J. Pharm. Pharmacol. 2000, 52: 1395–1403
Received March 16, 2000
Accepted June 12, 2000

Possible Underestimation of the Channel Conductance Underlying Pinacidil-induced K^+ Currents Using Noise Analysis in Pig Urethral Myocytes

NORIYOSHI TERAMOTO, ALISON F. BRADING* AND YUSHI ITO

*Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 812-8582, Japan and *University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK*

Abstract

Electrophysiological and pharmacological properties of the pinacidil-induced K^+ currents in isolated cells from pig urethra were investigated using patch-clamp techniques.

Pinacidil (100 μM) induced an outward current at −50 mV which gradually decreased. Under current-clamp conditions, 100 μM pinacidil induced a hyperpolarization that was sustained. This suggests that activation of only a few channels can hyperpolarize the membrane. At a holding potential of −50 mV, glibenclamide inhibited the pinacidil-induced current with a single exponential time course.

Unitary current recordings in symmetrical 140 mM K^+ conditions demonstrated that pinacidil activates a 43-pS, glibenclamide-sensitive K^+ channel (i.e. K_{ATP} channel). Analysis of the basal noise of the pinacidil-induced macroscopic currents from −90 mV to −30 mV yielded estimates of channel conductance (6 pS) which were much smaller, and probably an underestimate.

These results indicate that pinacidil induces a glibenclamide-sensitive K^+ current through only one type of K^+ channel (K_{ATP} channel) in pig urethra.

Pinacidil, one of the most well-known and potent K^+-channel openers, has been utilized for functional studies of channel-opening kinetics ever since its synthesis as an antihypertensive agent (Petersen et al 1978). In vascular smooth muscle, it has been clearly shown that pinacidil selectively activates either intracellular Ca^{2+}-activated large conductance K^+ channels (BK_{Ca}, maxi K^+ channels etc.) or intracellular Ca^{2+}-insensitive smaller conductance K^+ channels (ATP-sensitive K^+ channels: K_{ATP} channels, nucleoside-diphosphate-sensitive K^+ channels: K_{NDP} channels etc.; Quayle et al 1997). Recently, pinacidil has been described as a non-selective K^+-channel opener, targeting both maxi K^+ channels and K_{ATP} channels in human coronary artery (Bychkov et al 1997). Despite the common vasodilator action of pinacidil, it still remains uncertain whether or not the different target K^+ channels arise from different experimental conditions or are species-dependent even in vascular smooth muscle. The potent relaxant effects of pinacidil on nonvascular smooth muscles in intact tissues have also been widely investigated (guinea-pig ileum, Sun & Benishin (1994); rat intestine, Davies et al (1996); guinea-pig detrusor, Gopalakrishnan et al (1999); pig urethra, Teramoto & Ito (1999)) and a possible clinical role for pinacidil in several diseases has been suggested (Andersson 1992). Therefore, we believe that direct investigations of the effects of pinacidil are essential in non-vascular smooth muscle as well as in vascular smooth muscle.

This study was designed to identify the target K^+ channels for pinacidil in pig urethral smooth muscle. We have reported the presence in this tissue of ATP-sensitive 43-pS K^+ channels which are suppressed by glibenclamide (K_{ATP} channel; Teramoto et al 1997), and were interested to see if these were the target. We also wished to compare the time course of the pinacidil-induced K^+ currents in voltage-clamp experiments, with that of the pinacidil-induced hyperpolarization in current-clamp mode. Furthermore, we discuss the possibility that noise analysis may underestimate channel conductance of the pinacidil-induced K^+ currents in whole-cell configuration in comparison with the

Correspondence: N. Teramoto, Department of Pharmacology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, 812-8582, Japan.
E-Mail: noritera@linne.med.kyushu-u.ac.jp

direct measurement of the channel conductance in single-channel recordings.

Materials and Methods

Solutions and drugs

In whole-cell configuration, the following solutions were used: physiological salt solution (PSS) containing (mM): Na^+ 140, K^+ 5, Mg^{2+} 1·2, Ca^{2+} 2, glucose 5, Cl^- 151·4, HEPES 10, titrated to pH 7·35–7·4 with Tris base; pipette solution containing (mM): K^+ 140, Cl^- 140, 5 EGTA, 10 HEPES/Tris (pH 7·35–7·4). For single-channel recording, the pipette and bath solution was a high-K^+ solution (mM): K^+ 140, Cl^- 140, EGTA 5, glucose 5, HEPES 10/Tris (pH 7·35–7·4) producing symmetrical 140 mM K^+ conditions. Glibenclamide (Sigma-Aldrich Japan K.K., Tokyo, Japan), pinacidil (RBI Chemicals Ltd, MN) and levcromakalim (kindly provided by SmithKline Beecham Pharmaceuticals, Harlow, UK) were prepared daily in DMSO as a stock solution (100 mM; 0·1% DMSO did not affect the K^+ channels). Adenosine 5′-diphosphate (ADP), guanosine 5′-diphosphate (GDP), inosine 5′-diphosphate (IDP) and uridine 5′-diphosphate (UDP) were added as the relevant sodium salt and 100 mM stock solutions of these were titrated to pH 7·4 and frozen at −70°C. Dilution of the stock solution was made immediately before application. Nucleotide diphosphates were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan).

Cell preparation and recording procedure

Fresh female pig urethra, still attached to its bladder, was collected from a local abattoir and transported to the laboratory in cold PSS solution. The proximal region of the pig urethra (1–2 cm region from the bladder neck) was excised and the connective tissue and mucosa removed under a dissection microscope. An identical cell dispersion method was employed as described previously (Teramoto et al 1997). Relaxed spindle-shaped cells, length 200–400 μm, were isolated and stored at 4–6°C. The dispersed cells were normally used for experiments within 3 h of dispersion. The patch-clamp experimental system used was essentially the same as that described previously (21–23°C, Teramoto et al (2000)). In short, generation of voltage pulses was performed using an L/M-EPC 8 patch-clamp amplifier (HEKA, Lambrecht, Germany) in conjunction with a circuit board which had both an analogue-to-digital and a digital-to-analogue conversion function (ITC-16, Instrutech Corp., New York, US), using a Pulse software package (HEKA, Lambrecht, Germany). The sampled current data were filtered at 10 kHz and stored together with potential records on videotape using a digital data recorder (VR-10B, Instrutech Corp., New York, US) coupled to a video recorder (Panasonic, Tokyo, Japan) for subsequent off-line analysis. Junction potentials between bath and pipette solutions were measured using a 3 M KCl reference electrode and were < 2 mV, so that correction for these potentials was not made.

