.9±17.8	−5.58±0.19
Aprikalim (1 μM)	76.6±12.6⋆	−4.54±0.24⋆⋆
Aprikalim (30 μM)	49.3±14.1⋆⋆	#

†EC_{50} for K^+ is in mM. ⋆$P<0.05$, ⋆⋆$P<0.01$, ANOVA among three groups and scheffe's F-test compared with the control.
#The contraction in some rings was totally inhibited so no EC_{50} was available.

inadequate for maximal exercise [21] and hence causes a hypoperfusion syndrome occuring early in the patient's course; this situation may be worsened by high-dose-vasopressor therapy that could further reduce arterial graft flow [21, 22]. In addition, spasm of the arterial graft may occur [23] and antispastic drugs are necessary for arterial grafts during surgery [24, 25]. Therefore, there is a continuous search for appropriate vasodilators for arterial grafts.

The IMA has been demonstrated to be reactive to a variety of vasoconstrictors [7, 8, 9, 13]. In the present study, K^+, TXA_2 mimetic U46619, and α_1-adrenoceptor agonist PE were used to contract the IMA. During cardiopulmonary bypass TXA_2 has been measured and plasma concentrations increased [26]. This potent vasoconstrictor may constrict

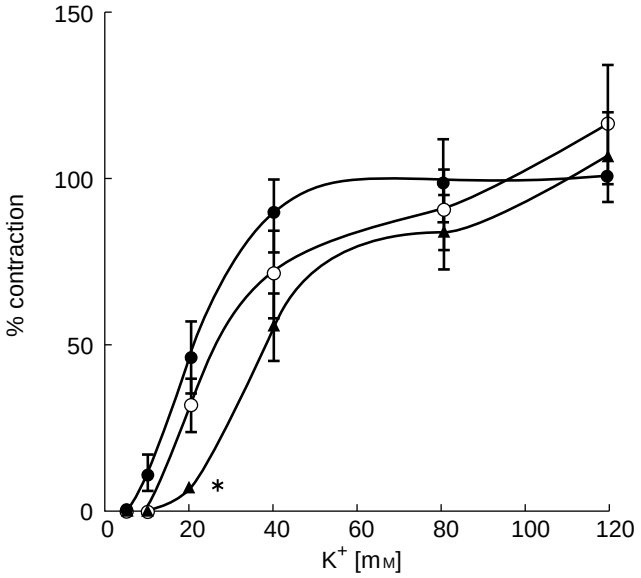

Figure 4 Mean concentration (−log M)-contraction (percentage of 100 mM K^+-induced contraction) curves for K^+. Control (●, $n=8$): without pretreatment of aprikalim. APK −6 (○, $n=6$) or APK −4.5 (▲, $n=6$): aprikalim (−6 or −4.5 log M) was added into the organ bath 10 min before K^+. Vertical error bars are 1 s.e.mean $P=0.2$ among the three groups at the maximal contraction (ANOVA). *$P=0.01$ among the three groups (ANOVA) and $P=0.01$ between the control and the rings treated with aprikalim −4.5 log M (Scheffe F-test).

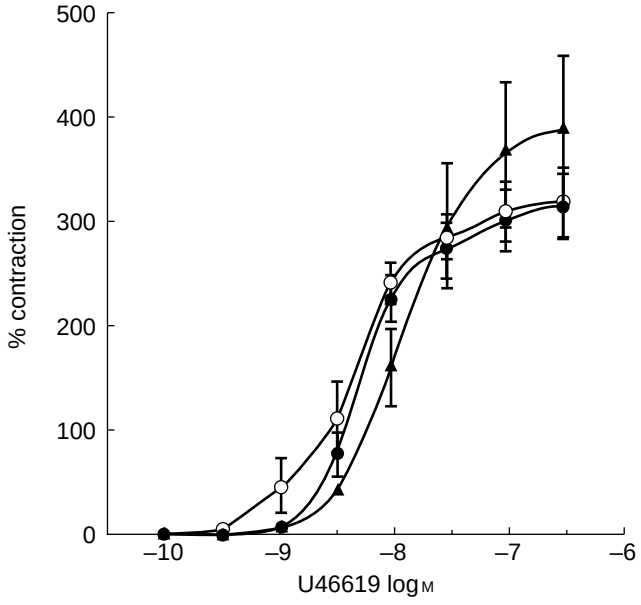

Figure 5 Mean concentration (−log M)-contraction (percentage of 100 mM K^+-induced contraction) curves for U46619. Control (●, $n=6$): without pretreatment of aprikalim. APK −6 (○, $n=6$) or APK −4.5 (▲, $n=6$): aprikalim (−6 or −4.5 log M) was added into the organ bath 10 min before U46619. Vertical error bars are 1 s.e.mean. $P=0.5$ among the three groups at the maximal contraction (ANOVA).

arterial grafts during perioperative period. In addition, exogenous and endogenous sympathomimetic amines have been demonstrated to be spasmogens for arteries [27, 28] and the α_1-adrenoceptor has been demonstrated to be predominant in the human IMA [8, 9].

