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Potentiation by nitric oxide of the ATP-sensitive K^+ current induced by K^+ channel openers in guinea-pig ventricular cells

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1 Modulation by nitric oxide (NO) of the ATP-sensitive K^+ channel (K_{ATP}) current ($I_{K\ (ATP)}$) was investigated in single ventricular cells dissociated from guinea-pig hearts. $I_{K\ (ATP)}$ was induced by 5-amino-*N*-[2-(2-chlorophenyl)ethyl]-*N*′-cyano-3-pyridinecarboxamidine (KRN4884) and cromakalim.

2 In the whole-cell patch clamp configuration, KRN4884 (0.1–3 μM) increased the outward current in a concentration-dependent manner with an EC_{50} value of 0.48 μM. This current was completely antagonized by glibenclamide (1 μM).

3 $I_{K\ (ATP)}$ induced by either KRN4884 (0.3 μM) or cromakalim (10 μM) was significantly enhanced by the additional application of a NO donor (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide (NOR3, 0.1 and 1 mM).

4 The potentiating effect was observed only when NOR3 solution was prepared just before experiments, when release of NO was considered to be on-going. The effect was significantly eliminated in the presence of the NO scavenger oxyhaemoglobin (3–10 μM). Also, oxidative metabolites of NO, such as NO_2^- or NO_3^-, were without effect.

5 8-Bromo-guanosine-3′:5′-cyclic monophosphate (8-Br-cyclic GMP, 0.1–0.5 mM) significantly decreased $I_{K\ (ATP)}$ induced by KRN4884.

6 In cell-attached patches, NOR3 (1 mM) potentiated the KRN4884-induced $I_{K\ (ATP)}$ in a way similar to that seen in whole-cell recordings. By contrast, NOR3 (1 mM) did not enhance the current in either inside-out or outside-out patches.

7 These results indicate that NO potentiates the action of K^+ channel openers on the K_{ATP} through a mechanism which remains to be determined.

Keywords: ATP-sensitive K^+ channel; nitric oxide; K^+ channel opener; KRN4884; cromakalim; NO donor; NOR3; 8-Br-cyclic GMP; patch-clamp; ventricular myocytes

Introduction

It has recently been shown that cardiac myocytes express not only an inducible (NOS2) (Schulz *et al*., 1992; Balligand *et al*., 1994) but also a constitutive isoform of nitric oxide synthase (NOS3) (Balligand *et al*., 1993). Induction of NOS2 may play a part in the pathogenesis of cardiac disease and NOS3 may be involved in the regulation of cardiac contractile functions (Kaye *et al*., 1996).

In cardiac muscle, activation of ATP-sensitive K^+ channels (K_{ATP}) shortens action potential duration and decreases contractility (Fosset *et al*., 1988; Nichols *et al*., 1991; Edwards & Weston, 1993; Grover, 1994). Furthermore, evidence is accumulating that K_{ATP} can be modulated by NO. In pancreatic β-cells, NO activates K_{ATP} via reduction of adenosine 5′-triphosphate (ATP) production (Tsuura *et al*., 1994), whilst in vascular smooth muscle, NO apparently activates K_{ATP} via a guanosine 3′:5′-cyclic monophosphate (cyclic GMP)-mediated mechanism (Kubo *et al*., 1994; Murphy & Brayden, 1995). It is, therefore, particularly important to know whether there is any interaction between NO and K_{ATP} in the heart.

In the present study with ventricular cells from guinea-pig hearts, K_{ATP} was activated by the K^+ channel openers, 5-amino-*N*-[2-(2-chlorophenyl)ethyl]-*N*′-cyano-3-pyridinecarboxamidine (KRN4884) or cromakalim, and effects of NO were investigated by the patch clamp method. The results show that NO enhances the effects of the K^+ channel openers.

Methods

Preparation

Single ventricular cells from guinea-pig hearts were obtained by an enzymatic dissociation procedure (Isenberg & Klöckner, 1982). Briefly, guinea-pigs weighing 250–550 g were deeply anaesthetized with pentobarbitone (50–60 mg kg^{-1}). Under artificial respiration, the heart was quickly removed from the thorax and hung on a Langendorff-type apparatus to start the coronary perfusion with a Ca^{2+}-free Tyrode solution. The heart was then further perfused with a Ca^{2+}-free Tyrode solution containing 0.04% (w/v) collagenase (200–230 units mg^{-1}; Wako Pure Chemical Industry, Osaka, Japan) for 25–35 min at 36°C. The heart was then rinsed with a high K^+, low Cl^- storage solution, the left ventricle was dissected, and stored in a storage solution at 4°C for later use.

A small piece of the ventricular tissues was dissected and gently agitated in the recording chamber (1.5 ml in volume) filled with normal Tyrode solution. After the cells had settled on the floor of the recording chamber, the chamber was perfused with normal Tyrode solution at 2–3 ml min^{-1}. Experiments were performed at 36–37°C on those rod-shaped quiescent single cells which had clear sarcomere striations.