Data analysis in patch-clamp experiments

For display purpose, the whole-cell current data were low-pass filtered at 500 Hz by a filter (E-3201B, NF Electronic Instruments, Yokohama, Japan), sampled at 25 ms and analysed on a computer by use of the MacLab 3.5.6. For measuring the current noise, we used programs provided by Dr J. Dempster (University of Strathclyde, UK) to measure mean current and variance of 250 ms segments of recording filtered at 2 kHz (8-pole Butterworth) and sampled at 4 kHz. For single-channel recording, the stored data were low-pass filtered at 2 kHz and sampled into the computer with an interval of 80 μs using the PAT program (kindly provided by Dr J. Dempster). The all-point amplitude histogram was obtained from a continuous recording of 2 min and fitted with the Gaussian distribution function using a least-squares fitting. Continuous traces in the figures (> 4 s) were obtained from records filtered at 500 Hz for presentation. Statistical analyses were performed with an analysis of variance test (two-factor with replication). Changes were considered significant at $P < 0{\cdot}01$, and data are expressed as mean ± s.d.

Results

Properties of the pinacidil-induced K^+ currents in pig urethra

The properties of the pinacidil-induced K^+ currents were studied using whole-cell voltage-clamp techniques. To obtain both the current–voltage (I–V) relationships in the absence and presence of pinacidil, and the reversal potential of the pinacidil-induced current, voltage ramps were applied (see inset in Figure 1A) and the extracellular K^+ concentration ($[K^+]_o$) was changed by the iso-osmotic substitution of Na^+. In the absence of pinacidil (control), I–V relationships were obtained first in 5 mM K^+ and then in 60 mM K^+ and then back in 5 mM K^+. Pinacidil (100 μM) was then added to the bath solution (5 mM K^+), causing a sustained outward current, which reversed to an inward current on raising $[K^+]_o$ to 60 mM. On returning to 5 mM

$[K^+]_o$, the pinacidil-induced current again became outward. The current was suppressed by application of 5 μM glibenclamide. Figure 1B shows the average of the six ramp currents before and during application of 100 μM pinacidil (5 mM K^+, 60 mM K^+) for the cell shown in Figure 1A. In each $[K^+]_o$ condition, the net membrane current activated by 100 μM pinacidil was obtained by subtraction of the averaged control current from the mean pinacidil-induced current (Figure 1C). The reversal potential of the pinacidil-induced membrane current in this cell was −76 mV in 5 mM K^+ (mean value from 6 experiments, -78 ± 3 mV), and −15 mV in 60 mM K^+ (mean value from 6 experiments, -17 ± 3 mV). These values were close to the theoretical K^+ equilibrium potential (E_K) in each $[K^+]_o$ condition (5 mM K^+, $E_K = -84{\cdot}2$ mV; 60 mM K^+, $E_K = -21{\cdot}4$ mV). These results suggest that the pinacidil-induced membrane currents are mainly carried by K^+, through channels which are sensitive to glibenclamide.

To investigate further the inhibitory effects of glibenclamide on the pinacidil-induced membrane currents, conventional whole-cell recordings were performed in symmetrical 140 mM K^+ conditions (bath solution 140 mM K^+ PSS, pipette solution 140 mM KCl containing 5 mM EGTA, $E_K = 0$ mV) at a holding potential of −50 mV to enhance the peak amplitude of the currents. Figure 2A shows an example of the effects of glibenclamide on the 100 μM pinacidil-induced inward membrane current. In an attempt to investigate the time course of the inhibitory action of glibenclamide, we plotted the current amplitude against time after application of 5 μM glibenclamide using a concentration-jump method. The inhibitory time course could be fitted with a single exponential (Figure 2B), with a time constant of 33 ± 9 s (n = 5).

