British Journal of Pharmacology (1998) 124, 985–991
http://www.stockton-press.co.uk/bjp

SUR2 subtype (A and B)-dependent differential activation of the cloned ATP-sensitive K^+ channels by pinacidil and nicorandil

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1 The classical ATP sensitive K^+ (K_{ATP}) channels are composed of a sulphonylurea receptor (SUR) and an inward rectifying K^+ channel subunit (BIR/Kir6.2). They are the targets of vasorelaxant agents called K^+ channel openers, such as pinacidil and nicorandil.

2 In order to examine the tissue selectivity of pinacidil and nicorandil, *in vitro*, we compared the effects of these agents on cardiac type (SUR2A/Kir6.2) and vascular smooth muscle type (SUR2B/Kir6.2) of the K_{ATP} channels heterologously expressed in HEK293T cells, a human embryonic kidney cell line, by using the patch-clamp method.

3 In the cell-attached recordings (145 mM K^+ in the pipette), pinacidil and nicorandil activated a weakly inwardly-rectifying, glibenclamide-sensitive 80 pS K^+ channel in both the transfected cells.

4 In the whole-cell configuration, pinacidil showed a similar potency in activating the SUR_{2B}/Kir6.2 and SUR_{2A}/Kir6.2 channels (EC_{50} of $\sim$2 and $\sim$10 μM, respectively). On the other hand, nicorandil activated the SUR_{2B}/Kir6.2 channel $>$100 times more potently than the SUR_{2A}/Kir6.2 (EC_{50} of $\sim$10 μM and $>$500 μM, respectively).

5 Thus, nicorandil, but not pinacidil, preferentially activates the K_{ATP} channels containing SUR_{2B}. Because SUR_{2A} and SUR_{2B} are diverse only in 42 amino acids at their C-terminal ends, it is strongly suggested that this short part of SUR_{2B} may play a critical role in the action of nicorandil on the vascular type classical K_{ATP} channel.

Keywords: ATP-sensitive K^+ channel; pinacidil; nicorandil; sulphonylurea receptor

Introduction

Adenosine 5′-triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels are inhibited by intracellular ATP and activated by intracellular nucleoside diphosphates, thereby serving as a link between metabolic condition and excitability in various cell types (Ashcroft, 1988; Terzic *et al*., 1995). K_{ATP} channels are associated with diverse biological responses such as vasodilatation, insulin secretion, ischaemia-induced cardiac preconditioning, neurotransmitter release and oocyte maturation (Ashcroft, 1988; Terzic *et al*., 1995).

K_{ATP} channels are activated by numerous compounds with distinct chemical structures such as benzothiadiazines, guanidine/thiourea derivatives and pyridine derivatives (Edwards & Weston, 1990). These agents, collectively termed as K^+ channel openers (KCOs), cause various biological effects through modulation of the different K_{ATP} channel-mediated responses (Terzic *et al*., 1995). Thus, KCOs can potentially be useful therapeutics. However, they have been rarely utilized for treatments of human diseases, probably because most of the agents non-specifically activate the K_{ATP} channels in different tissues and thus may cause undesirable side-effects (Ashcroft & Ashcroft, 1990). The KCOs which are selective for a specific organ or tissue might be more preferable for clinical usage. Actually, some of the KCOs do exhibit a certain tissue-specificity. For example, diazoxide activates the K_{ATP} channels in pancreas and vascular smooth muscle but not in heart (Trube *et al*., 1986; Escande, 1989; Faivre & Findlay, 1989). Pinacidil activates cardiac and smooth-muscle K_{ATP} channels but not those in pancreas (Escande *et al*, 1989; Standen *et al*., 1989; Terzic *et al*., 1995). It may be important to elucidate the molecular mechanism responsible for such 'tissue-specificity' of the KCOs for designing novel useful KCOs.

It is now established that the classical K_{ATP} channels are composed of two distinct subunits: a sulphonylurea receptor (SUR) and BIR (Kir6.2), an inwardly rectifying K^+ channel (Kir) subunit (Aguilar-Bryan *et al*., 1995; Inagaki *et al*., 1995b; 1996; Sakura *et al*., 1995; Isomoto *et al*., 1996). When coexpressed with Kir6.2, all of the three types of SUR identified so far (i.e., SUR_1, SUR_{2A} and SUR_{2B}) form the classical K_{ATP} channels with the same single channel characteristics but distinct KCO sensitivities (Inagaki *et al*., 1995b; 1996; Sakura *et al*., 1995; Isomoto *et al*., 1996). Thus, SURs may be responsible for the differential effects of KCOs on each K_{ATP} channel. Because SUR_1, SUR_{2A} and SUR_{2B} have been ascribed to the SUR of the pancreatic, cardiac and smooth-muscle K_{ATP} channels, respectively (Inagaki *et al*., 1995b; 1996; Sakura *et al*., 1995), the tissue-specificity of KCOs now can be examined *in vitro* by measuring the current responses to each drug of heterologously expressed K_{ATP} channels composed of different type SUR and Kir6.2.