Solutions

The normal Tyrode solution contained (in mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, HEPES 5.0 and glucose 5.5 (pH = 7.4, adjusted with NaOH). The storage solution contained (in mM): taurine 10, oxalic acid 10, L-glutamic acid 70, KCl 25, KH_2PO_4 10, ethylene glycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.5, glucose 11 and HEPES 10 (pH = 7.4, adjusted with KOH). In

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whole-cell voltage clamp experiments, L-type Ca^{2+} channels were inhibited by nifedipine (3 μM; Wako Pure Chemical Industry, Osaka, Japan). The pipette solution contained (in mM): K-aspartate 110, KCl 20, $MgCl_2$ 2, ATP dipotassium salt 4, GTP disodium salt 0.1, creatine phosphate dipotassium salt 6, EGTA 10 and HEPES 5 (pH = 7.0, adjusted with KOH). In cell-attached and inside-out patch clamp experiments, the normal Tyrode solution was used as the pipette solution. The bath solution was a high K^+ solution which contained (in mM): KCl 150, $MgCl_2$ 0.5, HEPES 5, EGTA 1 (pH = 7.2, adjusted with KOH). In outside-out patch clamp experiments, the pipette and bath solution were same as those used in whole-cell recordings.

Measurement of the concentration of NO_2^-

Immediately after dissolving NOR3 in the superfusing solution, NO release begins (Kita *et al*., 1994). Since NO is very unstable and susceptible to oxidation, NO release is followed by formation of the stable oxidative metabolites, NO_2^- and NO_3^-. Although a direct measurement of NO was not available, the time-course of formation of the oxidative metabolite, NO_2^-, provided an indicator of the amount of NO released from NOR3. We thus measured the concentration of NO_2^- in the solution by a diazotization assay (Archer, 1993). After dissolving NOR3 in the superfusing solution at 37°C, 1 ml of the solution was sampled at a given time and added to 1 ml of Griess reagents. After 10 min, the absorbance of a purple dye at 550 nm was measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan). Then the concentration of NO_2^- was calculated and plotted vs time (Figure 1). To obtain a standard curve, $NaNO_2$ (1 – 100 μM) was used under the same experimental conditions. It is evident that the concentration of NO_2^- increased with time and thus the NOR3 solution was prepared just before use, except in those experiments in which the effects of NOR3, prepared 24 h previously, were examined (Figure 4b).

Electrophysiological recordings

Currents were recorded with the patch-clamp technique (Hamill *et al*., 1981) by use of a patch-clamp amplifier (Nihon Kohden CEZ-2300, Tokyo, Japan). In whole-cell recordings, data were collected by and stored on an IBM-compatible 386 computer (Proside, Tokyo, Japan) running pClamp software (Axon Instrument, Foster City, CA, U.S.A.). To evaluate the current-voltage (*I*-V) relationship of the K_{ATP} current ($I_{K\,(ATP)}$), a slow depolarizing voltage ramp (15 mV s^{-1}) was applied every 2 min from a holding potential of −120 to 60 mV. Current signals were digitized with a sampling frequency of 25 Hz and stored on the computer hard disc.

In cell-attached patch recordings, the cells were superfused with the high K^+ solution (see above) to reduce the resting membrane potential of myocytes. The pipette potential was set to 0 mV, and $I_{K\,(ATP)}$ was recorded as outward current. With this protocol, $I_{K\,(ATP)}$ could be measured without interference from inwardly rectifying K^+ channels. In inside-out and outside-out patch recordings, $I_{K\,(ATP)}$ was also recorded as outward current at 0 mV. These data were stored on digital audio tape by a DAT recorder (RD-125T, TEAC, Tokyo, Japan) for later computer analysis.

Drugs

KRN4884 (Kirin Brewery Co., Ltd., Tokyo, Japan), cromakalim (Sigma Chemical, St. Louis, MO, U.S.A.) and glibenclamide (Hoechst, Frankfurt, Germany) were each dissolved in dimethyl sulphoxide (DMSO) to give a 10 mM stock solution, and were diluted to final concentrations with the superfusing solution. The final concentration of DMSO to which cells were exposed was less than 0.1%. (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide (NOR3, Dojin Laboratory, Kumamoto, Japan) (FK409, Fujisawa Pharmaceutical Co. Ltd. Osaka, Japan) was directly dissolved in the superfusing solution at a concentration of 0.1 or 1 mM. In the present study, we refer to this substance as NOR3. 8-Bromocyclic GMP (8-Br-cyclic GMP, Sigma Chemical, St. Louis, MO, U.S.A.) and oxyhaemoglobin (Funakoshi, Tokyo, Japan) were also directly dissolved in the superfusing solution to give the concentrations described in the text.

Data analysis

In the present study, the *I*-V relationships of whole-cell currents were recorded with ramp command pulses and the effects of drugs were examined 8 min or more after their application. The actions of KRN4884 were evaluated at 0 mV at which potential other current components were thought to be minimal. To obtain concentration-response curves for KRN4884, currents were normalized to the maximal response. The curve was fitted with the following logistic equation by the least squares method:

$$E = A^p/(A^p + K^p)$$

where E is the normalized effect, A is the drug concentration, K is the concentration for half maximal effect (EC_{50}) and p is the slope parameter.

In cell-attached, inside-out and outside-out patch recordings, data were reproduced, low-pass filtered at 1 kHz, and sampled at 2 kHz onto a computer (NEC-PC98Ap, Tokyo, Japan). The channel activity was expressed as NPoi, where N is the number of channels in the patch, Po is the open probability of channel and i is the unitary current. NPoi was calculated by integrating a 5 – 20 s continuous current record during the steady effect of each drug, with respect to the zero current level which was determined when all the channels were closed.

All values are expressed as mean ± s.e.mean. Comparisons between two groups were performed by use of a paired Student's *t* test. A value of $P < 0.05$ was considered significant.

