Characterization of outward K⁺ currents in isolated smooth muscle cells from sheep urethra

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Hollywood, M. A., K. D. McCloskey, N. G. McHale, and K. D. Thornbury. Characterization of outward K⁺ currents in isolated smooth muscle cells from sheep urethra. *Am J Physiol Cell Physiol* 279: C420–C428, 2000.—The perforated-patch technique was used to measure membrane currents in smooth muscle cells from sheep urethra. Depolarizing pulses evoked large transient outward currents and several components of sustained current. The transient current and a component of sustained current were blocked by iberiotoxin, penitrem A, and nifedipine but were unaffected by apamin or 4-aminopyridine, suggesting that they were mediated by large-conductance Ca²⁺-activated K⁺ (BK) channels. When the BK current was blocked by exposure to penitrem A (100 nM) and Ca²⁺-free bath solution, there remained a voltage-sensitive K⁺ current that was moderately sensitive to blockade with tetraethylammonium (TEA; half-maximal effective dose = 3.0 ± 0.8 nM) but not 4-aminopyridine. Penitrem A (100 nM) increased the spike amplitude and plateau potential in slow waves evoked in single cells, whereas addition of TEA (10 mM) further increased the plateau potential and duration. In conclusion, both Ca²⁺-activated and voltage-dependent K⁺ currents were found in urethral myocytes. Both of these currents are capable of contributing to the slow wave in these cells, suggesting that they are likely to influence urethral tone under certain conditions.

CaCl₂

Most researchers now agree that urethral smooth muscle plays an essential role in maintaining urinary continence (12). However, though it is clear that the smooth muscle generates spontaneous tone (2, 12, 28), the basis of this tone is poorly understood, largely because of a lack of electrophysiological data. Nevertheless, several studies with intracellular microelectrodes have clearly demonstrated that the urethra can generate electrical events that are coupled to contraction. These events have variously been described as “action potentials” or “slow waves” and consist of a spike of variable amplitude, followed by a prominent plateau (10, 13, 14, 17). Sucrose gap recordings suggest that the force of contraction depends on the amplitude and duration of the slow wave plateau (17), factors that are likely to affect the degree of Ca²⁺ influx into the smooth muscle cells.

Despite the likely importance of the slow wave in tone generation, there have been few attempts to identify the ionic conductances present in urethral myocytes. We have recently begun to do this using the patch- clamp technique and have demonstrated an L-type Ca²⁺ current and a prominent Ca²⁺-activated Cl⁻ current in sheep cells (9). Both currents contributed to the generation of spontaneous slow waves in isolated cells, similar in configuration to those found in whole tissue recordings (10, 13, 14, 17). However, with the exception of one group that has described an ATP-sensitive K⁺ channel in the pig (25–27), so far there has been no attempt to study the K⁺ currents present in the urethra. This is an important omission, because the shape of the action potential in other smooth muscles is modified by both voltage-dependent and Ca²⁺-dependent K⁺ currents that can act to attenuate the spike and shorten the plateau and, therefore, reduce contraction amplitude (21, 29, 33).

The aim of the present study was, therefore, to identify and characterize the K⁺ currents in isolated sheep urethral myocytes by using the patch-clamp technique. We have also examined the effects of blockers of these currents under current clamp to gain some insight into their potential role during the slow wave.

METHODS

Urethras of sheep of either sex were obtained from an abattoir ~15 min after slaughter and transported to the laboratory in Krebs solution at room temperature. The most proximal 1 cm of the urethra was removed, and 0.5-cm strips of smooth muscle were dissected free and cut into 1-mm³ pieces. The method for cell isolation was similar to that described previously (9). The tissue pieces were stored in Hanks’ Ca²⁺-free solution for 30 min, after which they were incubated in an enzyme medium containing (per 5 ml of Hanks’ Ca²⁺-free solution) 15 mg of collagenase (414 U/mg; type 1a, Sigma), 1 mg of protease (10 U/mg; type XXIV, Sigma), 10 mg of BSA (Sigma), and 10 mg of trypsin inhibitor (Sigma) for ~40 min at 37°C. They were then placed in Hanks’ Ca²⁺-free solution and stirred for a further 15–30 min to release single relaxed smooth muscle cells. These were

