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PROPERTIES OF STRETCH-ACTIVATED CHANNELS IN MYOCYTES FROM THE GUINEA-PIG URINARY BLADDER

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SUMMARY

1. Stretch-activated channels (SACs) were analysed on patches attached to myocytes isolated from the guinea-pig urinary bladder. At 22 °C application of −2 to −4 kPa to the patch electrode induced SACs at a density of one to two per patch (3–5 MΩ electrodes).

2. With electrodes containing 145 mM K^+, 20 mM TEA and 2 mM Mg^{2+}, the single channel current followed a linear *I–V* curve with a slope conductance of 39±5 pS (mean±S.D.) and a reversal potential of 2±6 mV. Substitution of chloride by aspartate ions left both parameters unchanged suggesting that the anions do not contribute to the currents.

3. Hyperpolarization from −30 to −80 mV did not open channels by itself but increased channel activity (NP_o; where N is the number of channels in the patch and P_o is the probability of the channel being open) twofold. The hyperpolarization-induced increase in NP_o can be attributed to a reduction of long closures. At positive patch potentials numerous blank records strongly diminished NP_o.

4. Inward currents through SACs can be carried by a variety of cations. In the presence of 2 mM Mg^{2+}, the respective channel conductance was 40±4 pS for 140 mM K^+ > 34±2 pS for 140 mM Na^+ ⩾ 33±6 pS for 140 mM Cs^+ > 19±2 pS for 110 mM Ba^{2+} > 17±2 pS for 110 mM Ca^{2+}.

5. Reduction of $CaCl_2$ from 110 to 10 mM did not change the conductance but shifted the reversal potential from +7 to −7 mV; the reversal potentials suggest that SACs are slightly more permeable for Ca^{2+} than for K^+.

6. In the absence of divalent cations, the conductance of K^+ was 82±4 pS for inward but 45 pS for outward currents. Addition of either 2 mM Ca^{2+} or 2 mM Mg^{2+} reduced the conductance for inward currents to 40 pS.

7. The change from 140 to 14 mM KCl plus 136 mM Tris-Cl reduced the conductance from 82 to 56 pS whereas the reversal potential shifted only from −4 to −9 mV. When 20 mM K^+ and 300 mM sucrose were applied, the conductance fell to 39 pS and the reversal potential shifted by −30 mV. The results suggest that $Tris^+$ can permeate through SACs when extracellular divalent cations are absent.

8. Channel openings were blocked by 200 or 20 μM Gd^{3+}. Gd^{3+} (5 μM) reduced the apparent single channel conductance and the mean lifetime of the open state.

9. We discuss the physiological importance of cell stretch; length changes due to filling of the urinary bladder could activate SACs and thereby contraction firstly by

Ca^{2+} influx through SACs which could increment cellular Ca^{2+} content directly or trigger Ca^{2+} release from intracellular stores. In addition, Na^+ influx through SACs depolarizes the membrane and activates Ca^{2+} influx through L-type Ca^{2+} channels.

INTRODUCTION

Single unloaded myocytes, isolated from the urinary bladder of the guinea-pig, have a stable resting potential of −50 mV (Klöckner & Isenberg, 1985*a*). This result contrasts with the measurements in multicellular strips from this tissue where the most negative potentials were −35 mV and spontaneous action potentials were superimposed (Creed, 1971). There are a number of important differences between the preparations, e.g. the presence or absence of nerve endings and neurotransmitter. In this paper we address the question of whether the mechanical conditions may be important for the electrical activity; whereas the isolated cells were sinking without external mechanical load to the coverslip, the multicellular strips were stretched to produce maximal force. More specifically, we ask: can the spontaneous electrical activity of the multicellular strips be attributed to currents through stretch-activated non-selective cation channels (SACs, cf. Sachs, 1990) that remained inactive in the isolated cell? If yes, can we activate these SACs in the isolated cells and estimate their importance for the *in-vivo* cell function?