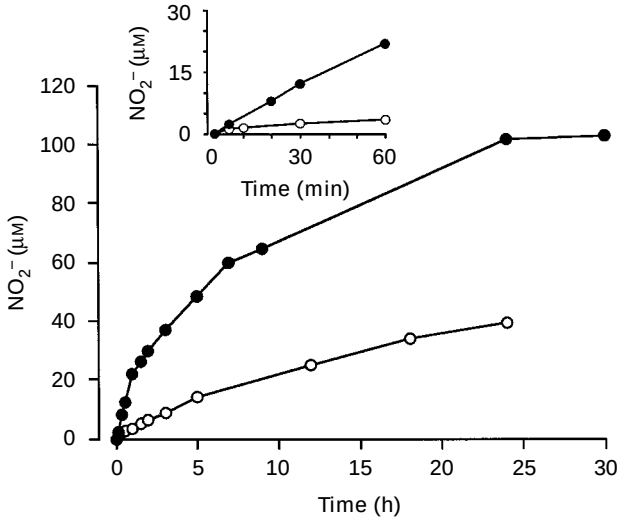

Figure 1 Time-dependent production of NO_2^- in Tyrode solution. NOR3 was dissolved in Tyrode solution at time 0 to give a concentration of 0.1 (○) and 1 mM (●). The solution was kept at 37°C. One ml of the solution was sampled at a given time and the NO_2^- concentration was measured by a diazotization assay. Inset: the initial part of the time-dependent production of NO_2^- is shown with an expanded time scale.

Results

Effects of KRN4884 on $I_{K\,(ATP)}$

In the presence of 3 μM nifedipine to inhibit L-Type Ca^{2+} channels, the membrane current evoked by a slow depolarizing

voltage-ramp (5 mV s^{-1}) from the holding potential of −120 to 60 mV was measured every 2 min. Under these conditions, the control *I*-V relationship was characteristically N-shaped with a negative slope around −50 to −20 mV (Figure 2a). The reversal potential was −93.5±1.3 mV ($n=9$), which is close to the calculated K^+ equilibrium potential in these experiments of −88.9 mV. Exposure to KRN4884 (0.1 μM) produced an increase of the outward current at potentials positive to the reversal potential. The outward current was further enhanced by increasing the concentration of KRN4884, the maximal effects of which were reached at approximately 3 μM. In the presence of KRN4884 at concentrations more than 1 μM, the N-shaped control *I*-V relationship became linear without affecting the reversal potential (control, −93.5±1.3; KRN4884 3 μM, −95.7±1.3 mV, $n=9$).

The KRN4884-induced outward current was measured at 0 mV and normalized to the maximal value. The concentration-response curve for KRN4884 could be fitted to a logistic equation with an EC_{50} value of 0.48 μM and slope parameter of 1.83 (Figure 2b). The outward current induced by KRN4884 (3 μM) was completely inhibited by 1 μM glibenclamide and the effects of a typical experiment are shown in Figure 2c. Essentially identical results were obtained in four other cells.

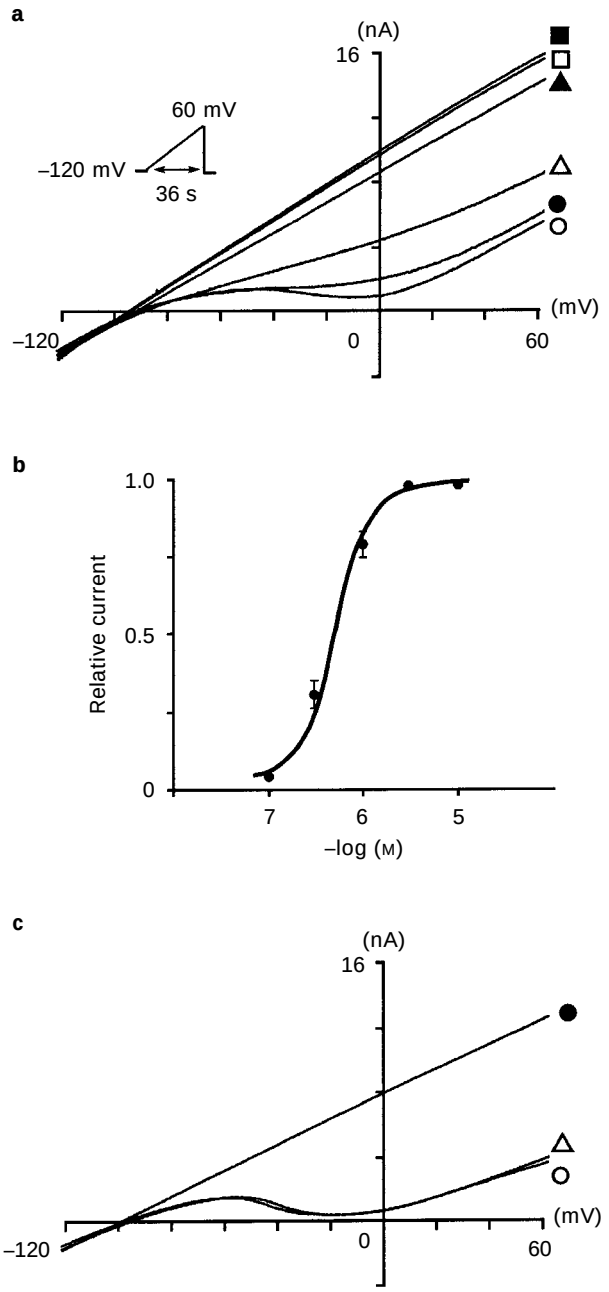

Figure 2 Activation of $I_{K\,(ATP)}$ by KRN4884. (a) Effect of KRN4884 on background current. A slow depolarizing voltage ramp (5 mV s^{-1}) was applied every 2 min from a holding potential of −120 to 60 mV. *I*-V relationships were obtained in the absence (○) and presence of 0.1 (●), 0.3 (△), 1 (▲), 3 (□) and 10 μM KRN4884 (■). (b) Concentration-response curve for the KRN4884-induced outward current determined at 0 mV. Each point represents the mean value of nine experiments and the s.e.mean is shown by vertical lines. A smooth curve was drawn by a least squares fit to a logistic equation with an EC_{50} value of 0.48 μM and a slope factor of 1.83. (c) Inhibition of the KRN4884-induced current by glibenclamide. After the control *I*-V curve (○) had been obtained 3 μM KRN4884 was applied to activate $I_{K\,(ATP)}$ (●), followed by additional application of 1 μM glibenclamide (△). Note that the KRN4884-induced current was almost completely inhibited by exposure to glibenclamide.

