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Potassium Channel Activity Recorded from the Apical Membrane of Freshly Isolated Epithelial Cells in Rat Caudal Epididymis[1]

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ABSTRACT

K^+ channels were recorded in excised, inside-out patches from the apical membrane of the freshly isolated tubule of the caudal portion of the rat epididymis. With asymmetric K^+ concentrations in bath and pipette (140 mM K^+_{in}/6 mM K^+_{out}), the channels had a slope conductance of 54.2 p*S* at 0 mV. The relative permeability of K^+ over Na^+ was about 171 to 1. The channels were activated by intracellular Ca^{2+} and by membrane depolarization. These channels belong to a class defined as "intermediate-conductance Ca^{2+}-activated K^+ channel." External tetraethylammonium ions (TEA^+) caused a flickery block of the channel with reduction in single-channel current amplitude measured at a range of holding membrane potentials (−40 to 60 mV). Activity of the K^+ channels was inhibited by intracellular ATP (K_D =1.188 mM). The channel activity was detected only occasionally in patches from the apical membrane (about 1 in 17 patches containing active channels). The presence of the intermediate-conductance Ca^{2+}-activated K^+ channels indicates that they could provide a route for K^+ secretion in a Ca^{2+}-dependent process responsible for a high luminal K^+ concentration found in the epididymal duct of the rat.

INTRODUCTION

Ca^{2+}-activated K^+ channels (K_{Ca} channels) have been recorded in a number of epithelial cells [1–4] using the patch-clamp technique. This type of K^+ channel is experimentally activated by raising the intracellular free Ca^{2+} level and by membrane depolarization. Conductance ranges from 40 to 350 p*S*. The channel displays a higher selectivity for K^+ ions than other monovalent cations.

Rat caudal epididymal fluid contains a high concentration (40 mM) of K^+ ions [5, 6]. High luminal K^+ concentration has been shown to inhibit sperm motility during sperm storage and maturation [7]. This high K^+ content is mainly caused by the absorption of water in the epididymis and probably in part by the active secretion of K^+ from the apical membrane [5]. More direct study showed that adrenaline stimulated $^{86}Rb^+$ efflux across the apical membrane of monolayer cultures of the rat epididymis [8]. K^+ channels have been identified on the apical membrane of rat epididymis [1] and human vas deferens [4] in primary culture. Ion channels were also recorded in the apical membrane of the isolated rat caudal epididymis [9]. In addition, K^+ secretion may involve apical K^+ channels in other epithelia, e.g., mammalian salivary and lacrimal glands [10] and renal cortical collecting duct [11–13].

In the present study, we used the patch-clamp technique to record K^+ channels directly from the apical surface of the epithelial cells in the freshly isolated caudal epididymal duct. We investigated the general characteristics of the K^+ channels of ~54 p*S* and demonstrated that extracellular tetraethylammonium ions (TEA^+) and intracellular ATP, but not glibenclamide, inhibited channel activity. The tissue preparation employed in this study offers one important advantage compared to primary cell culture, i.e., a K_{Ca} channel can be precisely identified from the apical membrane of epithelia. The existence of K_{Ca} channels and possibly other K^+ channels implies that they might play a role in maintaining a high K^+ level along the epididymal tubule in a regulated manner. This high content of K^+ is required for sperm storage in the epididymis [7].

MATERIALS AND METHODS

Preparation

Male Sprague-Dawley rats (400 g; Animal Resource Center, Canning Vale, Australia) were anesthetized with pentobarbital (40 mg/kg BW). The lower part of the abdomen was cut open to expose the vas deferens and caudal epididymis. The content of the caudal epididymis was flushed out with Krebs-Henseleit solution by cannulation of the caudal tubule and vas as previously described [14, 15]. A segment 5 mm in length was dissected from the caudal epididymal duct and slit open longitudinally. The flat tissue was mounted on a perspex platform (made by the Technical Support Unit, Chinese University of Hong Kong) with the luminal surface facing upward and was affixed in place with two small platinum bars. Recordings were made from the luminal side of the epithelial layer.

Single-Channel Recording

Single ionic channels were investigated with the patch-clamp technique [16] using the cell-attached and inside-out configurations. Patch pipettes were prepared from 1.5-mm o.d. borosilicate glass capillaries on a micropipette puller (Sutter Instrument Co., Novato, CA), coated with Sylgard elastomer, and fire-polished. The pipettes were filled with electrolyte, and tip resistance ranged between 10 and 20 MΩ. Seals were over 10 GΩ. Single channels were recorded using Axopatch 1D (Axon Instruments Inc., Foster City, CA) amplifier, stored in either a VCR or computer using pClamp software (5.51 version; Axon Instruments). According to convention, membrane voltages were defined as inside relative to outside, and outward K^+ current was represented by an upward deflection of current when membrane potential was held positive to the equilibrium potential estimated from the Nernst equation.

Recording Solutions

For experiments using the inside-out configuration, the epithelial layer was bathed in a K^+-rich solution comprising (in mM) 10 NaCl, 140 KCl, 1 $MgCl_2$, 5 EGTA, 10 Hepes, pH = 7.2, giving a desired free Ca^{2+} concentration with appropriate concentration of $CaCl_2$ (calculated by EQCAL; Biosoft, Cambridge, UK). The pipette-filling solution con-

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tained (in mM) 140 NaCl, 5.5 KCl, 2 $MgCl_2$, 2.5 $CaCl_2$, 0.5 KH_2PO_4, 4.2 $NaHCO_3$, 10 Hepes, 11.1 glucose, pH = 7.4. For the ion-replacement experiments, internal K^+ was replaced with an equimolar concentration of Na^+ to maintain a constant ionic strength. The osmolarity of bath and pipette solutions was adjusted to 300 mOsm/L with mannitol.

