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Effects of KRN2391 on ionic currents in rabbit femoral arterial myocytes

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1 The effects of KRN2391, an ATP-sensitive K^+ channel opener (KCO) which also acts as a nitrate, on ionic membrane currents in rabbit femoral arterial myocytes were examined.

2 Under whole-cell clamp conditions where cells were superfused with physiological salts solution containing 5.9 mM K^+, KRN2391 elicited an outward current at a holding potential of −30 mV. KRN2391-induced current had a reversal potential of −78 mV and was abolished by glibenclamide (glib). KRN2391 was approximately 25 times more potent than nicorandil to activate an ATP-sensitive K^+ current (I_{KATP}). On the other hand, 10 μM KRN2391 did not affect either voltage-dependent Ca^{2+} or delayed rectifier K^+ channel currents.

3 In the inside-out patch configuration, KRN2391 activated 47 pS K^+ channels in the presence of nucleotide diphosphates (NDPs) under the symmetrical 140 mM K^+ conditions. Glib and intracellular ATP reversibly inhibited the activity of the 47 pS K^+ channels.

4 The 47 pS K^+ channels activated by KRN2391 are similar in their conductance and other properties to NDP-sensitive K^+ channels (K_{NDP} channels) described in other smooth muscles and the cloned channels. KRN2391 is a potent activator of the 47 pS K^+ channels and the activation can contribute to the KRN2391-induced vasodilation in arterial muscles.

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Keywords: K^+ channel opener; KRN2391; ATP-sensitive K^+ channels; rabbit femoral artery; nucleotide diphosphates

Abbreviations: ADP, adenosine diphosphate; 4-AP, 4-aminopyridine; ATP, adenosine triphosphate; BK channels, large conductance Ca^{2+}-dependent K^+ channels; gK, single K^+ channel conductance; glib, glibenclamide; I_{Ba}, inward Ba^{2+} current through VDCCs; $I_{K\text{-}ATP}$, ATP-sensitive K^+ current; I_{Kd}s, delayed rectifier K^+ currents; K_{ATP} channels, ATP-sensitive K^+ channels; KCO, ATP-sensitive K^+ channel opener; K_{NDP} channels, NDP-sensitive K^+ channels; KRN2391, N-cyano-N′-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethansulphonate; NDPs, nucleotide diphosphates; NPo, open-state probability of channels; RFAMs, rabbit femoral arterial myocytes; TEA, tetraethylammonium; UDP, uridine diphosphate; VDCCs, voltage-dependent Ca^{2+} channels

Introduction

Opening of K^+ channels in vascular smooth muscle cells causes membrane hyperpolarization toward the equilibrium potential of K^+, resulting in closure of voltage-dependent Ca^{2+} channels (VDCCs) and hence vasodilation (Nelson & Quayle, 1995; Lawson, 1996; Quayle *et al*., 1997; Kuriyama *et al*., 1998). A number of synthetic openers of ATP-sensitive K^+ channels (K_{ATP} channels) KCOs, have been developed for a clinical use in the treatment of systemic hypertension, coronary angina pectoris and congestive heart diseases (Weston, 1989; Ligtenberg *et al*., 1995; Lawson, 1996). Nicorandil, a pyridine derivative type KCO, is more beneficial than other KCOs because nicorandil is a balanced arterial- and veno-dilator with a hemodynamic profile which should make it effective for the treatment of coronary angina (Knight *et al*., 1995). KRN2391, N-cyano-N′-(2-nitroxyethyl)-3-pyridinecarboximidamide monomethansulphonate, is a novel vasodilator which has both a K^+ channel opening action as a KCO and a stimulatory effect on guanylate cyclase as a nitrate (Kashiwabara *et al*., 1991; Miwa *et al*., 1993; Ogawa, 1994). Since KRN2391 reduces a vascular tone more potently than nicorandil, it is expected that KRN2391 has more advantages than nicorandil for clinical uses (Ogawa, 1994). However, despite a large number of studies, a target channel of KRN2391 has not yet been elucidated.