In the literature, most studies on SACs were performed by application of a negative pressure (i.e. suction) to the open end of the patch pipette. Sachs (1990) suggested that the mechanical deformation of the membrane patch exerts a shear stress of the cytoskeleton that is transferred to the SAC channel protein. Here, we consider this assumption as justified and use the suction method as a routine technique that can activate SACs in a reproducible and reversible way. Part of the work has been published in abstract form (Wellner & Isenberg, 1992).

METHODS

Patch clamp techniques were used to record single channel currents from smooth muscle cells from the urinary bladder. Guinea-pigs were killed by cervical dislocation and myocytes were enzymatically dissociated from the urinary bladder using the collagenase method (Klöckner & Isenberg, 1985*a*). Until final use, the cells were stored in a Kraft–Brühe (KB) medium (Isenberg & Klöckner, 1982) composed of (mM): 60 KCl, 30 K_2PO_4, 1 EGTA, 5 $MgCl_2$, 5 creatine, 20 taurine, 2 glucose, 5 succinic acid, 5 glutamic acid, 1 g/l fatty acid-free albumin, adjusted with ≈ 5 mM KOH to a pH of 7·4. A drop of KB medium containing the cell suspension was placed in the experimental chamber (volume ≈ 0·1 ml). After the cells had settled down, the KB medium was replaced by a solution composed of (mM): 140 KCl, 2 $MgCl_2$, 2 EGTA, 10 Hepes, ≈ 5 KOH (pH 7·4) which effectively zeroed the membrane potential and kept the cells relaxed. To minimize mechanical disturbance, perfusion was halted before attempting to form gigaohm seals between the patch pipette and the cell membrane. The temperature of the experiments was between 22 and 25 °C.

Patch pipettes were pulled from 2 mm borosilicate glass to tips of approximately 1·5 μm outer diameter (3–5 MΩ tip resistance). For symmetrical recording conditions, the patch pipettes were filled with the electrode solution composed of (mM): 140 KCl, 1 EGTA, 2 $MgCl_2$ (free Mg^{2+} 1·8 mM), 10 Hepes, ≈ 5 KOH (pH 7·4). TEA (20 mM) was added to block potassium channels; the presence of TEA is indicated in the text. Ca^{2+} was removed in order to avoid the possibility of mistaking a Ca^{2+} channel. Variations in the electrode solution are specified in the text, e.g. 110 mM $[Ca^{2+}]_p$ represents 110 mM $CaCl_2$ solution in the patch pipette. The single channel currents were recorded with an RK 300 patch amplifier (Biologic, Echirolles, France) which allowed compensation for

small leakage currents (seals with input resistances less than 5 GΩ were discarded). Inward currents are shown as downward deflections. The input amplifier was connected through an CED-1401 interface (Cambridge Electronics, Cambridge, UK) to an IBM-compatible host. The currents were low-pass filtered (1 kHz), sampled at 2·5 kHz, digitized and stored in records of either 2 or 20 kB. To document the experiment and for illustration purposes, the currents were also on-line recorded with a thermo-writer (Tarr 220, Gold inc., OH, USA). The channel activity NP_o, the product of the number of channels in the patch (N) and the probability that the channel is open (P_o), was calculated as in Markwardt & Isenberg (1992). Statistical significance was calculated using Student's t test ($p < 0{\cdot}05$).

The negative pressure was calibrated using a water manometer and converted into kilopascals and generated by a syringe and applied to the patch pipette through a computer command to a magnetic valve (P/N 225P011-21 NC, NResearch, Maplewood, NJ, USA). In this study, all single channel recordings were made in the cell-attached patch mode.

RESULTS

Single channel currents due to negative pressure on the patch electrode

Patches were clamped to a potential of −40 mV (whole cell convention), usually 'spontaneous' channel activity was absent. Figure 1 shows that application of a negative pressure of −3·2 kPa (32·6 cmH_2O) induced the appearance of single channel inward currents that reached an amplitude of −1·7 or −3·4 pA (second level). In general, the channel activity (NP_o) increased with enhanced negative pressure. When different pressures were applied to the same patch clamped to −50 mV, NP_o was 2·2% for −3·2 kPa, 3·2% for −3·9 kPa and 12·2% for −4·9 kPa.

