



Characterization of ATP-sensitive potassium channels in human corporal smooth muscle cells

SW Lee¹, H-Z Wang² and GJ Christ^{2,3*}

¹Department of Urology, Sungkyunkwan University, College of Medicine, #50, Ilwon-Dong Kangnam-Ku, Seoul, Korea, 135-710; ²Departments of Urology; and ³Physiology & Biophysics, Albert Einstein College of Medicine, Bronx, New York, 10461, USA

Potassium (K) channels play a significant role in modulating human corporal smooth muscle tone, and thus, erectile capacity. Recent pharmacological studies indicate that the metabolically-regulated K channel (K_{ATP}) may be an important modulator of human penile erection with significant therapeutic potential. The goal of these initial studies, therefore, was to utilize patch clamp techniques to characterize the putative K_{ATP} subtype(s) present in cultured and freshly isolated human corporal smooth muscle cells. In the cell-attached patch mode, two distinct unitary K^+ currents were identified whose respective conductance values were similar in cultured and freshly isolated smooth muscle cells. In cultured myocytes, the measured conductance values in symmetric KCl (140 mM) solutions were 59.1 ± 2.7 pS and 18.4 ± 2.1 pS ($n = 5$ cells). Under identical experimental conditions in freshly isolated myocytes, corresponding conductance values were 59.2 ± 3.7 pS and 18.5 ± 2.4 pS, respectively ($n = 4$ cells). I-V curves constructed during step depolarization (-60 to $+80$ mV), revealed a linear I-V relationship for both unitary conductances. Single channel records documented that both conductances were reversibly inhibited by the application of ATP (1–3 mM) to the bath solution in the inside-out attached patch configuration. The unitary activity of both K channel subtypes was significantly increased by the application of pinacidil (10 μ M) and levromakalim (10 μ M). Whole cell patch recordings documented a glibenclamide-sensitive, pinacidil- and levromakalim-induced increase in the whole cell outward K^+ current during step depolarization (-70 mV to $+130$ mV) of $105 \pm 37\%$, $139 \pm 42\%$, respectively. These data confirm and extend our previous observations, and provide the first evidence for the presence of K_{ATP} channel subtypes in human corporal smooth muscle cells.

Keywords: patch clamp studies; human corporal smooth muscle; cultured cells; freshly isolated cells; pinacidil; levromakalim; K_{ATP} ; relaxation

Introduction

Decreased penile vascular resistance induced by arterial and corporal smooth muscle relaxation is a critical step in penile erection. As such, it is not surprising that heightened tone and/or impaired relaxation of the corporal smooth muscle cell is considered to be a major cause of impotence.^{1–3} In this regard, modulation of corporal smooth muscle tone is a complex process requiring the integration of a host of intracellular events and extracellular signals. Unequivocally, however, nonjunctional ion channels such as potassium (K) and calcium (Ca) channels play a major and functionally antagonistic

role, in modulating corporal smooth muscle tone. In fact, K and Ca channels serve as the final common mediators of the actions of a diverse array of physiologically relevant endogenous neurotransmitters, neuromodulators and hormones.^{4,5}

Functionally, K channels are important regulators of smooth muscle membrane potential in response to depolarizing stimuli, and thus, they balance the activity of the voltage-dependent Ca channels that are responsible for ensuring continuous transmembrane calcium flux, and therefore, sustained corporal smooth muscle contraction during flaccidity. Consistent with these facts, K channels are known to play a fundamental role in both the physiologic and pathophysiologic regulation of smooth muscle tone in diverse tissues.^{6,7} Among the several subtypes of potassium channels present in smooth muscle, the calcium-sensitive (K_{Ca} ; or maxi-K channel) and K_{ATP} channel subtypes are thought to be among the most important modulators of human corporal smooth muscle tone.^{8,9}

*Correspondence: Dr GJ Christ, Laboratory of Molecular & Integrative Urology, Room 744, Forchheimer Building, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

Received 10 December 1998; revised 7 January 1999; accepted 14 January 1999

Since their identification in cardiac muscle,¹⁰ *K_{ATP}* channels have been found in a diverse number of cell types, including smooth muscle cells.^{11–14} In vascular smooth muscle, *K_{ATP}* channels have several physiological roles. For example, *K_{ATP}* channels are the target of a number of vasoactive factors and are also involved in regulating basal blood flow in response to changes in metabolic demand. With respect to their physiological role in penile erection, pharmacological studies have demonstrated that the class of compounds known as K channel modulators (pinacidil and levcromakalim) can relax human corporal smooth muscle.⁵ Preliminary patch clamp experiments on cultured human corporal smooth muscle cells have documented that pinacidil elicits a two-fold increase in the whole cell outward *K⁺* currents. Such observations provide further support for the physiological importance of the *K_{ATP}* channel to the modulation of human corporal smooth muscle tone. However, there is still little information available concerning the electrophysiological properties of the *K_{ATP}* channel subtype(s) present in this tissue. The major goal of this investigation therefore, was to utilize the patch clamp technique to begin characterizing the *K_{ATP}* channel subtype(s) present in human corporal smooth muscle.

Material and methods

Explant cell cultures

All studies were performed according to a protocol approved by the Internal Review Board of the Albert Einstein College of Medicine/Montefiore Medical Center. Human erectile tissue was obtained from the corpus cavernosum of patients undergoing surgery for implantation of penile prostheses. Homogeneous explant cell cultures of human corporal smooth muscle cells were prepared as previously described.^{17–20} Briefly, radial sections $\approx 3 \times 3 \times 10$ mm were excised from the mid-penile shaft of each patient; these specimens consisted exclusively of smooth muscle, endothelium, and connective tissue, with occasional nerve fibers. Tissue was washed, cut into 1–2 mm pieces, and placed in tissue culture dishes with a minimal volume of Dulbecco's medium (DMEM; GIBCO) with 20% fetal calf serum (FCS). After the explants attached to the substrate (usually 1–2 d), additional medium was added. Smooth muscle cells migrated from the explant and underwent division. Cells were subsequently detached using a trypsin/ethylenediamine tetraacetic acid (EDTA) protocol to establish secondary cultures from the explants. These cultures were morphologically homogeneous, and furthermore, we did not observe cobblestone morphologies

characteristic of endothelial cells or the very flattened and spread out shapes characteristic of fibroblasts. Cellular homogeneity was further verified by the presence of smooth muscle specific α -actin immunoreactivity. Cultures were maintained for no more than four passages; importantly, during this time all measured pharmacological and molecular properties observed in the intact tissue are retained in culture; for example, cAMP formation,^{18,19} calcium mobilization,²⁰ expression or function of the gap junction protein connexin 43.¹⁷