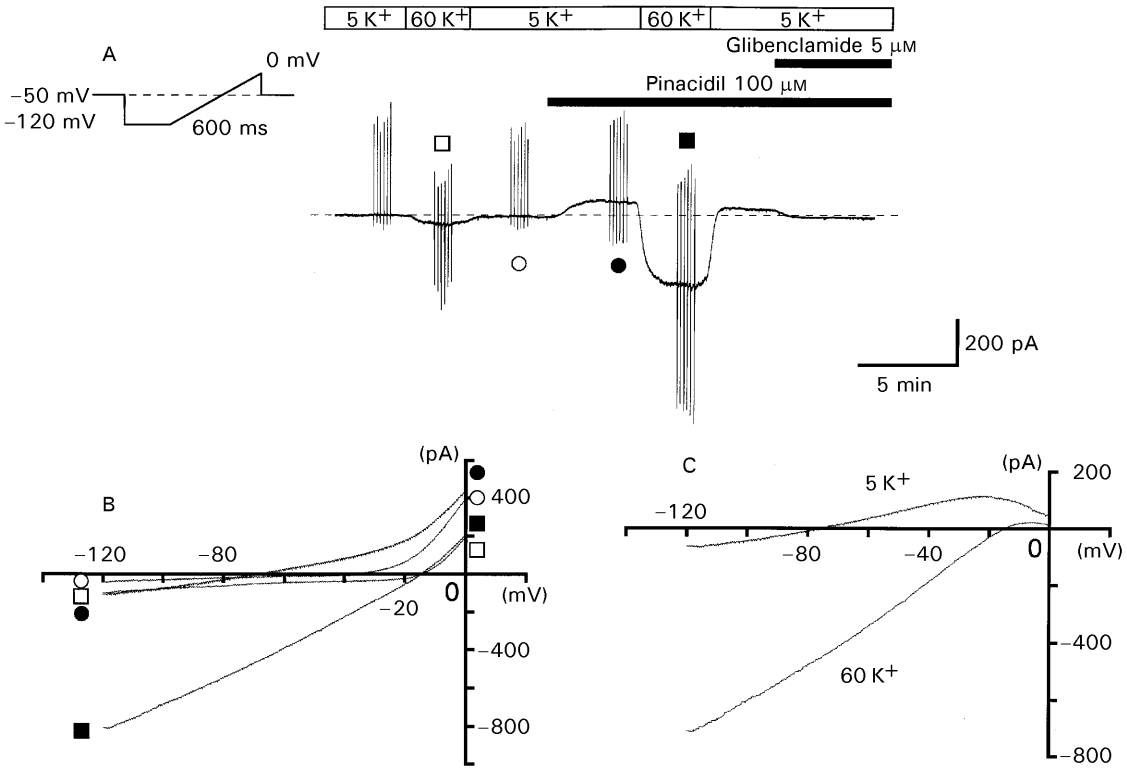

Figure 1. Properties of the pinacidil-induced membrane current in whole-cell recording at −50 mV. A. Membrane currents recorded in PSS and in 60 mM K^+ PSS, in the absence and presence of 100 μM pinacidil. Vertical lines indicate currents induced by ramp potentials from −120 mV to 0 mV for 600 ms, after a 300 ms conditioning pulse to −120 mV (see inset). Pinacidil in PSS induced an outward current, which became inward when $[K^+]_o$ was raised from 5 mM (●) to 60 mM (■), indicating a selective K^+ permeability. Application of 5 μM glibenclamide suppressed the 100 μM pinacidil-induced current. The dashed line indicates the zero-current level. Similar observations were obtained in five other cells. B. The mean ramp membrane currents on an expanded time scale in several conditions. Each symbol is the same as in A. Pinacidil (100 μM) shifted the potential at which the averaged ramp current crossed the zero current level from −35 mV (○) to −68 mV (●). When $[K^+]_o$ was raised to 60 mM, the mean ramp current changed sign at −19 mV (□). In the presence of pinacidil, the averaged ramp membrane current intersected at −21 mV (■). C. Net membrane currents evoked by pinacidil when $[K^+]_i$ was either 5 mM or 60 mM. The reversal potential of pinacidil-induced current in 60 mM K^+ was −15 mV.

Different time courses between the pinacidil-induced outward current and hyperpolarization in pig urethra

The outward current induced by pinacidil will lead to membrane hyperpolarization under normal conditions. We compared the time courses of the pinacidil-induced outward current and hyperpolarization using the conventional whole-cell configuration in quasi-physiological K^+ conditions (pipette solution, 140 mM KCl containing 5 mM EGTA; bath solution, 5 mM K^+ PSS), and voltage-clamp mode to measure membrane current and current-clamp mode to measure membrane potential. In voltage-clamp mode, application of 100 M pinacidil using a concentration-jump technique caused an immediate outward current (Figure 3A). The peak amplitude of the pinacidil-induced outward currents increased in a concentration-dependent manner (10 μM, 34 ± 13 pA, $n = 8$; 100 μM, 95 ± 20 pA, $n = 18$). After reaching a peak, the amplitude of the current at -50 mV gradually decreased even in the presence of pinacidil, reaching a value of $22 \pm 6\%$ ($n = 8$) of the peak amplitude after approximately 20 min. In current-clamp mode, 100 μM pinacidil induced a large hyperpolarization from approximately -37 mV (control, -37 ± 2 mV, $n = 16$) to -82 mV, a value close to E_K under the present experimental conditions. In contrast to the decreasing current, the hyperpolarization was relatively stable. As shown in Figure 3B, the membrane potential had only fallen by about 6% to -77 mV (-78 ± 4 mV, $n = 8$) even after 20 min. Application of 10 μM pinacidil caused a small membrane hyperpolarization (to -52 ± 5 mV, $n = 6$) and the hyperpolarization increased in a concentration-dependent manner (30 μM, -70 ± 3 mV, $n = 4$). The pinacidil-induced hyperpolarization remained stable as long as pinacidil was present.

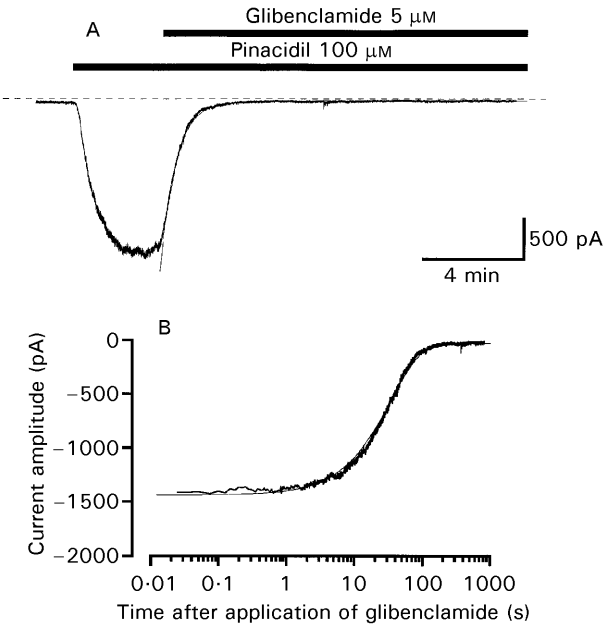

Figure 2. Inhibitory time course of the glibenclamide-sensitive inward currents in pig urethra. A. Inhibitory effects of 5 μM glibenclamide on the 100 μM pinacidil-induced inward current at -50 mV in symmetrical 140 mM K^+ conditions. Inhibitory effects of 5 μM glibenclamide on the 100 μM pinacidil-induced inward current. The dashed line indicates the zero current level. B. The expanded fitting trace of the glibenclamide-induced inhibitory time course. A single exponential (solid line) was fitted to the current decay between time zero (start of application of glibenclamide) and time = 1116 s; $I(t) = I_0 + I_a \exp(-t/\tau_a)$; where $I_0 = -29$ pA, $I_a = -1410$ pA and $\tau_a = 35$ s, respectively. The solid line indicates the exponential fitting. The abscissa shows the amplitude of the inward current (pA) and the ordinate the duration of application of glibenclamide (s) on a logarithmic scale. The membrane current was filtered through 2 kHz and the duration of the sampling time was 25 ms.