Data Collection and Analysis

Single-channel data were stored on videotape and then played back at a filtering rate of 1 kHz before computer acquisition at 10 kHz. Data analyses were performed on pClamp software. Amplitude histograms were made after separation of open and closed states of the channels using the threshold set at half of the open-channel amplitude. A nonlinear Levenberg-Marquardt least-squares curve-fitting procedure was used for fitting Gaussian curves. The open state probability (P_{open}) was calculated as the total open time divided by the total observation time. NP_{open} (N, the number of the channels in the patch estimated from the maximum number of simultaneous openings; P_{open}, the open state probability of an individual channel) was calculated over 90 sec as $\Sigma N_{j=1}\ t_j j/TN$, where t_j is the time spent with $j = 1,2, \ldots N$ channels open.

Since E_K and E_{Na} are opposite in polarity and E_{Cl} is approximately zero in the symmetric Cl^- solutions used in the present study, the relative permeability (P_K/P_{Na}) was estimated using one of two limiting theoretical formulas [17]:

$$E_{rev} = (RT/F)\mathrm{In}\frac{P_K[K^+]_o + P_{Na}[Na^+]_o}{P_K[K^+]_i + P_{Na}[Na^+]_i} \qquad (1)$$

where P_K and P_{Na} are the permeabilities of a channel to K^+ and Na^+. RT/F = 25.43 mV at 22°C.

To examine the inhibitory effect of internal ATP, the concentration dependence of ATP-induced inhibition of the channel activity can be estimated using the following equation:

$$P_b = \frac{P_c}{1 + ([ATP]/K_D)^n} \qquad (2)$$

where P_c and P_b are the NP_{open} in the absence and presence of internal ATP. K_D is the dissociation constant for ATP inhibition, and n is the Hill coefficient.

Chemicals and Drugs

The following drugs were used in the present study: ATP (Na_2-ATP), tetraethylammonium ions, diazoxide, and glibenclamide (Sigma Chemical Co., St. Louis, MO). Diazoxide and glibenclamide were dissolved in dimethyl sulfoxide. ATP stock was prepared in an intracellular K^+-rich solution containing 10 μM Ca^{2+} and pH was adjusted to 7.2.

RESULTS

All recordings were made of single channels in either excised inside-out or cell-attached membrane patches after the formation of high-resistance seals (about 10 GΩ). A single group of ion channels with intermediate conductance was detected. However, we were unable to make either whole-cell current recordings or outside-out patches from the apical membrane of the freshly isolated epithelial sheet in the rat caudal epididymis.

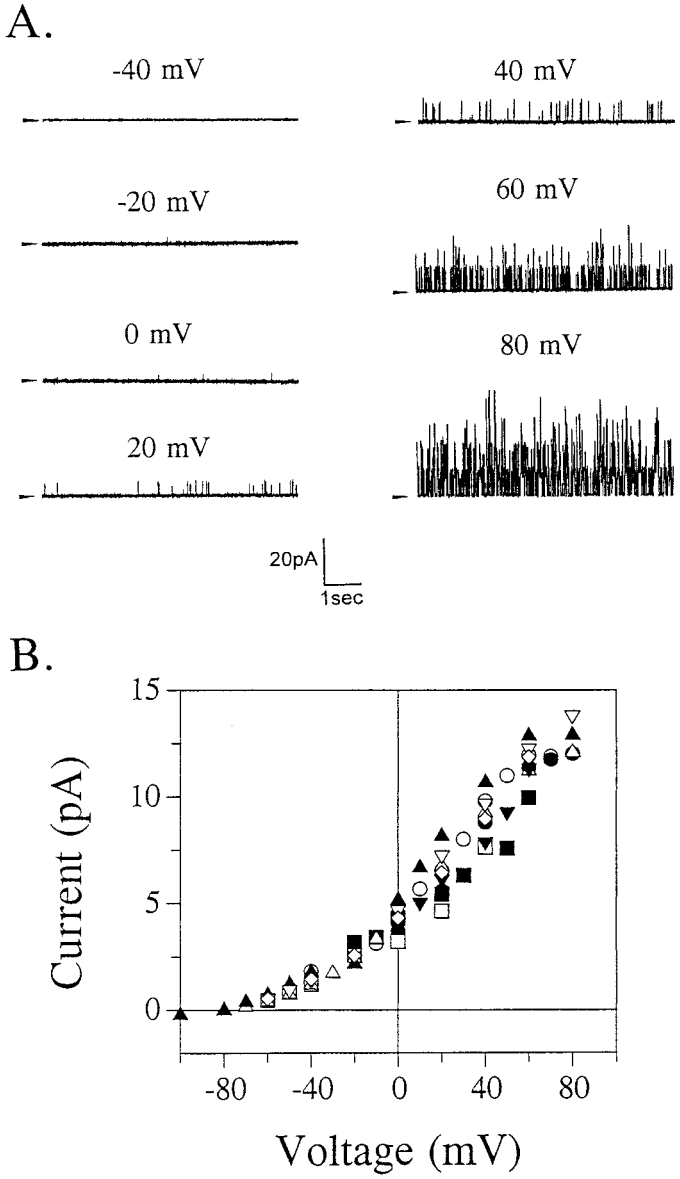

FIG. 1. **A**) Single-channel current recorded at various holding membrane potentials from an inside-out patch excised from the apical membrane of the freshly isolated epithelial layer of the rat caudal epididymis. The pipette solution contained 6 mM KCl. The cytoplasmic surface of the patch was exposed to a bath solution containing 140 mM KCl with 1 μM free Ca^{2+} buffered with EGTA. Single-channel records were filtered at 1 kHz and sampled at 10 kHz. Arrowheads to the left indicate the current levels where all channels are shut. Calibration bars apply to all current traces. **B**) Current-voltage relation of the single channel obtained in the solution given in **A.** Symbols show results from 11 patches.