Based upon biophysical and pharmacological investigations, it has been proposed that two types of K_{ATP} channels which are sensitive to KCOs are present in smooth muscle cells (Quayle *et al*., 1997): small and intermediate conductance K_{ATP} channels (30–50 pS) and large conductance K_{ATP} channels (100–150 pS). NDPs such as ADP, UDP and GDP effectively activate the small and intermediate K_{ATP} channels, and have been described as K_{NDP} channels (Beech *et al*., 1993a; Fujita & Kurachi, 2000). Moreover, it has been found that a channel subunit, Kir 6.1, could form a smooth muscle K_{NDP} channel with a member of sulphonylurea receptor, SUR2B (Satoh *et al*., 1998; Yamada *et al*., 1997; Fujita & Kurachi, 2000). It is of importance to identify and characterize the properties of the channels which are sensitive to KRN2391 and to compare them with those of K_{ATP} channels in various smooth muscle clones. In the present study, effects of KRN2391 on ionic membrane currents in rabbit femoral arterial myocytes (RFAMs) were examined

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and the potency to activate ATP-sensitive K^+ current ($I_{K\text{-}ATP}$) was compared with that of nicorandil. The K_{ATP} channel which was a target of KRN2391 was also identified for the first time and the properties were compared with those of K_{ATP} channels in smooth muscles and the cloned channels.

Methods

Cell isolation

All experiments were carried out in accordance with guiding principles for the care and use of laboratory animals (the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture) and also with the approval of the ethics committee in Nagoya City University. Single smooth muscle cells were enzymatically isolated from the femoral artery of the rabbit. The procedures for cell-isolation were similar to those previously described (Imaizumi *et al*., 1989). Briefly, male New Zealand White rabbits, weighing 1.5–2.0 kg, were anaesthetized with sodium thiopental (70 mg kg^{-1} i.v.). The isolated femoral artery (diameter: approximately 100 μm) was cleaned of fat and connective tissues, and cut open longitudinally. Two pieces of arterial segments were incubated in Ca^{2+}, Mg^{2+}-free Krebs' solution (no EGTA) for 20 min at 37°C and then placed in the Ca^{2+}, Mg^{2+}-free Krebs' solution containing 0.4% collagenase, 0.1% papain, 0.1% dithiothreitol and 0.1% trypsin inhibitor for 15–20 min. After the solution was replaced with Ca^{2+}, Mg^{2+}-free and enzyme-free Krebs' solution, the segments were gently agitated with a glass pipette having a fire-polished tip.

Electrical recordings

Whole-cell or single channel current recordings were made from a single arterial myocyte or a membrane patch, respectively, using an EPC-7 (List, Germany) or CEZ-2300 amplifier (Nihon-Koden, Tokyo, Japan). The resistance of microelectrodes filled with the pipette solution was approximately 2–5 MΩ for whole-cell and inside-out patch configurations. The series resistance was partially compensated electrically under the whole-cell clamp conditions. The cell capacitance value was determined from the capacity current elicited by a square hyperpolarizing voltage step from −60 to −70 mV. All experiments were carried out at room temperature (25±1°C).

Data storage and analysis

Membrane currents and voltage signals were monitored on a storage oscilloscope (VC-6041, Hitachi Tokyo, Japan) and stored on videotapes after digitized by PCM-recording system (modified to get DC signal, PCM 501ES; SONY, Tokyo, Japan). The data on the tape were replayed later and loaded into a computer (IBM-AT compatible machine) through an A-D converter (Data translation, DT2801A). Data-acquisition and analysis for whole-cell and single channel currents were carried out using AQ/Cell-soft, developed in the laboratory of Dr Wayne Giles, University of Calgary, and Single channel current analysis program V7.0C (PAT) developed by Dr John Dempster, University of Strathclyde, respectively. Membrane current signals were occasionally printed out using a thermal array recorder (RTA-1200; Nihon-Koden, Tokyo, Japan). In some experiments, ramp waveform pulses were applied as a voltage-clamp command using a multi-pulse generator (FS-1915; NF Electronics, Tokyo, Japan). All current records were filtered at 1 kHz (4-pole Bessel filter, NF Electronics).

The relative open-state probability of channels (NPo) was calculated using the following equation:

$$NPo = \sum_{i=0}^{N} (i^* t_i)/T$$

where i is the number of channels open, t_i is the time spent by i channels in the open state, N is the maximum number of open channels observed in the patch and T is the sampling time. Since we did not define the total number of channels present in each patch membrane, we assumed the maximum number of unitary current levels observed in a patch to be equal to the number of active channels in the patch. The single channel data were sampled into a computer by using the PAT program. Single channel events were detected using a half amplitude criterion and the all-point amplitude histogram was fitted with the Gaussian distribution function.