Cell isolation protocol

Corporal tissue was dissected as described above and placed in ice-cold DMEM solution. The cell isolation protocol was modified from that previously described.²¹ Tissues were washed and placed in 100 mm culture dishes containing 20 mls of physiological saline solution (PSS in mM: NaCl 137, KCl 5.6, MgCl₂ 1, Na₂HPO₄ 0.42, NaH₂PO₄ 0.44, NaHCO₃ 4.2, HEPES 10, pH set to 7.4 with NaOH) with 0.1% bovine serum albumin. Tissues were cut into ≈ 1 mm³. Approximately 10 pieces of tissue were placed into 2 ml plastic tubes containing 1 ml PSS with 0.1% bovine serum albumin, 45U Papain, 0.1% dithioerythritol (DTT). Tissues were then incubated at 37°C for 35 min. This solution was removed at the end of incubation period, and replaced by 1 ml PSS containing 0.1% bovine serum albumin, 0.1% Collagenase Type 4, 0.05% Elastase, and 0.1% Soybean trypsin inhibitor. The tissue was then incubated for an additional 25 min at 37°C. Once again, the enzyme containing solution was gently removed, and replaced by 2 ml of a fresh PSS solution containing albumin. The tissue was then allowed to settle down for 10 min, after which the solution was gently replaced by 2 ml of fresh PSS solution containing albumin. The tissue was then gently and repeatedly pipetted with a Pasteur pipette. It was during this final washing that the individual smooth muscle cells were isolated. The freshly isolated cells were then transferred to 35 mm culture dishes for the patch clamp experiments.

Solutions for patch clamp studies

Bath and pipette solutions with the following compositions were used in the whole cell, cell-attached and cell-detached patch modes (in mM): (1) 140 KCl, 1 EGTA, 10 HEPES, pH 7.4 (KCl solution); and (2) 140 NaCl, 5.4 KCl, 10 HEPES, pH 7.4 (NaCl solution). For recording single channel recording, KCl solution was present in the bath and pipette. For recording whole cell currents, the NaCl solution and KCl solution were present in the bath and

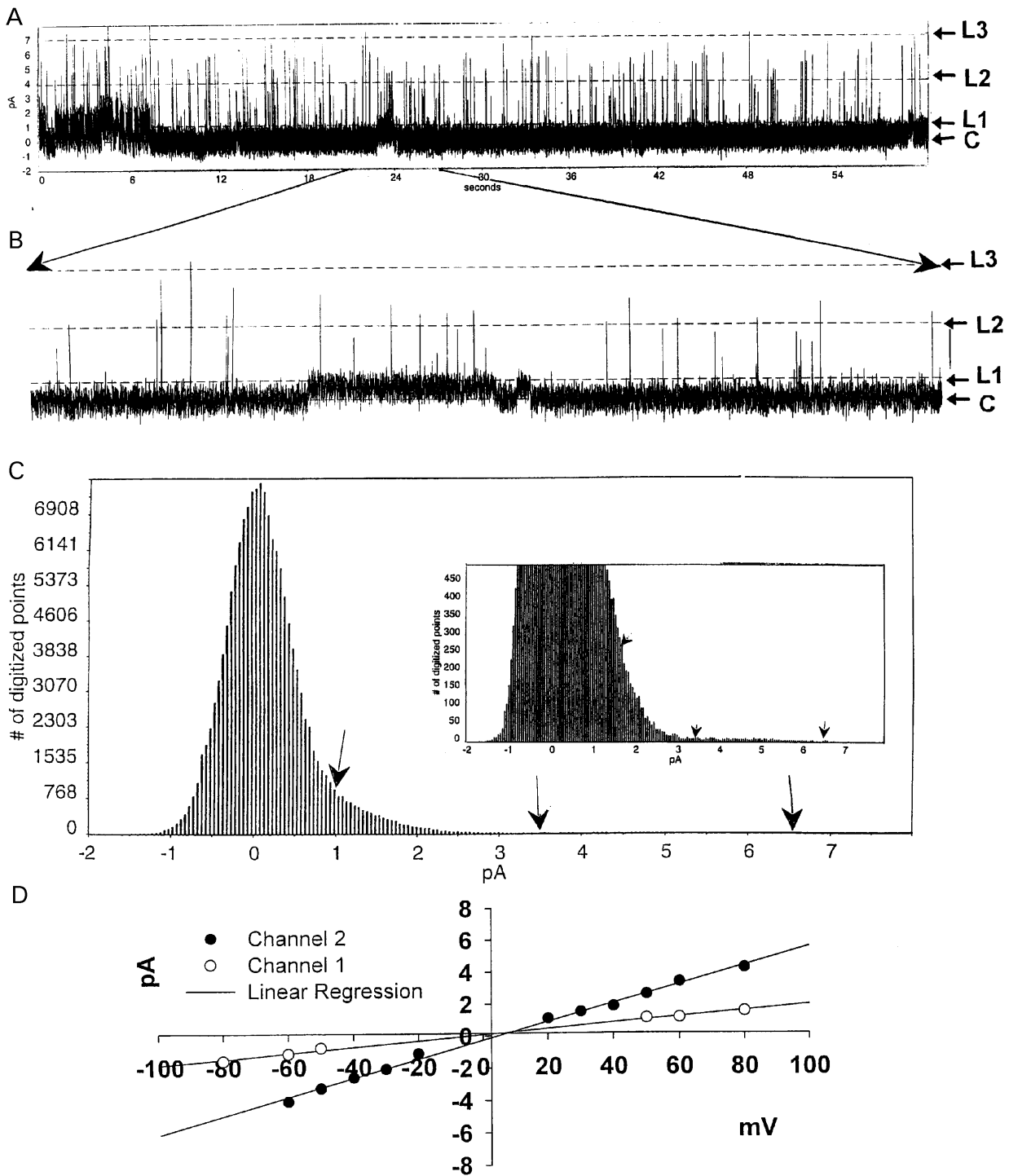


Figure 1 Two distinct unitary K⁺ currents were observed in cultured human corporal smooth muscle cells. (A) A 60 s current recording in the cell-attached mode at a membrane potential of 60 mV. As illustrated by the dashes lines (c-closed, L₁-level 1, L₂-level 2, o-open) in the current trace, two distinct conductances are clearly observable in this record. Data were filtered at 1 kHz and sampled at 200 μs. 140 mM KCl solution was present in both the pipette and bath. (B) Blow-up of the portion of the record demarcated by the arrows, in order to better visualize unitary channel transitions. (C) All points amplitude histogram constructed from the entire 60 s recording (A), and another inset blow-up to permit better resolution of the individual current peaks, as indicated by arrows corresponding to each current levels shown in (A). (D) Representative current-voltage relationship for each of the two distinct unitary K⁺ currents observed in a different cultured human corporal smooth muscle cell recorded in the detached patch mode. The corresponding slope conductance values of the two channels detected in this experiment were 58 pS and 19 pS, respectively. To block contamination of the records by K_{Ca} channel activity, 1 mM tetraethylammonium (TEA) was always present in the pipette solution.

pipette, respectively. To block K_{Ca} channel activity, 1 mM tetraethylammonium (TEA) was always present in the pipette solution.