To investigate the discrepancy in time course of hyperpolarization and outward current, both the peak amplitude of the membrane current and the value of the membrane potential were measured using the same cells (Figure 3C). In this experiment, 100 μM pinacidil was applied for 3 min repeatedly at -50 mV, evoking an outward current, the peak amplitude of which declined progressively with each application. During each application, when the amplitude of the pinacidil-induced outward current had reached a sustained value, the recording was temporarily shifted to current-clamp mode for 20 s to measure mean membrane potential in the presence of pinacidil. On removal of pinacidil, the membrane current gradually recovered to the control level. During the first three applications of pinacidil, the mean amplitude of the pinacidil-induced hyperpolarization remained at approximately -72 mV, in spite of the dramatic decline in the peak current amplitude.

Noise analysis of the pinacidil-induced inward membrane currents in pig urethra

Noise analysis of macroscopic currents has frequently been used to estimate the unitary conductance of channels that underlie the macroscopic current. We applied this procedure to estimate channel conductance of the unitary currents which underlie the pinacidil-induced macroscopic currents. Increase in current noise was evoked by application of pinacidil in a conventional whole-cell configuration (symmetrical 140 mM K^+ conditions). Figure 4A shows a typical trace of the pinacidil-induced noisy inward membrane current at a holding potential of -70 mV. Pinacidil-induced changes in variance and mean current were obtained by subtracting values of these two para-