Solutions

Ca^{2+}, Ma^{2+}-free Krebs' solution for cell-isolation (mM): NaCl 112, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 14. The Ca^{2+}, Mg^{2+}-free Krebs' solution was gassed with mixture of 95% of O_2 and 5% CO_2. External solution for whole-cell recording (mM): NaCl 137, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.2, glucose 14, HEPES 10. To record inward Ba^{2+} current through VDCCs (I_{Ba}), 45 mM NaCl in the external solution was replaced with 30 mM $BaCl_2$. In some experiments, high K^+ external solution (140 mM) was used to obtain a large $I_{K\text{-}ATP}$. External solution for inside-out patch configuration (mM): NaCl 2.9, KCl 140, $MgCl_2$ 1.2, glucose 14, HEPES 10, EGTA 1. The pH in each external solution was adjusted to 7.4 with 10 N NaOH. Pipette solution for whole-cell recording (mM): KCl 140, $MgCl_2$ 4, HEPES 10, ATP-2Na 1, EGTA 10. When delayed rectifier K^+ currents (I_{kd}s) were recorded, concentration of ATP in the pipette solution was increased to 5 mM to reduce $I_{K\text{-}ATP}$. To record I_{Ba}, 140 mM KCl was replaced with equimolar CsCl and 5 mM ATP was added to the pipette solution. Pipette solution for inside-out patch configuration (mM): NaCl 2.9, KCl 140, $MgCl_2$ 1.2, glucose 14, HEPES 10, $CaCl_2$ 2.2. In some experiments, to reduce the activity of large conductance Ca^{2+}-dependent K^+ channels (BK channels) or VDCCs, 100–300 nM iberiotoxin or 0.1 mM $CdCl_2$, respectively, was added to the pipette solution. The pH of pipette solutions was adjusted to 7.2 using 10 N KOH.

Drugs

The following drugs were used in this study: KRN2391 (supplied from Kirin Brewery Corp., Tokyo, Japan), nicorandil (Kirin Brewery Corp., Tokyo, Japan), glibenclamide (glib, Sigma, St Louis, U.S.A.), tetraethylammonium (TEA, Tokyo Kasei, Tokyo, Japan), 4-aminopyridine (4-AP, Tokyo Kasei,

Tokyo, Japan), adenosine diphosphate (ADP, Oriental, Tokyo, Japan), adenosine triphosphate (ATP, Oriental, Tokyo, Japan), uridine diphosphate (UDP, Yamasa, Tokyo, Japan), iberiotoxin (Peptide Inc., Osaka, Japan).

Statistics

Pooled data are expressed as mean ± s.e.mean in text and figures, and *n* indicates number of cells used. Statistical significance was examined using Student's *t*- or Scheffe's test for two or multiple groups, respectively. In figures, * and ** indicate statistical significance at *P* values of 0.05 and 0.01, respectively.

Results

KRN2391-induced membrane current in RFAMs

Effects of KRN2391 on a holding current across the cell membrane were examined in RFAMs under a physiological gradient of K^+ concentration (5.9 : 140 mM) in Figure 1. When a RFAM was voltage-clamped at a holding of −30 mV, application of 10 μM KRN2391 elicited a slowly developing outward current ($I_{KRN2391\text{-}out}$, left panel in Figure 1, 44.4 ± 8.1 pA at −30 mV, $n=11$). Addition of 10 μM glib, a specific inhibitor of K_{ATP} channels, to the external solution abolished the $I_{KRN2391\text{-}out}$. To obtain a current and voltage (I–V) relationship of $I_{KRN2391\text{-}out}$, a triangular ramp waveform was applied as voltage command pulses before and during the activation of the current (see the inset in Figure 1). After subtracting the current obtained at the time indicated by '1' from that by '2', $I_{KRN2391\text{-}out}$ was plotted against ramp potentials as shown in the right panel in Figure 1. $I_{KRN2391\text{-}out}$ reversed at −78.3 ± 3.9 mV ($n=5$), suggesting that $I_{KRN2391\text{-}out}$ is caused by activation of a K^+ current. In addition, effective inhibition of $I_{KRN2391\text{-}out}$ by glib indicates that $I_{KRN2391\text{-}out}$ is an $I_{K\text{-}ATP}$.

In Figure 2, the potency of KRN2391 to activate $I_{K\text{-}ATP}$ was compared with that of nicorandil. Since the amplitude of $I_{KRN2391\text{-}out}$ under the physiological K^+ gradient was not large enough at −60 mV for the quantitative analysis, K^+ concentration in the external solution was elevated to 140 mM. Cumulative application of nicorandil or KRN2391 at the concentration ranges of 10–1000 μM or 0.01–30 μM, respectively, elicited inward currents in a concentration-dependent manner (Figure 2Aa, Ab). These inward currents were abolished by the addition of 10 μM glib. Figure 2Ba shows concentration-response relationships of nicorandil and KRN2391. The current density was obtained by dividing the amplitude of the inward current with that of each cell-capacitance and plotted against concentrations of drugs. A maximum current density induced by nicorandil was not significantly different from that by KRN2391 (9.2 ± 2.6 pA pF^{-1}, $n=6$ vs 7.0 ± 1.7 pA pF^{-1}, $n=7$, $P>0.05$). However, apparent EC_{50}s of nicorandil and KRN2391 to activate the inward current were 50 and 2 μM, respectively, indicating that KRN2391 was approximately 25 times more potent than nicorandil (Figure 2Bb).