Electrophysiological methods

Cell-attached, isolated inside-out and whole-cell patch recording modes were used in the experiments. The seal resistance between the patch and

the pipette was 10–30 G Ω . Liquid junction potentials were corrected for by ensuring that the bath and pipette solutions contained sufficient chloride concentrations; previous studies have shown that under these conditions the potential liquid junction artifacts are reduced to ≤ 3 mV.²² For whole-cell current measurements, PCLAMP software (version 6.0; Axon Instruments, Inc., Foster City, CA) and the associated analog-to-digital converter and interface were used. The signals were digitized and stored directly on an IBM PC compatible computer. For

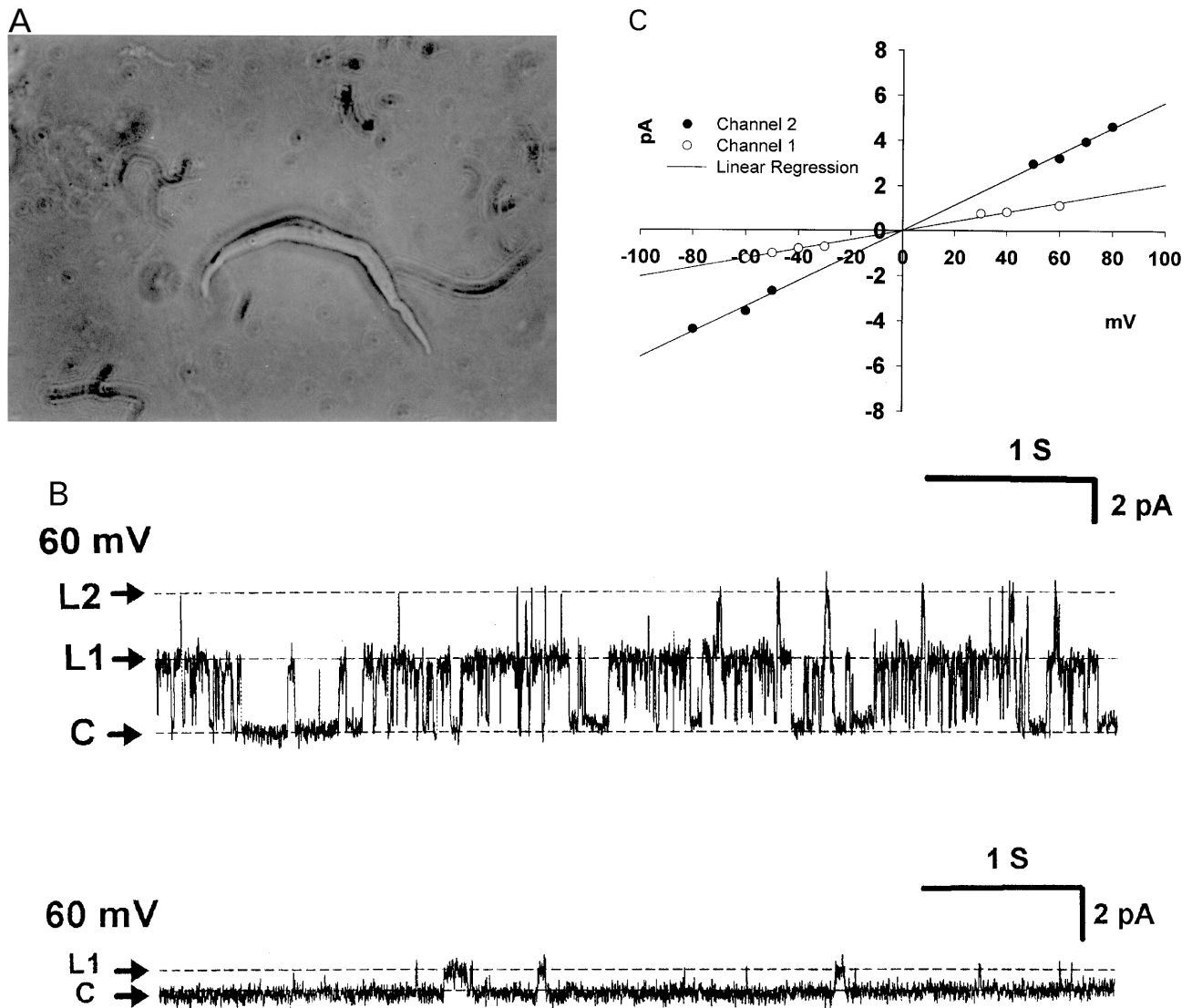


Figure 2 Two distinct unitary K^+ currents were also observed in freshly isolated human corporal smooth muscle cells. (A) Morphology of isolated human corporal smooth muscle cells typically observed following enzymatic dissociation from human corporal tissue strips using a modified protocol (see Methods). This technique yielded cells that had the typical spindle shape characteristic of the corporal myocytes (magnification is 400 \times). (B) Two distinct current traces from different myocytes. Both records were obtained at a 60 mV membrane potential in the inside-out detached patch recording mode. The pipette and bath solutions, as well as the recording conditions were identical to that described in Figure 1. The upper trace shows unitary activity of the larger channel, while the lower trace shows unitary activity of the smaller conductance. (C) Representative current-voltage relationship for each of the two different unitary K_{ATP} currents observed records obtained from distinct freshly isolated human corporal myocytes in the detached patch mode. Slope conductances of the larger (channel 2-filled circle) and the smaller (channel 1-open circle) channels as determined from this experiment were 57 pS and 20 pS, respectively. To block K_{Ca} channel activity, 1 mM tetraethylammonium (TEA) were used in all pipette solution. (c: closed channels; s: seconds; L1: level 1; L2: level 2).

single-channel current measurements, the signal output from an Axon Instrument model ID patch clamp amplifier was recorded on magnetic videotape with a video tape recorder via a Neurocoder analog-to-digital converter (Model DR 384, Neuro Data Instruments Corp., NY). The bandwidth of the amplifier was set at 10 kHz.