The effect of suction started promptly, i.e. the delay between application of suction and appearance of SACs was less than 0·2 s. The effect of suction could be repeated several times (compare Fig. 3) and was reversible, i.e. upon removal of suction the currents through SACs disappeared promptly. During the closures between the individual channel openings, the current trace returned to the same baseline as in the absence of suction (Figs 2*A* and 4*A*), i.e. the effect of suction cannot be attributed to a non-specific leakage.

In a total of 202 patches, 16% of the trials were failures; 56% of the trials were successful with one channel in the patch, and in 28% patches two or more channels were activated. The results suggest that the density of SACs in the membrane of urinary bladder cells is approximately one channel per 2 μm^2, when a membrane patch of approximately 2 μm^2 is assumed (Sokabe, Sachs & Jing, 1991).

Evaluation of the single channel conductance (2 mM $[Mg^{2+}]_p$)

In the presence of continuous suction (e.g. −3·8 kPa in Fig. 2), the patch potential was clamped to a variety of negative potentials between −30 and −90 mV. From the stored data, the amplitude of the unitary current was estimated either from histograms fitted by Gaussian curves, or by visual inspection of the channel records on the computer display. The open channel current was plotted over the patch potential and the data could be fitted by straight lines (linear I–V curves). The slope of the linear regression yielded the open channel conductance. With an electrode solution containing 145 mM K^+ and 2 mM Mg^{2+}, the slope conductance was 40 ± 4 pS (mean ± S.D., $n = 5$). A very similar value (39 ± 5 pS, $n = 5$) was obtained when the solution was complemented with 20 mM TEA as expected from a current that does not flow through TEA-sensitive K^+ channels.

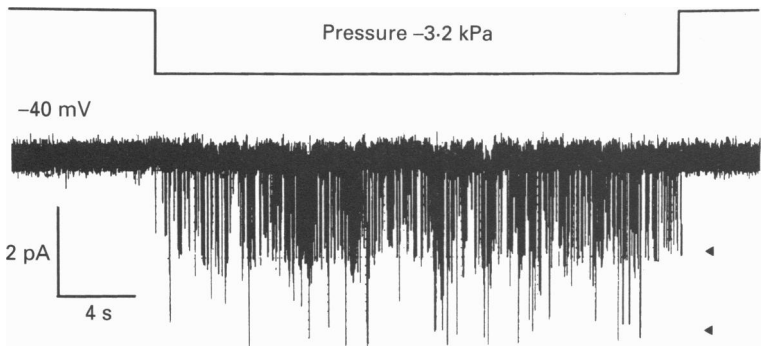

Fig. 1. Basic phenomenon of suction-induced channel activity, on-line pen recording. The cell was superfused with a Ca^{2+}-free zeroing solution containing 140 mM KCl, 2 mM EGTA and 2 mM $MgCl_2$. The patch electrode contained 140 mM KCl, 1 mM EGTA and 2 mM $MgCl_2$. The cell attached patch was clamped to −40 mV throughout. Application of suction (−3·2 kPa) by opening of the valve activated two channels; currents superimpose to amplitudes of either −1·7 or −3·4 pA (marked by arrowheads). Flow of K^+ ions from the electrode into the cell is shown as negative current deflection.