Effects of KRN2391 on I_{Kd}s and I_{Ba} were also examined in RFAMs. In the presence of 0.1 mM Cd^{2+}, a voltage jump from a holding potential of −60 mV to potentials between −70 and +40 mV (for 300 ms) elicited outward currents which were assumed to be I_{kd}s. The treatment with 10 μM KRN2391 had little effect on I_{Kd}s (−10 mV: 104.4 ± 26.4 pA vs 107.1 ± 22.0 pA, +10 mV: 207.3 ± 54.2 pA vs 219.0 ± 47.8 pA, +30 mV: 327.4 ± 87.3 pA vs 347.1 ± 74.9 pA in the presence and absence of 10 μM KRN2391, respectively, $P>0.05$), whereas TEA and 4-AP, non-selective K^+ channel blockers, significantly inhibited the I_{kd}s (not shown). Moreover, the voltage-dependent Ca^{2+} channel current which was measured as I_{Ba} was not affected by 10 μM KRN2391 (I_{Ba} at +10 mV, 1.02 ± 0.07 of the control, $n=5$, $P>0.05$).

Single channel current activated by KRN2391 in RFAMs

Characteristics of the single channel currents activated by KRN2391 are shown in Figure 3. After the inside-out patch configuration was established under the symmetrical high K^+

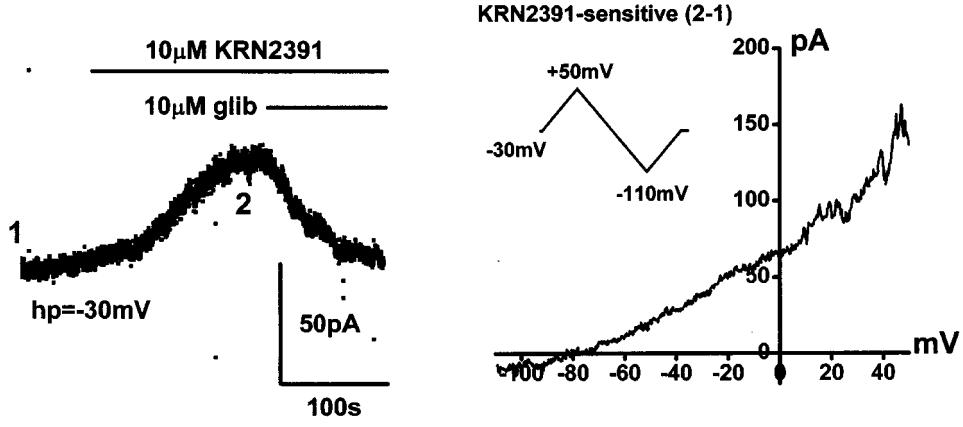

Figure 1 KRN2391-induced outward current in RFAMs. At a holding potential of −30 mV, 10 μM KRN2391 was applied to a cell in the physiological salt solution containing 5.9 mM K^+ followed by 10 μM glib. The pipette was filled with solution which mainly contained 140 mM KCl and 1 mM ATP. The left panel shows a typical current trace. Before and during the activation of the outward current, triangular ramp waveform pulses (see the inset in right panel) were applied at the time indicated by 1 and 2. A KRN2391-sensitive current was obtained by subtracting the current recorded at 1 from that at 2 and plotted against the ramp potentials (the right panel).