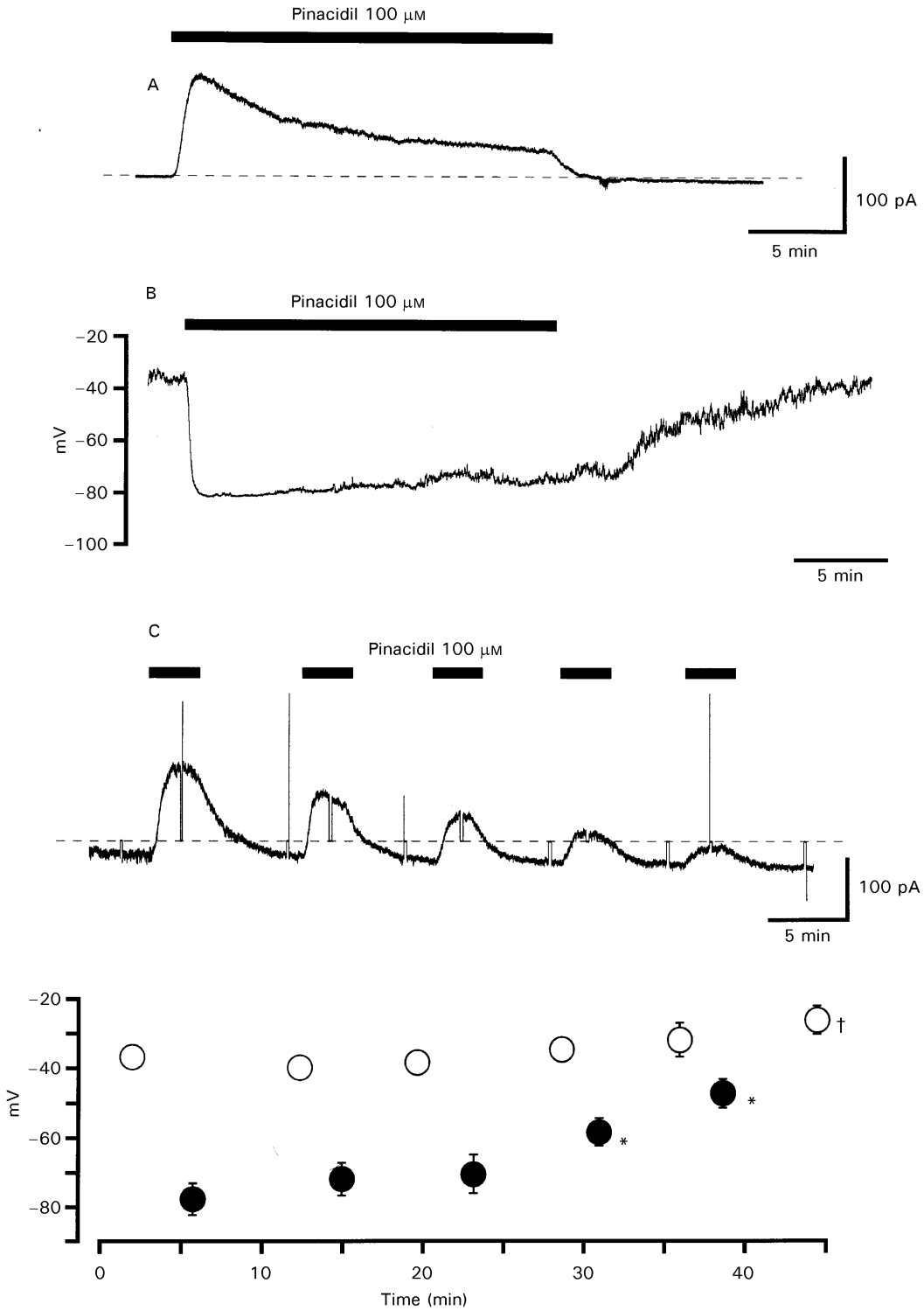

Figure 3. The time course of 100 μM pinacidil-induced outward membrane current and hyperpolarization with two different modes (current-clamp mode and voltage-clamp configuration) in single isolated smooth muscle cells of pig proximal urethra. A. Effects of 100 μM pinacidil (20 min duration of application) on membrane potential in current-clamp mode. B. Effects of 100 μM pinacidil (20 min duration) on membrane current in voltage-clamp configuration at −50 mV. The dashed line indicates the zero-current level. C. The effects of repeated application of 100 μM pinacidil (3 min) on both the membrane current and membrane potential in the same urethral myocytes. Just before application of pinacidil (control; ○), average membrane potential was obtained by use of current-clamp mode for 20 s, indicated by the vertical deflections. At nearly peak amplitude of membrane current, voltage-clamp mode was shifted to current-clamp mode for 20 s (the vertical deflections) to record the membrane potential (●) in the presence of pinacidil. The dashed line indicates the zero-current level. Time 0 indicates the start of the membrane current recording. Each symbol shows the mean of 4–5 observations with s.d. when greater than the size of the symbol. *$P < 0{\cdot}01$, vs the first application of pinacidil; †$P < 0{\cdot}01$, vs control.

meters made in the absence of pinacidil from those made in its presence, and fitting with the hyperbolic function of the form:

$$\sigma^2 = iI - I^2/N \quad (1)$$

where i is the unitary current, σ^2 is the variance of the macroscopic current, I is the mean macroscopic current, and N is the number of channels. With this symmetrical K^+ gradient, the estimated mean unitary current was 0·4 pA at a holding membrane potential of −70 mV (n = 7, Figure 4B). Similar experiments were performed at each holding membrane potential (10 mV increments from −90 mV to −30 mV, n = 5–7), obtaining a slope conductance from the I-V relationships (6 pS, Figure 4C).

Single-channel recordings of pinacidil-activated glibenclamide-sensitive K^+ channel in pig urethra

The unitary-channel conductance estimated from noise analysis was much less than the single-channel conductance of the K_{ATP} channel we had previously studied in pig urethra using single-channel recordings (Teramoto et al 1997). We therefore used cell-attached patches to examine more directly the single-channel properties of the pinacidil-activated channels.

Figure 5A shows the channel records at the indicated membrane potentials. Figure 5B shows the I–V relationships, revealing that the reversal potential is approximately 0 mV (i.e., symmetrical 140 mM K^+ conditions, $E_K = 0$ mV) and that the conductance of the small amplitude channel is approximately 43 pS (43·2 ± 0·6 pS, n = 8). Additional application of 10 μM glibenclamide reversibly suppressed the activity of the channel (Figure 5C), while excision of the membrane patch resulted in a rapid run-down in the opening activity of channels at −50 mV. To investigate the effects of nucleotide diphosphate on the glibenclamide-sensitive 43-pS K^+ channel, we utilized uridine 5′-diphosphate (UDP), one of the most potent nucleotide diphosphates for activating the K_{ATP} channel (Teramoto et al 1997). Figure 5D shows that, after the run-down was complete, application of 1 mM UDP to the inner surface of the membrane

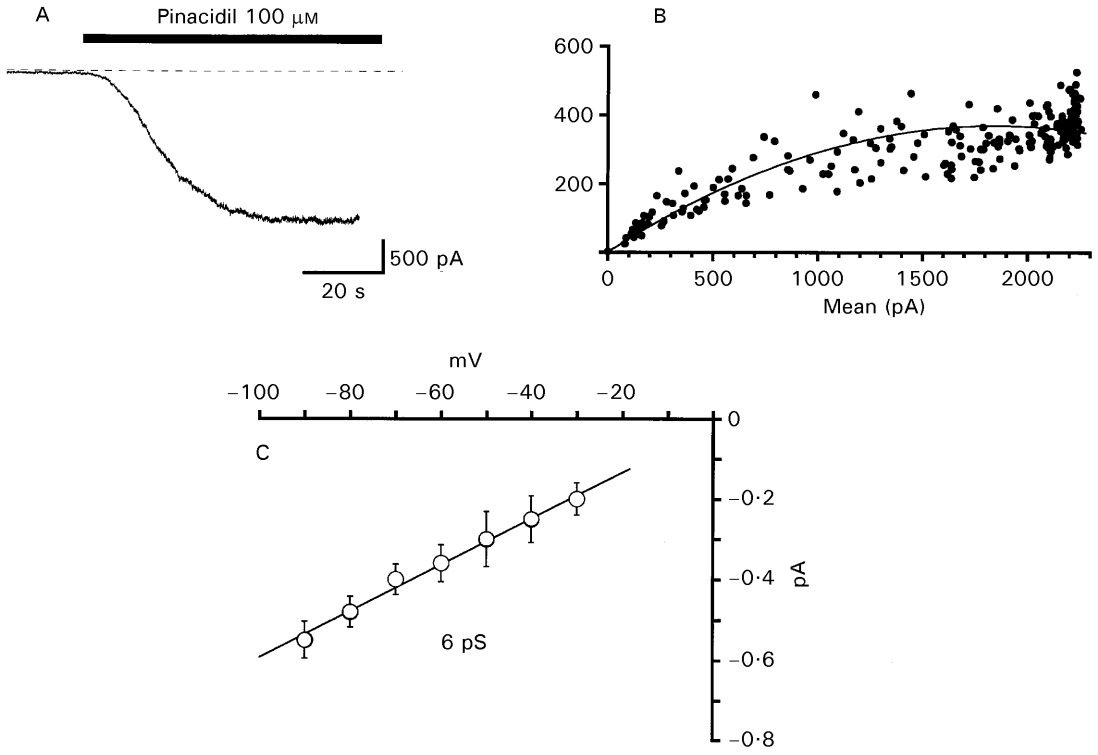

Figure 4. Noise analysis of the pinacidil-activated K^+ currents (conventional whole-cell recording, symmetrical 140 mM K^+ conditions. A. Trace of pinacidil-induced membrane current at −70 mV. The dashed line indicates the zero-current level. B. Analysis of the current noise seen in A when 100 μM pinacidil was present in the bath. Current was split into 250-ms blocks, sampled at 4 kHz, and digitally filtered at 2 kHz. The fitting curve (see text) gave estimates for i of 0·4 pA and a value for N of 9191 by the least-squares method. C. I-V relationships of the K^+ channel underlying the pinacidil-induced K^+ currents. Mean amplitude of the unitary current at each membrane potential was obtained from noise analysis of the pinacidil-induced macroscopic currents (n = 5–7). The line was fitted by the least-squares method. A roughly estimated slope conductance was approximately 6 pS.