Intermediate-Conductance Ca^{2+}-Activated K^+ Channels

Active channels were seldom seen in the cell-attached patches. After excision, several channels were usually active in the patches with the cytoplasmic side of the membrane facing 1 μM Ca^{2+}. Figure 1A shows single-channel current traces recorded at a range of membrane holding potentials. In this patch the potential at which channel opening first appeared was −20 mV. Figure 1B shows the current-voltage relationship of the single channel. The single-channel conductance at 0 mV was 54.2 ± 2.8 pS (n = 11), assuming that the reversal potential was −80 mV estimated from the Nernst equation.

The K^+ selectivity of the channel in inside-out patches

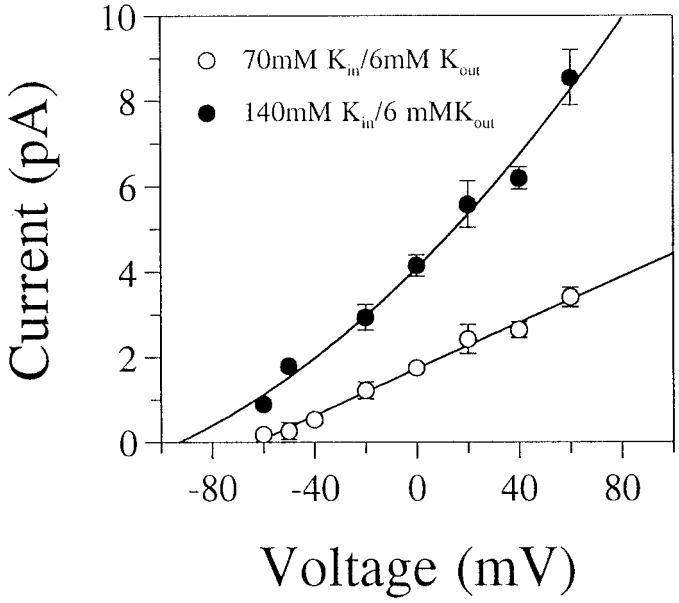

FIG. 2. Current-voltage relationship of the single-channel current recorded in an inside-out membrane patch at two different K^+ gradients: 6 mM $[K^+]_{out}$:140 mM $[K^+]_{in}$ (solid circles) and 6 mM $[K^+]_{out}$:70 mM $[K^+]_{in}$ (open circles). Continual lines are the least-squares fits according to the Goldman-Hodgkin-Katz constant field equation. The reversal potentials were extrapolated. Reduction of intracellular K^+ concentration is compensated for by Na^+ to maintain the same ionic strength. Results are mean ± SEM of four experiments.

