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Action of nicorandil on ATP-sensitive K^+ channel in guinea-pig ventricular myocytes

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1 Patch-clamp techniques were used to study the effects of nicorandil (2-nicotinamiodethyl nitrate) on the adenosine 5'-triphosphate (ATP)-sensitive K^+ channel current ($I_{K.ATP}$) in guinea-pig ventricular myocytes.

2 Nicorandil activated the time-independent outward current. This effect was dependent on intracellular ATP concentration ($[ATP]_i$) showing a larger effect at 2 mM than at 10 mM $[ATP]_i$. The nicorandil-induced outward current was inhibited by application of 0.3 μM glibenclamide.

3 In the inside-out patch configuration, 0.3–1.0 mM nicorandil increased the open-state probability of $I_{K.ATP}$ without a change in its conductance value (about 90pS). This effect was inhibited by glibenclamide. Analysis of the open and closed time distributions showed that nicorandil had no effect on open and closed distributions shorter than 5 ms. On the other hand, nicorandil increased the life time of bursts and decreased the interburst intervals.

4 The inward rectifier K^+ channel current was not influenced by internal application of nicorandil.

5 Therefore, we conclude that $I_{K.ATP}$ is the only K^+ current activated by nicorandil, and the main effect of nicorandil is on the kinetics of the $I_{K.ATP}$ bursting behaviour. These actions are similar to that of pinacidil on this preparation.

Keywords: Nicorandil; ATP-sensitive K^+ channel; guinea-pig ventricular myocytes

Introduction

The vasodilators, cromakalim, nicorandil and pinacidil have the ability to relax vascular smooth muscle and to hyperpolarize the membrane potential (Hamilton *et al.*, 1986). Hyperpolarization is considered to be caused by activation of certain types of K^+ channels. Because of their hyperpolarizing action, these vasodilators are claimed to be K^+ channel openers (Cook, 1988). The K^+ channel openers shorten action potential duration (APD) and sometimes hyperpolarize the resting membrane potential of heart muscle (Yanagisawa & Taira, 1981; Imanishi *et al.*, 1983; Taira, 1987; Smallwood & Steinberg, 1988). Recently, the target of the K^+ channel openers in the heart has been clarified by use of the single channel recording technique. A common action of the three agents, cromakalim, nicorandil and pinacidil was shown to be activation of the ATP-sensitive K^+ channel ($I_{K.ATP}$) in the heart (Escande *et al.*, 1988; 1989; Sanguinetti *et al.*, 1988; Osterrieder, 1988; Hiraoka & Fan, 1989; Arena & Kass, 1989a,b; Fan *et al.*, 1990). Standen *et al.* (1989) subsequently demonstrated the existence of $I_{K.ATP}$ in arterial smooth muscle cells and showed that cromakalim actually opened this channel.

In the previous study (Hiraoka & Fan, 1989), we demonstrated that nicorandil activated an outward K^+ current dependent on intracellular ATP concentration ($[ATP]_i$) and showed the current activated by nicorandil was $I_{K.ATP}$ at the single channel level in mammalian ventricular myocytes. However, the detailed action of nicorandil on $I_{K.ATP}$ remained unclarified. We subsequently studied the action of another K^+ channel opener, pinacidil on $I_{K.ATP}$ at the single channel level (Fan *et al.*, 1990; Nakayama *et al.*, 1990). Pinacidil activated the $I_{K.ATP}$ current in a dose-dependent and $[ATP]_i$-dependent manner. Kinetic analysis of the channel transitions revealed that pinacidil and $[ATP]_i$ act antagonistically on the opening and closing behaviour of $I_{K.ATP}$. To obtain further insight into the activation mechanism of $I_{K.ATP}$ of the heart by the K^+ channel openers, we have studied the actions of nicorandil on the whole cell current at various $[ATP]_i$ quantitatively and on the kinetics of single channel currents in guinea-pig ventricular myocytes. Part of this study was presented as an abstract (Hiraoka *et al.*, 1990).

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Methods

Single myocytes were isolated enzymatically from guinea-pig ventricles as described elsewhere (Hirano & Hiraoka, 1988).

Solutions

In the whole cell configuration the bath solution was Tyrode solution, the composition of which was (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES 5.0; the pH was adjusted to 7.3–7.4 by addition of NaOH. The composition of the pipette solution was (mM): KCl 120, K_2ATP (Sigma Chemical Co., St. Louis, MO, U.S.A.) 5.0, HEPES 5.0 and K_4BAPTA (Dojin Co., Kumamoto, Japan) 5.0; the pH was adjusted to 7.2 with KOH. In pipette solutions with different ATP contents, the concentration of KCl was varied so as to maintain the final K^+ concentration constant at 150 mM.

In the case of single-channel recordings, the bath solution (intracellular solution) contained (mM): KCl 140, glucose 5.5, EGTA 2 and HEPES 5; the pH was adjusted to 7.3 with KOH. The drug was dissolved in the bath solution at the concentration indicated in the text. The composition of the pipette solution (extracellular medium) was (mM): KCl 140, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES 5; the pH was adjusted to 7.3 by addition of KOH.

Whole-cell experiments

The whole cell configuration of the patch-clamp technique (Hamill *et al.*, 1981) was used to record membrane currents with a patch-clamp amplifier (Model 8900, Dagan Corp., Min-

neapolis, MN, U.S.A.). The recording technique and the data acquisition systems have been described previously (Hirano & Hiraoka, 1988). When the ramp voltage-clamp method was employed, an intelligent arbitrary function synthesizer (Model 1731, NF Inst., Yokohama, Japan) was used to supply the command pulse.

The temperature of the bath chamber was maintained at 34–35°C. Before contacts of the electrode and the cell, the junction potential was adjusted to zero at the level of the bath solution. At the end of each experiment the junction potential was checked again and, if the differences between the 1st and the 2nd measurements exceeded more than ±2 mV, the value of the membrane potential was corrected accordingly.