In this study, we compared the effects of pinacidil and nicorandil on the heterologously expressed SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in HEK293T cells. We show that nicorandil $>$100 times more potently activates the SUR_{2B}/Kir6.2 channel than the SUR_{2A}/Kir6.2, while pinacidil activates both channels with similar potency. This result may indicate that nicorandil, compared with pinacidil, acts more specifically on vascular smooth muscle than on heart. Because SUR_{2A} and SUR_{2B} are divergent only in the last 42 amino acid sequence at their carboxyl-terminal ends, this short portion of SUR_{2B} may play a critical role in nicorandil-action on the smooth muscle K_{ATP} channel.

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Methods

Cloning and functional expression of SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels

BIR (Kir6.2) was cloned from a rat brain cDNA library (Stratagene, La Jolla, CA, USA), while mouse SUR_{2A} and SUR_{2B} were from a mouse heart cDNA library (Stratagene, La Jolla, CA, U.S.A.) as previously described (Isomoto *et al*., 1996). The nucleotide sequences of all these cDNAs were fully verified and have been reported to the GenBankTM/EBI Data Bank with the accession numbers of D86039, D86037 and D86038, respectively (Isomoto *et al*., 1996). The coding region of each cDNA was individually subcloned into an expression vector pcDNA3 (Invitrogen, San Diego, CA, U.S.A.). The plasmid containing Kir6.2 was cotransfected with either SUR_{2A} or SUR_{2B} cDNA into HEK293T cells, a human embryonic kidney cell line, by using LipofectAMINE (Gibco, BRL) according to the manufacturer's instructions. Electrophysiological measurements were usually conducted 2–4 days after transfection.

Electrophysiological recordings

The channels expressed in the HEK293T cells were analysed with the cell-attached and the whole-cell variants of the patch-clamp method (Hamill *et al*., 1981). In either of the configurations, pinacidil, nicorandil and glibenclamide were applied to the bath. The tip of the electrodes had a resistance of 2–5 MΩ after coated with silicon and fire polished. Currents were measured with a patch clamp amplfier (Axopatch 200A, Axon Instruments, Inc., Foster City, CA, U.S.A.) and monitored throughout experiments with an analogue-storage oscilloscope (Dual Beam Storage Oscilloscope, Tektronix, Inc., Beaverton, Ore, U.S.A.). For subsequent analyses, currents were recorded on videocassette tapes by using a PCM recorder (VR-10B, Instrutech Corp., Great Neck, NY, U.S.A.). For analysis, the data were reproduced, low pass filtered at 1.0 kHz (-3 dB) with an 8-pole Bessel filter (Frequency Devices, Harverhill, MA, U.S.A.), and digitized at 3 or 5 kHz with an AD converter (ITC-16, Instrutech corp., NY, U.S.A.). These data were analysed off-line on a computer (Macintosh Quadra 700, Apple Computer Inc., Cupertino, CA, U.S.A.) with commercially available programmes, i.e. Pulse Program (HEKA electronik, Lambrecht, Germany) and Patch Analyst Pro (MT Corporation, Hyogo, Japan). Single channel amplitude was estimated from amplitude histograms. The response of the whole-cell current to pinacidil or nicorandil was measured by subtracting the basal current from that in the presence of these agents. The subtracted current at each concentration of the agent was normalized to that induced by 100 μM pinacidil in each cell, which was 100 ± 10 and 100 ± 30 pA pF^{-1} (mean ± s.e.mean, $n = 10$) at −30 mV in the cells expressing the SUR_{2A}/Kir6.2 or SUR_{2B}/Kir6.2 channels, respectively (5.4 mM extracellular K^+). No significant difference was detected between these two values ($P = 0.405$).

Statistical data are all expressed as mean ± s.e.mean. Significance of differences between two data were assessed with the Student's unpaired t test, and a P value < 0.05 was taken to be significant.