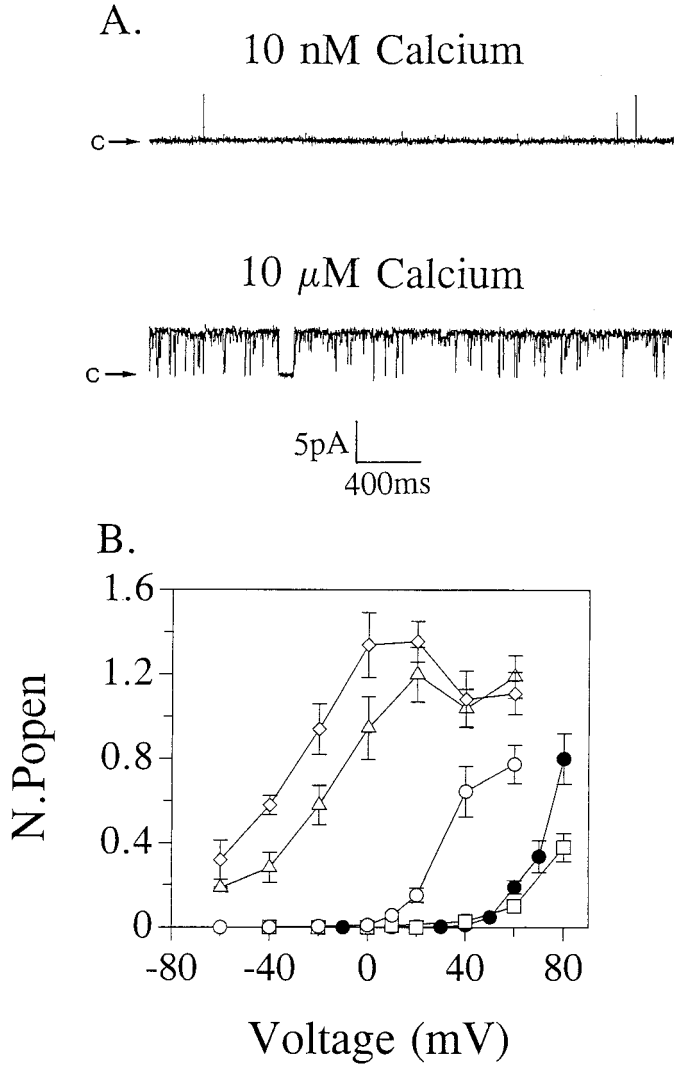

FIG. 3. **A**) Intracellular Ca^{2+} sensitivity (10 nM and 10 μM Ca^{2+}) of the single channel recorded at 0 mV in an inside-out patch configuration. All traces were filtered at 1 kHz. Arrows mark the closed level of the channel. **B**) Mean NP_{open} as a function of holding potential of the cell-attached (solid circles, n = 5) and inside-out patches at different Ca^{2+} concentrations facing the endoplasmic side of the channel (squares, n = 4 in 10 nM Ca^{2+}; open circles, n = 4 in 169 nM Ca^{2+}; triangles, n = 4 in 1 μM Ca^{2+}; diamonds, n = 5 in 10 μM Ca^{2+}). Results are mean ± SEM of n experiments.

was examined by changing the K^+ concentration in the bath. Figure 2 shows the current-voltage relationship with two different K^+ concentrations in the bath. For these experiments KCl was replaced with an equimolar amount of NaCl, and $[Cl^-]$ was symmetric. The conductance at 0 mV at 140 mM KCl_{in}/6 mM KCl_{out} was 51.6 ± 4.6 p*S* (n = 4), and the reversal potential of −86.3 ± 5.7 mV (n = 4) was extrapolated from current-voltage relation fitted to the Goldman-Hodgkin-Katz equation. When the K^+ concentration in the bath was reduced by half (70 mM), the conductance at 0 mV was 29.4 ± 4.2 p*S* (n = 4) and the reversal potential was −61.7 ± 4.8 mV (n = 4). The reversal potentials were close to the calculated values from the Nernst equation at the given K^+ concentration gradients. A mean value for P_K/P_{Na} of 171 ± 24 (n = 4) was calculated from equation 1.

Internal Ca^{2+} Sensitivity of the Channel

The K^+ channels recorded from the apical membrane of the isolated rat epididymal tubule displayed modest sensitivity to internal Ca^{2+}. This is demonstrated in original current recordings shown in Figure 3A. Occasional openings of a channel in this patch were seen at 10 nM $[Ca^{2+}]_i$ (NP_{open} = 0.0005). When $[Ca^{2+}]_i$ was increased to 10 μM, NP_{open} increased to 0.951. The Ca^{2+} sensitivity was reversible. Figure 3B shows the effect of membrane potential on NP_{open} of the channels at various $[Ca^{2+}]_i$. NP_{open} shows a sigmoid voltage dependence with increasing NP_{open} upon depolarization. NP_{open} at low $[Ca^{2+}]_i$ (10 nM) in the inside-out patches was similar to that measured in the cell-attached patches. Increase in $[Ca^{2+}]_i$ caused a leftward shift in the NP_{open}-voltage curve. At high $[Ca^{2+}]_i$ (10 μM), NP_{open} increased even at negative potentials and reached a maximum at 0 mV but decreased at potential more positive than +20 mV.

Effect of External TEA^+

The blocking effect of external TEA^+ was investigated using an inside-out configuration. Figure 4A shows single-channel currents recorded at 0 mV with 10 μM $[Ca^{2+}]_i$ in the absence and presence of external TEA^+. TEA^+ at 0.1 mM, when included in the pipette solution, induced a flickery block of the single K^+ channel current. It is apparent that TEA^+ frequently interrupted long openings. TEA^+ blocked the channel by reducing the single-channel current amplitude (4.52 pA in control and 2.83 pA at 0.1 mM TEA^+, Fig. 4B), and open-channel noise increased in comparison with that of the control trace. The increased noise level in the open channel was caused by the partial time resolution of the channel activity. The mean current-voltage relationship in control patches and in patches with 0.1 mM TEA^+ is shown in Figure 4C. In four experiments with 0.5 mM TEA^+ included in the pipette solution, the single-chan-