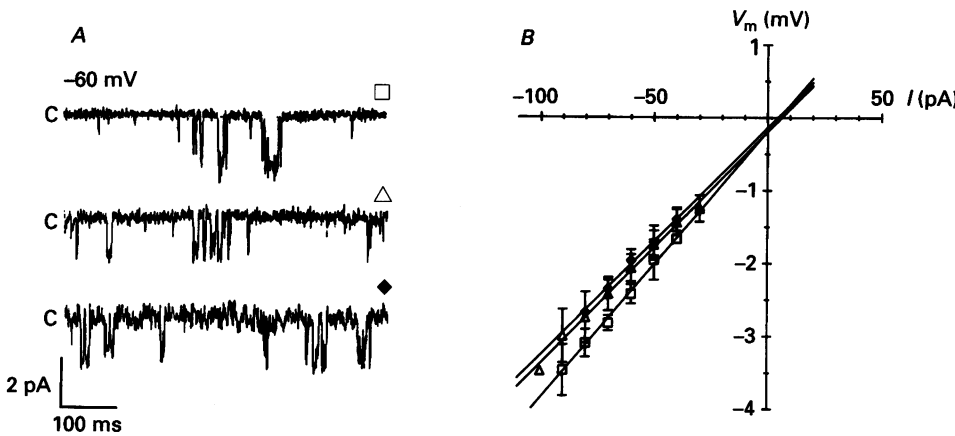

Fig. 2. Evaluation of the single channel conductance activated by suction. *A*, computer playback of single channel currents at −60 mV. The following electrode solutions were used: 140 mM KCl plus 20 mM TEA (□), 140 mM NaCl (◆), 140 mM CsCl (△), 1 mM EGTA and 2 mM $MgCl_2$ in all three solutions. Closed state is marked by C. *B*, *I–V* curves. V_m, membrane potential. Open channel currents (mean ± S.D., $n = 5$) plotted against patch potential. Symbols as in *A*.

Hyperpolarization increases the open probability

Although the open channel current does not rectify, SACs may contribute a hyperpolarization-activated component to the whole-cell current if the open probability (P_o) would increase with more negative membrane potentials. Hyperpolarization alone did not induce SAC-like single channel currents in the guinea-pig urinary bladder myocytes (tested up to −120 mV), that is, the activation of the channel required suction or other mechanical deformation of the patch as a prerequisite. In this respect, the present results differ from those reported for myocytes from the toad stomach (Kirber, Walsh & Singer, 1988). However, once the

channel was activated, its activity (NP_o) could be increased by hyperpolarization. In reference to the high NP_o at −80 mV, NP_o was approximately 50% at −40 mV and approximately 30% at −20 mV. Evaluation of the data in histograms indicated no significant effect of the membrane potential on the open times or on the short shut

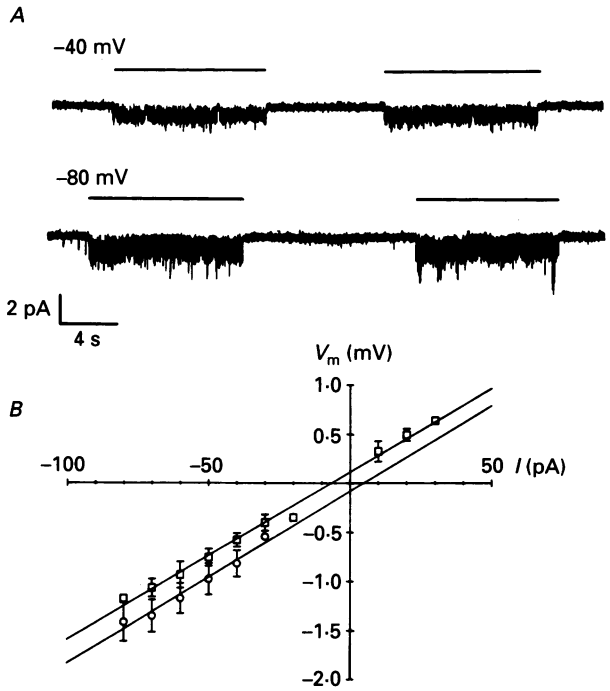

Fig. 3. SACs recorded with 110 mM Ca^{2+} in the pipette; suction by −3·8 kPa is marked by horizontal bar. *A*, on-line pen recording at −40 and −80 mV showing reversibility and reproducibility of channel activation by suction. *B*, *I–V* curve (mean ± S.D., $n = 5$) using 110 (○) or 10 (□) mM $CaCl_2$ in the patch pipette. The slope conductance was 17·4 ± 2·2 and 18·0 ± 1·5 pS, respectively, and the current reversal shifted from +7 ± 7 to −7 ± 5 mV, respectively.

times. The closures longer than 100 ms became shorter with membrane hyperpolarization, however, the number of available long closures was insufficient for fits with exponentials. At positive potentials, NP_o was further reduced due to the appearance of many failures. At +20 mV, the percentage of those blank records (500 ms) was approximately 80%.