Analysis of single channel current data

The data were played back through a 1 kHz (corner frequency) filter and a Neurocoder analog-to-digital converter. Current distribution histograms were generated using point-by-point analysis. Sampling rate was 200 μ s per point. Details of the method of analysis can be found in Ramanan and Brink.²³ The mean open and closed times were calculated according to the method of Ramanan *et al*²⁴ and Brink *et al*.²⁵ For statistical comparison of groups of interest, the Student's *t*-test for paired samples was used. Data are expressed as means \pm s.d. (standard deviation).

Results

Identification of two distinct K⁺ currents in cultured human corporal smooth muscle cells

As shown in the representative current tracing displayed in Figure 1, two distinct unitary K⁺ currents were routinely visualized in the cell-attached patch mode in cultured human corporal smooth muscle cells. Transitions of the unitary currents are visible in the enlargement displayed in Figure 1B, and the corresponding peaks for each of these channels are noted in the amplitude histogram (Figure 1C) derived from the 60 s record shown in Figure 1A. Consistent with the single channel observations, as shown in the representative example displayed in Figure 1D, the current-voltage relationships of the two distinct channels in inside-out patches of cultured myocytes were linear in the range -60 to $+100$ mV. The corresponding mean \pm s.d. slope conductance values for each channel subtype obtained during several similar experiments were 59.1 ± 2.7 pS and 18.4 ± 2.1 pS respectively ($n=5$ cells). Note that these conductance values are similar to those previously reported

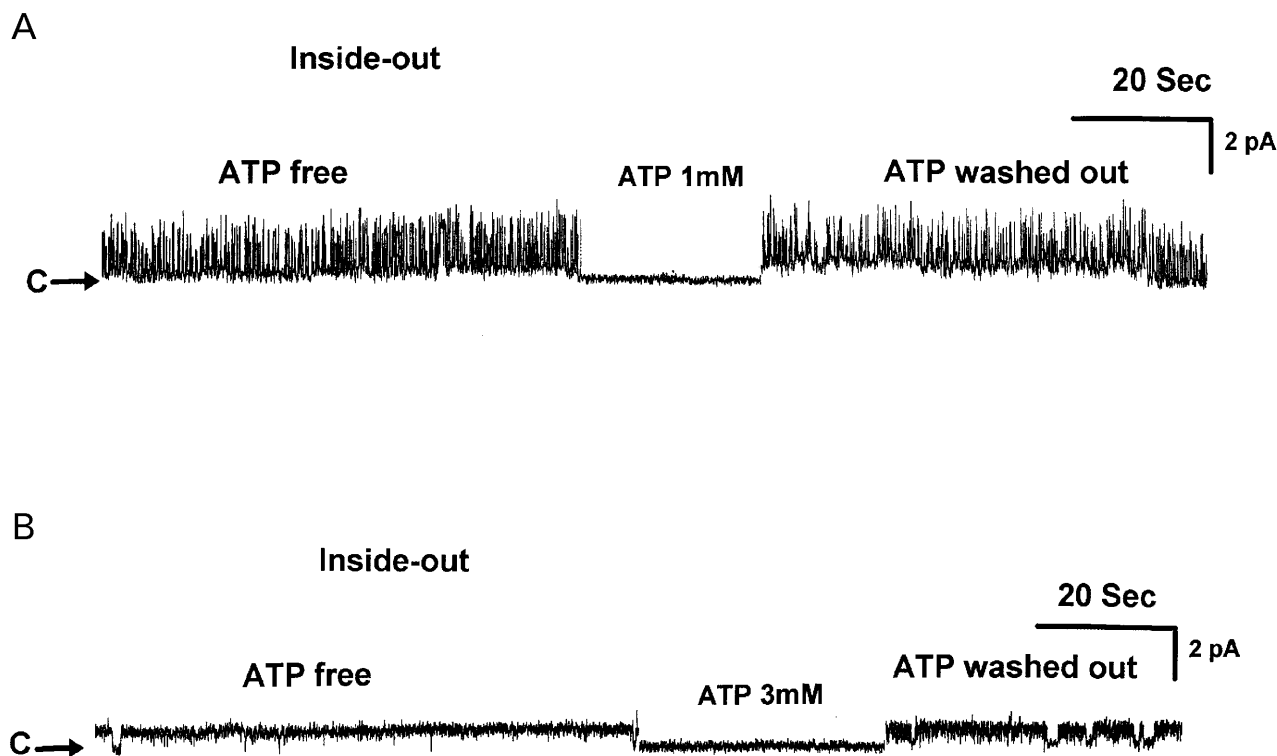


Figure 3 ATP inhibition of each of the distinct K_{ATP} currents. (A) Current tracing showing the effect of perfusion of 1 mM ATP on the activity of the larger K channel in an inside-out patch at a 50 mV membrane potential. As shown, channel activity was completely ablated in the presence of 1 mM ATP. (B) Effect of 3 mM ATP on unitary activity of the smaller K channel in an inside-out patch at 50 mV membrane potential. The smaller channels were in fact less sensitive to ATP inhibition. Once again, contamination of the records by the presence of the K_{Ca} channel activity was prevented by the presence of 1 mM tetraethylammonium (TEA) in the pipette solution. (c: closed).

for K_{ATP} channels in smooth muscle cells using symmetric KCl solutions.^{7,26}

Identification of K^+ currents in freshly isolated human corporal smooth muscle cells

Two distinct K^+ currents were also resolved on freshly isolated corporal myocytes using identical recording techniques. Figure 2A shows a representative example of the myocytes typically isolated using a modification of a previously described enzymatic dissociation protocol.²¹ Figure 2B shows current tracings of the two distinct conductances

routinely observed on freshly isolated myocytes. In this case, the unitary currents for the larger (top trace) and smaller (lower trace) conductance were observed on distinct smooth muscle cells, at the same membrane potential as described above for the cultured corporal myocytes (60 mV). Again, as shown in the representative example shown in Figure 2C, the current-voltage relationships for these two distinct channels in freshly isolated corporal myocytes were also linear over the voltage range from -60 to $+100$ mV. The corresponding mean \pm s.d. slope conductance values for each channel subtype obtained during several similar experiments were 59.2 ± 3.7 pS and 18.5 ± 2.4 pS, respectively ($n = 4$ cells).