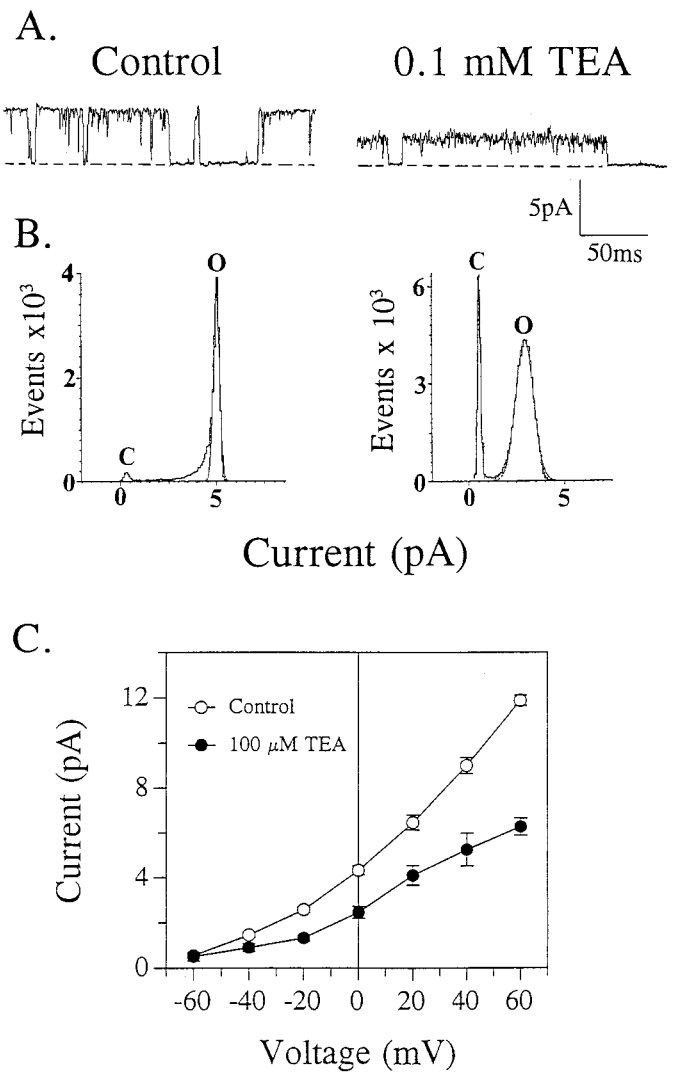

FIG. 4. **A**) Recordings from 2 inside-out patches with cell membrane held at 0 mV. The pipette solution contained no TEA^+ (left) and 0.1 mM TEA^+ (right). Dashed lines indicate the closed level in each trace. **B**) Histogram of current amplitude in the same patches as shown in **A.** C and O, closed and open current levels. **C**) Current-voltage relationship in the absence (open circles) and presence (solid circles) of external TEA^+ (0.1 mM). Each point represents means of SEM of 5–6 measurements. Data were filtered at 1 kHz and sampled at 10 kHz.

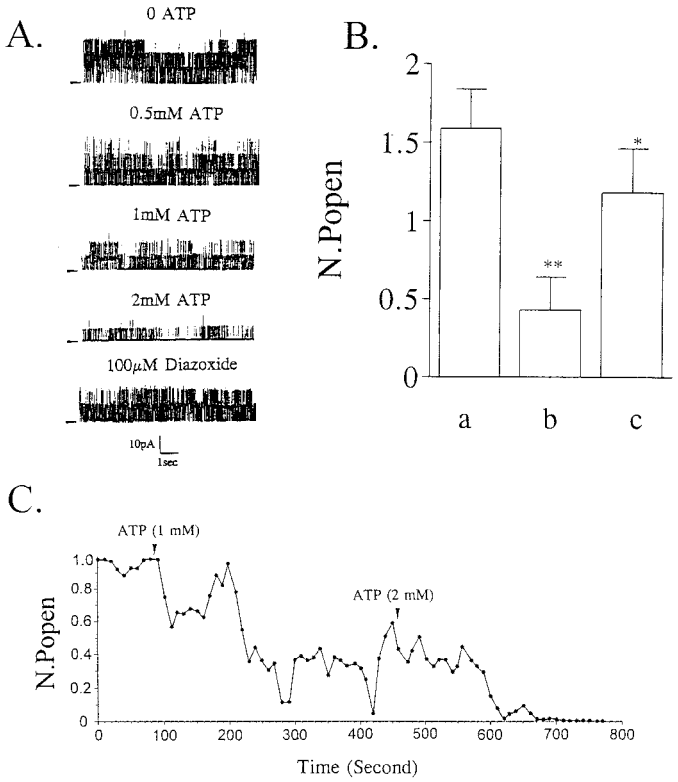

FIG. 5. Records in **A** show the concentration dependence of the intracellular ATP-induced inhibition of channel activity and partial reversal of ATP effect by diazoxide. **B**) NP_{open} calculated at 2 min after each addition of drugs was plotted against drug concentration (a, control; b, 2 mM ATP; c, 100 μM diazoxide; n = 4). **C**) The ATP-inhibitable channel from another experiment. This patch contained one active channel that was open maximally at 0 mV with 10 μM $[Ca^{2+}]_i$. NP_{open} was calculated over a 30-sec interval. Stock of ATP (100 mM) was dissolved in the bath solution and pH was adjusted to 7.2.

nel current amplitude at 0 mV was reduced from 4.32 ± 0.19 pA (n = 5) in the control to 1.45 ± 0.13 pA (n = 4). The remaining channel activity in the presence of TEA^+ was completely eliminated by addition of 0.5 mM EGTA. By contrast, when included in the pipette solution, glibenclamide (10 μM) did not affect the activity of K^+ channels with 10 μM $[Ca^{2+}]_i$. Mean NP_{open} and single-channel current amplitude at 0 mV were 1.42 ± 0.32 and 4.82 ± 0.43 pA (n = 3), respectively. These values were not different from control values (NP_{open}: 1.34 ± 0.18, n = 5; mean amplitude: 4.50 ± 0.23 pA, n = 11; $p > 0.05$ in unpaired data).