Permeability for monovalent cations in the presence of 2 mM $[Mg^{2+}]_p$ *or 2 mM* $[Ca^{2+}]_p$

In a series of experiments the composition of the electrode solution was varied. In the constant presence of 145 mM K^+, 130 mM chloride was substituted by 130 mM aspartate, this substitution did not significantly change the single channel conductance (36 ± 6 pS, $n = 9$). With the assumption that the large aspartate is impermeable, the result suggests that chloride ions do not contribute to the single channel current, that is, that the channel is a cation channel.

With 140 mM NaCl (plus 2 mM $[Mg^{2+}]_p$) in the electrode, the current amplitudes

were somewhat smaller than in the case of 140 mM KCl. Correspondingly, the slope conductance (34 ± 2 pS, $n = 8$) was moderately smaller (significance at $p < 0{\cdot}05$). The current reversed polarity at a reversal potential $E_{rev} = 4 \pm 1$ mV, this value is not significantly different from the $E_{rev} = 2 \pm 6$ mV obtained with 140 mM KCl

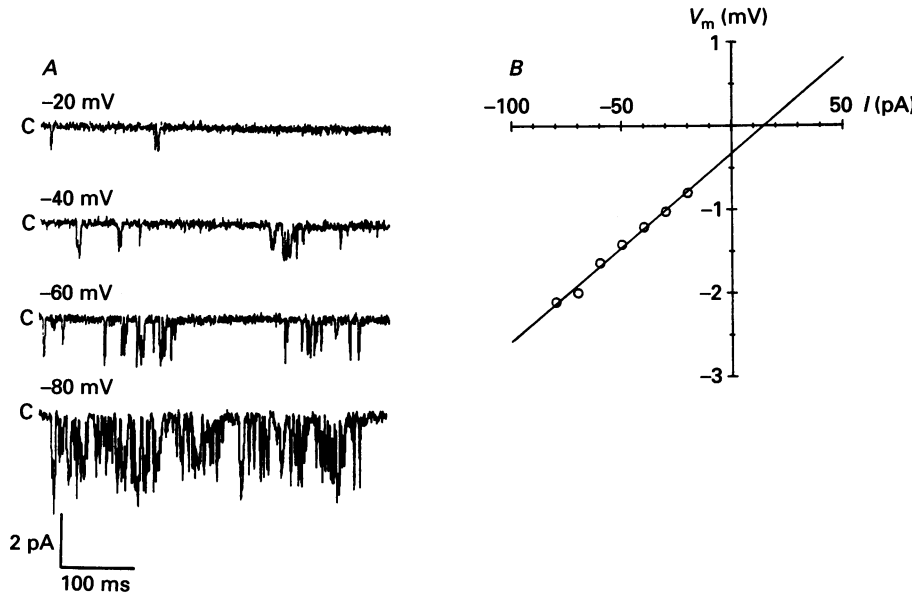

Fig. 4. SACs recorded with 110 mM Ba^{2+} in the pipette, suction $-4{\cdot}0$ kPa. *A*, computer playbacks for indicated patch potentials; note the short lifetime of the individual channel openings. *B*, data evaluated from the experiment of *A* in an *I–V* curve with a slope conductance of 22 pS.

(20 mM TEA present). With 140 mM NaCl plus 2 mM $CaCl_2$ the conductance was 33 ± 4 pS and reversal potential, E_{rev}, was -1 ± 6 mV ($n = 5$). With 140 mM CsCl in the pipette, the reversal potential was 8 ± 8 mV and the conductance was 33 ± 6 pS ($n = 6$), the conductance being different from the conductance for K^+ ions ($p < 0{\cdot}05$) but not from the conductance for Na^+ ions (see Fig. 2*B*). In general, the conductances were ranked in the order of $K^+ > Na^+ \geqslant Cs^+$. The conductances and the reversal potentials were very close to each other suggesting that the SAC is a channel that does not discriminate well between the monovalent cations when 2 mM $[Ca^{2+}]_p$ or 2 mM $[Mg^{2+}]_p$ are present.