Solutions and chemicals

In the cell-attached configuration, the pipette was filled with a solution containing (in mM): KCl 140, $MgCl_2$ 1, $CaCl_2$ 1 and HEPES-KOH 5 (pH 7.4), while the bath was perfused with 'internal' solution containing (in mM): KCl 140, $MgCl_2$ 2, EGTA-KOH 5 and HEPES-KOH 5 (pH 7.3). For whole-cell recordings, the pipette was filled with internal solution containing 3 mM ATP and 100 μM GTP with the concentration of free Mg^{2+} adjusted to 1.4 mM according to the stability constant of the Mg-nucleotide complexes (Dawson *et al*., 1986). The bath was perfused with a control bathing solution containing (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES-NaOH 5.5 (pH 7.4). Nicorandil was dissolved at 2 M in a glacial acetic acid, pinacidil at 100 mM in 0.1 M HCl and glibenclamide at 10 mM in dimethylsulphoxide. These vehicles on their own did not induce any significant effect on the whole-cell current of the non-transfected and transfected HEK293T cells even at the maximum concentrations used in this study ($n = 5$, for each). These agents were diluted to the desired concentrations with the internal or the control bathing solution for the cell-attached and the whole-cell recordings, respectively. Nicorandil was a gift from Chugai-Pharmaceutical Company (Tokyo, Japan). Pinacidil was purchased from RBI (Natick, MA, U.S.A.). ATP and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and GTP from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and materials were obtained from commercial sources.

Results

Single channel characteristics of the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the presence of pinacidil or nicorandil

Figure 1a shows the cell-attached recordings obtained from the HEK293T cells cotransfected with Kir6.2 plus SUR_{2A} (the upper row) or with Kir6.2 plus SUR_{2B} (the lower row) in the presence of pinacidil (the left column) or nicorandil (the right column). The channel opened in bursts at various membrane potentials and the reversal potential was 0 mV under the symmetrical 150 mM K^+ conditions. Figure 1b depicts the single-channel current-voltage (*I*-V) relationships obtained from the curent records shown in Figure 1a. These *I*-V curves were identical and all exhibited ~80 pS of the single-channel conductance at negative membrane potential in the presence of 150 mM external K^+. The channel kinetics in the bursts under application of pinacidil or nicorandil were analysed in SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels. Both of the open and closed time histograms could be fitted with a single exponential curve. The time constants for open events (τ_o) and for closed events (τ_c) obtained from the open and closed time histograms are shown in Table 1 ($n = 3 \sim 5$, for each). The τ_o and τ_c of the two channels in the presence of pinacidil or nicorandil were practically identical at various potentials.

In the presence of effective concentrations of pinacidil or nicorandil, we observed these channel currents in more than ~80% of the cell-attached patch membranes either of the cotransfected cells ($n = 15$ and 18 for the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 combinations, respectively). In the presence of higher concentrations of the KCOs, more than tens of the channels sometimes simultaneously opened, leading to quasi-macroscopic current measurement in the cell-attached configuration. Even under these conditions, we could clearly see the activation of the channels with the same single-channel characteristics shortly after application or sufficiently after washout of the agents when the open probability of the channels was low. In contrast, these KCOs never induced any

currents in non-transfected HEK293T cells ($n=3$) or those transfected with either of Kir6.2 ($n=4$), SUR_{2A} ($n=5$) and SUR_{2B} ($n=5$) alone (data not shown). From these data, we concluded that the channel currents shown here were mediated by the expressed SUR_{2A}/Kir6.2 or SUR_{2B}/Kir6.2 channels, consistent with previous findings (Inagaki *et al*., 1996; Isomoto *et al*., 1996). In addition, the single-channel characteristics of the SUR_{2A}/Kir6.2 or SUR_{2B}/Kir6.2 channels were identical in the presence of pinacidil or nicorandil. These channel currents were completely inhibited by glibenclamide (2 μM), an inhibitor of K_{ATP} channels (data not shown, $n=4$), as previously found (Inagaki *et al*., 1996; Isomoto *et al*., 1996).

Effects of pinacidil on the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the whole cell configuration

Figure 2 shows the effect of different concentrations of pinacidil on the whole-cell current of HEK293T cells expressing the SUR_{2A}/Kir6.2 (Figure 2a) or the SUR_{2B}/Kir6.2 channel (Figure 2b). The cells were held at -30 mV. In both of the transfected cells, pinacidil at concentration >1 μM induced outward K^+ currents in a concentration-dependent manner. The K^+ currents were completely inhibited by glibenclamide (2 μM) (Figure 2b). Figure 2c shows the relationship between the concentration of pinacidil and the whole-cell SUR_{2A}/Kir6.2 or SUR_{2B}/Kir6.2 channel currents. Pinacidil (0.1 – 100 μM) increased both channel currents in a concentration-dependent manner. High concentrations (>100 μM) of pinacidil showed a weak inhibitory effect on both the channel currents. The relationships were fitted with the modified Hill equation using the least-squares method:

$$\text{Relative current} = A/\{1 + (K/[\text{Pinacidil}])^{n_H}\} \qquad (1)$$

where the relative current is the current normalized to that induced by 100 μM pinacidil in the same cells; A is the maximum relative current attained with pinacidil; K is the apparent dissociation constant of pinacidil; [Pinacidil], the concentration of pinacidil; and n_H the Hill coefficient. The