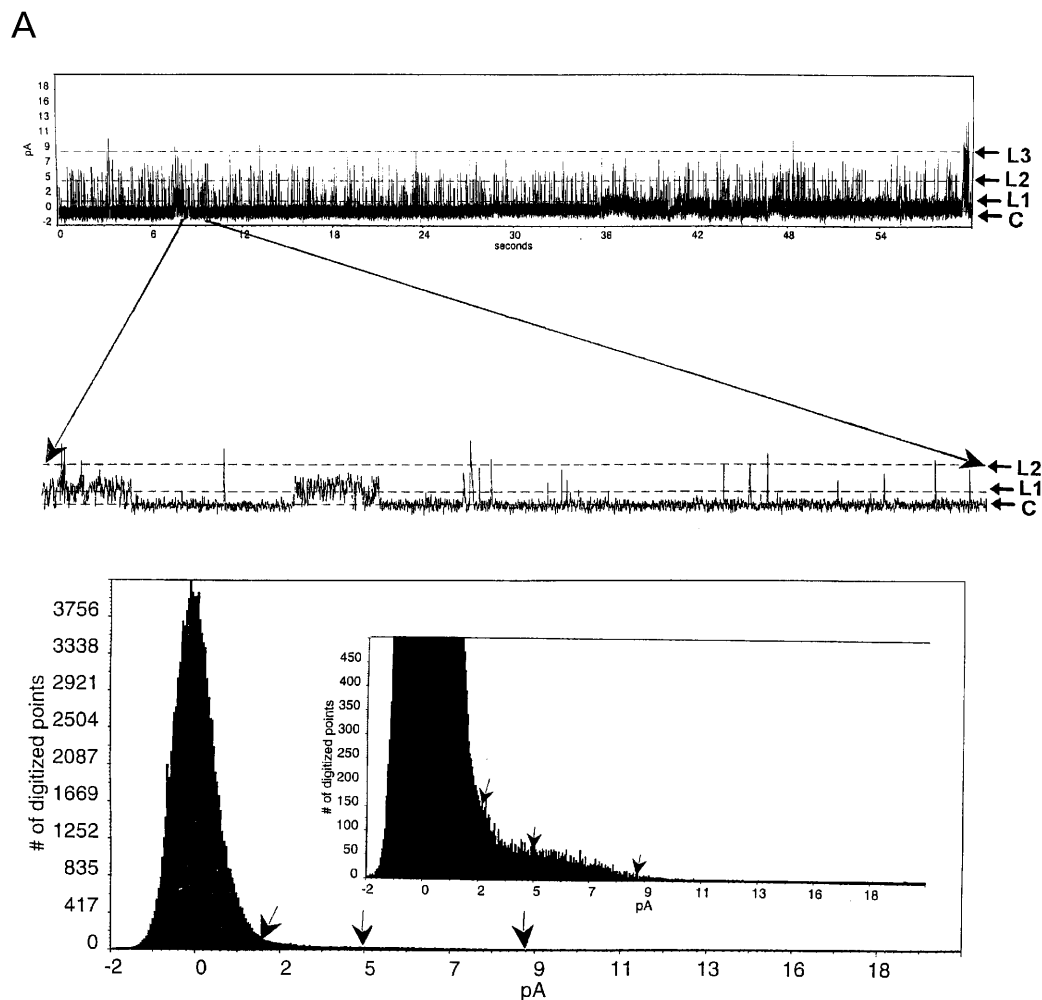


Figure 4 Pinacidil-induced activation of the two distinct unitary K_{ATP} channels present in cultured human corporal smooth muscle cells. (A) Current trace of single channel recordings (60 s) in the cell-attached patch configuration at a membrane potential of 80 mV. Center panel shows a blow-up of the portion of the record demarcated by the arrows to permit a better view of channel transitions. The bottom panel shows all-points amplitude histogram constructed from the entire 60 s record (top), where the insert indicates the individual current peaks (arrows). (B). Another 60 s record take from the same cell-attached patch at the same membrane potential (80 mV), 5 min after application of $10 \mu\text{M}$ pinacidil to the bath (see Methods). Compared with the control record displayed in (A), the activities of both the smaller and larger conductance K_{ATP} channels were increased by pinacidil, and this is reflected in the all-points amplitude histogram displayed in the bottom panel in (B). Consistent with the obvious change in the distribution of the amplitude histogram, the open probability of both channels increased (see text). Once again, the potential contribution of K_{Ca} channel activity to this record was prevented by the presence of 1 mM tetraethylammonium (TEA) in the pipette solution.

Identification of the K⁺ currents as K_{ATP} channels following inhibition of unitary activity by ATP, and activation by pinacidil and levcromakalim

Importantly, as illustrated in Figure 3, application of 1–3 mM ATP to the bath solution in inside-out patches caused a complete, albeit reversible, ablation in the activity of both K channel subtypes. Such observations clearly indicate that these K channel subtypes are K_{ATP} channels. However, in order to further explore the characteristics of these K channel subtypes, we examined the effects of the two well-documented K channel modulators, pinacidil and levcromakalim, respectively, on the unitary activity of these two putative K_{ATP} channel subtypes. In cell-attached patches, application of pinacidil (10 μM) or levcromakalim (10 μM) to the bath solution was associated with increased activity of both K_{ATP} channel subtypes (Figure 4). This increase in channel activity was paralleled by changes in the channel open probability, but not channel conductance. Specifically, using previously described fit-

ting routines,^{22–25} estimates of channel conductance and open probability were obtained. For the record shown in Figure 4, these estimates, in the absence and presence of pinacidil, respectively, were for the smaller channel, ≈ 25 pS and ≈ 0.01, and ≈ 25 pS and ≈ 0.4, and for the larger channel ≈ 0.01 and ≈ 60 pS and ≈ 0.03 and ≈ 60 pS. Therefore, it is clear that the activity of the smaller channel is increased by more than an order of magnitude, while the activity of the larger channel is increased 2–3 fold. Qualitatively similar observations were made in four other experiments with pinacidil, as well as five additional experiments with levcromakalim.