Measurements of cell surface area

The area of the cell surface was estimated from the cell capacitance assuming a specific membrane capacitance of 1 μF cm^{-2}. The cell capacitance was calculated from current responses induced by 1 mV depolarizing pulse from a holding potential of −90 mV (Lindar & Neher, 1988).

Single-channel recordings

Single-channel current recordings were made at room temperature in the inside-out patch configuration (Hamill *et al.*, 1981) with the same patch-clamp amplifier as in the whole-cell experiments. The current signals were stored on a video cassette recorder (BR-6400, Victor, Tokyo, Japan) through a PCM converter system (RP-882, NF Inst., Yokohama, Japan) at a conversion rate of 40 kHz. The recorded signals were filtered off-line through an 8-pole Bessel filter (48 dB/octave, FV-665, NF Inst., Yokohama, Japan) and digitized at 30 kHz on the disc of a computer (IBM-PS/2) using an analog-to-digital converter (TL-1 DMA interface, Axon Inst., Burlingame, CA, U.S.A.).

Single channel data analysis

Single channel records were analysed by the programme of the software package, pCLAMP 5.5 on a computer (IBM-PS/2). Briefly, the unitary current amplitude of $I_{K.ATP}$ channel was measured from the all point histograms through Gaussian fitting. When the inward rectifier K^+ current (I_{K1}) was induced together with $I_{K.ATP}$ at a potential negative to 0 mV (Trube & Hescheler, 1984), $I_{K.ATP}$ was identified by larger amplitude, current fluctuation during channel opening, and blockade by increased $[ATP]_i$. Each apparent distribution of open time, burst duration or closed time was formed using different cut-off frequencies (f_c) of the filter and bin-widths as described in the text and figure legends. At first, for measurement of the lifetime of the open- and closed state, 10 kHz f_c was used for detecting all events. From these data, mean open time and the shortest closed time constants were obtained. Then a burst duration and closed-state distribution were analysed using 0.1 kHz f_c. Under this f_c, almost all the fast closing events less than 1.6 ms which appeared under the 10 kHz f_c were filtered out leaving the open state, that is burst (Yellen, 1984). On the other hand, when the closed-time distribution analyses were carried out using the 0.1 kHz f_c, less than 5% of gaps between bursts were underestimated. Finally, a critical closed-time of 600 ms was chosen to separate one cluster from another.

Data were expressed as mean ± s.e. statistical comparison was made using non-paired or paired t test. A P value of less than 0.05 was considered significant.

Drugs

Nicorandil (2-nicotinamidoethyl nitrate; a gift from Chugai Pharmaceutical Co., Tokyo, Japan) was freshly prepared before every experiment and diluted into the test solution to obtain the final concentrations indicated in the text. Glibenclamide (a gift from Hoechst Japan, Tokyo) was dissolved as 0.2 mM stock solution in 2% dimethyl sulphoxide (DMSO) and diluted into the test solution appropriately before study. The final concentration of DMSO contained in the test solution was less than 0.01%. Our recent study confirmed that DMSO, at this concentration, had no effect on $I_{K.ATP}$ (Nakayama *et al.*, 1990).

Results

Effect of internal ATP concentration on nicorandil-activated outward current

We have already reported that nicorandil activates a time-independent outward current and this is suppressed by increasing $[ATP]_i$ (Hiraoka & Fan, 1989). Therefore, we examined quantitatively the nicorandil-activated current in different $[ATP]_i$. A ramp voltage clamp method was used to produce a slow depolarization (200 mV. 30 s^{-1}) from −120 mV to 80 mV. Figure 1 presents typical results obtained under

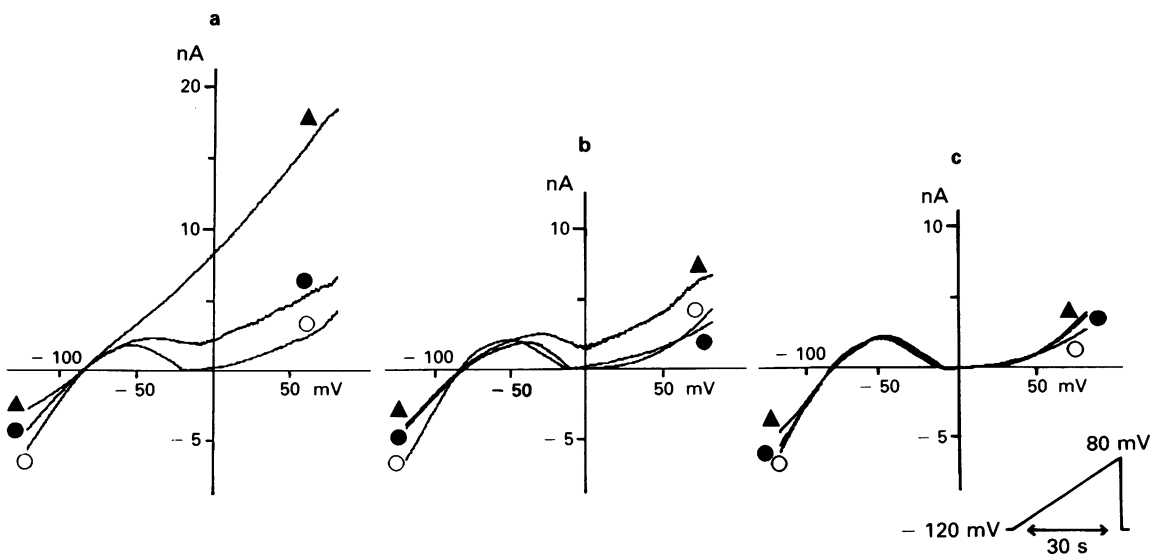

Figure 1 Effects of nicorandil on the background current induced by a 30 s depolarizing ramp voltage clamp pulse from −120 mV to +80 mV at different intracellular ATP concentrations ($[ATP]_i$). $[ATP]_i$ was 2 mM in (a); 5 mM in (b); and 10 mM in (c). (○) Control current before the application of nicorandil; (●) current in the presence of 0.3 mM nicorandil; (▲) current in the presence of 1.0 mM nicorandil.