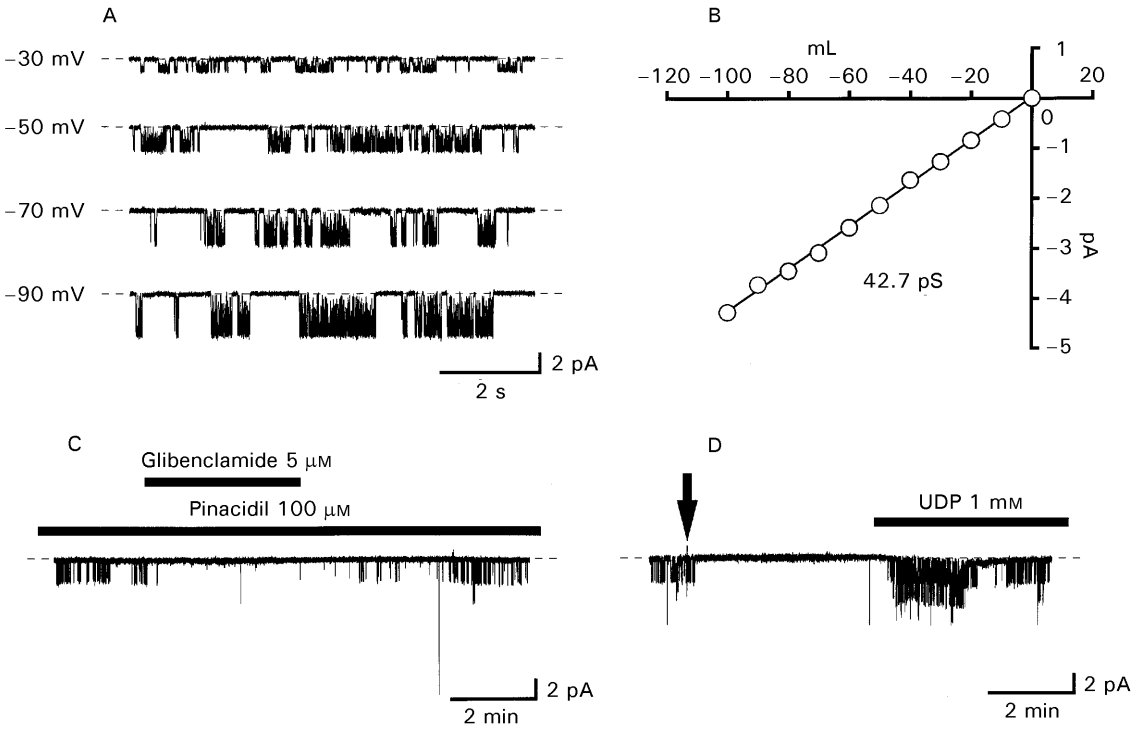

Figure 5. Characterization of the 100 μM pinacidil-induced K^+ channel using a cell-attached patch in symmetrical 140 mM K^+ conditions. The membrane potential was held at −50 mV. The dashed line indicates the current base line where the channel is not open. A. Traces are channel activities recorded from the same membrane patch at the indicated membrane potentials in the presence of 100 μM pinacidil. B. Relationships between the holding membrane potential and the amplitude of the single-channel current. The amplitude of the K^+-channel current was taken from the all-points amplitude histograms for 30 s. The line was fitted by the least-squares method. The channel conductance was 42·7 pS (43·2 ± 0·6 pS, n = 8). C. In the presence of 100 μM pinacidil, 10 μM glibenclamide reversibly inhibits the pinacidil-induced K^+ channel. D. After patch excision (inside-out configuration) at the arrow, run-down of the 43-pS K^+ channel immediately occurred even in the presence of 100 μM pinacidil (in the bath). UDP (1 mM) reactivated the channel.

patch reactivated the channel when 100 μM pinacidil was present in the bath. Other nucleotide diphosphates (such as ADP, GDP, IDP etc. at 1 mM) reactivated the same amplitude K^+ channel in a similar manner (data not shown). The properties of the pinacidil-activated channels are thus identical to those of K_{ATP} channels.

Discussion

The effects of pinacidil on the glibenclamide-sensitive K_{ATP} currents in smooth muscle

Using the results of single-channel recording, it has been reported that pinacidil activates K^+ channels that demonstrate a wide variation of pharmacological and physiological properties, and in vascular smooth muscle these can be subdivided into three main groups by the size and properties of channel conductance: intracellular Ca^{2+}-activated large conductance K^+ channels (200–250 pS in symmetrical 140 mM K^+ conditions, neonatal rat azygos vein (Hermsmeyer 1988a), rat caudal artery (Hermsmeyer 1988b), rat basilar artery (Stockbridge et al 1991), rabbit aorta (Gelband & McCullough (1993), human coronary artery (Bychkov et al 1997)), intracellular Ca^{2+}-insensitive smaller conductance K_{ATP} channels (26–50 pS in symmetrical 140 mM K^+ conditions, rabbit portal vein (Kamouchi & Kitamura 1994), rat portal vein (Zhang & Bolton 1996), human coronary artery (Bychkov et al 1997)) and intracellular Ca^{2+}-insensitive smaller conductance K_{ATP} channels (20 pS, rat mesenteric artery (Zhang & Bolton 1995), 22 pS, middle K^+ channel (MK), rat portal vein (Zhang & Bolton 1996)). One of the common pharmacological properties shared by most of these channels is their sensitivity to inhibition by glibenclamide (10–100 μM). Recent molecular biological and molecular genetic studies of K_{ATP} channels have revealed that native K_{ATP} channels appear to be a complex of at least two regulatory proteins containing a sulphonylurea receptor (SUR)-binding site(s) for sulphonylurea drugs and an inward-rectifying K^+ channel (K_{ir} channel) serving as a pore-forming subunit (Inagaki et al 1995). Thus, it is generally established that sul-