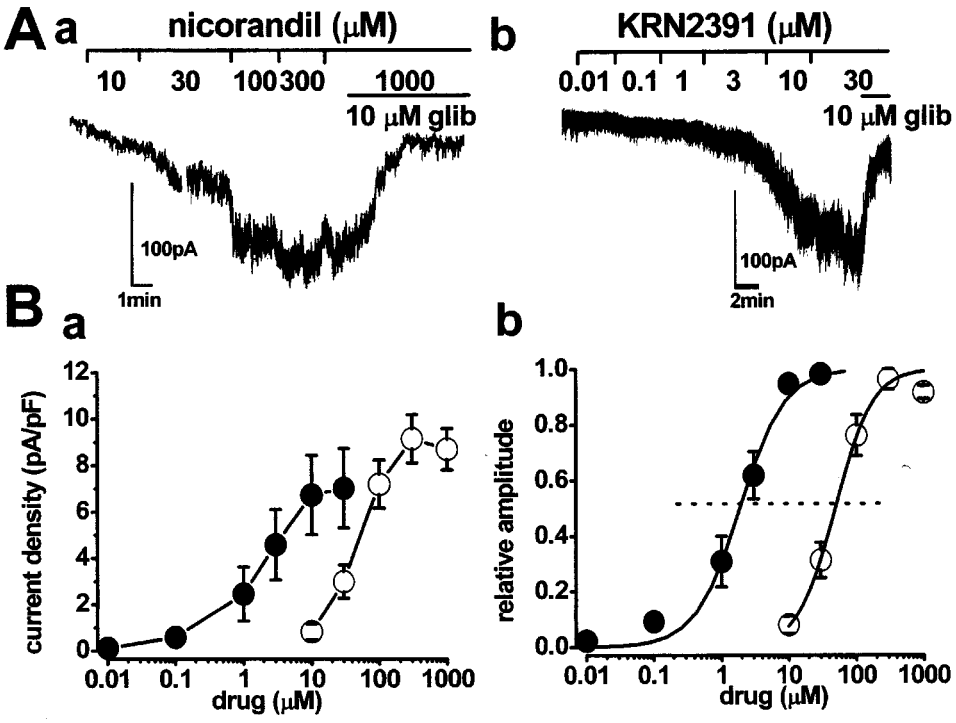

Figure 2 Activation of $I_{K\text{-}ATP}$ by nicorandil and KRN2391 in high K^+ solution. Pipette and external solution contained mainly 140 mM K^+. Nicorandil (Aa) or KRN2391 (Ab) was cumulatively applied and thereafter, 10 μM glib was added to the external solution. The holding potential was -60 mV. (B) Concentration-response relationships of nicorandil (open circles) and KRN2391 (closed circles). Current densities obtained by dividing the current amplitude with each cell-capacitance were plotted against drug-concentrations (a). The relative current densities normalized to the maximum were expressed in (b).

conditions (140 : 140 mM K^+), the simultaneous application of 10 μM KRN2391 and 1 mM ADP activated a single channel current whose unitary amplitude was approximately 2 pA at -40 mV (a in Figure 3A). In the lower panel in Figure 3A, the channel activity was expressed as NPo against time. The recordings shown in Figure 3A (upper panel) were obtained at the time shown correspondingly by a, b, c and d in the lower panel. Exposure to 10 μM KRN2391 without ADP reduced the channel activity to low levels (b in Figure 3A) and reapplication of both KRN2391 and ADP restored the activity (c in Figure 3A). The withdrawal of KRN2391 in the presence of 1 mM ADP abolished the channel activity (d in Figure 3A).

In Figure 3B, the channel activity under various experimental conditions is summarized. NPo over 30 s was calculated and the pooled data averaged. Under control conditions in the absence of either KRN2391 or a NDP, the channel activity was consistently low (NPo: 0.00032 ± 0.00032, $n=5$). Moreover, the application of 10 μM KRN2391 alone had little effect on the channel activity (NPo: 0.00039 ± 0.00039, $n=7$). Addition of 1 mM ADP in the presence of KRN2391 markedly increased the NPo (0.14 ± 0.05, $n=6$; $P<0.05$ vs control). The addition of 1 mM UDP in the presence of KRN2391 was also effective in a manner similar to that of ADP (0.24 ± 0.06, $n=3$, $P<0.01$ vs control). In contrast, 1 mM ADP itself did not affect channel activity in the absence of KRN2391 (NPo: 0.0023 ± 0.0023, $n=2$).

Figure 4 shows single channel current recordings at different potentials (A) and the I–V relationships of the KRN2391-sensitive channel (B). Both 10 μM KRN2391 and 1 mM NDP (ADP or UDP) were added to the external high K^+ solution. The amplitude of the unitary current was plotted against each holding potential and the single channel conductance (gK) was determined by fitting the data points to a regression line. Averaged gKs were 47.0 ± 1.9 pS ($n=5$); 47.4 ± 2.6 pS ($n=3$; $P>0.05$), when cells were superfused with solution containing KRN2391 plus either 1 mM ADP or UDP, respectively. Additionally, both I–V relationships were reversed at around 0 mV.