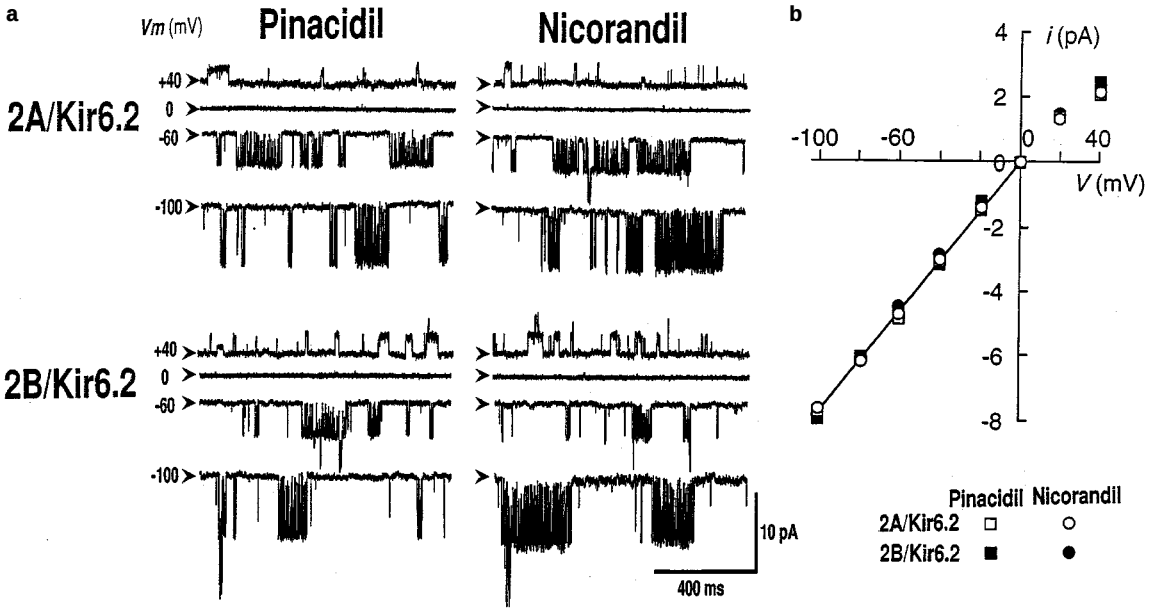

Figure 1 Single channel characteristics of the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the presence of pinacidil or nicorandil. (a) Cell-attached recordings obtained from HEK293T cells cotransfected with Kir6.2 plus SUR_{2A} (the upper row) or with Kir6.2 plus SUR_{2B} (the lower row) at different membrane potentials in the presence of pinacidil (100 μM) (the left column) or nicorandil (100 μM) (the right column) in the bath. The pipette contained 145 mM K^+. Arrow heads indicate the zero current level. The membrane potentials are indicated to the left of trace. (b) The single-channel current-voltage relationship measured from the cell-attached recordings shown in (a). The data were obtained from the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the presence of 100 μM pinacidil or nicorandil. The line is the regression line to fit the data between -100 and 0 mV, which had the slope of 78 pS.

Table 1 The time constants for intraburst open (τ_0) and closed (τ_c) events of SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the presence of pinacidil or nicorandil

	Membrane potential (mV)	*Pinacidil*		*Nicorandil*	
		τ_o (ms)	τ_c (ms)	τ_o (ms)	τ_c (ms)
SUR_{2A}/Kir6.2	+40	9.1±1.2	0.23±0.04	8.4±1.4	0.20±0.05
	−60	2.4±0.8	0.20±0.02	2.2±0.7	0.19±0.04
	−100	1.3±0.6	0.17±0.02	1.3±0.6	0.16±0.03
SUR_{2B}/Kir6.2	+40	8.5±1.0	0.22±0.06	8.6±1.4	0.21±0.04
	−60	2.1±0.6	0.20±0.02	2.3±0.7	0.21±0.03
	−100	1.2±0.7	0.14±0.03	1.3±0.8	0.18±0.04

All these data were estimated from the dwell time histograms for open and closed times in bursts constructed from the traces shown in Figure 1a. Both the open and closed time histograms were satisfactorily fitted with a single exponential function under each condition. The values indicate the mean±s.e.mean. The number of observations is 3–5 for each of the conditions.

values of A, K and n_H were 1.05, 9.8 μM and 1.24 for the SUR_{2A}/Kir6.2 channel; and 1.03, 1.4 μM and 1.42 for the SUR_{2B}/Kir6.2 channels.