phonylureas (such as tolbutamide, glibenclamide etc.) have been considered a selective blocker for SURs in K_{ATP} channels. However, Zhang & Bolton (1996) reported the presence of two significantly different types of pinacidil-induced K^+ channels in rat portal vein, which were inhibited by glibenclamide with a different potency. Therefore, there still remains considerable uncertainty about which pinacidil-induced K^+ channels are responsible for the functional and pharmacological properties of pinacidil (such as intracellular Ca^{2+} sensitivity, nucleotide-diphosphate-sensitivity and ATP-sensitivity) even in vascular smooth muscles.

In our experiments, the effects of pinacidil on the membrane currents in pig urethra are similar to those reported for levcromakalim in several respects (Teramoto et al 1997). Firstly, the pinacidil-induced K^+ current is blocked by subsequent application of 5 μM glibenclamide. Secondly, pinacidil selectively activates the 43-pS K_{ATP} channel. Thirdly, application of nucleotide diphosphate to the internal side of the excised membrane patch reactivates the K_{ATP} channel after run-down is complete, demonstrating a clear nucleotide diphosphate-sensitivity, which is one of the most important channel properties in K_{ATP} channels (reviewed by Quayle et al 1997). These direct observations strongly suggest that pinacidil induces the activation of K_{ATP} channels in pig urethra. Furthermore, the fact that the time course of the inhibitory effects of glibenclamide on the pinacidil-induced inward membrane current at -50 mV were well-fitted by a single exponential suggests that glibenclamide is binding to a single site, inhibiting a single function of the sulphonylurea receptor in pig urethra. Similar results were observed in the levcromakalim-induced membrane currents in pig urethra (Teramoto et al 1998). Thus, although pinacidil activates two different types of K^+ channel in some vascular smooth muscle cells (rat portal vein (Zhang & Bolton 1996); human coronary artery (Bychkov et al 1997)), it is likely that in pig urethra, K_{ATP} channel is the only target K^+ channel for pinacidil at the resting membrane potential. Our results are also supported by our previous observations that the pinacidil-induced relaxation in pig urethra was selectively suppressed by additional application of glibenclamide in tension recordings (Teramoto & Ito 1999).

Different time course between pinacidil-induced outward K^+ currents and pinacidil-induced hyperpolarization

In conventional whole-cell recording, the gradual decay of K_{ATP} channel opener-induced membrane current has been commonly observed in a wide variety of dispersed smooth muscle cells including pig urethra (rabbit portal vein (Russell et al 1992), guinea-pig urinary bladder (Bonev & Nelson 1993), human coronary artery (Bychkov et al 1997), pig urethra (Teramoto et al 1997)). On the other hand, the K_{ATP}-channel-opener-induced membrane current was better maintained with nystatin perforated patch whole-cell recording, suggesting the possibility that some intracellular organic molecules or metabolic regulators, lost by diffusion during whole-cell recordings, may be necessary to maintain the membrane current evoked by a K_{ATP}-channel opener in pig urethra (Teramoto et al 1997). In these experiments, we have demonstrated that despite the current decay, the pinacidil-induced hyperpolarization was sustained (-82 to -75 mV) even in the same urethral myocytes. Since in the same cells, the peak amplitude of the pinacidil-induced K_{ATP} current was significantly reduced without loss of hyperpolarization, despite the fact that the total number of the pinacidil-stimulating K_{ATP} channel may decrease or run-down, the results strongly suggest that a stable and maximal hyperpolarization may be maintained by only a small number of the pinacidil-induced K_{ATP} channels in pig urethra.

Channel conductance and the channel density underlying K^+_{ATP} channel-opener-induced K^+ currents by use of noise analysis

The unitary current conductance and density of the channels underlying K_{ATP} channel opener-induced macroscopic current have been estimated from the basal noise of the membrane current recording in a wide variety of smooth muscle cells (urinary bladder (Bonev & Nelson 1993), coronary artery (Bychkov et al 1997), A10 cells, a cell line derived from rat thoracic aorta (Russ et al 1997)). It is well-known that K_{ATP} channels are inwardly rectifying in their unitary current amplitude (reviewed by Quayle et al 1997). Nevertheless, most of the noise analyses have been performed using the fluctuations of K_{ATP} channel opener-induced outward K^+ currents. Since the peak amplitude of K_{ATP} channel opener-induced K^+ currents in the above reports was quite small (less than 50 pA), it is somewhat difficult to accurately estimate channel conductance simply from the traces of outward K^+ currents with a liner fitting of mean amplitude. We have reported a significant discrepancy of the estimated channel density between the noise analysis of whole-cell recording and the direct measurement of single-channel recording in pig urethra (levcromakalim (Teramoto et al 1997)). In our experiments, to compare further

the two methods, we utilized the same experimental solutions in two different configurations with the same cells (symmetrical 140 mM K^+ conditions). The channel conductance estimated from the basal noise of the pinacidil-induced macroscopic currents was much smaller than the value obtained from the direct measurement of the unitary current recordings in pig urethra. Similarly, Dart & Standen (1993) reported that noise analysis of the adenosine-activated current gave an estimate for the underlying urinary current which was lower than the amplitude measured directly in pig coronary artery. We are not certain why there is this discrepancy between the different modes, but it is well-known that intracellular ions (such as Mg^{2+}, Ca^{2+} etc.) and intracellular pH readily modify the size of the conductance in K_{ATP} channels. Moreover, since the measured variance can be reduced both by low-pass filtering and by an insufficient sample duration relative to the gating kinetics of the underlying channels, noise analysis usually gives an underestimate of unitary current (Silberberg & Magleby 1993). Thus, it seems plausible to assume that the channel conductance estimated from noise analysis in pig urethra may be too low.