Effects of NOR3 on $I_{K\,(ATP)}$

As shown in Figure 3a, KRN4884 (0.3 μM) increased $I_{K\,(ATP)}$, an effect which was enhanced in the presence of NOR3 (0.1 and 1 mM). At 0 mV, the mean increase in $I_{K\,(ATP)}$ produced by NOR3 was 34.9±10.8% ($n=8$, $P<0.01$) and 47.4±9.5% ($n=5$, $P<0.01$) for NOR3 concentration of 0.1 and 1 mM, respectively. These effects of KRN4884 and of NOR3 were completely inhibited by glibenclamide (1 μM) (Figure 3a).

NOR3 (0.1 mM) also increased $I_{K\,(ATP)}$ elicited by cromakalim (10 μM) (Figure 3b) and at a higher concentration of 1 mM the effects of NOR3 were even greater (data not shown). These effects were completely inhibited by glibenclamide (1 μM).

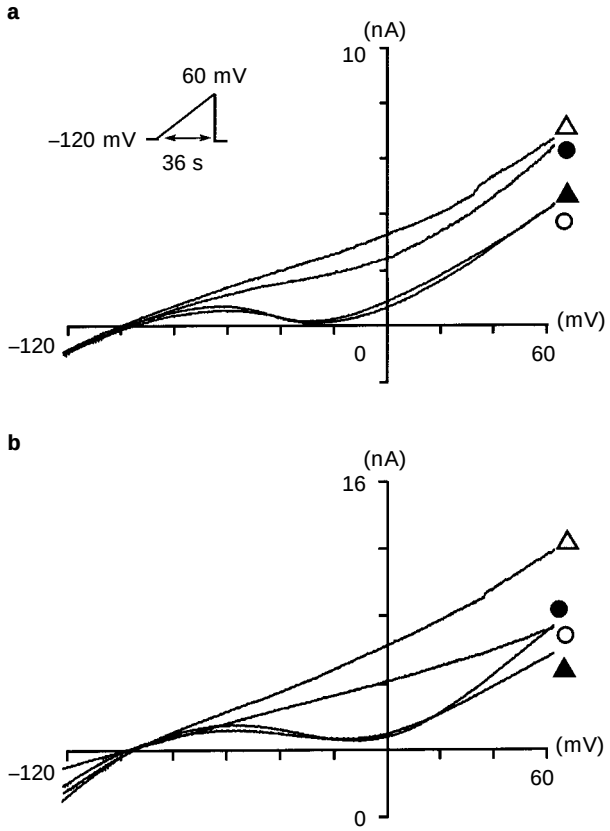

Figure 3 Potentiation by NOR3 of $I_{K\,(ATP)}$ induced by KRN4884 (a) and cromakalim (b). NOR3 (0.1 mM) was dissolved in Tyrode solution just before the experiment. After the control *I*-V relationship (○) had been obtained, either 0.3 μM KRN4884 (a) or 10 μM cromakalim (b) was applied to activate $I_{K\,(ATP)}$ (●), followed by additional application of 0.1 mM NOR3 (△). Note that the KRN4884- and cromakalim-induced current and the NOR3-accelerated outward current were almost completely inhibited by 1 μM glibenclamide (▲).

In two out of four cells, NOR3 (0.1 mM) slightly decreased the background current (Figure 4a), while in the other two cells, the current was slightly enhanced. The average change produced by NOR3 (0.1 mM) was $4.2 \pm 18.1\%$ at 0 mV ($n=4$). When the NOR3 concentration was increased to 1 mM, a clear decrease in the background current was observed ($n=2$, data not shown).

The potentiating effects of NOR3 (0.1 and 1 mM) on $I_{K\,(ATP)}$ induced by KRN4884 were only elicited by freshly-dissolved NOR3 (see methods section). NOR3 (1 mM) dissolved 24 h before the experiments decreased $I_{K\,(ATP)}$ (Figure 4b).

Oxyhaemoglobin, a NO scavenger, greatly suppressed the potentiating effect of NOR3 (Figure 4c). In the presence of oxyhaemoglobin (3–10 μM), NOR3 (1 mM) increased $I_{K\,(ATP)}$ by only $10.8 \pm 9.5\%$ ($n=5$) at 0 mV and thus the effect of NOR3 was significantly smaller than that in the absence of oxyhaemoglobin ($47.4 \pm 9.5\%$, $n=5$).