Activation of whole cell outward K⁺ currents by pinacidil and levcromakalim

To further explore the nature and identity of the unitary currents observed in the attached patch mode, outward K⁺ currents were also studied in

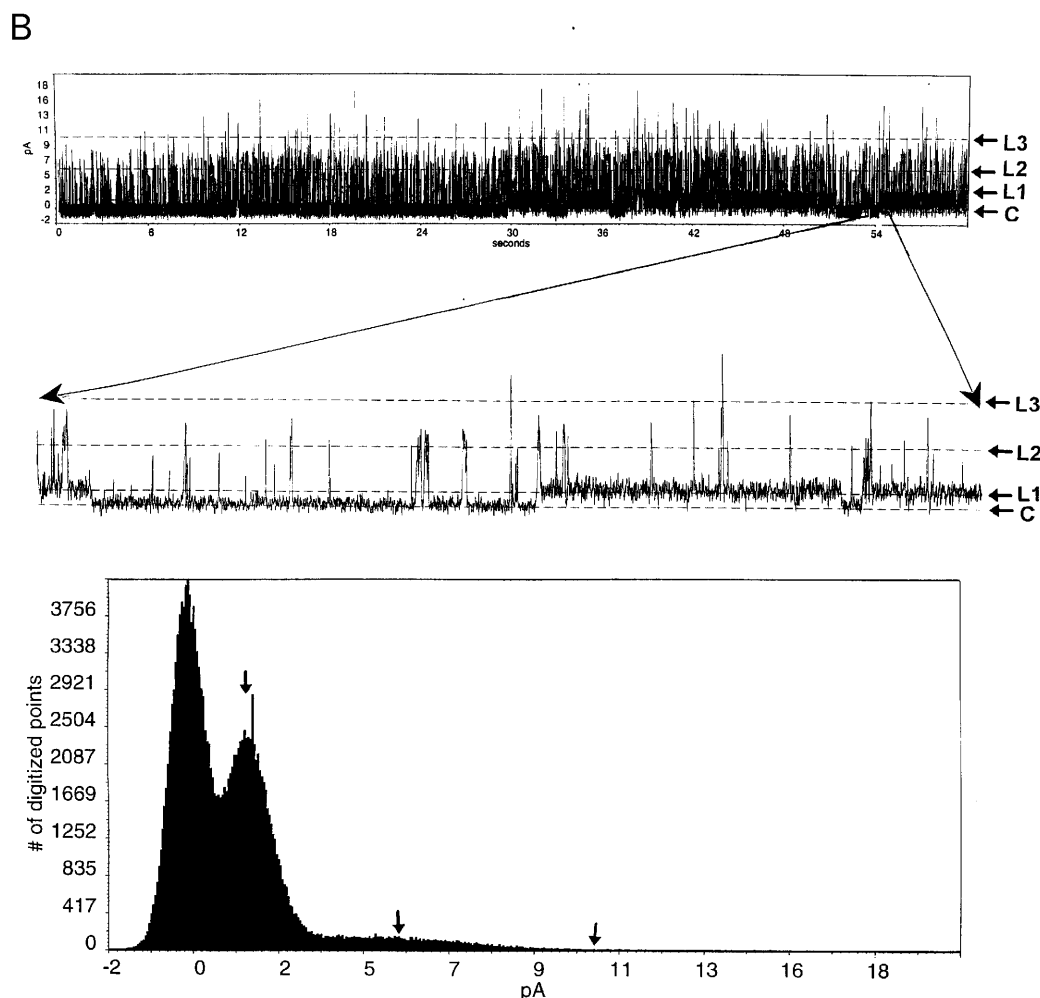


Figure 4 continued from previous page.

the whole-cell mode. Outward currents were recorded during 200 ms pulses from a holding potential of -70 mV to potentials of $+140$ mV in consecutive 10 mV steps. After running a control protocol (Figure 5A), pinacidil ($10 \mu\text{M}$) or levcromakalim ($10 \mu\text{M}$) was added to the bath solution (Figure 5B), and 5 min later, the same voltage protocol was repeated. As illustrated, there was a consistent and dramatic increase in the magnitude of the outward K⁺ current by levcromakalim ($139 \pm 42\%$, $n = 4$ cells, $P < 0.05$), which was blocked by the subsequent addition of the specific K_{ATP} channel subtype blocker glibenclamide ($10 \mu\text{M}$; Figure 5C). Figure 5D shows the corresponding I-V curve for the data depicted in panels A–C. Addition of pinacidil also increased the magnitude of the whole cell outward K⁺ currents ($105 \pm 37\%$, $n = 4$, $P < 0.05$), and this increase was also blocked by glibenclamide ($10 \mu\text{M}$).

Discussion

The recent emphasis on understanding the physiological relevance of the K_{ATP} channel to the regulation of smooth muscle tone derives in large part from the fact that these channels are implicated as the putative target for the relaxing effects of the class of compounds known as K channel modulators/openers, for example, cromakalim and pinacidil.^{31–33} In this regard, both pinacidil and levcromakalim have been shown to directly activate K_{ATP} channels in physiologically diverse vascular smooth muscle cell types.⁷ Of additional interest is the fact that K_{ATP} channels are activated when the intracellular ATP concentration is low, a condition nominally relevant to metabolically-regulated contractile responses in smooth muscle.^{34,35} In fact, it is