Acknowledgements

We are grateful to the Sir Edward P. Abraham Foundation (University, Oxford, UK) for financial support. This work was also supported in part by the grants-in-aid from the Kaibara Morikazu Medical Science Promotion Foundation (Fukuoka, Japan), awarded to Noriyoshi Teramoto. We also thank Dr R. Inoue for his helpful discussions.

References

Andersson, K.-E. (1992) Clinical pharmacology of potassium channel openers. Pharmacol. Toxicol. 70: 244–254

Bonev, A. D., Nelson, M. T. (1993) ATP-sensitive potassium channels in smooth muscle cells from guinea pig urinary bladder. Am. J. Physiol. 264: C1190–C1200

Bychkov, R., Gollasch, M., Ried, C., Luft, F. C., Haller, H. (1997) Effects of pinacidil on K^+ channels in human coronary artery vascular smooth muscle cells. Am. J. Physiol. 273: C161–C171

Dart, C., Standen, N. B. (1993) Adenosine-activated potassium current in smooth muscle cells isolated from the pig coronary artery. J. Physiol. 471: 767–786

Davies, M. P., McCurrie, J. R., Wood, D. (1996) Comparative effects of K^+ channel modulating agents on contractions of rat intestinal smooth muscle. Eur. J. Pharmacol. 297: 249–256

Gelband, C. H., McCullough, J. R. (1993) Modulation of rabbit aortic Ca^{2+}-activated K^+ channels by pinacidil, cromakalim, and glibenclamide. Am. J. Physiol. 264: C1119–C1127

Gopalakrishnan, M., Whiteaker, K. L., Molinari, E. J., Davis-Taber, R., Scott, V. E., Shieh, C. C., Buckner, S. A., Milicic, I., Cain, J. C., Postl, S., Sullivan, J. P., Brioni, J. D. (1999) Characterization of the ATP-sensitive potassium channels (K_{ATP}) expressed in guinea pig bladder smooth muscle cells. J. Pharmacol. Exp. Ther. 289: 551–558

Hermsmeyer, K. (1988a) Ion channel effects of pinacidil in vascular muscle. Drugs 36 (Suppl. 7): 29–32

Hermsmeyer, R. K. (1988b) Pinacidil actions on ion channels in vascular muscle. J. Cardiovasc. Pharmacol. 12 (Suppl. 2): S17–S22

Inagaki, N., Gonoi, T., Clement, J. P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., Bryan, J. (1995) Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science 270: 1166–1170

Kamouchi, M., Kitamura, K. (1994) Regulation of ATP-sensitive K^+ channels by ATP and nucleotide diphosphate in rabbit portal vein. Am. J. Physiol. 266: H1687–H1698

Petersen, H. J., Nielsen, C. K., Arrigoni-Martelli, E. (1978) Synthesis and hypotensive activity of N-alkyl-N″-cyano-N′-pyridlguanidines. J. Med. Chem. 21: 773–791

Quayle, J. M., Nelson, M. T., Standen, N. B. (1997) ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol. Rev. 77: 1165–1232

Russ, U., Metzger, F., Kickenweiz, E., Hambrock, A., Krippeit-Drews, P., Quast, U. (1997) Binding and effects of K_{ATP} channel openers in the vascular smooth muscle cell line, A10. Br. J. Pharmacol. 122: 1119–1126

Russell, S. N., Smirnov, S. V., Aaronson, P. I. (1992) Effects of BRL 38227 on potassium currents in smooth muscle cells isolated from rabbit portal vein and human mesenteric artery. Br. J. Pharmacol. 105: 549–556

Silberberg, S. D., Magleby, K. L. (1993) Preventing errors when estimating single channel properties from analysis of current fluctuations. Biophys. J. 65: 1570–1584

Stockbridge, N., Zhang, H., Weir, B. (1991) Effects of K^+ channel agonists cromakalim and pinacidil on rat basilar artery smooth muscle cells are mediated by $Ca(^{z+})$-activated K^+ channels. Biochem. Biophys. Res. Commun. 181: 172–178

Sun, Y. D., Benishin, C. G. (1994) K^+ channel openers relax longitudinal muscle of guinea pig ileum. Eur. J. Pharmacol. 271: 453–459

Teramoto, N., Ito, Y. (1999) Comparative studies on the relaxing action of several adenosine 5′-triphosphate-sensitive K^+ channel openers in pig urethra. J. Smooth Muscle Res. 35: 11–22

Teramoto, N., McMurray, G., Brading, A. F. (1997) Effects of levcromakalim and nucleoside diphosphates on glibenclamide-sensitive K^+ channels in pig urethral myocytes. Br. J. Pharmacol. 120: 1229–1240

Teramoto, N., Brading, A. F., Ito, Y. (1998) Glibenclamide-sensitive K^+ channels underlying levcromakalim-induced relaxation in pig urethra. Eur. J. Pharmacol. 365: 291–300

Teramoto, N., Nakashima, T., Ito, Y. (2000) Properties and pharmacological modification of ATP-sensitive K^+ channels in cat tracheal myocytes. Br. J. Pharmacol. 130: 625–635

Zhang, H., Bolton, T. B. (1995) Activation by intracellular GDP, metabolic inhibition and pinacidil of a glibenclamide-sensitive K-channel in smooth muscle cells of rat mesenteric artery. Br. J. Pharmacol. 114: 662–672

Zhang, H.-L., Bolton, T. B. (1996) Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. Br. J. Pharmacol. 118: 105–114