From this study, aprikalim has a vasorelaxant effect in conduit arteries. It relaxed the contraction induced by all of the three vasoconstrictors to a certain extent (Figure 1). The maximal relaxation was significantly greater for U46619 and PE than for K^+ ($P<0.05$). This demonstrates vasoconstrictor-selective relaxation. In addition, there was a significant difference ($P<0.05$) with regard to the EC_{50}, which was 2.6-fold higher for K^+ than for U46619 and 10.7-fold higher for K^+ than for PE ($P<0.05$). These results show that the vasorelaxant effect of aprikalim is selective with greater potency to receptor-mediated than depolarizing-mediated contraction. To our knowledge, such a vasoconstrictor-selective relaxant effect of aprikalim has not been reported previously. Theoretically, the mechanism of KCO-induced relaxation is to repolarize or hyperpolarize the smooth muscle membrane. In contrast, K^+ depolarizes the membrane. It is therefore possible that the K^+-induced contraction, due to higher membrane potential [4, 5], may make it more difficult for subsequent KCOs to repolarize or hyperpolarize the membrane [4, 5]. In our study, there is evidence that the contraction induced by a low-concentration of K^+ (at 20 mM) was effectively inhibited by aprikalim (Figure 4). To ensure that the incomplete vasorelaxant effect of aprikalim in the human IMA is not due to our methodology, we also tested its effect in porcine coronary arteries. In these experiments, aprikalim fully relaxed the coronary artery (100%) contracted by U46619 (30 nM) and relaxed the coronary artery to 75% in K^+ (25 mM)-induced contraction (data not shown). Therefore, the incomplete relaxation seen in the human IMA by aprikalim is due to the vessel-selective effect of aprikalim.

Further evidence for the vasoconstrictor-selective effect of aprikalim from our study is the selective inhibition of contraction by pretreatment with aprikalim. The maximal contraction induced by either K^+ or U46619 was not reduced by the pretreatment of aprikalim even at a high concentration (30 μM) although there was a rightward shift of the concentration-contraction curve (Figures 4 and 5), which suggests the inhibitory effect of aprikalim on the contraction (Figures 4 and 5, Table 1). In contrast, pretreatment with aprikalim, either at 1 or 30 μM, significantly depressed the maximal contraction to PE in addition to the rightward shift of the curve (Figure 3). These results demonstrate that although pretreatment with aprikalim has inhibitory effects on all three vasoconstrictors, it selectively inhibits α_1-adrenoceptor-mediated contraction with a greater potency.

Aprikalim is suggested to be a K_{ATP} opener [1–3]. In the present study, glibenclamide, a specific K_{ATP} inhibitor, significantly inhibited the relaxation induced by aprikalim in either K^+ or PE-induced contraction (Figure 2). This suggests that the relaxation effect of aprikalim is mainly related to the K_{ATP}.

Since aprikalim has not been used in CABG patients, the direct clinical implications of the findings from the present study are unclear. However, our study provides some basic information for the future use of this KCO in various situations in which the use of KCOs may be indicated.

K_{ATP} openers were initially developed for antihypertensive therapy [2]. It was then discovered that KCOs can protect the ischemic myocardium at concentrations that cause very little cardiodepression [2]. This is probably due to the

findings that K_{ATP} is involved in vasodilation during hypoxia [29] and that K_{ATP} openers inhibit the development of myocardial contracture, reduce the release of lactate dehydrogenase, and preserve intracellular ATP content during ischaemia [30–33]. Therefore, apart from its vasorelaxant effect which may be clinically useful, KCOs have a potential advantage for cardiac protection in myocardial infarction [34] or as cardioplegia during open-heart surgery [35]. Those potential clinical usages of aprikalim may enhance the importance of the findings from the present study.

In conclusion, the results of our study suggest that aprikalim has vasorelaxant effects in human conduit arteries contracted by a variety of vasoconstrictors and its effects are vasoconstrictor-selective with greater potency to α_1-adrenoceptor agonists than to the depolarizing agent K^+. These findings provide information for the possible use of this KCO in various clinical settings.

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