Effects of glib and ATP on the activity of the KRN2391-sensitive K^+ channel were examined in the inside-out patch configuration (Figure 5). The same pipette and external solutions which mainly contained 140 mM K^+ as those in Figures 3 and 4 were used. As shown in Figure 5Aa, the presence of 10 μM KRN2391 and 1 mM UDP induced the channel activity whose unitary current was approximately 1.3 pA at a holding potential of $+20$ mV. Occasionally, transient openings of BK channels which had a large unitary current (>5 pA) were detected (large transient lines in the upper panel in Figure 5A). In a lower panel in Figure 5A, the channel activity expressed as NPo was plotted against time. Upon the addition of 10 μM glib to the external solution, the channel activity almost disappeared (Figure 5Ab). However, the withdrawal of glib followed by the reapplication of 10 μM KRN2391 and 1 mM UDP reactivated the channel (Figure 5Ac). On the other hand, as illustrated in Figure 5Bb, the addition of intracellular 1 mM ATP reduced the channel activity, which was stimulated by the presence of 10 μM KRN2391 and 1 mM UDP. The removal of ATP restored the channel activity (Figure 5Bc).

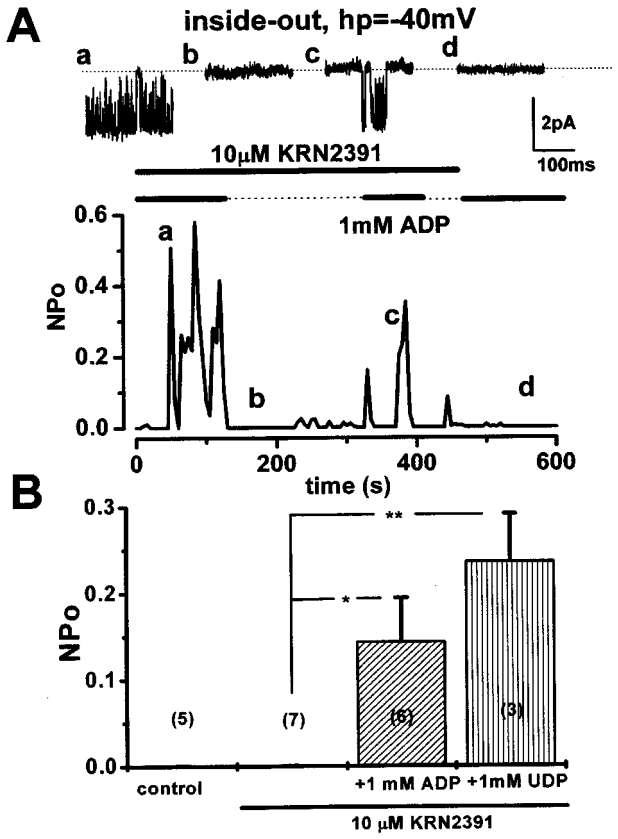

Figure 3 (A) KRN2391-sensitive 47 pS K^+ channel current in RFAMs. After the establishment of inside-out patch configuration, 10 μM KRN2391 and 1 mM ADP were applied to a membrane patch voltage-clamped at -40 mV. The upper panel shows the single channel current traces recorded at the corresponding time indicated by a, b, c and d in the lower panel. (B) Summarized data describing the activity of KRN2391-sensitive 47 pS K^+ channels under various experimental conditions. Number in parentheses indicates the number of cells used.

Discussion

The present study clearly shows that KRN2391 activates glib-sensitive currents approximately 25 times more potently than nicorandil in RFAMs. KRN2391, however, had neither an effect on I_{Ba} through VDCCs nor on I_{Kd}s. A target K^+ channel activated by KRN2391 is susceptible to glib, NDPs and ATP, strongly suggest that KRN2391-sensitive K_{ATP} channels have similar biophysical and pharmacological properties to those of K_{NDP} channels in other smooth muscles.

KRN2391-induced outward currents in RFAMs

It had been expected that KRN2391 would open K_{ATP} channels as a KCO and dilate vascular smooth muscles (Kashiwabara *et al*., 1991; Miwa *et al*., 1993; Ogawa, 1994). The KRN2391-induced vasodilation was effectively blocked by glib (Kashiwabara *et al*., 1991; Ogawa, 1994). It has been reported that KRN2391-induced relaxation was more extensive when vessels were pre-contracted with hormonal agonists than when pre-contracted with high K^+ solution (Kashiwabara *et al*., 1991). Additionally, an increase in $^{86}Rb^+$-efflux from vessels and the hyperpolarization of cells have been observed in the presence of KRN2391 (Kashiwabara *et al*., 1991; Okada *et al*., 1993). In the present study, under the whole-cell clamp conditions where cells were superfused with the solution containing 5.9 mM K^+, application of KRN2391 elicited an outward current at the holding potential of -30 mV. The outward current had a reversal potential of -78 mV which was close to an equilibrium potential of K^+ under the physiological conditions and 0 mV in symmetrical high K^+ solution, and was abolished by an exposure to glib, directly indicating that KRN2391 activates $I_{K\text{-}ATP}$ and acts as a KCO in rabbit femoral artery.