Both relationships were very similar but significantly different at 1, 3 and 10 μM pinacidil ($P=0.0013$, 0.0017 and 0.027, respectively). Therefore, pinacidil may activate the SUR_{2B}/Kir6.2 channel slightly more potently than the SUR_{2A}/Kir6.2 channel. However, there was a tendency for the SUR_{2B}/Kir6.2 channel to become desensitized to some extent by high concentrations (i.e., 10 and 100 μM) of pinacidil more frequently than the SUR_{2A}/Kir6.2 channel (Figure 2a and b). Thus, if desensitization had not occurred, the maximum value of SUR_{2B}/Kir6.2 channel activity induced by 100 μM pinacidil would have been larger than that actually measured. To avoid the influence of desensitization as far as possible, the current values obtained at each concentration of pinacidil were normalized to the peak current value induced by 100 μM pinacidil in each patch. Nevertheless, the data shown in Figure 2c could have been biased to some extent by such desensitization. However, in such a case the relative current values observed in the presence of lower concentrations of pinacidil may have been overestimated. Therefore the real difference in the sensitivity to pinacidil between the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels might be even smaller than that shown in Figure 2c.

Effects of nicorandil on the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels in the whole-cell configuration

Figure 3 shows the effect of nicorandil on the whole-cell SUR_{2A}/Kir6.2 (Figure 3a) and SUR_{2B}/Kir6.2 channel currents (Figure 3b). In the cells expressing the SUR_{2A}/Kir6.2 channels, nicorandil induced an outward current at concentrations >100 μM. The current induced by 1 mM nicorandil was only $\sim 30\%$ of that induced by 100 μM pinacidil. Glibenclamide (2 μM) completely inhibited the pinacidil-current. On the other hand, the SUR_{2B}/Kir6.2 channel was effectively activated by 1–30 μM nicorandil in a concentration-dependent manner. Nicorandil (30 μM) activated the channel approximately to the same extent as 100 μM pinacidil. Figure 3c shows the relationships between the concentration of nicorandil and the whole-cell SUR_{2A}/Kir6.2 or SUR_{2B}/Kir6.2 channel currents. In this graph, the current induced by each concentration of nicorandil was normalized to that induced by 100 μM pinacidil in each cell. The threshold concentration of nicorandil to activate the SUR_{2A}/Kir6.2 channel was 10 μM. The current evoked by 1 mM nicorandil was only $\sim 37 \pm 14$ ($n=3$) % of that induced by 100 μM pinacidil. On the other hand, nicorandil (1–100 μM) effectively activated the SUR_{2B}/Kir6.2 channel. The maximum current of SUR_{2B}/Kir6.2 channel induced by nicorandil was approximately the same as that by 100 μM pinacidil. At concentrations higher than 100 μM, nicorandil showed some inhibitory effect on the SUR_{2B}/Kir6.2 channel. The concentration-response relationship for the nicorandil-activation of SUR_{2B}/Kir6.2 channel was fitted with the following modified Hill equation:

$$\text{Relative current} = A/\{1 + (K/[Nicorandil])^{n_H}\} \qquad (2)$$

where the relative current is the current normalized to that induced by 100 μM pinacidil in the same cells; A is the maximum relative current induced by nicorandil; and [Nicorandil], the concentration of nicorandil. The values of A, K and n_H were 1.05, 9.2 μM and 1.30, respectively.