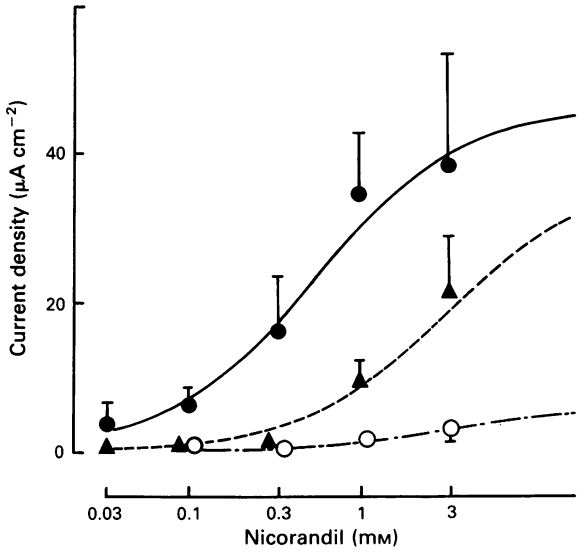

Figure 2 Dose-response curves of the nicorandil-activated current at different $[ATP]_i$. The ordinate scale indicates current density and the abscissa scale, the nicorandil concentration. Current density was calculated from the background current values at 0 mV. Each value represents a mean of four or five measurements from different preparations; bars indicate ±s.e. (●) 2 mM $[ATP]_i$; (▲) 5 mM $[ATP]_i$; (○) 10 mM $[ATP]_i$. A sigmoidal curve was fitted to the equation: $I = I_{max}/(1 + (Kd/[Nico])^n)$ where I = current density, I_{max} = maximum current density, K_d = apparent dissociation constant, n = slope parameter (Hill's coefficient) = 1, using a least squares method. Values of K_d were 0.5 mM, 2.3 mM and 2.9 mM for 2, 5 and 10 mM $[ATP]_i$, respectively.

conditions of different $[ATP]_i$. Nicorandil dose-dependently produced an increased outward current at voltages positive to −80 mV, and decreased the inward current negative to this voltage (see Discussion). These effects were most prominent at 2 mM $[ATP]_i$, while nicorandil had almost no effect on the outward current at 10 mM $[ATP]_i$. A quantitative estimation of the nicorandil-activated outward current was achieved by expressing the dose-response curve as current density compensating cell surface area (Figure 2). The current density was calculated using the current level at 0 mV from the type of experiments shown in Figure 1, since it was near zero during the control. At 2 mM $[ATP]_i$, the current density showed a sigmoidal dependence on the nicorandil concentration and was nearly saturated at 3 mM ($37.9 \pm 17.4\,\mu A\,cm^{-2}$, $n = 4$). At 5 mM $[ATP]_i$, the current density activated by nicorandil was decreased in comparison to 2 mM $[ATP]_i$ ($21.3 \pm 5.5\,\mu A\,cm^{-2}$ at 3 mM nicorandil; $n = 4$). At 10 mM $[ATP]_i$, the effect of nicorandil was further decreased to $3.06 \pm 1.61\,\mu A\,cm^{-2}$ at 3 mM nicorandil ($n = 4$). We did not examine the effects of nicorandil at concentrations higher than 3 mM, because such high concentrations could not be dissolved in the test solution.

Block of the nicorandil-activated current by glibenclamide

The effect of glibenclamide, a specific blocker of $I_{K.ATP}$ (Fosset *et al.*, 1988) on the nicorandil-activated current was examined. Since we have shown that nicorandil activates $I_{K.ATP}$ at the single channel level (Hiraoka & Fan, 1989), the nicorandil-activated outward current should be blocked by glibenclamide. Figure 3 shows a complete block by 0.3 μM glibenclamide of the nicorandil-activated current. The wash-out of glibenclamide caused a slow recovery and it took more than 10 min partially to reverse the effect. The effects of glibenclamide were confirmed in three myocytes.

Effect of nicorandil on the single channel current

The effects of nicorandil on the single channel current were examined by the inside-out patch configuration (Figure 4).

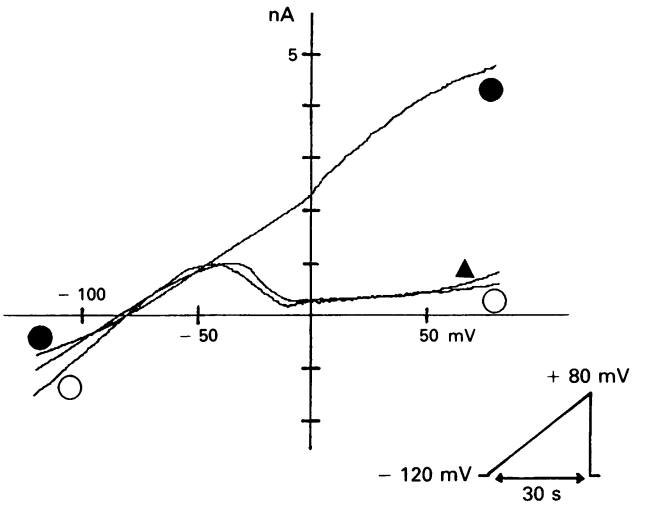

Figure 3 Effect of glibenclamide on the nicorandil-activated background current. Current was induced by 30 s depolarizing ramp pulse from −120 to +80 mV. $[ATP]_i$ was 2 mM. Note that 0.3 μM glibenclamide (▲) completely suppressed the nicorandil-activated background current (●); (○) control.