SACs conduct Ca^{2+} and Ba^{2+} ions

Figure 3 shows single channel currents that were recorded with an electrode solution that contained 110 mM $CaCl_2$ as the charge carrier. The pen recording shows four consecutive activations by $-3{\cdot}8$ kPa, at -40 and -80 mV. The amplitude of Ca^{2+} currents through SACs was evaluated from computer playbacks, and the slope of the linear *I–V* curves (Fig. 3*B*) yielded a Ca^{2+} conductance of $17{\cdot}4 \pm 2$ pS ($n = 5$). The experiments with 110 mM $BaCl_2$ in the electrode gave similar currents. The *I–V* curve of Fig. 4*B* indicates a single channel conductance of 22 pS, the mean value was $19{\cdot}4 \pm 2{\cdot}4$ pS ($n = 5$). The conductances measured with 110 mM Ca^{2+} or Ba^{2+} were

significantly smaller than those for the monovalent cations. Ca^{2+} currents through SACs could be recorded also with solutions of lower concentrations of $CaCl_2$, $Tris^+$ or *N*-methyl-D-glucamine$^+$ being used as a substitute for keeping osmolarity constant. At a $[Ca^{2+}]_p$ of 10 mM the slope conductance was $17{\cdot}9 \pm 5$ pS, i.e. not different from the value obtained with 110 mM $CaCl_2$. With 5 mM $CaCl_2$, a conductance of 12 pS was estimated. The dependence of conductance on $[Ca^{2+}]_p$ could be described with a steep saturation curve yielding a Hill coefficient of 3 and a dissociation constant (K_D) value of 4 mM.

Permeability ratio $P_{Ca}:P_K$

The reversal potential of the single channel currents (E_{rev}) might be used for evaluation of the ratio of Ca^{2+} to K^+ permeability, provided the Ca^{2+} and K^+ ions moved independently (cf. Hille, 1992). Figure 3*B* shows for 110 mM $[Ca^{2+}]_p$ an E_{rev} of $+7 \pm 7$ mV. Assuming an intracellular $[K^+]$ of 150 mM (Isenberg, Wendt-Gallitelli & Ganitkevich, 1992), the E_{rev} of 7 mV suggests a permeability ratio $P_{Ca}:P_K \approx 0{\cdot}6$. The experiment was repeated with 10 mM $[Ca^{2+}]_p$ plus 140 mM $Tris^+$, and an E_{rev} of -7 ± 5 mV suggested a permeability ratio $P_{Ca}:P_K$ of 2. One may conclude that Ca^{2+} and K^+ ions move through SACs with similar permeabilities. However, it might well be feasible that these estimates are incorrect because of the erroneous assumption of the independence principle.

Suppression of single channel conductance by Ca^{2+} or Mg^{2+}

In the case of voltage-gated Ca^{2+} channels, the permeating Ca^{2+} ions are thought to bind to the channel protein thereby reducing the permeation of the monovalent Na^+ ions (e.g. Hess, Lansman & Tsien, 1986). For the 'non-selective' SACs, a similar situation may apply. Figure 5 shows *I–V* curves obtained in the absence of divalent cations. With 145 mM K^+, the conductance of SACs for inward currents was $81{\cdot}5 \pm 4$ pS which is twice as high as the 40 ± 4 pS that was recorded in the presence of 2 mM Mg^{2+}. At positive patch potentials the conductance for outward current was only 45 pS, that is, the SACs rectified. We postulate that the outward current drives blocking Mg^{2+} ions from the cytosol into the pore.

As already reported above, addition of 2 mM Mg^{2+} to the electrode solution reduced the conductance from 81·5 to 40 pS. Addition of 2 mM Ca^{2+} to the Mg^{2+}-free KCl solution reduced the conductance to 40 ± 4 pS, the effect of Ca^{2+} was not distinguishable from the Mg^{2+} effect. In the presence of both 2 mM Ca^{2+} and 2 mM Mg^{2+}, the K^+ conductance was $39{\cdot}4 \pm 5$ pS, i.e. identical to the one reported for Mg^{2+} alone. Addition of 2 mM Ca^{2+} to the pipette solution which contained 145 mM Na^+ resulted in a conductance of 33 ± 4 pS which is close to the 34 ± 2 pS recorded with 145 mM Na^+ plus 2 mM Mg^{2+}. The results are compatible with the idea that the presence of divalent cations hinders the permeation of the monovalent K^+ cations.