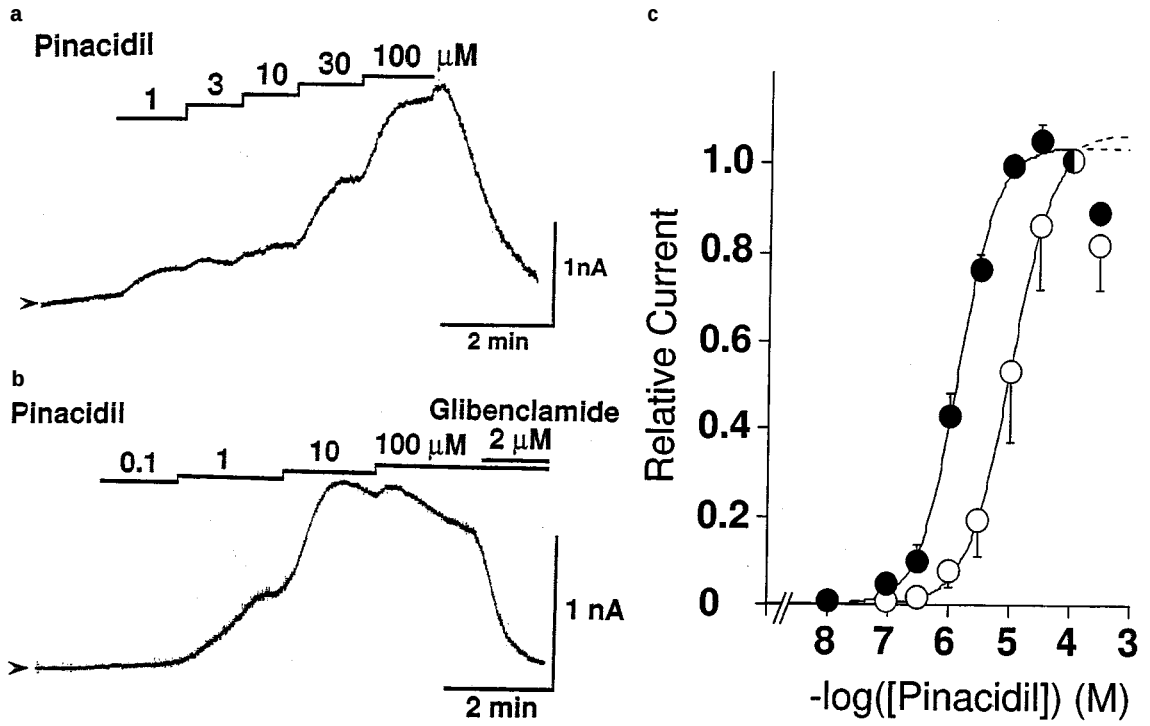

Figure 2 Concentration-dependent effect of pinacidil on the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels. Concentration-dependent effect of pinacidil on the SUR_{2A}/Kir6.2 (a) and the SUR_{2B}/Kir6.2 channels (b) were measured at -30 mV with 5.4 mM external K^+ in the whole-cell configuration. Above each trace, the perfusion protocol is indicated. Arrow heads indicate the zero current level. (c). The relationship between the concentration of pinacidil and the whole-cell current of the SUR_{2A}/Kir6.2 (open circles) and SUR_{2B}/Kir6.2 channels (solid circles). The current amplitude induced by each concentration of pinacidil was normalized to the pinacidil (100 μM)-induced current in the same cell. Each symbol and vertical lines indicate the mean and s.e.mean, respectively. The number of observations at each point was 5. The lines are the fit of the data with Eq.1 (see text) at concentrations lower than 100 μM.