When the intracellular solution did not contain ATP, the channel stayed in the open state almost all the time (open-state probability (P_{open}) was 0.94) (Figure 4a). The P_{open} was decreased to 0.32 when 0.5 mM ATP was applied to the intracellular solution (Figure 4b), while addition of 1 mM nicorandil increased P_{open} to 0.54 without apparent changes in the unitary current amplitude (Figure 4c). The increased channel activity was largely inhibited by 0.3 μM glibenclamide, the P_{open} decreased to 0.07 (Figure 4d). The effect of glibenclamide, however, was not easily reversed and incomplete recovery was seen during the wash-out period of more than 10 min. In nine out of twelve examined patches, application of 1 mM nicorandil caused channel activation similar to that shown in Figure 4c. The effects of nicorandil did not last long and, 5–10 min after application, the increased channel activity gradually declined. Furthermore, $I_{K.ATP}$ activity decreased with time in the low $[ATP]_i$, the process known as 'run-down' of the channel (Ashcroft, 1988). Once the channels were completely inactivated in the low $[ATP]_i$ due to run-down, nicorandil up to 3 mM could not restore the channel activity as reported recently ($n = 10$; data not shown) (Takano & Noma, 1990). Therefore, we assume that the patches in which nicorandil did not activate the channels, contained no $I_{K.ATP}$ channels or these channels were completely inactivated.

The conductance properties of the nicorandil-activated channel were examined in more detail (Figure 5). Amplitude histogram analysis of all the data points observed in 0.5 mM ATP solution, and 0.5 mM ATP plus 1 mM nicorandil solution showed that there was one Gaussian peak distribution at positive voltages. When the membrane potential was held at negative voltages, there were two peaks of amplitude histogram observed. The smaller amplitude channel showed long-lasting openings, had a single channel conductance of 30 pS and was not recorded at positive voltages probably due to inward rectification. Because of the magnitude of conductance and strong inward-rectification, this channel was identified as I_{K1} (Kameyama *et al.*, 1983; Sakmann & Trube, 1984). The other channel had a larger amplitude, and showed rapid opening and closing behaviour and it was sensitive to ATP applied intracellularly. Application of 1 mM nicorandil increased the open-state probability of this larger amplitude current. The current-voltage relationship (I–V) of this channel is shown in Figure 5B. The current recorded in the presence of 0.5 mM ATP had a reversal potential of −2.8 mV. The I–V curve at negative voltages was linear with a slope conductance of 92.9 pS ($n = 5$). On the basis of the slope conductance, the reversal potential and ATP sensitivity, the current recorded at 0.5 mM ATP was identified as $I_{K.ATP}$. The nicorandil-activated

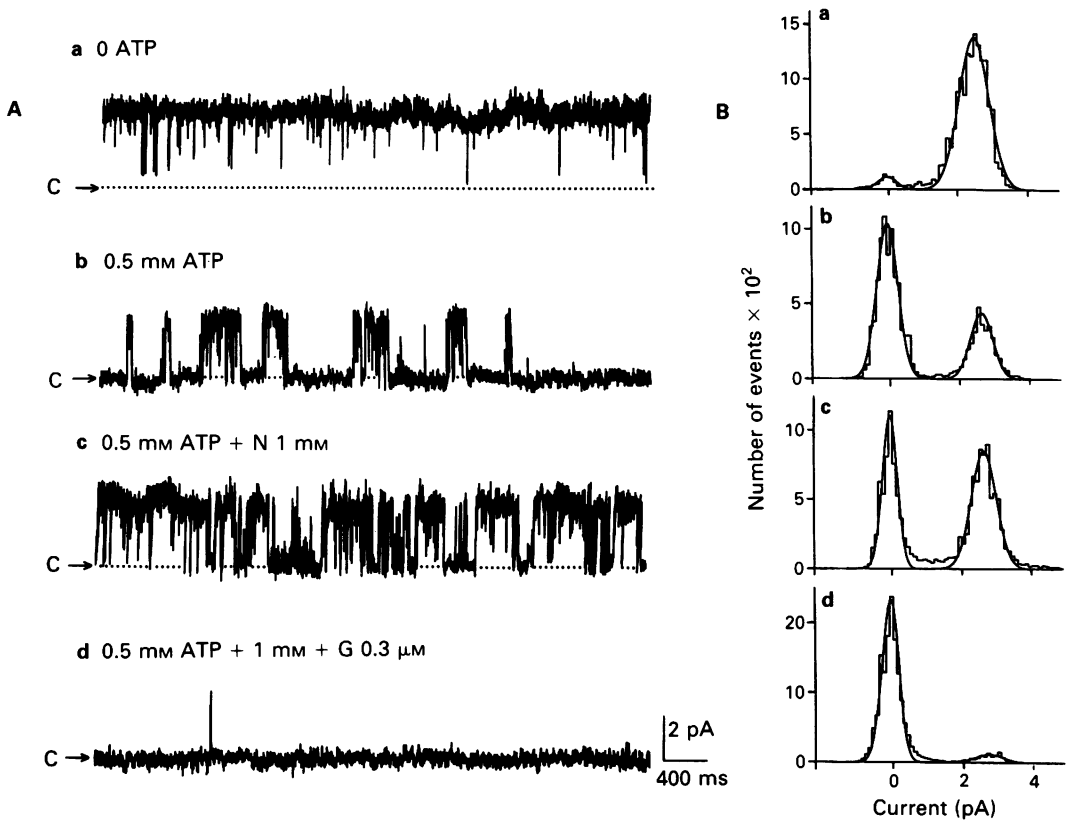

Figure 4 Effect of nicorandil (N) and glibenclamide (G) on the ATP-sensitive single-channel current. (A) Current traces recorded from a single patch. Membrane potential was held at +40 mV in the inside-out patch configuration. C, Closed level. (a) Channel activity in the absence of ATP (0 ATP) in the intracellular solution. There were marked openings of the channel, so that the channel mostly stayed at the open state (P_{open} = 0.94). (b) Presence of 0.5 mM ATP in the intracellular solution. P_{open} was decreased to 0.32. (c) Application of 0.5 mM ATP and 1 mM nicorandil in the intracellular solution. Marked openings of the channel were again observed (P_{open} = 0.54). (d) Further application of 0.3 μM glibenclamide in the internal solution, resulted in suppression of channel activity (P_{open} = 0.07). (B) Amplitude histograms recorded from the same patch in panel (A) in the corresponding conditions of (a)–(d).