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plated in petri dishes containing Hanks’ solution (100 μM Ca^{2+}) and stored at 4°C for use within 8 h. The solutions used were of the following composition (in mM): 1) Hanks’ Ca^{2+}-free solution: 141 Na^{+}, 5.8 K^{+}, 130.3 Cl^{-}, 15.5 HCO_{3}^{-}, 0.34 HPO_{4}^{2-}, 0.44 H_{2}PO_{4}^{-}, 10 dextrose, 2.9 sucrose, and 10 HEPES, pH adjusted to 7.4 with NaOH; 2) PSS: 130 Na^{+}, 5.8 K^{+}, 135 Cl^{-}, 4.16 HCO_{3}^{-}, 0.34 HPO_{4}^{2-}, 0.44 H_{2}PO_{4}^{-}, 1.8 Ca^{2+}, 0.9 Mg^{2+}, 0.4 SO_{4}^{2-}, 10 dextrose, 2.9 sucrose, and 10 HEPES, pH adjusted to 7.4 with NaOH; and 3) pipette solution: 133 K^{+}, 1 Mg^{2+}, 55 Cl^{-}, 80 gluconate, 0.5 EGTA, and 10 HEPES, pH adjusted to 7.2 with KOH.

Recordings were made using the amphotericin B perforated-patch method as described previously (9). Briefly, this consisted of dipping the tips of the patch pipettes in amphotericin-free pipette solution for a few seconds and then backfilling with pipette solution containing 0.6 mg/ml amphotericin B (Sigma). After gigaseals were obtained, the series resistance fell over a 10- to 15-min period to 10–15 MΩ and remained stable for up to 1 h. Series resistance was partially compensated by the circuitry provided in the patch-clamp amplifier, voltage-clamp commands were delivered with an Axopatch 1D patch-clamp amplifier (Axon Instruments), and currents were recorded by means of a 12-bit analog-to-digital/digital-to-analog converter (Labmaster, Scientific Solutions) interfaced to an AT-type computer running pCLAMP software (Axon Instruments). The junction potential between the pipette solution and the bath was found to be less than −3 mV and was uncorrected. During experiments the dish containing the cells was superfused with bath solution. In addition, the cell under study was continuously superfused by means of a close delivery system consisting of a pipette (tip diameter 200 μm) placed ~300 μm away. This could be switched, with a dead space time of <5 s, to a solution containing a drug.

In the single-channel experiments, voltage commands were applied using pCLAMP ramped potentials. This allowed more efficient measurement of channel activation by intracellular Ca^{2+} concentration ([Ca^{2+}]) and voltage than by conventional step depolarizations (6). Activation curves were calculated by averaging current responses to 15 potential ramps and dividing each data point of the averaged current by the single-channel amplitude at that holding potential after leakage current correction. The rate of change of the applied ramp potentials was sufficiently slow (100 mV/s) so that the activation curves were not distorted by the time constants of activation or deactivation (6). Both single-channel and whole cell experiments were carried out at 37°C.

The following drugs were used: nifedipine (Bayer), apamin, tetraethylammonium (TEA), 4-aminopyridine (4-AP), penitrem A, and iberiotoxin (all from Sigma). Data are presented as means ± SE, and statistical differences were compared using a paired or unpaired t-test, as appropriate, with the P < 0.05 level taken as significant.

RESULTS

Ca^{2+}-activated K⁺ current. Voltage-clamp experiments were performed using K⁺-filled pipettes to characterize the K⁺ currents evoked by depolarization. In the typical examples shown in Fig. 1, families of currents were evoked by holding at −60 mV and stepping to potentials ranging from −80 to +40 mV for 500 ms. This resulted in large transient outward currents at potentials positive to −30 mV, and these were followed by noisy, sustained current that declined slowly during the 500-ms pulse (Fig. 1, A–C). In the majority of cells, repolarization back to −60 mV resulted in an inward tail current (Fig. 1B and Fig. 2), which we have previously shown to be due to Ca^{2+}-activated Cl⁻ current (9). We next set out to identify the other components of current evoked during the 500-ms depolarization steps. The fast activation and inactivation of the transient current (full activation, 10 ms; inactivation, 50–100 ms) at first suggested that this might be an “A” current similar to that described in the ureter (20). To test this idea, we examined the effect of 4-AP (1 mM) on the transient current. As the example in Fig. 1A shows, this blocker had very little effect on the transient current, thus excluding the possibility that it was an A current. In seven cells the transient current evoked by a step to 0 mV was 334 ± 76 pA before and 316 ± 45 pA after 4-AP (1 mM; P = 0.59). In contrast, the transient current was reduced by nifedipine (1 μM) from 452 ± 92 to 209 ± 40 pA (n = 6, P < 0.01) as typified by the example shown in Fig. 1B. This result suggested the possibility that the transient current was another
Ca\(^{2+}\)–dependent current. The two most obvious candidates seemed to be small-conductance (SK) and large-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK) currents, because both of these have been demonstrated in renal pelvis smooth muscle (21). To test whether SK channels were involved, we examined the effect of apamin (1 μM), and an example is shown in Fig. 1C. Despite its high concentration, apamin had almost no effect on the amplitude of the current, suggesting that it was not mediated by SK channels (however, see Ref. 16). In four cells the transient current evoked by a step to 0 mV was 390 ± 70 pA before compared with 418 ± 95 pA after apamin (P = 0.36).