Effect of Internal ATP on the Channel

Figure 5 shows the inhibition of these intermediate-conductance K^+ channels by internal ATP. In the inside-out patch shown in Figure 5A, ATP induced a concentration-dependent reduction of NP_{open} by reducing the mean open time and number of active channels in the patch. Diazoxide at 100 μM reactivated the channel that had been inhibited by ATP. In four patches, 2 mM ATP decreased NP_{open} (2 min measurement) from a mean control value of 1.589 ± 0.252 to 0.432 ± 0.21, and diazoxide (100 μM) increased the NP_{open} to 1.18 ± 0.28. However, the single-channel conductance was unaffected (4.21 pA in control, 4.08 pA in 2 mM ATP, and 4.43 pA in diazoxide, measured for the first opening level at 0 mV). Figure 5B shows NP_{open} as a function of concentration of intracellular ATP and diazoxide. In another inside-out patch containing only one active channel, mean NP_{open} was calculated at 10-sec intervals and plotted against the time in seconds shown in Figure 5C. In the control at 10 μM $[Ca^{2+}]_i$, NP_{open} at 0 mV was close to 1. ATP at 2 mM completely inhibited the channel activity when applied to the bath. A lag of 1–2 min for ATP inhibition was probably due to a slow diffusion process, and a similar delay was seen in Ca^{2+} activation of the channel. Figure 6 shows the dependence of P_b/P_c on ATP concentration at 0 mV. The points are closely fitted by equation 2 with a K_D of 1.188 ± 0.342 mM (fitted by a least-squares algorithm to data points, n = 4). This K_D value is much greater than that for ATP-sensitive K^+ channels in rat β-pancreatic cells (IC_{50} = 15 μM [18]), in frog skeletal muscle (IC_{50} = 135 μM [18]), and in rat central neurones (K_D < 1 mM, [19]).

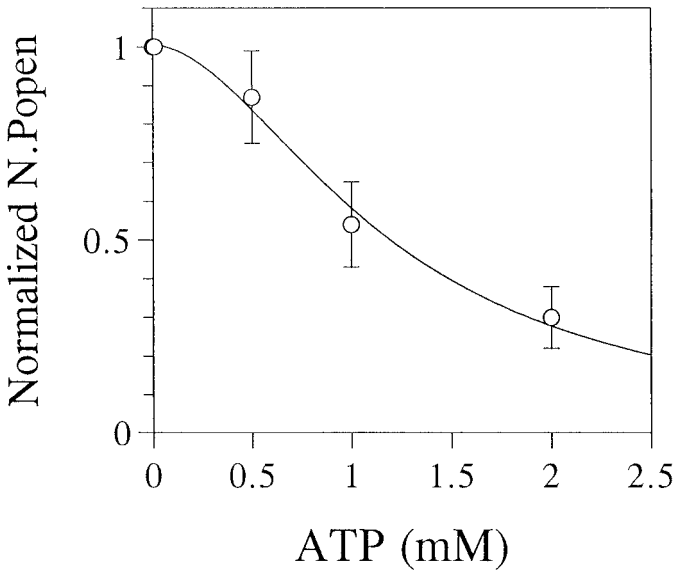

FIG. 6. Plot of normalized NP_{open} (NP_{open} in the presence of ATP divided by NP_{open} in the absence of ATP) at 0 mV against ATP concentration. Curve is drawn to Eq. 2 with dissociation constant (K_D) of 1.188 mM and Hill coefficient (n) of 1.875. Results are mean ± SEM of n experiments.

DISCUSSION

In the present study we recorded intermediate-conductance ion channels from the apical membrane of an isolated epithelial sheet of the rat caudal epididymis. The intermediate-conductance (54 p*S*) channel of the apical membrane is highly selective for K^+ ions. The intracellular $[K^+]_{in}$ dependence of the reversal potential can be predicted by Nernst equation. The P_K/P_{Na} value of about 171 is similar to those for K_{Ca} channels in other secretory cells [20]. It is evident that activity of the K^+ channel was dependent upon $[Ca^{2+}]_i$ and membrane potential. In cell-attached patches, the channel opening was absent at holding potential more negative than +40 mV. At higher $[Ca^{2+}]_i$ (10 μM), the voltage dependence of NP_{open} shifted to the negative potential range. The channel activity (NP_{open}) decreased on strong depolarization. Similar observations were also made in intermediate-conductance K^+ channels recorded from the epithelial membrane of rat cortical collecting duct [3].

TEA^+ has been shown to inhibit K_{Ca} channels in a number of epithelial cells [3, 21]. TEA^+ when included in recording pipettes caused a flickery block of single-channel current through K^+ channels in the apical surface of the epididymal epithelium. The blocking and unblocking events were too fast to be resolved. The external TEA^+ block appeared as a reduction in open-channel unitary current in a range of holding membrane potentials. TEA^+ at 100 μM reduced amplitude of the channel current by approximately 43%, while the K_D values for a 50% reduction in single-channel current for external TEA^+ block of K_{Ca} channels in chromaffin cells and smooth muscle range between 100 and 300 μM [22, 23].

Internal ATP inhibited the intermediate-conductance K^+ channels in a concentration-dependent fashion. ATP reduced the NP_{open} without effect on the single-channel current amplitude. ATP has been found to be an effective inhibitor for the ATP-sensitive K^+ channels in many types of cells including epithelia [2, 18, 24, 25]. In non-epithelial cells, the ATP-sensitive K^+ channels and K_{Ca} channels have distinct pharmacological properties [23]. We did not detect any difference in the single-channel current amplitude and voltage dependence of ATP-inhibitable channels from the K^+ channel activated by $[Ca^{2+}]_i$. In view of the important role of intracellular ATP in ion transport on epithelial membranes, it is possible that intracellular ATP may be a modulator for the K^+ channel in epithelia. Similar findings were reported in airway smooth muscle, where cytoplasmic ATP inhibited the large-conductance K_{Ca} channel with K_i of 0.2 mM [26]. In three recordings, diazoxide, a K^+ channel opener, reactivated the channel previously inhibited by ATP_i. Diazoxide has been found previously to promote activity of K_{Ca} channels [27]. Furthermore, glibenclamide, a selective blocker for ATP-sensitive K^+ channels, failed to influence channel activity. These results indicate that internal ATP might not act at ATP-sensitive K^+ channels.