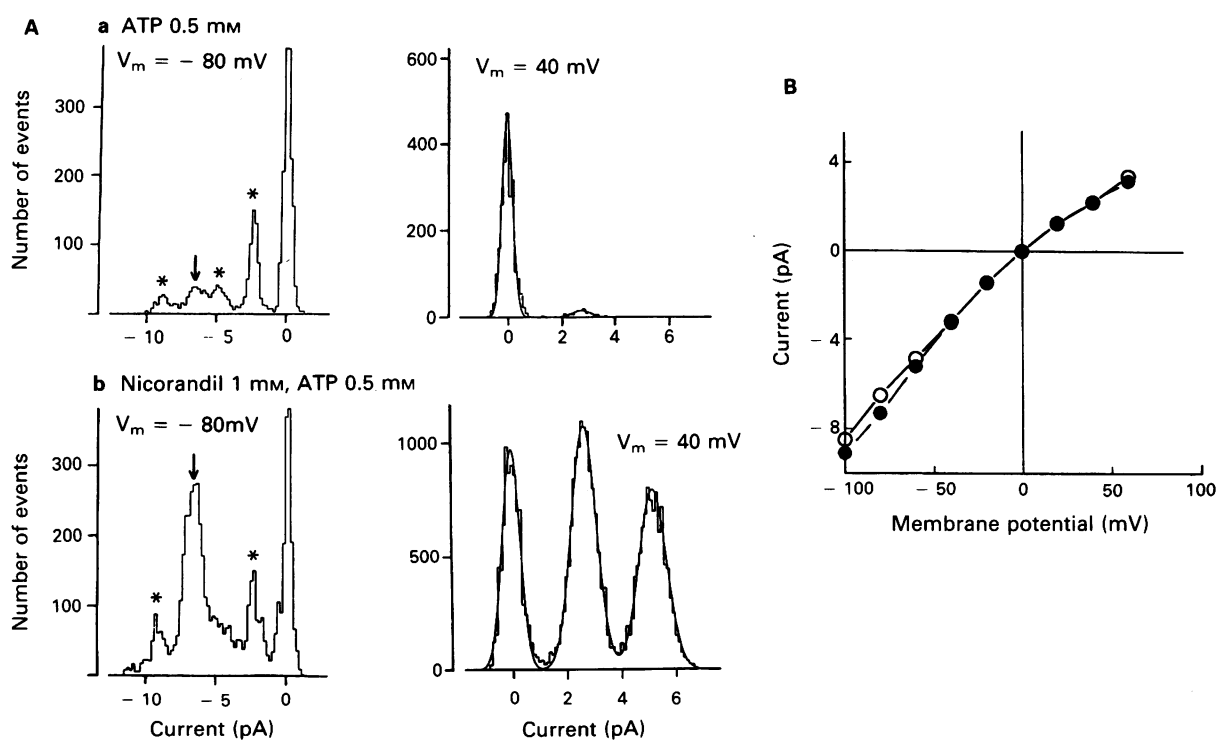

Figure 5 Effects of nicorandil on activation of ATP-sensitive K^+ channel currents recorded from an inside-out membrane patch. (A) (a) Amplitude histograms of the single-channel current at V_m = −80 mV (left panel) and V_m = +40 mV (right panel) obtained in the solution containing 0.5 mM ATP. (A) (b) Amplitude histograms of the single-channel current at V_m = −80 mV (left panel) and V_m = +40 mV (right panel) obtained in a solution containing 1 mM nicorandil and 0.5 mM ATP. In (A) ↓: ATP sensitive K^+ channel; *: the inward rectifier K^+ channel. Note that the amplitude of both channels was not changed by nicorandil, but the numbers of events of the larger amplitude (↓) were increased at V_m = −80 mV. (B) Current-voltage relationship (*I*–V) and the effect of nicorandil on it. Nicorandil did not change the conductance of this channel current.

current had a reversal potential of −2.3 mV and a slope conductance of 88.9 pS (n = 4). There was no significant difference in the reversal potential or slope conductance between $I_{K.ATP}$ and the nicorandil-activated current. The linearity of the I–V curves in both cases was lost at voltages positive to +40 mV, indicating weak inward rectification at high positive voltages.