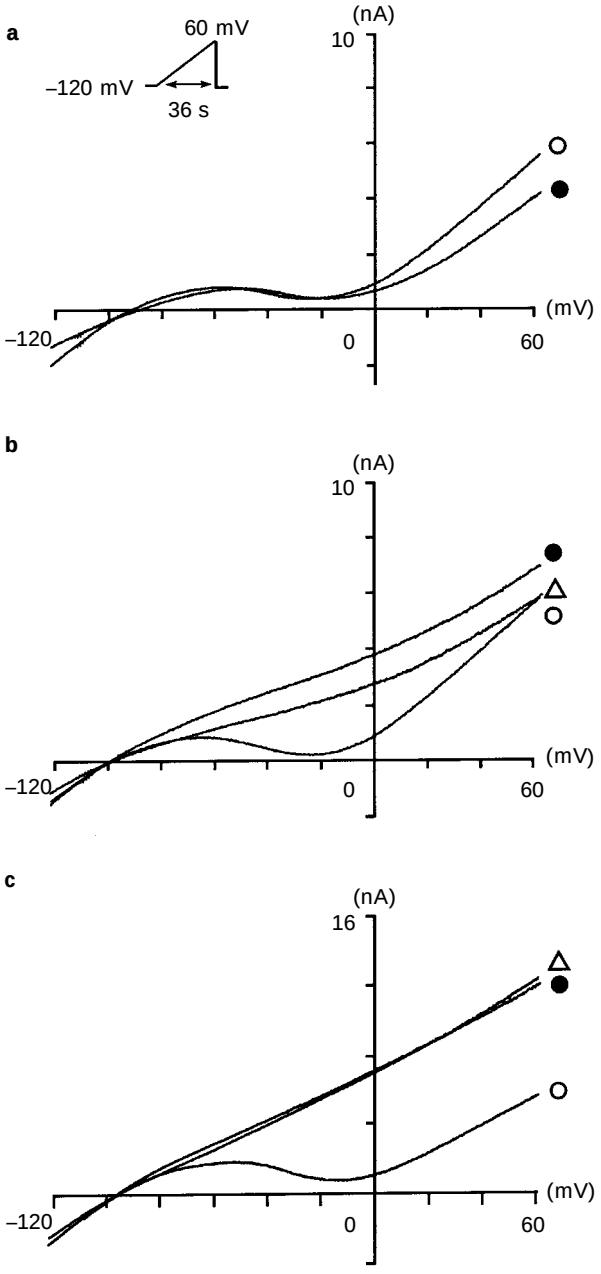

Figure 4 (a) Effect of NOR3 on background current. *I*-V curves obtained in the absence (○) and presence (●) of 0.1 mM NOR3 are superimposed. (b) Effect of 1 mM NOR3 solution prepared 24 h before the experiment on the KRN4884-induced $I_{K\,(ATP)}$. This solution suppressed the current. (○) Control; (●) 0.3 μM KRN4884; and (△) 0.3 μM KRN4884 plus 1 mM NOR3. (c) Effect of NOR3 on KRN4884-induced $I_{K\,(ATP)}$ in the presence of oxyhaemoglobin. (○) Control; (●) 0.3 μM KRN4884 plus 10 μM oxyhaemoglobin and (△) 0.3 μM KRN4884 plus 10 μM oxyhaemoglobin plus 1 mM NOR3.

Effects of NO_2^- and NO_3^- on $I_{K\,(ATP)}$

The effects of NO_2^- and NO_3^-, two oxidative metabolites of NO on KRN4884-induced $I_{K\,(ATP)}$ were also examined. As shown in Figure 5a, $NaNO_2$ (30 μM) slightly decreased the mean KRN4884-induced $I_{K\,(ATP)}$ (at 0 mV, $-11.6 \pm 12.8\%$; $n=4$), but this change was not significant. $NaNO_3$ (0.1 mM) had no effect on the KRN4884-induced $I_{K\,(ATP)}$ (-5.9 ± 12.6, $n=4$).

Effects of 8-Br-cyclic GMP on $I_{K\,(ATP)}$

NO activates soluble guanylate cyclase, which in turn generates cyclic GMP to bring about a number of cellular changes (Moncada *et al.*, 1991). NOR3-induced potentiation of $I_{K\,(ATP)}$ could be mediated by this mechanism, a view supported by the finding that isosorbide dinitrate activates K_{ATP}, an effect mimicked by 8-Br-cyclic GMP (Kubo *et al.*, 1994). In the present study, 8-Br-cyclic GMP (0.1 mM) suppressed $I_{K\,(ATP)}$ produced by KRN4884 by $33.3 \pm 9.0\%$ ($P<0.01$, $n=4$; Figure 5b). However, even under these conditions, NOR3 (1 mM) was still able to increase the KRN4884-induced $I_{K\,(ATP)}$. Higher concentrations of 8-Br-cyclic GMP (0.3 and 0.5 mM) produced an even greater inhibition of the current (not illustrated).

Effects of NOR3 on $I_{K\,(ATP)}$ in cell-attached patches

After giga-seal formation, the pipette potential was set to 0 mV and the bath solution was switched from the normal Tyrode