Evidence that a major part of the current was mediated by BK channels is illustrated by the experiments shown in Fig. 2, in which the effects of supramaximal concentrations of two potent BK channel blockers, iberiotoxin (11) and penitrem A (19), were examined. In Fig. 2, A and B, families of currents were evoked by stepping from −60 mV to potentials ranging from −80 to +50 mV. Control currents resembled those described in Fig. 1 in having an early transient component followed by a sustained current. Addition of iberiotoxin (300 nM; Fig. 2A) or penitrem A (100 nM; Fig. 2B) completely blocked the transient current and unmasked an initial L-type Ca\(^{2+}\) current. These substances also reduced the sustained current, the remainder of which turned out to be a combination of Ca\(^{2+}\)-activated Cl\(^{−}\) current and delayed rectifier potassium current (see Voltage-dependent K\(^{+}\) currents). The BK current was dissected out from the other currents by examining difference currents obtained by subtracting the currents in the presence of blocker from the control currents (Fig. 2, A and B). Here the transient nature of the BK current may be seen clearly, with the current activating fully within 10 ms and then decaying within 50 ms to a steady level of 20–30% of peak. Penitrem A-sensitive difference currents from six cells are summarized in Fig. 2C, which shows that both the transient and sustained currents activated at steps to −30 mV or more positive. Results similar to these were obtained with iberiotoxin (300 nM) in three cells (data not shown).
Single Ca\(^{2+}\)-activated K\(^+\) channels. Experiments were carried out to determine whether BK channels could be demonstrated in excised patches. Inside-out patches were studied with symmetrical 140 mM K\(^+\) solutions at a temperature of 37°C. Under these conditions openings of high-conductance single channels could be seen (Fig. 3A). The single-channel conductance was estimated by stepping to potentials of 20, 40, 60, 80, and 100 mV and plotting unitary currents against the appropriate potentials. The current-voltage relationship was fitted with a straight line to give a mean conductance of 309 ± 3 pS (n = 6) and reversal potential of −5 ± 1 mV, typical of BK channels in smooth muscle. Penitrem A has previously been shown to be an effective blocker of BK channels when applied to the inside of the membrane (8, 19), thus conferring an advantage over iberiotoxin, which is ineffective when applied to inside-out patches. The single-channel openings were completely and irreversibly blocked by penitrem A (100 nM) in six experiments. An example is shown in Fig. 3A where frequent openings of a single large-conductance channel could be seen during the control period. When penitrem A (100 nM) was added to the bath, the channel activity ceased after 25 s. Penitrem A also appeared to transiently activate the channels before completely blocking them. This was a consistent finding and is similar to a previous observation in lymphatic myocytes (8).

The voltage and Ca\(^{2+}\) sensitivities of channel activation were examined simultaneously using voltage-ramp protocols as described in METHODS. The inner surface of the patch was exposed to different [Ca\(^{2+}\)]\(_i\), with at least 15 ramps being recorded at each concentration. The averaged data points derived from such an experiment are shown in Fig. 3B where [Ca\(^{2+}\)]\(_i\) was 0.1, 0.25, and 0.5 μM. This procedure produced activation curves that were then fitted with the Boltzmann function

\[
NP_o = \frac{n}{1 + \exp(-K(V - V_{1/2}))}
\]