We recorded the intermediate-conductance K^+ channels in about 6% of a total of 432 membrane patches excised from the apical membrane of freshly isolated epithelia from the rat caudal epididymis. This indicates that there is a low density of active K^+ channels situated on the apical membrane and that the activity of apical K^+ channels may be too low to make a significant contribution to membrane K^+ conductance and basal K^+ secretion in unstimulated cells. In contrast, a higher success rate of channel detection was reported in cultured epithelial cells from the rat epididymis and human vas deferens [1, 4]. This discrepancy in channel density might be a result of differing treatment of the epithelia. However, the K^+ channel observed in this study is modestly activated by increasing $[Ca^{2+}]_i$ above 100 nM. Many intracellular Ca^{2+}-mobilizing agonists would readily raise $[Ca^{2+}]_i$ above this level and promote channel activity.

In a general model for electrogenic Cl^- secretion in the epithelia, the excess intracellular K^+ brought in by Na-K-2Cl symport must be removed to maintain an environment favorable for Cl^- exit through the apical membrane down the electrochemical gradient. Both basolateral and apical K^+ channels should provide effective routes for K^+ efflux if they are subjected to similar regulation. Marty et al. [10] proposed a model of fluid secretion in mammalian salivary and lacrimal glands based on their work on the stimulatory effect of Ca^{2+}-mobilizing agonist on large-conductance K_{Ca} channels. During the initial phase of secretion, both Ca^{2+}-activated K^+ and Cl^- channels are activated, and K^+ and Cl^- ions leave the cell on both apical and basolateral membranes through the respective channels. During the steady state phase, ion extrusion occurs only on the apical side because Cl^- efflux through the basolateral membrane is inverted and basolateral K^+ channels are assumed to be inhibited. At the apical membrane, entry of NaCl leads to activation of the Na-K pump and subsequent entry of KCl into the cell [10]. This model clearly indicates a significant role of apical K^+ channels in fluid secretion, and it also explains the K^+-rich secretions found both in salivary [28, 29] and lacrimal [30] glands in response to low levels of agonist stimulation. Moreover, a positive role for the apical K^+ channels in Cl^- secretion has been indicated through computer modeling of the epithelial cells [31]. Placing 10–20% of the total cell K^+ conductance on the apical membrane would increase the fluid secretion driven by secondary active transport of Cl^- [31]. K^+ secretion may be another function of the apical K^+ channel. The model presented by Cook and Young [31] predicts that apical K^+ channels do not produce an especially K^+-rich primary fluid and that the luminal K^+ concentration is not much higher than 40 mM. This value is in agreement with that calculated by Marty et al. [10]. During sustained fluid secretion in

exocrine glands, the likely luminal K^+ concentration is 40 mM. Passive excretion of KCl requires that intracellular K^+ concentration remain high (140 mM) in order for E_K to be more negative than E_{Cl} on the apical membrane [10]. Actually, the primary fluid K^+ concentrations are all in this range in micropuncture experiments on various exocrine glands [32] and in rat caudal epididymis [15, 33]. In view of the high luminal K^+ concentration (40 mM) measured at the caudal portion of the rat epididymis [15, 33], it is possible that the K^+ channel may play a part in K^+ secretion through the apical membrane, assuming that the amount of K^+ released from spermatozoa is insignificant. Indeed, adrenaline caused an increase in the rate of $^{86}Rb^+$ efflux from the apical membrane of cultured rat epididymal epithelium [8] and activated the apical K^+ channels in primary culture [1]. In addition, noradrenaline increased intracellular Ca^{2+} concentration in the same preparation [34]. However, K^+ secretion through the apical membrane is likely to be regulated by secretagogues, since activity of the channel is extremely low at the resting membrane potential.

In summary, we detected an intermediate-conductance K_{Ca} channel on the apical membrane of the rat caudal epididymis. The K^+ channels were activated by internal Ca^{2+} and membrane depolarization but inhibited by external TEA^+ and internal ATP. These channels may be involved in both fluid secretion and K^+ secretion. The functional role of these channels needs to be further investigated.