Modulation of channel kinetics by nicorandil

The open and closed time distributions were analysed to determine the action of nicorandil on the kinetic properties of $I_{K.ATP}$. Probability density histograms of the open and closed times measured at −80 mV are shown in Figures 6 and 7. The

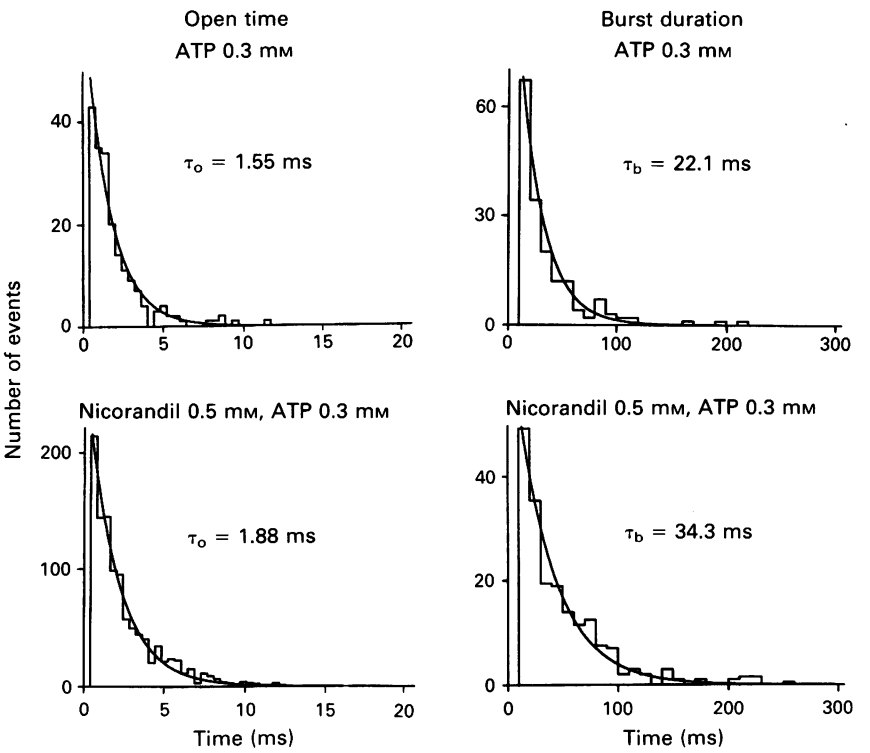

Figure 6 Effects of nicorandil on the open time of the ATP-sensitive single-channel current recorded from an inside-out patch membrane. Histograms of open time, analysed with high f_c = 10 kHz (left column) and histogram of life-time of bursts analysed with low f_c = 0.1 kHz (right column), with 0.3 mM ATP (upper panel) and 0.5 mM nicorandil plus 0.3 mM ATP (lower panel) in the internal solution. The time constants (τ_0) of the open time and that of the bursts (τ_b) obtained under the two different conditions were best expressed by single exponentials. Nicorandil increased τ_b without affecting τ_0.

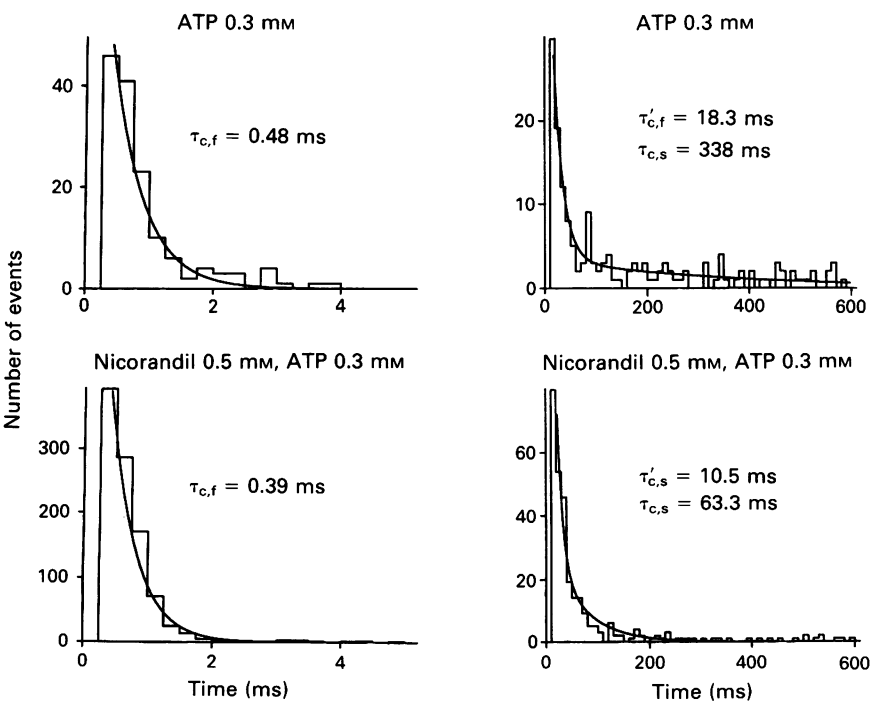

Figure 7 Effects of nicorandil on closed time distributions of the ATP-sensitive channel current recorded from an inside-out patch membrane. The left panel shows analysis of closed time shorter than 5 ms at an f_c of 10 kHz and the right panel shows the analysis of those up to 600 ms at an f_c of 0.1 kHz. The presence of nicorandil did not alter the time constants of the fast components ($\tau_{c,f}$ or $\tau'_{c,f}$) in distributions of short (left panel) or long (right panel) closed times. The time constant of the slow component ($\tau_{c,s}$) in the distribution of long closed times (right panel) was shortened by nicorandil. Data obtained from same patch as Figure 6 showing only a single level of channel activity throughout the two conditions.

open time histogram, which was analysed from the current record filtered at f_c of 10 kHz, revealed a single exponential distribution with a time constant of 1.55 ms in 0.3 mM ATP. In 0.3 mM ATP plus 0.5 mM nicorandil, the open time constant did not differ substantially from that in the control condition (τ_0 = 1.88 ms). The life time of a burst was defined as an opening period observed in the records filtered at f_c of 0.1 kHz (see, Methods). The histogram of burst duration consisted of a single exponential distribution. Its time constant, designated as τ_b was markedly prolonged by nicorandil.