where N is the number of channels in the patch, P\(_o\) is the single-channel open probability, n is the maximal N\(_p\), K\(^{-1}\) is the steepness of the voltage-dependent activation (change in potential necessary to cause an e-fold increase in activation), and V\(_{1/2}\) is the voltage at which there is half-maximal activation. In this experiment (Fig. 3B) V\(_{1/2}\) was +89 mV when [Ca\(^{2+}\)]\(_i\) = 0.1 μM, and this value decreased to +46 and −7 mV as [Ca\(^{2+}\)]\(_i\) was increased to 0.25 and 0.5 μM, respectively. Data derived from four to eight patches for each value of [Ca\(^{2+}\)]\(_i\) are summarized in Table 1. The results indicate that, whereas increasing [Ca\(^{2+}\)]\(_i\) shifted V\(_{1/2}\) in the hyperpolarizing direction, there was no obvious pattern to the changes in K\(^{-1}\), and in most cases the shifts in the activation curve were essentially parallel. The shift in V\(_{1/2}\) per 10-fold change in [Ca\(^{2+}\)]\(_i\) was over 120 mV, ranking these among the most Ca\(^{2+}\)-sensitive BK channels (5).

The whole cell experiments showed that the BK current was transient in nature, suggesting that the channels may inactivate (32). To test whether urethral BK channels demonstrate inactivation, we performed step protocols in excised inside-out patches. Figure 4A shows an example of a patch stepped from −100 mV to +50 mV for 500 ms. There was little evidence to suggest that the BK channels had a higher P\(_o\) at the beginning of the depolarizing step. The ensemble average trace from 15 sweeps in Fig. 4B confirms this finding, showing a current that developed over 50 ms and then remained constant for the remainder of the depolarizing step. Figure 4C shows a summary of ensemble average currents in five patches, where current was plotted at 10 ms and then 50, 100, and 150 ms, etc. These data show that when [Ca\(^{2+}\)]\(_i\) was clamped (to 0.5 μM), the BK current did not inactivate.

Voltage-dependent K\(^+\) currents. We studied the remaining K\(^+\) current after blockade of the BK current and the Cl\(^-\) current with a combination of penitrem A (100 nM) and Ca\(^{2+}\)-free bath solution (Mg\(^{2+}\) substituted, 5 mM EGTA). Penitrem A was preferred to iberiotoxin because it was the more effective blocker of
whole cell BK current (compare the remaining noise in Fig. 2, A and B, middle). Under these conditions another smaller component of K⁺ current was revealed (Fig. 5A). This current activated rapidly and then slowly inactivated, reaching a steady state by the end of the 500-ms pulse (Fig. 5A). A summary current-voltage plot for six experiments in which this current was recorded under these conditions is shown in Fig. 5B. This current activated at around −50 mV and developed an 8- to 20-pA sustained current in the range of potentials (−40 to −20 mV) corresponding to the slow wave plateau.

The time-dependent relaxation of the current was examined in these six experiments, stepping from a holding potential of −60 mV. At test potentials below 10 mV, the decay was variable and generally difficult to fit. At test potentials of +10, +20, +30, +40, and +50 mV, the decay could be well fitted with single exponentials (R > 0.97), giving mean time constants of 60 ± 9, 66 ± 7, 69 ± 11, 59 ± 9, and 75 ± 8 ms, respectively, indicating that there was little or no voltage dependence of the time-dependent inactivation. However, this current did demonstrate the property of voltage-dependent inactivation over more negative potentials. This was studied by holding the cell at conditioning potentials ranging from −100 to +10 mV for 2s, before stepping to a test potential of +40 mV (Fig. 6A; every second step was omitted for clarity). As the cell was progressively held at more positive conditioning potentials, the outward current evoked at +40 mV was reduced. Figure 6B shows the voltage-dependent inactivation curve obtained by plotting the normalized peak current (I/I_max) evoked at +40 mV

<table>
<thead>
<tr>
<th>[Ca²⁺]o, μM</th>
<th>V_1/2, mV</th>
<th>K⁻¹, mV</th>
<th>No. of patches</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>109 ± 9</td>
<td>20 ± 2</td>
<td>8</td>
</tr>
<tr>
<td>0.25</td>
<td>62 ± 6</td>
<td>18 ± 3</td>
<td>7</td>
</tr>
<tr>
<td>0.50</td>
<td>37 ± 7</td>
<td>17 ± 2</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>4 ± 5</td>
<td>13 ± 1</td>
<td>6</td>
</tr>
<tr>
<td>1.00</td>
<td>−14 ± 17</td>
<td>16 ± 1</td>
<td>4</td>
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Values for voltage at half-activation (V_1/2) and the slope of the voltage-dependent activation (K⁻¹) are means ± SE. [Ca²⁺]o, intracellular Ca²⁺ concentration.