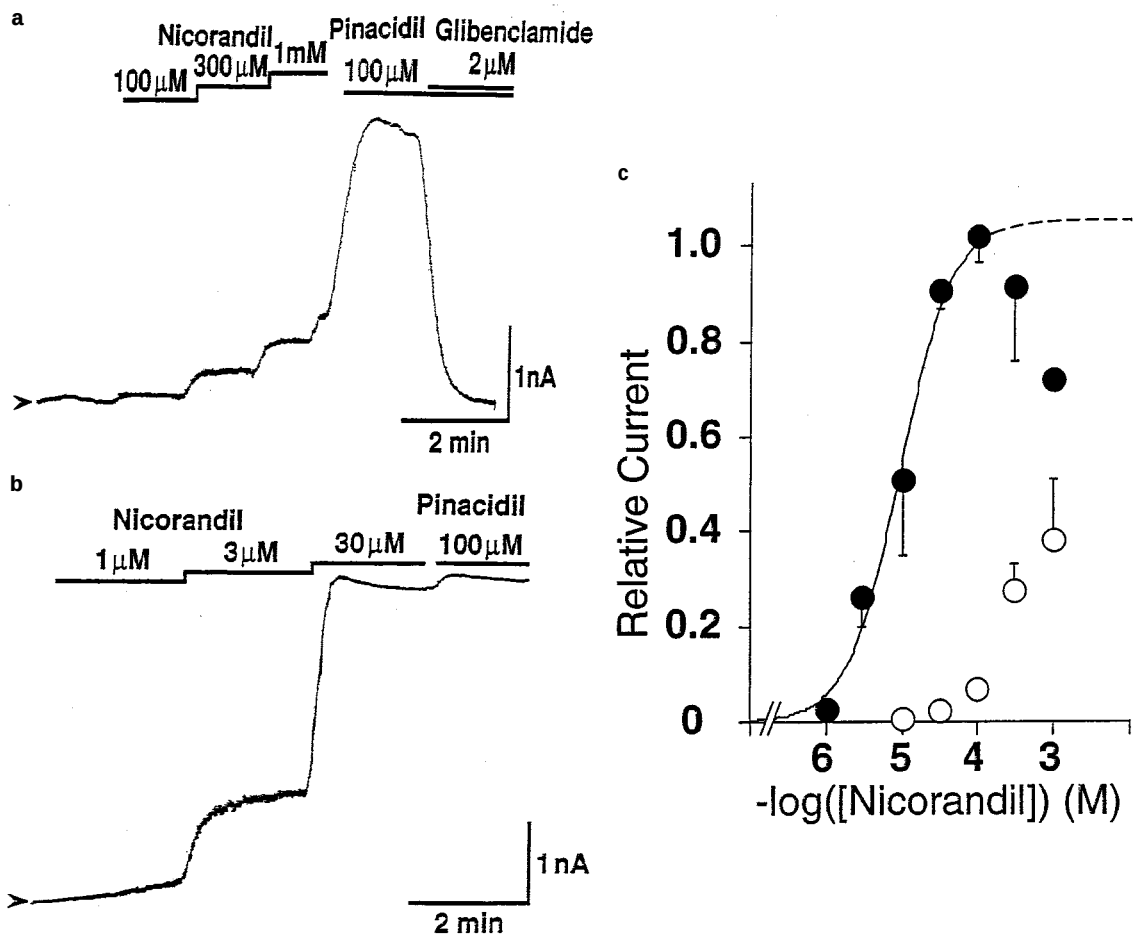

Figure 3 Concentration-dependent effect of nicorandil on the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels. Concentration-dependent effect of nicorandil on the whole-cell current of the SUR_{2A}/Kir6.2 (a) and SUR_{2B}/Kir6.2 channels (b) at −30 mV with 5.4 mM external K^+. Arrow heads indicate the zero current level. The perfusion protocol is indicated above each trace. (c) Relationship between the concentration of nicorandil and the whole-cell current of the SUR_{2A}/Kir6.2 (open circles) and SUR_{2B}/Kir6.2 channels (solid circles). The current amplitude induced by each concentration of nicorandil was normalized to the pinacidil (100 μM)-induced current in the same cell. Each symbol and vertical lines indicate the mean and s.e.mean, respectively. The number of the observations at each point was 5. The line is the fit of the data with Eq.2 (see text) at concentrations lower than 100 μM.

Therefore, nicorandil showed almost the same potency and efficacy as pinacidil in activating the SUR_{2B}/Kir6.2, but was more than 100 times less potent than pinacidil in activating the SUR_{2A}/Kir6.2 channels.

Discussion and conclusions

We showed for the first time that nicorandil, but not pinacidil, activates the SUR_{2B}/Kir6.2 channel >100 times more potently than the SUR_{2A}/Kir6.2 channel. The partition coefficient of pinacidil in octanol/phosphate buffer (pH 7.4) is 0.99 (from Dr Erling N. Petersen, Leo Pharmaceutical Products Ltd.), while that of nicorandil in octanol/phosphate buffer (pH 7.5) is 0.78 (from Chugai Pharmaceutical Company). Therefore, pinacidil is more hydrophobic than nicorandil at neutral pH, raising the possibility that the two agents may achieve different concentrations at the receptor sites of the channels if applied to the bathing solution at the same concentration. However, this does not affect our conclusion because we compared the sensitivities of the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels to each drug, but not the effects of pinacidil and nicorandil on each type of channel.

SUR_{2A} and SUR_{2B} are the splicing variants of the same gene, both of which are composed of 1546 amino acids and differ only in the last 42 amino acid residues in their carboxyl-terminal ends (i.e. aa. 1505–1546) (Isomoto *et al.*, 1996). Therefore, the carboxyl-terminal regions of SUR_{2A} and SUR_{2B} seem to play a critical role in the different sensitivity of the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels to nicorandil.