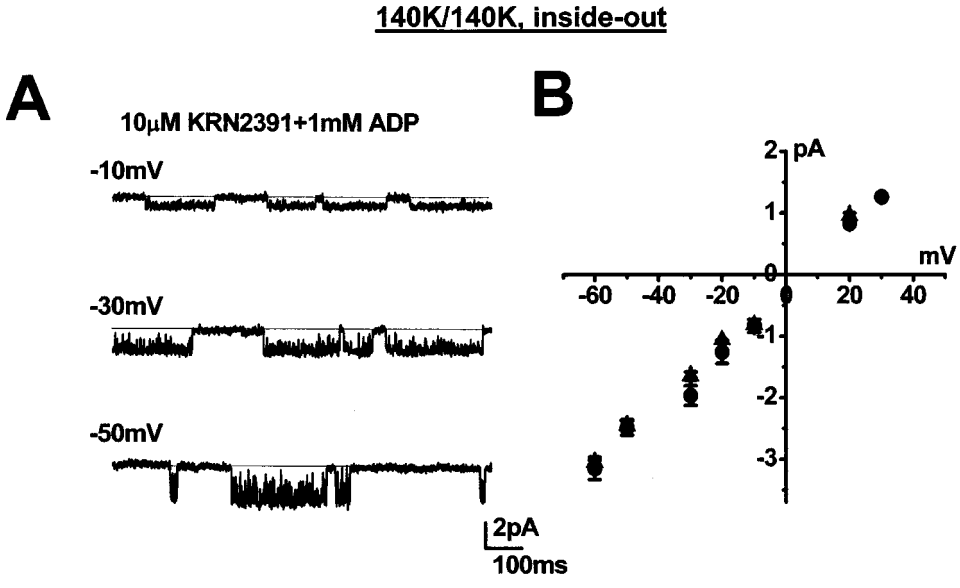

Figure 4 I–V relationships of KRN2391-sensitive 47 pS K^+ channels in the inside-out patch configuration. In the presence of 10 μM KRN2391 and NDPs (1 mM ADP: circles, 1 mM UDP: triangles), single channel currents were recorded at various holding potentials (A) and these amplitudes plotted against each potential (B).

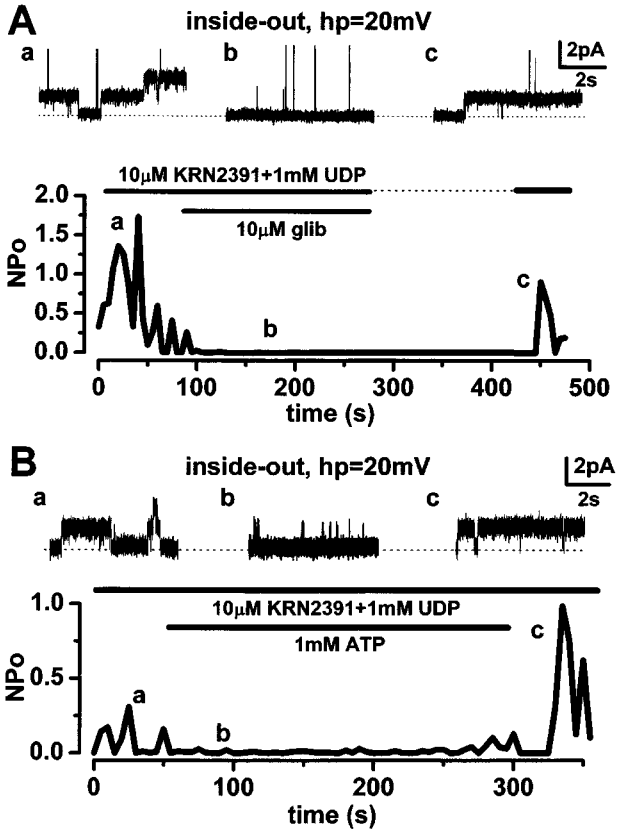

Figure 5 Effects of glib (A) and ATP (B) on the activity of KRN2391-sensitive 47 pS K^+ channels. The channel activity was induced by application of 10 μM KRN2391 and 1 mM UDP in the inside-out-patch configuration. The lower panels in (A) and (B) indicate NPo over 30 s, which was plotted against time. The traces illustrated in the upper panels were obtained at the corresponding times indicated by a, b and c in the lower panels, respectively.