Fig. 5. Unmasking the voltage-sensitive K⁺ current. A: family of currents recorded in the presence of penitrem A to block the BK current (left) and then in Ca²⁺-free solution to block the Ca²⁺-current and the Ca²⁺-activated Cl⁻ current (right). B: mean current-voltage relationship of the delayed rectifier K⁺ current in 6 cells. Protocol was the same as in A, inset. Data points indicate the mean ± SE of the peak current measured near the beginning of the 500-ms test potential.

Fig. 4. BK channels do not inactivate in off-cell (inside-out) patches. A: single-channel currents evoked by a step from −100 to +50 mV (symmetrical 140 mM KCl solutions, [Ca²⁺]o = 0.5 μM, T = 37°C). B: ensemble average currents from the same patch used in A (15 sweeps). C: summary of the ensemble average currents (as in B) in 5 patches.

Table 1. Effect of Ca²⁺ on voltage-dependent activation of channels in inside-out patches
against the previous conditioning potential in five cells. These data were fitted with a Boltzmann function of the form

\[ I/I_{\text{max}} = \frac{1}{1 + \exp[-K(V - V_{1/2})]} + C \]

which gives a \( V_{1/2} \) of \(-55 \pm 7 \) mV, a slope factor of \(-14 \pm 4 \) mV, and a residual (noninactivating) fraction of current (\( C \)) of 30 \pm 6\%.

The voltage-dependent \( \mathrm{K}^+ \) current was quite insensitive to 4-AP, with a concentration of 10 mM only producing an inhibition between 5 and 15\% (\( n = 4 \)), but was moderately sensitive to blockade with TEA. An example of the response to increasing concentrations of TEA is shown in Fig. 7A, where the cell was repeatedly stepped to +40 mV from a holding potential of -80 mV. The effect of TEA was to depress the current in a concentration-dependent manner. A summary concentration-effect curve from four cells in which this protocol was applied is shown in Fig. 7B. The data points are means of the peak current recorded during the appropriate concentration of TEA (\( I_{\text{drug}} \)) normalized to the peak control current (\( I_{\text{control}} \)). These data were fitted with a Langmuir equation of the form

\[ I_{\text{drug}}/I_{\text{control}} = \frac{(1 - C)}{[1 + ([\text{drug}]/ED_{50})]} + C \]

where \( ED_{50} \) is the half-maximal effective dose and \( C \) is the residual current at supramaximal concentrations. This gave an \( ED_{50} \) of 3.0 \pm 0.8 mM and a \( C \) value 13 \pm 5\% of control.

**Current-clamp experiments.** To investigate slow waves, cells were studied in current-clamp mode, and under the conditions of these experiments [\( \mathrm{K}^+ \)-filled pipettes; \( \mathrm{Cl}^- \) equilibrium potential (\( E_{Cl} \)) = -24 mV] they had a mean resting potential of \(-30 \pm 3 \) mV. This was more depolarized than the value reported for whole tissue urethral recordings, where the resting potential was found to be near -60 mV (13, 14). For reasons not clearly understood, the resting potential of single smooth muscle cells may be more depolarized than that measured in whole tissue (30). Therefore, we injected a small background hyperpolarizing current (2–20 pA) to bring the resting potential of the cells close to -60 mV. Cells were then stimulated by injecting 40-ms depolarizing currents of 50–100 pA. In cells that developed an initial net inward current under voltage clamp, this procedure evoked slow waves sim-
similar to those illustrated in Fig. 8A. These consisted of 1) a rapid spike, 2) an initial repolarization, and 3) an afterdepolarization (or “plateau”) of variable duration and amplitude. In 11 cells the spike amplitude was 65 ± 4 mV, the peak plateau potential was −38 ± 4 mV, and the initial repolarization reached a value of −48 ± 3 mV. Plateau duration, measured from the initial repolarization to the point at which the cell had repolarized by 80%, was variable, ranging from 250 ms to 5 s, with a mean of 1,266 ± 397 ms. All of these features were completely blocked with nifedipine (1 μM; n = 7; not shown) to leave a passive membrane response, indicating that the evoked slow wave was dependent on L-type Ca\(^{2+}\) current. The role of the BK current was assessed by examining the effect of penitrem A (100 nM) present in the bath to block the BK current. Addition of TEA clearly increased the amplitude and duration of the plateau, and this effect was reversed following a 20-s wash.