The histogram of closed time within bursts was best fitted to a single exponential function. This analysis was performed with closed times longer than 5 ms to be discarded, and filtered at f_c of 10 kHz. The time constant of the closed time within bursts was designated at $\tau_{c,f}$. The value of $\tau_{c,f}$ was not changed markedly by application of nicorandil. The closed time histogram analysis using records filtered at f_c of 0.1 kHz and discarding closed times longer than 600 ms consisted of two exponentials with time constants of a fast ($\tau'_{c,f}$) and a slow component ($\tau_{c,s}$). $\tau'_{c,f}$ was equivalent to $\tau_{c,f}$ filtered at f_c of 10 kHz which was distorted by heavy filtering. The value of $\tau'_{c,f}$ was not influenced by nicorandil. $\tau_{c,s}$ represented the time constant of the interburst durations. The value of $\tau_{c,s}$ was 338 ms in 0.3 mM ATP. This value was markedly decreased to 63.3 ms by application of 0.5 mM nicorandil. These findings suggest that nicorandil increases the open-state probability of $I_{K.ATP}$ by an increase in burst durations and a decrease in interburst intervals. Similar results were obtained from three other patches at different concentrations of nicorandil and $[ATP]_i$. In a previous study (Fan *et al.*, 1990), we analysed the effects of $[ATP]_i$ and pinacidil on the clusters separated by longer closed times than 600 ms. In the present study, however, as the frequencies of such clusters were relatively low and the nicorandil effects were short-lived as stated above, we did not examine the drug actions on the clusters separated by closed times longer than 600 ms.

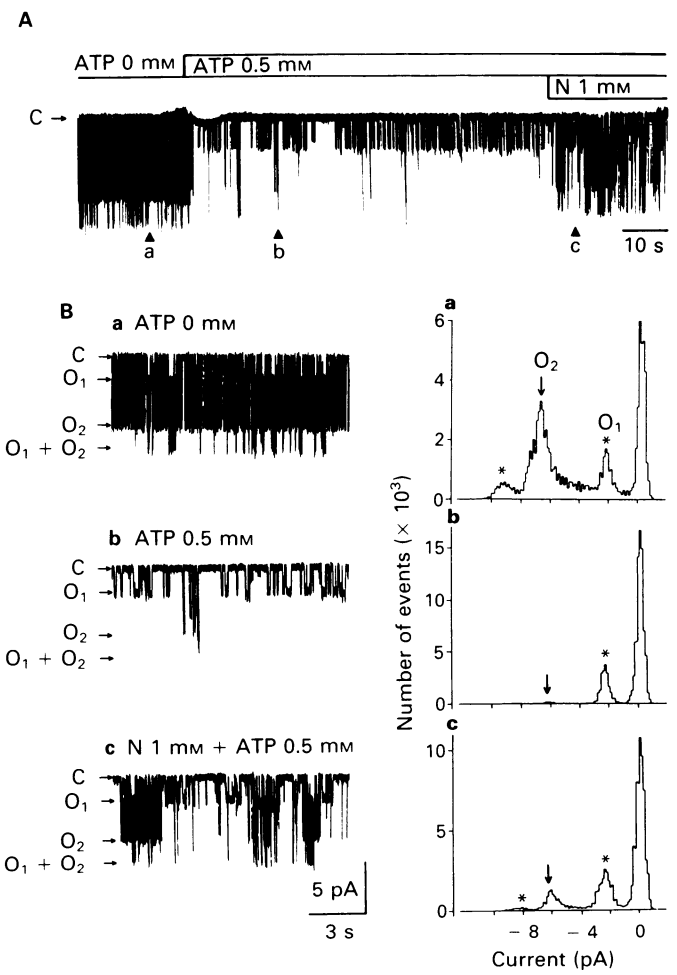

Figure 8 Effects of nicorandil on the inward rectifier K^+ channel current recorded from an inside-out patch membrane. (A) Continuous record at a holding potential of −80 mV. At the top, the ATP concentration and application time of nicorandil in the internal solution are shown. (B) Expansions of the records (left panel) and amplitude histograms (right panel) at the times marked by (▲) are shown. O_2 (↓) represents the ATP-sensitive K^+ channel current and O_1(*) represents the inward rectifier K^+ channel current. Note that the inward rectifier K^+ channel current was not influenced by nicorandil and no other current was activated by the drug under these recording conditions.

Effect of nicorandil on the inward rectifier K^+ channel

Kakei *et al.* (1986) reported nicorandil activated a small-conductance, time-independent K^+ channel (4.6 ± 1.7 pS) estimated from the relation between the variance and the mean of whole-cell current in guinea-pig ventricular myocytes. Therefore, we examined the effects on a K^+ channel other than $I_{K.ATP}$ present in these cells. Figure 8 shows a typical example for estimation of the nicorandil effect on a single I_{K1} channel in an inside-out patch. At a holding potential of −80 mV, two kinds of channels were observed, $I_{K.ATP}$ and I_{K1} as already revealed in Figure 5. $I_{K.ATP}$ was suppressed by 0.5 mM ATP and activated by 1 mM nicorandil. On the other hand, the open-state probability (P_0) of the I_{K1} channel was 23.7 ± 8.4% at 0 mM ATP (n = 3), and was not changed by 0.5 mM ATP (P_0 = 21.7 ± 5.2%) and 1 mM nicorandil (P_0 = 28.6 ± 8.6%). Neither ATP nor nicorandil had any effects on the unitary amplitude of I_{K1}. We did not see any channel activity having a unitary current amplitude smaller than those of $I_{K.ATP}$ and I_{K1} in the presence of 0.5 mM ATP and of nicorandil up to 1 mM in 15 patches examined.

Discussion

The present study shows that a coronary vasodilator, nicorandil, activates the time-independent outward current in ventricular myocytes, in a manner dependent on $[ATP]_i$. The dose-response curve of nicorandil had a sigmoid dependence on $[ATP]_i$ and the curve was shifted to the right with increasing $[ATP]_i$. The nicorandil-activated outward current was inhibited by application of glibenclamide. Single channel current recordings confirmed an activation of $I_{K.ATP}$ by nicorandil without changing the unitary amplitude of the current. The mode of the channel activation was to prolong the burst duration and to shorten the interburst interval. Therefore, in ventricular myocytes nicorandil activates $I_{K.ATP}$ channel by increasing its open probability via modification of the bursting behaviour of the channels.