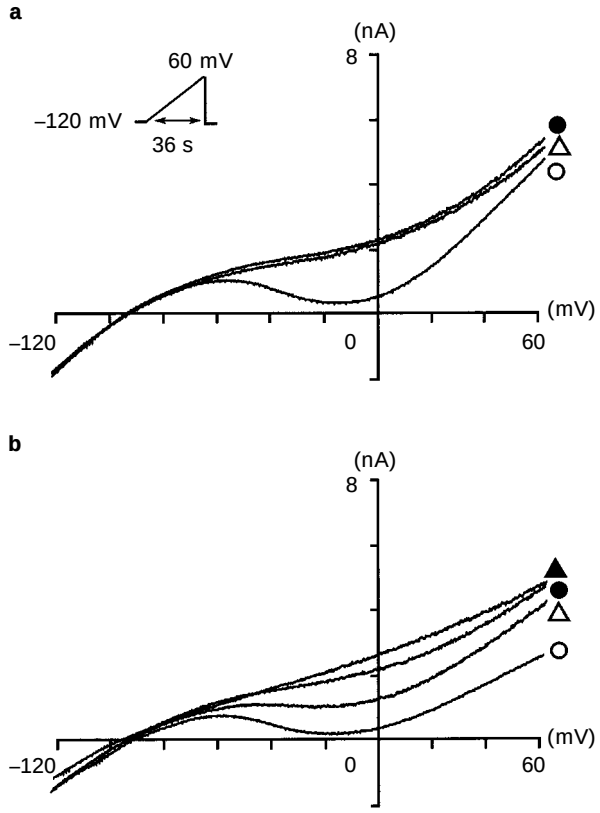

Figure 5 (a) Effect of $NaNO_2$ on the KRN4884-induced $I_{K\,(ATP)}$. (○) Control; (●) 0.3 μM KRN4884 and (△) 0.3 μM KRN4884 plus 30 μM $NaNO_2$. (b) Effect of 8-Br-cyclic GMP on the KRN4884-induced $I_{K\,(ATP)}$. (○) Control; (●) 0.3 μM KRN4884; (△) 0.3 μM KRN4884 plus 0.1 mM 8-Br-cyclic GMP and (▲) 0.3 μM KRN4884 plus 0.1 mM plus 0.1 mM 8-Br-cyclic GMP plus 1 mM NOR3. Note that 8-Br-cyclic GMP (0.1 mM) inhibited the KRN4884-induced $I_{K\,(ATP)}$, whereas NOR3 (1 mM) potentiated the current.

solution to the high K^+ solution (see methods). Under these conditions, no channel activity was recorded (Figure 6a). Subsequent bath application of KRN4884 (0.3 and 3 μM) activated an outward current in a concentration-dependent manner with a single-channel current amplitude of approximately 3 pA. These effects were almost completely inhibited by glibenclamide (3 μM) (Figure 6a, $n=4$), confirming that the KRN4884-induced outward current was derived from K_{ATP}. Potentiation of the KRN4884-induced $I_{K\,(ATP)}$ by NOR3 (1 mM) was observed in five patches, in which NPoi increased from 2.4 ± 1.5 to 5.1 ± 2.0 pA, ($P<0.01$, $n=5$; Figure 6b). This potentiation was reversed by washout of NOR3 (Figure 6b). Single channel current amplitude was not changed by NOR3 (0.3 μM KRN4884, 2.6 ± 0.1 pA; 0.3 μM KRN4884 plus 1 mM NOR3, 3.1 ± 0.1 pA; $n=4$). On the other hand, NOR3 (1 mM) dissolved 24 h before the experiment did not enhance KRN4884-induced $I_{K\,(ATP)}$ (0.3 μM KRN4884, 4.0 ± 1.4 pA; 0.3 μM KRN4884 plus 1 mM NOR3, 1.9 ± 1.0 pA; $n=3$).

Effects of NOR3 on $I_{K\,(ATP)}$ *in cell-free patches*

The effects of NOR3 on KRN4884-induced $I_{K\,(ATP)}$ were examined in either inside-out or outside-out patch configurations to minimize interference from cellular metabolic changes and regulatory enzymes. In inside-out patches, where the pipette contained the normal Tyrode solution and the patch membrane was exposed to the high K^+ solution containing 1 mM ATP, NOR3 (1 mM) caused little change in KRN4884-induced $I_{K\,(ATP)}$ at 0 mV (Figure 7a). In five patches, the mean NPoi was 7.6 ± 5.0 pA for 0.3 μM KRN4884 and 3.1 ± 1.4 pA for 0.3 μM KRN4884 plus 0.1 or 1 mM NOR3.

No potentiation was observed in outside-out patches either, where the pipette and bath solutions were the same as those used in the whole-cell recordings (Figure 7b). The membrane potential was held at 0 mV. In seven patches, the mean NPoi was 0.61 ± 0.3 pA for 0.3 μM KRN4884 and 0.44 ± 0.2 pA for 0.3 μM KRN4884 plus 1 mM NOR3 (NS, $P>0.05$).

Discussion

In the present study, we have demonstrated that NO facilitates the opening of K_{ATP} produced by K^+ channel openers, KRN4884 and cromakalim, in both the whole-cell and cell-attached patch clamp configurations. This conclusion is reached from the following findings: (1) a NO donor, NOR3, was effective only immediately after it had been dissolved into the solution, where NO release was expected to be on-going. NOR3 dissolved 24 h before the experiment failed to potentiate $I_{K\,(ATP)}$ activated by K^+ channel openers. (2) NOR3 by itself did not increase $I_{K\,(ATP)}$. (3) The potentiating effect of NOR3 was suppressed by oxyhaemoglobin. (4) Oxidative metabolites, such as NO_2^- or NO_3^-, could not mimic the effect of NOR3. (5) 8-Br-cyclic GMP could not mimic the effect of NOR3. (6) In cell-attached patches, NOR3 increased the open probability of K_{ATP} while the single channel amplitude remained unchanged.