We previously found that diazoxide, a potent activator of the SUR_1/Kir6.2 channel (Inagaki *et al.*, 1995b), activates the SUR_{2B}/Kir6.2, but not SUR_{2A}/Kir6.2, channel (Isomoto *et al.*, 1996; Inagaki *et al.*, 1996). Because the amino acid sequence within the alternative region of mouse SUR_{2B} is 74% and 33% identical to those of the corresponding regions of rat SUR_1 and mouse SUR_{2A}, respectively, it was postulated that the carboxyl-terminal region is involved in differential activation of SUR/Kir6.2 complex channels by diazoxide (Isomoto *et al.*, 1996). Diazoxide does not activate the SUR_{2A}/Kir6.2 channel, while neither nicorandil nor pinacidil can activate SUR_1/Kir6.2 channel (Inagaki *et al.*, 1995b; data not shown). Therefore, the region shared by SUR_{2A} and SUR_{2B} (i.e. aa. 1–1504) appears to be crucial for the action of both pinacidil and nicorandil. Taken together, the whole sequence of SUR_{2B} is necessary for nicorandil-

action, while the carboxyl-terminal region of SUR_{2B} may enhance the sensitivity of the SUR/Kir6.2 complex channels to nicorandil. Alternatively, it is also possible that the divergent part of SUR_{2A} suppresses the nicorandil action. The carboxyl-terminal regions of SUR_{2A} and SUR_{2B} may not be involved in the action of pinacidil.

However, unknown is how this short segment of the C-terminus of SUR_{2B} is involved in actions of nicorandil and diazoxide on the SUR/Kir6.2 channels. This region might be a part of the receptor sites for the KCOs, or might indirectly affect the function of the receptor sites for the KCOs located in other parts of SUR. In this regard, it should be noted that the SUR_{2B}/Kir6.2 channel is more effectively inhibited by Mg^{2+}-free than -bound forms of ATP, while the SUR_{2A}/Kir6.2 channel is equally sensitive to both forms of ATP (Isomoto *et al*., 1996). Recently, Tucker *et al*. (1997) showed that the inhibitory ATP-binding site may be a lysine residue (K185) locating in the proximal region of the C-terminus of Kir6.2. The SUR (SUR_1 in their study) interacting with Kir6.2 increases the sensitivity of the channel to intracellular ATP. Thus, SURs modulate the sensitivity of the interaction between the lysine residue in Kir6.2 and ATP. It was postulated that the 42 amino acids at the C-termini of SUR_{2s} may play important roles in differential regulation of the ATP-binding site of Kir6.2 by Mg-free and Mg-bound forms of ATP (Isomoto *et al*., 1996). It is also known that most of KCOs activate K_{ATP} channels by modulating the sensitivity of channels to intracellular nucleotides (Terzic *et al*., 1995). Therefore, there might be a similar basis for the action of some KCOs and intracellular ATP. However, further studies are necessary to elucidate the molecular mechanism by which the carboxyl-termini of SUR_{2A} and SUR_{2B} produce the different sensitivities to nicorandil of the SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.2 channels.

SUR_{2A} forms the cardiac or skeletal-muscle type classical K_{ATP} channel with Kir6.2 (Inagaki *et al*., 1996). On the other hand, SUR_{2B} is likely to form a vascular K^+ channel *in vivo* by coupling with Kir6.1 (Yamada *et al*., 1997), another Kir subunit whose amino acid sequence is $\sim$70% identical to that of Kir6.2 (Inagaki *et al*., 1995a). This vascular K^+ channel, often referred to as the nucleotide diphosphate-dependent K^+ (K_{NDP}) or the small conductance K_{ATP} channel, serves as the major receptor site for KCOs in vascular smooth muscle (Kajioka *et al*., 1991; Beech *et al*., 1993; Kamouchi & Kitamura, 1994; Zhang & Bolton, 1996). The SUR_{2B}/Kir6.1 channel shows the same pharmacological properties as the SUR_{2B}/Kir6.2 channel (Yamada *et al*., 1997). It is, however, difficult to compare quantitatively the tissue specificity of various KCOs using SUR_{2A}/Kir6.2 and SUR_{2B}/Kir6.1 channels, because they differ in the single channel characteristics and nucleotide regulations (Isomoto *et al*., 1996; Yamada *et al*., 1997). The present data show that a quantitative comparison of the effects of various KCOs can be performed *in vitro* by using heterologously expressed K_{ATP} channels composed of different types of SUR and Kir6.2. This method, when combined with site-directed mutagenesis, will more precisely delineate the molecular mechanism responsible for the subtype specific action of KCOs, and thus will provide a future strategy to develop novel KCOs.

We thank Dr Lutz Pott (Institute of Physiology, School of Medicine, Ruhr-University, Bochum, Germany) for critical reading of the manuscript. This work was supported by the grants from the Ministry of Education, Science, Sports and Culture of Japan and the 'Research for the Future' Program from The Japan Society for the Promotion of Science (JSPS-RFTF96L00302).

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(Received August 14, 1997
Revised April 1, 1998
Accepted April 2, 1998)