In porcine coronary artery, the relaxing effect of KRN2391 against agonist-induced contractions was 10–20 times greater than that of nicorandil (Fukata *et al*., 1993; Kasai *et al*., 1993; Jinno *et al*., 1992; Miwa *et al*., 1993). In the present study, KRN2391 and nicorandil elicited a glib-sensitive current in a concentration-dependent manner. The maximum amplitude of the current obtained in the presence of KRN2391 was similar to that of nicorandil (7–9 pA pF^{-1}), whereas EC_{50}s of KRN2391 and nicorandil were approximately 2 and 50 μM, respectively, clearly indicating that KRN2391 is 25 times more potent than nicorandil to activate $I_{K\text{-}ATP}$ as a KCO.

No inhibitory effects of KRN2391 on I_{Ba} *and* I_{Kd}*s*

Cromakalim, the first KCO, has inhibitory effects on VDCCs and I_{kd}s in vascular smooth muscle cells (Okabe *et al*., 1990). It is, therefore, possible that multiple actions of KRN2391 on ion channels contribute to its various pharmacological effects on the vasculature. On the other hand, 10 μM KRN2391 did not affect I_{Ba} under the conditions where 30 mM Ba^{2+} was used as the charge carrier through VDCCs. Moreover, I_{Kd}s were not affected by treatment with 10 μM KRN2391. However, we cannot completely rule out the possibility that KRN2391 acts on I_{kd}s because a part of $I_{K\text{-}ATP}$ activated in the presence of KRN2391 may mask the effect on I_{kd}s under the present experimental conditions even though 5 mM ATP was added to the pipette solution.

Characteristics of KRN2391-sensitive single channel current

K_{ATP} channels which were originally found in cardiac myocytes are widely distributed in tissues including pancreatic β-cells, skeletal muscle, smooth muscle and neurons. Despite the similarity in pharmacology, K_{ATP} channels in vascular smooth muscle exhibits different single channel characteristics and a distinct mechanism of nucleotide regulation (Kajioka *et al*., 1991; Beech *et al*., 1993a,b; Zhang & Bolton, 1996; Fujita & Kurachi, 2000). Vascular K_{ATP} channels have less than one-half of the single channel conductance of the classical K_{ATP} channels observed in cardiac and skeletal muscle cells or pancreatic β-cells. Moreover, the classical K_{ATP} channels open spontaneously when intracellular ATP is removed, while the vascular K_{ATP} channels require NDPs to open. Consistently, K_{ATP} channels in RFAMs had a 47 pS conductance under the symmetrical 140 mM K^+ conditions and their activity was NDP-dependent, indicating that K_{ATP} channels in RFAMS are a certain type of K_{NDP} channels. Although such differences between the vascular and the classical K_{ATP} channels causes some confusion about the identity of the vascular K_{ATP} channels, it has been found that a channel subunit, Kir 6.1, may form a smooth muscle K_{NDP} channel with a member of sulphonylurea receptor, SUR2B (Satoh *et al*., 1998; Yamada *et al*., 1997; Fujita & Kurachi, 2000). Co-expression of SUR2B and Kir6.1 formed a functional K^+ channel with the features of a K_{NDP} channel: a single channel conductance of 33 pS in symmetric 145 mM K^+ solution, NDP-dependent activation and no spontaneous activation in the absence of internal ATP (Yamada *et al*., 1997; Satoh *et al*., 1998).

Pharmacological properties of K_{ATP} channels that respond to KCOs may be determined by SURs coupled to a Kir channel subunit. When the Kir6.2 subunit was co-expressed with SUR2A or SUR2B, the EC_{50} of nicorandil in activating the K_{ATP} channels was 500 and 10 μM, respectively (Shindo *et al*., 1998). On the other hand, the EC_{50} of nicorandil to activate $I_{K\text{-}ATP}$ in RFAMs was 50 μM. Hence, it is expected that mainly SUR2B is expressed in RFAMs, while expression of different SUR isoform cannot be ruled out. Further molecular biological studies are necessary to elucidate the type of Kir channel as well as SUR subunits in RFAMs.

In conclusion, KRN2391 potently activates $I_{K\text{-}ATP}$ in rabbit femoral artery, whereas KRN2391 affects neither voltage-dependent Ca^{2+} nor delayed rectifier K^+ channel currents. The potency of KRN2391 as a KCO is significantly higher (approximately 25 times) than that of nicorandil. The 47 pS K^+ channels which are activated by KRN2391 are susceptible to glib, ATP and NDPs, and therefore underlie the $I_{K\text{-}ATP}$ observed under whole-cell clamp conditions. The potent opening of K_{ATP} channels by KRN2391 most likely contributes to the vasodilation by KRN2391.

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