Direct evidence for $I_{K.ATP}$ as a target of K^+ channel openers was first described in cardiac myocytes at the single channel level (Escande *et al.*, 1988; 1989; Hiraoka & Fan, 1989; Arena & Kass, 1989b). Recently, several studies have indicated that the target is also $I_{K.ATP}$ in vascular smooth muscle cells (Standen *et al.*, 1989) and pancreatic β cells (Dunne, 1990). Therefore, K^+ channel openers seem to have a common target in different tissues, although effectiveness and sensitivity to these openers were not equal among different preparations. In addition, conflicting reports have been presented as to the target of the K^+ channel openers in vascular smooth muscle cells at the single channel level (Standen *et al.*, 1989; Gelband *et al.*, 1989; Inoue *et al.*, 1989; Kajioka *et al.*, 1990). It is not known whether these conflicting results represent the diversity of K^+ channels in vascular smooth muscles from different organs, species differences or any other unspecified reasons. At present, it is not known whether, or not, the K^+ channel activated by the K^+ channel openers in vascular smooth muscles is identical to $I_{K.ATP}$ in the heart and pancreatic β cells.

The general characteristics of the target of K^+ channel openers in the heart and pancreatic β cells are similar. For example, the target channel is inhibited by increased $[ATP]_i$ and by glibenclamide and has a single channel conductance of

60–90 pS under symmetrical K^+ conditions (Escande *et al.*, 1988; 1989; Arena & Kass, 1989a; Fan *et al.*, 1990; Dunne, 1990). These characteristics are similar to those of $I_{K.ATP}$ reported by others (see, Ashcroft, 1988).

The properties of $I_{K.ATP}$ described in cardiac and skeletal muscles, and in pancreatic β cells are similar to those recorded in the present study (Ashcroft, 1988). The single channel conductance recorded during control experiments using a low (0.5 mM) ATP concentration is about 93 pS, a little larger than that of our previous report (about 80 pS; Fan *et al.*, 1990). This difference may arise from different systems of the patch clamp amplifiers used and somewhat different sampling frequencies of the low-pass filter in both experiments.

There is general agreement that at least one or two open states and two closed states are required to account for $I_{K.ATP}$ channel kinetics (Ashcroft, 1988). In our previous report (Fan *et al.*, 1990), we proposed the following channel kinetic scheme for $I_{K.ATP}$:

$$C_3 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1$$

where the rate constant k_{-1} was considered to be much faster than k_{-2}. For the present analysis, we used different f_c values and discarded the critical times to discriminate the different states, as we did in a previous report (Fan *et al.*, 1990). A single open state was proposed, because the open time distribution was best fitted to a single exponential function when data were collected using an f_c value of 10 kHz. When f_c was set at 0.1 kHz, the briefer closures than 1.6 ms were filtered out so that the transition from state O to state C_2 could be observed, i.e. the burst duration. For closed time analysis, we also employed different f_c value and divided the shut time events of the channel into different time scales. Using an f_c value of 10 kHz and the closed time duration up to 5 ms, the transition from state C_1 to state O was analysed. When f_c was reduced to 0.1 kHz and the closed time durations up to 600 ms were included, the histogram was best fitted to two exponentials which were considered to reflect the transitions from C_1 to O and C_2 to O. The closed time longer than 600 ms was taken to separate clusters of bursts from one another. This revealed another closed state, C_3 (see, Fan *et al.*, 1990).

The transitions between state O and C_1 are insensitive to nicorandil. Nicorandil seems to affect the transition between state O and C_2, that is increases in burst duration. This effect of nicorandil on $I_{K.ATP}$ kinetics was similar to that of pinacidil and opposite to increasing the ATP concentration (Fan *et al.*, 1990), although the nicorandil effect on the transition between C_2 and C_3 was not evaluated. Gillis *et al.* (1989) analysed the effects of sulphonamides on $I_{K.ATP}$ kinetics in β cells. They showed that glibenclamide and tolbutamide shortened the $I_{K.ATP}$ burst duration and lengthened the closed time between the bursts, and this effect is similar to increasing the concentration of extracellular glucose recorded in cell-attached patch. From all of these data, many $I_{K.ATP}$ modulators, including ATP, influence the same aspect of channel kinetics.

Nicorandil has been shown to shorten APD and to hyperpolarize depolarized resting membrane potentials (Yanagisawa & Taira, 1981; Imanishi *et al.*, 1983). It was suggested that the mechanism responsible for these findings was activation of the background K^+ conductance (Taira, 1987) and delayed outward K^+ current (I_K) (Imanishi *et al.*, 1983). In our previous report, we ruled out the possible involvement of I_K in drug-induced outward current. In the present study we have excluded the effect of nicorandil on I_{K1} at the single channel level using an inside-out patch, and did not see any evidence for activation of a small conductance K^+ channel as described by Kakei *et al.* (1986). We also showed the complete block of nicorandil-activated outward current by glibenclamide, a specific $I_{K.ATP}$ blocker (Fosset *et al.*, 1988). So far, $I_{K.ATP}$ seems to be the only K^+ current activated by nicorandil. In the whole current configuration, nicorandil decreased the inward current at potentials negative to −90 mV, a finding that does not agree with the explanation of $I_{K.ATP}$ as the only K^+ current affected by nicorandil. The most likely explanation for this is that nicorandil has an inhibitory action on I_{K1} from the outer surface of the membrane, since it did not affect I_{K1} with the internal application (Figure 8). Further studies are necessary to understand the whole drug effects on ventricular cells including outside-out membranes patches.

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