In smooth muscle cells, cultured from the rat thoracic aorta, isosorbide dinitrate activated K_{ATP} via an increase of intracellular cyclic GMP and the effect was mimicked by 8-Br cyclic GMP (Kubo *et al*., 1994). In addition, NO hyperpolarized rabbit mesenteric arteries by activating K_{ATP} with the

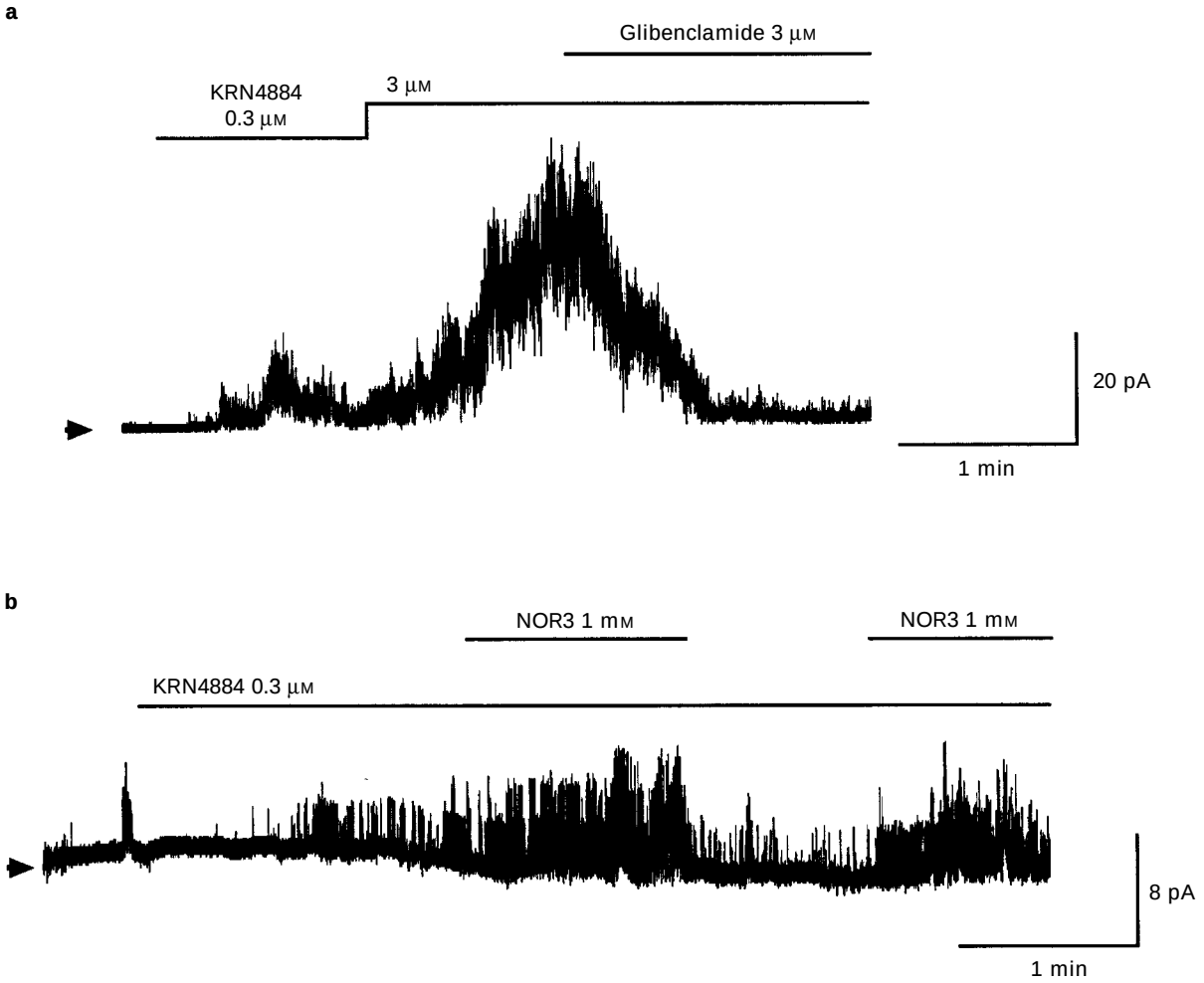

Figure 6 Potentiation by NOR3 of the KRN4884-induced $I_{K\,(ATP)}$ in cell-attached patches. (a) Activation of $I_{K\,(ATP)}$ by KRN4884. (b) Effect of NOR3 (1 mM). The membrane potential was held at 0 mV. The arrowheads indicate the zero current level. The protocols are shown as bars at the top of each trace. The low-filter was set at 300 Hz for trace reproduction.