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REFERENCES

1. Huang SJ, Cook DI, Jones AO, Wong PYD, Young JA. The Ca^{2+}- and voltage-sensitive potassium channels in the rat epididymis. In: Wong PYD, Young JA (eds.), Exocrine Secretion II. Hong Kong: Hong Kong University Press; 1990: 65–68.
2. Wang W, Giebisch G. Dual modulation of renal ATP-sensitive K^+ channel by protein kinase A and C. Proc Natl Acad Sci USA 1991; 88:9722–9725.
3. Hirsch J, Schlatter E. K^+ channels in the basolateral membrane of rat cortical collecting duct. Pflueg Arch Eur J Physiol 1993; 424:470–477.
4. Sohma Y, Harris A, Wardle CJC, Gary MA, Argent BE. Maxi K^+ channels on human vas deferens epithelial cells. J Membr Biol 1994; 141:69–82.
5. Levine N, Marsh DJ. Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. J Physiol 1971; 213:557–570.
6. Turner TT, Hartmann PK, Howards SS. In vivo sodium, potassium, and sperm concentrations in the rat epididymis. Fertil Steril 1977; 8: 191–194.
7. Wong PYD, Lee WM. Potassium movement during sodium-induced motility initiation in the rat caudal epididymal spermatozoa. Biol Reprod 1983; 28:206–212.
8. Fu WO, Huang SJ, Wilson SM, Wong PYD. Adrenaline stimulate K^+ ($^{86}Rb^+$) efflux across the apical and basolateral membranes of monolayer cultures of the rat epididymis. J Physiol 1990; 423:50P.
9. Cook DI, Huang SJ, Wilson SM, Wong PYD, Young JA. Ion channels in the apical plasma membrane of the rat cauda epididymides. J Physiol 1990; 423:57P.
10. Marty A, Tan YP, Trautmann A. Three types of calcium-dependent channel in rat lacrimal glands. J Physiol 1984; 357:293–325.
11. Hunter M, Lopes AG, Boulpaep E, Giebisch G. Regulation of single potassium channels from apical membrane of rabbit collecting tubule. Am J Physiol 1986; 251:F725-F733.
12. Gitter AH, Beyenbach KW, Christine CW, Gross P, Minuth WW, Fromter E. High-conductance K^+ channel in apical membrane of primary cell cultured from rabbit renal cortical collecting duct anlagen. Pflueg Arch Eur J Physiol 1987; 408:282–290.
13. Ling BN, Hinton CF, Eaton DC. Potassium permeable channels in primary culture of rabbit cortical collecting tubule. Kidney Int 1991; 40:441–452.
14. Wong PYD, Yeung CH. Absorptive and recretory functions of the perfused rat cauda epididymidis. J Physiol 1978; 275:13–26.
15. Wong PYD, Lee WM. Effects of spironolactone (aldosterone antagonist) on electrolyte and water content of the cauda epididymidis and fertility of male rats. Biol Reprod 1982; 27:771–777.
16. Hamill OPA, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflueg Arch Eur J Physiol 1981; 391:85–100.
17. Hille B. Ionic Channels of Excitable Membranes. Sunderland, MA: Sinauer Associates Inc.; 1984.
18. Ashcroft FM. Adenosine 5′-triphosphate-sensitive potassium channels. Annu Rev Neurosci 1988; 11:97–118.
19. Ashford MJL, Sturgess NC, Trout NJ, Gardner NJ, Hales CN. Adenosine 5′-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. Pflueg Arch Eur J Phyiol 1988; 412:297–304.
20. McNicholas CM, Fraser G, Sandle GI. Properties and regulation of basolateral K^+ channels in rat duodenal crypts. J Physiol 1994; 477: 381–392.
21. Segal Y, Reuss L. Ba^{2+}, TEA^+, and quinine effects on apical membrane K^+ conductance and maxi K^+ channels in gallbladder epithelium. Am J Physiol 1990; 259:C56-C68.
22. Yellen G. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. J Gen Physiol 1984; 61:357–359.
23. Langton PD, Nelson MT, Huang Y, Standen NB. Block of calcium-activated potassium channels in mammalian arterial myocytes by tetraethylammonium ions. Am J Physiol 1991; 260:H927-H934.
24. Ashcroft SJH, Ashcroft FM. Properties and functions of ATP-sensitive K^+ channels. Cell Signal 1990; 2:197–214.
25. Tsuchiya K, Wang W, Giebisch G, Welling PA. ATP is a coupling modulator of parallel Na-K-ATPase-K channel activity in the renal proximal tubule. Proc Natl Acad Sci USA 1992; 89:6418–6422.
26. Groschner K, Silberberg SD, Gelband CH, van Breemen C. Ca^{2+}-Activated K^+ channels in airway smooth muscle are inhibited by cytoplasmic adenosine 5′-triphosphate. Pflueg Arch Eur J Physiol 1991; 417:517–522.
27. Trieschmann U, Pichlmaier M, Klockner U, Isenberg G. Vasorelaxation due to K-agonists. Single channel recordings from isolated vascular myocytes. Pflueg Arch Eur J Physiol 1988; 411:R119.
28. Burgen ASV. The secretion of potassium in saliva. J Physiol 1956; 132:20–39.
29. Young JA, Schögel E. Micropuncture investigation of sodium and potassium excretion in rat submaxillar saliva. Pflueg Arch Eur J Physiol 1966; 291:85–98.
30. Alexander JH, van Lennep EW, Young JA. Water and electrolyte secretion by the exorbital lacrimal gland of the rat studied by micropuncture and catheterization techniques. Pflueg Arch Eur J Physiol 1972; 337:299–309.
31. Cook DI, Young JA. Effect of K^+ channels in the apical plasma membrane on epithelial secretion based on secretory active Cl^- transport. J Membr Biol 1989; 110:139–146.
32. Young JA, van Lennep EW. Transport in salivary and salt glands: I. Salivary glands. In: Giebisch G, Tosteson DC, Ussing HH (eds.), Membrane Transport in Biology. Vol. 4B, Transport Organs. Berlin: Springer-Verlag; 1979: 563–674.
33. Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neill J (eds.), The Physiology of Reproduction. New York: Raven Press Ltd.; 1988: 999–1080.
34. Leung AYH, Wong PYD. Biphasic short-circuit current response to noradrenaline mediated by Ca^{2+} and cAMP in cultured rat epididymal epithelium. Pflueg Arch Eur J Phyiol 1994; 426:396–401.