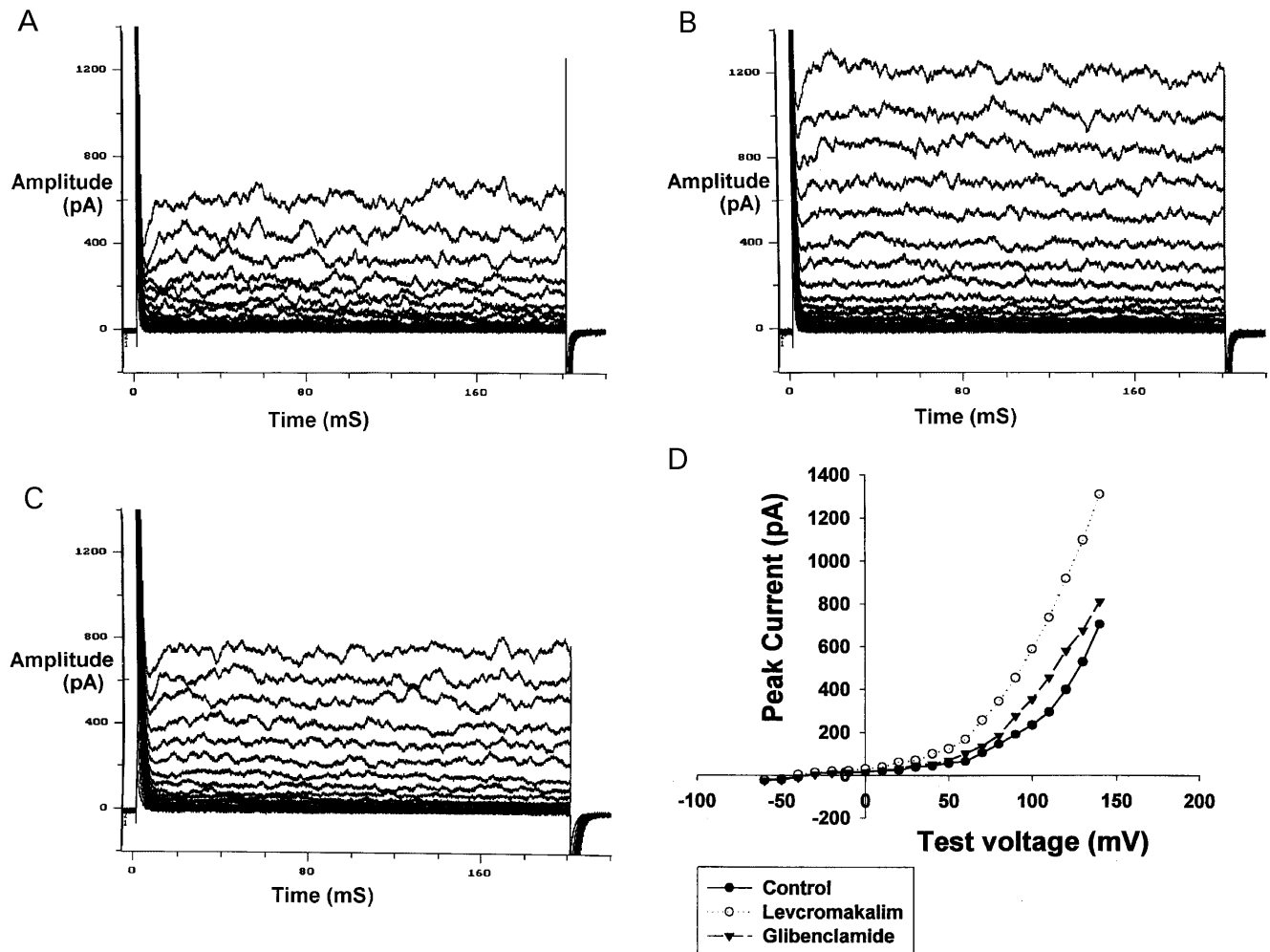


Figure 5 Whole cell outward K⁺ currents recorded from a cultured human corporal smooth muscle cell. The cell was voltage clamped at -70 mV and the currents were recorded using a 10 mV voltage step protocol, beginning at -60 mV and increasing sequentially to a maximum of $+140$ mV. Bath solution (in mM): 140 NaCl, 5.4 KCl, 10 HEPES, pH 7.4 (NaCl solution) and pipette solution: 140 KCl, 1 EGTA, 10 HEPES 1 TEA, pH 7.4 (KCl solution). (A) Control. (B) 5 min after addition of $10 \mu\text{M}$ levcromakalim in bath solution. (C) 5 min after addition of $10 \mu\text{M}$ glibenclamide in bath solution. (D) The quantitative current-voltage (I-V) curve for data depicted in panels A, B and C. Note that increased current was blocked by glibenclamide. Again, in order to block K_{Ca} channel activity, 1 mM tetraethylammonium (TEA) was present in the pipette solution.

clear that this latter phenomenon may be of particular relevance to the regulation of tonically contracted smooth muscle, such as the corporal smooth muscle of the penis.

With respect to penile erection, K channel modulators are known to relax the corporal smooth muscle of human, monkey, cat and rabbit penis.^{5,15,27–29} More specifically, intracavernous injection of pinacidil produced tumescence, or erection, in 16 out of 17 monkeys, a result similar to that obtained with papaverine.¹⁵ In cat, levcromakalim, nicorandil, and pinacidil all caused erections in a dose dependent manner when injected intracavernosally.³⁰ Whereas in humans, addition of pinacidil and levcromakalim to phenylephrine precontracted human corporal tissue strips produced a glibenclamide-sensitive and complete relaxation of all tissues examined.¹⁶ Taken together, such observations further support the view that K_{ATP} channels are of functional importance to the regulation of corporal smooth muscle tone. Despite these facts, there has been no rigorous characterization of the K⁺ channel subtype(s) that might be responsible for mediating these relaxing effects of the K channel modulators/openers in human corpora. As a first step in this direction, the goal of these preliminary studies therefore, was to utilize patch clamp techniques to characterize the putative K_{ATP} channel subtype(s) present in both cultured and freshly isolated human corporal smooth muscle cells.

In this regard, a recent and parsimonious categorization of the single channel conductances of K_{ATP} channels recorded in smooth muscle has placed the K_{ATP} conductance values into two general subgroups⁷: (1) small and intermediate conductance channels (≈ 20 – 50 pS); and (2) large conductance channels (≈ 100 – 200 pS). Consistent with the rather considerable variation in the single channel conductances reported for K_{ATP} channels in smooth muscle,⁷ we report here the identification of two distinct K_{ATP} channel subtypes. However, both of the observed conductances fall into the same small/intermediate conductance category.

We classify these channels as K_{ATP} channels based on the following criteria: (1) both conductances were ATP-inhibited (Figure 3); and (2) pinacidil and levcromakalim increased the unitary activity of these channels (Figure 4), as well as the whole cell outward currents, and the latter were glibenclamide-sensitive (Figure 5). Therefore, we describe here, for the first time, the presence of a larger (≈ 60 pS; Figures 1 and 2) and smaller conductance (≈ 20 pS; Figures 1 and 2) K_{ATP} channel subtype, respectively, in human corporal smooth muscle cells. It is worth noting that the detected conductance values are similar to the conductance values previously reported for K_{ATP} channels in other smooth muscle cell types, using similar recording conditions; that is, symmetric KCl solutions.⁷

Since two distinct conductances, with apparently similar electrophysiological characteristics, are found in both our short term explant cultured cells and the freshly isolated smooth muscle cells, these data provide further support that many aspects of cellular physiology and pharmacology are conserved in our cultured myocytes. Such observations, in turn, further validate the use of our smooth muscle cell cultures under appropriate experimental conditions. Therefore, as reported for other tissues,^{7,13} the present data confirms and extends our previous observations^{8,16} to suggest that K_{ATP} channel activation by drugs and neuropeptides may make an important contribution to membrane hyperpolarization and vasorelaxation in the human corpora, and therefore, erectile capacity.

Conclusion

Since these channels are nominally present on corporal smooth muscle cells *in vivo*, they may play a significant role not only in the physiological modulation of corporal smooth muscle tone, but perhaps also in the etiology of erectile failure. Consistent with this latter possibility, a recent study has shown a statistically significant and diabetes-related decrease in the sensitivity of isolated human corporal tissue strips to relaxation by pinacidil and levcromakalim; that is, a 1/2-log unit shift to the right in the concentration response curve of equivalently phenylephrine precontracted corporal tissue strips. Additionally, these data provide further support for the concept that K channels in general, and the K_{ATP} channel in particular, provide an attractive molecular target for the therapeutic manipulation of human corporal smooth muscle tone. However, it is clear that further investigation is required in order to determine the relative contribution of these two distinct K_{ATP} channels to the regulation of corporal smooth muscle tone, the pathophysiology of erectile dysfunction, and their corresponding therapeutic potential.