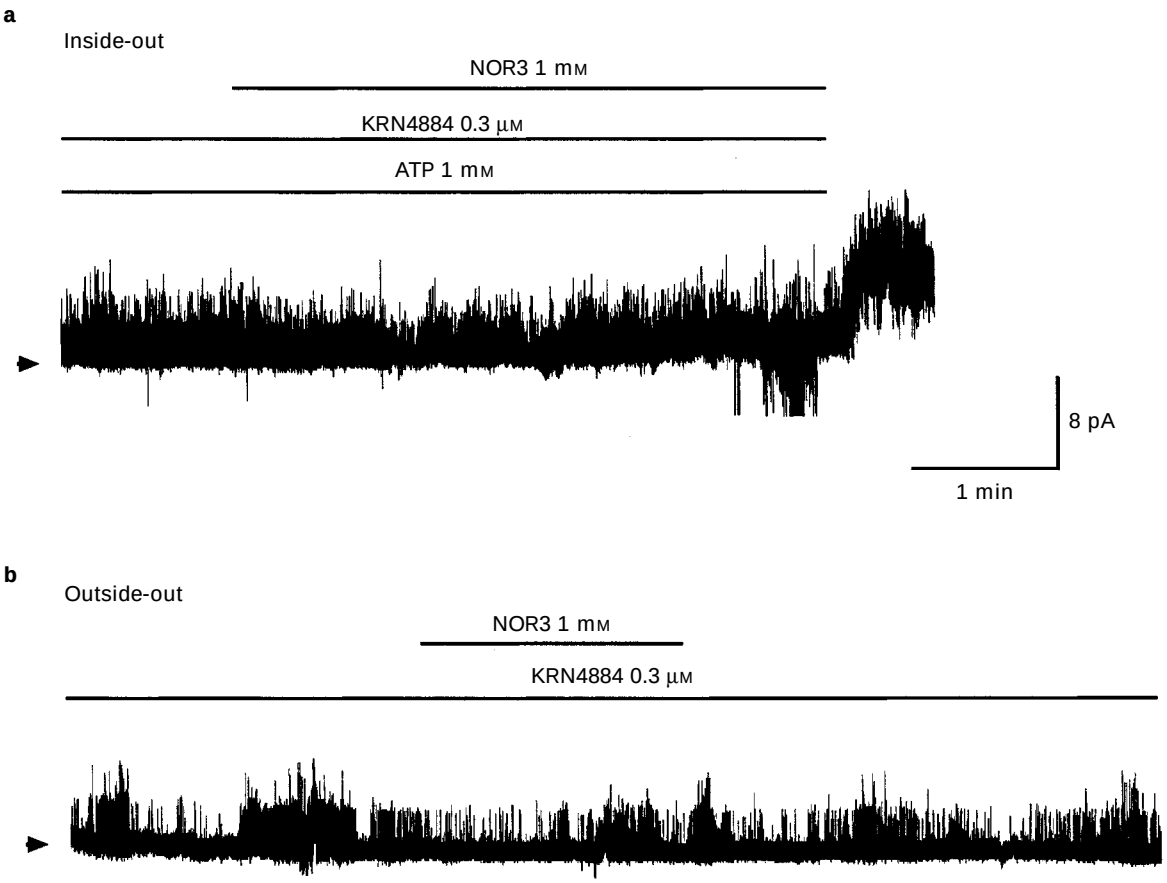

Figure 7 Effects of NOR3 on KRN4884-induced $I_{K\ (ATP)}$ in inside-out (a) and outside-out (b) patches. The membrane potential was held at 0 mV. The arrowheads indicate the zero current level. The low-filter was set at 300 Hz for trace reproduction.

accumulation of cyclic GMP as an intermediate step (Murphy & Brayden, 1995). However, in the present study, 8-Br-cyclic GMP (0.1 – 0.5 mM) significantly inhibited KRN4884-induced $I_{K\ (ATP)}$. Furthermore, even under these conditions, NO facilitated KRN4884-induced $I_{K\ (ATP)}$. These results suggest that the potentiating action of NO is not mediated by an increase in intracellular cyclic GMP in the heart.

In rat pancreatic β cells, NO activates K_{ATP} via a reduction of ATP production, an effect which was attributed to suppression of phosphofructokinase activity by Tsuura *et al.* (1994). However, in the present study, NO enhanced $I_{K\ (ATP)}$ in spite of 4 mM ATP in the pipette and 5.5 mM glucose in bath solution, conditions which should have been sufficient to avoid any possible decrease of ATP levels during the course of the experiments. Thus, it is not likely that the potentiating action of NO is mediated by a change in ATP levels. In fact, NO failed to ativate K_{ATP} in the heart even when the myocytes were exposed to partial metabolic inhibition (Tsuura *et al.*, 1994).

Direct effects of NO on ion channels have been documented in other systems. Bolotina *et al.* (1994) showed that NO directly activates Ca^{2+}-dependent K^+ channels in cell-free membrane patches from rabbit aortic smooth muscle without requiring cyclic GMP. Koh *et al.* (1995) obtained direct activation of NO on two different K^+ channels in canine colonic muscle cells. However, in the present study, NO failed to increase KRN4884-induced $I_{K\ (ATP)}$ both in inside-out and outside-out patches. This contrasted markedly with the consistent enhancement of this current in whole-cell and cell-attached patch configurations. These results may indicate the presence of soluble mediatiors which transduce the signal from NO to the enhancement of the $I_{K\ (ATP)}$.

NOS3 is expressed both in the endothelial cells (Marsden *et al.*, 1992) and in the myocytes of the heart (Balligand *et al.*, 1993). This implies that myocytes are exposed to NO under physiological conditions. In fact, recent studies have shown that NO may play an important role in mediating muscarinic cholinoceptor and β-adrenoceptor mediated regulation of voltage-dependent Ca^{2+} current and contraction (Balligand *et al.*, 1993; 1995; Han *et al.*, 1994; 1995). In addition, NOS2 is expressed in cardiac cells after stimulation with inflammatory mediators such as endotoxin and cytokines (Schulz *et al.*, 1992; Balligand *et al.*, 1994), resulting in a decrease in the contractility of both cardiac myocytes (Brady *et al.*, 1992) and papillary muscles (Finkel *et al.*, 1992). Furthermore, NO levels increase dramatically in the ischaemic heart to reduce both coronary vascular tone and the extent of the ischaemia (Node *et al.*, 1995). Furthermore, NO can protect the heart against ischaemia-induced reperfusion injury (Hoshida *et al.*, 1995). We therefore suggest that the K_{ATP} is modulated physiologically and pathophysiologically by NO in the heart. Thus the effects of K^+ channel openers would be amplified substantially, depending on the prevailing level of NO.

We have thus demonstrated that NO can potentiate the action of K^+ channel openers in cardiac cells. This potentiation cannot be explained by either an increase of intracellular cyclic GMP concentrations or metabolic inhibition, two mechanisms which have been linked with the action of NO in other preparations. Further studies are necessary to elucidate the underlying mechanism.

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