In the absence of divalent cations SACs may be permeable for $Tris^+$

In the experiments shown in Fig. 5, $[K^+]_p$ was reduced from 145 to 14 or to 20 mM. To keep the osmolarity constant, 136 mM $Tris^+$ was substituted for K^+ (Fig. 5*A*). Reduction of $[K^+]_p$ reduced the slope conductance from $81{\cdot}5 \pm 4$ to 56 ± 7 pS. In

addition, there was a shift in the reversal potential. The difference between E_{rev} = −4 mV at 140 mM KCl and E_{rev} = −9 mV at 14 mM KCl plus 140 mM Tris-Cl was −5 mV which is by far smaller than the −59 mV that are expected if only intra- and extracellular K^+ ions are permeable. The only other available cations were $Tris^+$ ions.

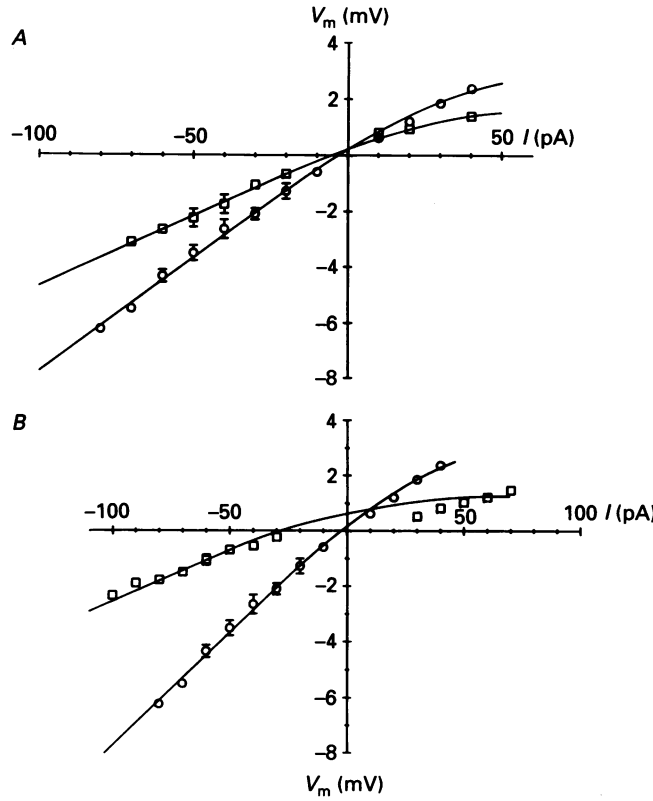

Fig. 5. *I–V* curves measured in the absence of divalent cations and the effect of reduced $[K^+]_p$. *A*, $Tris^+$ substitution, 145 mM K^+ (○) or 14 mM KCl plus 136 mM $Tris^+$ (□) are compared. *B*, sucrose substitution, 145 mM K^+ (○) is compared with 10 mM KCl, 10 mM K^+-Hepes, 300 mM sucrose (□).

Hence we suggest that the unexpectedly small change in E_{rev} is the result of a permeation of $Tris^+$ ions through SACs. With this assumption, one can estimate a permeability ratio $P_{Tris}:P_K \approx 0{\cdot}2:1$. Addition of 2 mM Mg^{2+} reduced the conductance to 16±2 pS ($n = 5$) and shifted E_{rev} to −27±4 mM suggesting that the high $Tris^+$ permeability occurs only in the absence of divalent cations.