This work was supported in part by NIH grants DK46379 and DK42027. The authors gratefully acknowledge the helpful comments and suggestions of Dr P Brink.

References

- 1 Lerner SE, Melman A, Christ GJ. A review of erectile dysfunction: new insights and more questions. *J Urol* 1993; **149**: 1246–1255.
- 2 Christ GJ. The penis as a vascular organ: The importance of corporal smooth muscle tones in the control of erection. *Urol Clin North Am* 1995; **22**: 727–745.
- 3 Saenz De Tejada I *et al.* Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. *N Engl J Med* 1989; **320**: 1020.

- 4 Christ GJ, Melman A. Molecular studies of human corporal smooth muscle: Implications for the understanding, diagnosis, and treatment of erectile dysfunction. *Mol Urol* 1997; **1**: 45–54.
- 5 Andersson KE, Wagner G. Physiology of penile erection. *Physiol Rev* 1995; **75**: 191–239.
- 6 Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 1995; **268**: C799–C822.
- 7 Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev* 1997; **77**: 1165–1232.
- 8 Christ GJ, Spray DC, Brink PR. Characterization of K currents in cultured human corporal smooth muscle cells. *J Androl* 1993; **14**: 319–328.
- 9 Fan S-F, Brink PR, Melman A, Christ GJ. An analysis of the Maxi-K⁺ (K_{Ca}) channel in cultured human corporal smooth muscle cells. *J Urol* 1995; **153**: 818–825.
- 10 Noma A. ATP-regulated K⁺ channels in cardiac muscle. *Nature Lond* 1986; **305**: 147–148.
- 11 Nelson MT. Ca²⁺-activated potassium channels and ATP-sensitive potassium channels as modulators of vascular tone. *Trends Cardiovasc Med* 1993; **3**: 54–60.
- 12 Aguilar-Bryan L et al. Toward understanding the assembly and structure of K_{ATP} channels. *Physiol Rev* 1998; **78**: 227–245.
- 13 Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle cell tone. *Am J Physiol* 1990; **259**: C3–C18.
- 14 Standen NB et al. Hyperpolarizing vasodilators activate ATP-sensitive K⁺ channels in arterial smooth muscle. *Science (Washington DC)* 1989; **245**: 177–180.
- 15 Giraldi A, Wagner G. Effects of pinacidil upon penile erectile tissue, in vitro and in vivo. *Pharmacol Toxicol* 1990; **67**: 235–238.
- 16 Giraldi A, Zhao W, Gondre CM, Murray FT, Christ GJ. Differential relaxation of human corpus cavernosum smooth muscle by potassium channel openers: Evidence that relaxation is both agonist dependent and latered by diabetes mellitus. *Int J Impotence Res* 1995; **7**: O10.
- 17 Brink PR, Ramanan SV, Christ GJ. Human connexin43 gap junction channel gating: evidence for mode shifts and/or heterogeneity. *Am J Physiol* 1996; **271**: C321–C331.
- 18 Palmer L et al. Characterization of cAMP accumulation in cultured human corpus cavernosum smooth muscle cells. *J Urol* 1994; **152**: 1308–1314.
- 19 Cahn DJ et al. Forskolin: A promising new adjunct to intracavernous pharmacotherapy. *J Urol* 1996; **155**: 1789–1794.
- 20 Zhao W, Christ GJ. Endothelin-1 as a putative modulator of erectile dysfunction II. Calcium mobilization in cultured human corporal smooth muscle cells. *J Urol* 1995; **154**: 1571–1579.
- 21 Jackson WF, Huebner JM, Rusch NJ. Enzymatic isolation and characterization of single vascular smooth muscle cells from cremasteric arterioles. *Microcirculation* 1977; **4**: 35–50.
- 22 Brink PR, Fan SF. Patch clamp recordings from membranes which contain gap junction channels. *Biophys J* 1989; **56**: 579–593.
- 23 Ramanan SV, and Brink PR. Multichannel recordings from membranes which contain gap junctions. II. Substates and conductance shifts. *Biophys J* 1993; **65**: 1387–1395.
- 24 Ramanan SV, Fan SF, and Brink PR. Model invariant method for extracting single-channel mean open and closed times from heterogeneous multichannel records. *J Neurosci Methods* 1992; **42**: 91–103.
- 25 Christ GJ, Brink PR. An analysis of the presence and physiological relevance of subconducting states of connexin-43-derived gap junction channels in cultured human corporal vascular smooth muscle cells. *Circ Res* 1999; **84**: (In press).
- 26 Zhang HL, Bolton TB. Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. *Br J Pharmacol* 1996; **118**: 105–114.
- 27 Holmquist FYE, Andersson M, Fovaeus H, Hedlund KE. KI-channel openers for relaxation of isolated penile erectile tissues from rabbit. *J Urol* 1990; **144**: 146–151.
- 28 Holmquist FKE, Andersson H, Hedlund KE. Effects of pinacidil on isolated human corpus cavernosum penis. *Acta Physiol Scand* 1990; **138**: 463–469.
- 29 Andersson KE. Clinical pharmacology of potassium channel openers. *Pharmacol Toxicol* 1992; **70**: 244–254.
- 30 Hellstrom WJG, R Wang, PJ Kadowitz, FR Domer. Potassium channel agonists cause penile erection in cats. *Int J Impot Res* 1992; **4**: 35–43.
- 31 Videbaek LM, Aalkjaer C, Mulvany MJ. Pinacidil opens K⁺-selective channels causing hyperpolarization and relaxation of noradrenaline contractions in rat mesenteric resistance vessels. *Br J Pharmacol* 1988; **95**: 103–108.
- 32 Longman SD, Hamilton TC. Potassium channel activator drugs: Mechanism of action, pharmacological properties, and therapeutic potential. *Med Res Rev* 1992; **12**: 73–148.
- 33 Edwards G, Weston AH. K_{ATP} fact or artifact? New thoughts on the mode of action of the potassium channel openers. *Cardiovasc Res* 1994; **28**: 735–737.
- 34 Kleppisch T, Nelson MT. ATP-sensitive K⁺ currents in cerebral smooth muscle: pharmacological and hormonal modulation. *Am J Physiol* 1995; **269**: H1634–H1640.
- 35 Quayle JM, Bonev AD, Brayden JE, Nelson MT. Pharmacology of ATP-sensitive K⁺ currents in smooth muscle cells from rabbit mesenteric artery. *Am J Physiol* 1995; **269**: C1112–C1118.