**DISCUSSION**

Though the mechanism of urethral smooth muscle tone generation is poorly understood, it is now generally believed to contribute significantly to urinary continence (for discussion, see Ref. 12). Although it is highly likely that ion channels in the membranes of the smooth muscle cells modulate the contractile state of the cells, there have been remarkably few studies of isolated urethral myocytes under voltage clamp. In the present study we have, for the first time, systematically studied the voltage-sensitive and Ca\(^{2+}\)-dependent K\(^{+}\) currents in sheep urethral myocytes. Because there are similarities in the behavior of human and sheep urethras at the whole tissue level such as the development of tone, the presence of a dual adrenergic and cholinergic excitatory innervation, and relaxation produced by nitrergic nerves (28), it is reasonable to use sheep cells as a basis to provide basic background knowledge of the behavior of urethral myocytes in general before carrying out necessarily much more difficult human studies in the future. However, because so far there have been no electrophysiological studies of the human urethra, the validity of different animal models remains to be established. The only other single studies that have been carried out were in the pig, in which an ATP-sensitive K\(^{+}\) current has been found (25–27). There is evidence that this current may contribute to the resting membrane conductance (27). However, because this current was neither voltage nor Ca\(^{2+}\) sensitive, it is unlikely to play a part in determining the shape of the slow wave, which in any case has not been demonstrated in the pig (26).

We have now characterized two further components of outward K\(^{+}\) current in the urethra. One component was Ca\(^{2+}\) and voltage sensitive and had characteristics in common with BK currents found in other smooth muscle preparations (5), whereas the other component was a voltage-activated, Ca\(^{2+}\)-insensitive current similar to delayed rectifier (DK) current in other smooth muscles. The BK current consisted of a large, rapid transient phase...
followed by a sustained phase that lasted throughout the 500-ms depolarizing step. In some cells (e.g., Fig. 1B) fluctuations were observed on the sustained component of current, resembling the STOCs (spontaneous transient outward currents) described in other smooth muscles (5).

At first we thought that the transient phase was an example of the A current found in a wide variety of preparations including several types of smooth muscle (1, 20). However, the transient component was identified as a BK current on the basis of its insensitivity to 4-AP (5), complete blockade by penitrem A and iberiotoxin, and dependence on Ca\(^{2+}\). Also, there was little contribution from apamin-sensitive SK channels (Fig. 1C), although a contribution from apamin-insensitive channels (e.g., SK1) cannot be excluded (16). Our findings in whole cell experiments were supported by data from excised patches, which showed that the cells expressed a high density of large-conductance (~300 pS) Ca\(^{2+}\)- and voltage-activated channels that were blocked by penitrem A.

The transient BK current of the present study contrasts with that recorded in a pig urethral myocyte (see Fig. 4 of Ref. 25), where only a nondecaying component was evident. Although this current was not characterized further, it had some of the features of BK current, including superimposed STOCs. The time courses of BK currents reported in other smooth muscle preparations were also enormously variable, ranging from fairly slowly activating sustained currents in dog colon and mouse gallbladder (7, 18) to transient currents in rabbit ileum (24), guinea pig vas deferens, and guinea pig bladder (15). It is not clear whether these represent genuine species- or tissue-specific differences or reflect variations in experimental conditions. Inactivating BK channels have been described in adrenal chromaffin cells, insulinoma tumor cells, and hippocampal neurons (see Ref. 32). The current in chromaffin cells superficially resembled that described in the present study in that it followed a similar time course of inactivation. Recently this behavior was attributed to the presence of a newly described \(\beta_3\)-subunit that not only confers the property of inactivation but also enhances the Ca\(^{2+}\) sensitivity and decreases the charybdotoxin sensitivity (32). However, the fact that the single BK channels of the present study do not inactivate when studied in off-cell patches, where \([\text{Ca}^{2+}]_i\) was clamped, suggests that these channels do not demonstrate true inactivation but, rather, that the transient time course in whole cell experiments reflects changes in subsarcolemmal \([\text{Ca}^{2+}]_i\). This is in marked contrast to the BK channels of adrenal chromaffin cells, which do inactivate in off-cell patches (32). Thus it seems unlikely that a specialized \(\beta_3\)-subunit was contributing to the behavior of the BK channels in urethral myocytes.

In the present study, several methodological factors may have contributed to the rapid time course of the BK current. First, recordings were made using the perforated patch so that no exogenous Ca\(^{2+}\) buffers were present intracellularly. This is in contrast to the study in pig urethra (25), in which cells were dialyzed with 5 mM EGTA. Buffers could potentially distort the time course of the current, resulting in either suppression or enhancement of the various phases of the current depending on the characteristics of the buffers and the coupling of the BK channels to their source of Ca\(^{2+}\) (22, 23). A second factor that may have increased the initial phase of the BK current in our experiments was the recording temperature of 37°C. Recording at physiological temperatures has been shown to greatly increase the size of the L-type Ca\(^{2+}\) current (31), and this may have been reflected by the BK current. Finally, in our experiments a more accurate time resolution was facilitated by examining difference currents, thereby avoiding contamination by L-type Ca\(^{2+}\) current, which would “drag down” the BK current.

The other K\(^+\) current described in the present study was voltage sensitive, but Ca\(^{2+}\) insensitive, and was only clearly visible when both the BK and Cl\(^-\) currents were blocked by a combination of Ca\(^{2+}\)-free conditions and penitrem A. Voltage-activated K\(^+\) currents in smooth muscle fall into two broad types: 1) a small group of transient, highly 4-AP-sensitive, TEA-insensitive A currents (1, 20), and 2) a larger group of DK currents. The latter are distinguished by slower inactivation kinetics, making them appear less “transient,” and are usually sensitive to millimolar concentrations of TEA but also variably sensitive to 4-AP (3, 18, 29, 39). The current described in the present study may be classified with the latter group on the basis of its sensitivity to TEA and relatively slow inactivation kinetics. However, in several smooth muscles the DK current has been successfully subdivided into two or more components, suggesting that its classification is complex (3, 8). Because of the lack of specific blockers, no further attempt was made to resolve the urethra DK current into possible subcomponents in the present study, although it is possible that it was mediated by more than one type of K\(^+\) channel.

Though the K\(^+\) current can be divided into BK and DK components in many other smooth muscles, the relative size, activation range, and time course of each component varies considerably, suggesting that the relative contribution of each component to excitability may vary according to muscle type. In the colon and gallbladder, blockade of the DK current with 4-AP resulted in prolongation of the slow wave plateau, but BK blockers had little effect (4, 29, 33). In these tissues a model was put forward whereby the plateau represented a quasi-stable membrane potential that resulted from a balance of sustained inward Ca\(^{2+}\) current and outward DK current with little proposed role for either a BK current (at least under resting conditions; see Ref. 4) or a Cl\(^-\) current. The situation, however, appears to be quite different in urinary tract muscle, where blockade of BK current with charybdotoxin prolonged the action potential in the renal pelvis (21) and Ca\(^{2+}\)-activated Cl\(^-\) current seemed to “clamp” the membrane potential near \(E_{\text{Cl}}\) during the slow wave plateau in the urethra (9, 13, 14).

In the present study, isolated urethral cells fired slow waves very similar to the ones observed in whole tissue (10, 13, 14). These were modulated by both penitrem A and TEA, suggesting that both the BK and
the DK current contributed to the slow wave. Penitrem A had the effect of increasing the spike amplitude and blocking the early repolarization. This is consistent with a role for the transient BK current during the early phase of the slow wave. Also, the sustained current seemed to contribute significantly to the overall membrane conductance during the plateau of the slow wave because the plateau was significantly enhanced by penitrem A. Though it was more difficult to assess the role of the DK current because of the lack of a selective blocker, TEA further enhanced the spike and plateau after the BK current had been blocked with penitrem A. This suggests that the DK current can also contribute to conductance changes during the slow wave. However, we still require much more detailed information regarding all the ionic conductances during the slow wave in the urethra; therefore, further analysis was not attempted at this stage.

In conclusion, the two K⁺ currents described in the present study have voltage- and time-dependent characteristics that suggest that they oppose depolarization during both the spike and plateau phases of the slow wave. Such effects are likely to modulate Ca²⁺ entry and may therefore play a part in regulating urethral tone.

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