The hypothesis that the removal of extracellular divalent cations can induce an abnormal $Tris^+$ permeability is further supported by another series of experiments where $[K^+]_p$ was reduced from 145 to 20 mM and 300 mM sucrose was used to balance osmolarity (Fig. 5*B*). The conductance fell from 81·5 to 39±7 pS, i.e. the conductance in the presence of 20 mM $[K^+]_p$ and 300 mM sucrose was lower than the conductance with 14 mM $[K^+]_p$ and 140 mM $Tris^+$, as if $Tris^+$ permeation contributed to the

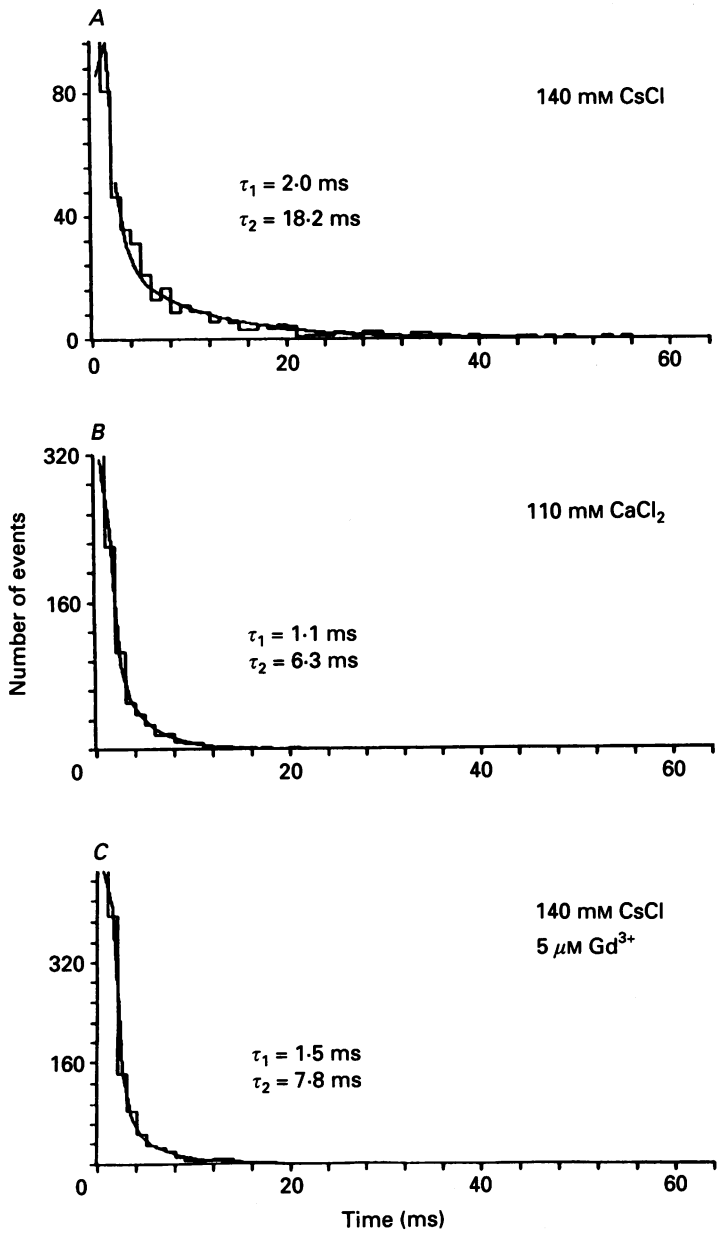

Fig. 6. Open time histograms of SACs. Bin size 1 ms. The electrodes were filled with (mM): *A*, 140 CsCl plus 2 $MgCl_2$; *B*, 110 $CaCl_2$; and *C*, 140 CsCl, 2 $MgCl_2$ and 5 μM Gd^{3+}. The time constants of the bi-exponential fit are labelled. Holding potential, −50 mV.

conductance. In the presence of sucrose, reduction of $[K^+]_p$ from 145 to 20 mM shifted the current reversal by −30 mV. The substitution of KCl by sucrose strongly reduces the ionic strength, which could be the reason why the measured shift (−30 mV) did not reach the expected one (−51 mV).

Extracellular divalent cations reduce the open channel time

Ca^{2+} or Ba^{2+} currents through SACs differed from the currents carried by monovalent cations not only in regard to the significantly smaller conductance, but in addition, the SACs spent a shorter time in the open state. The channel open time

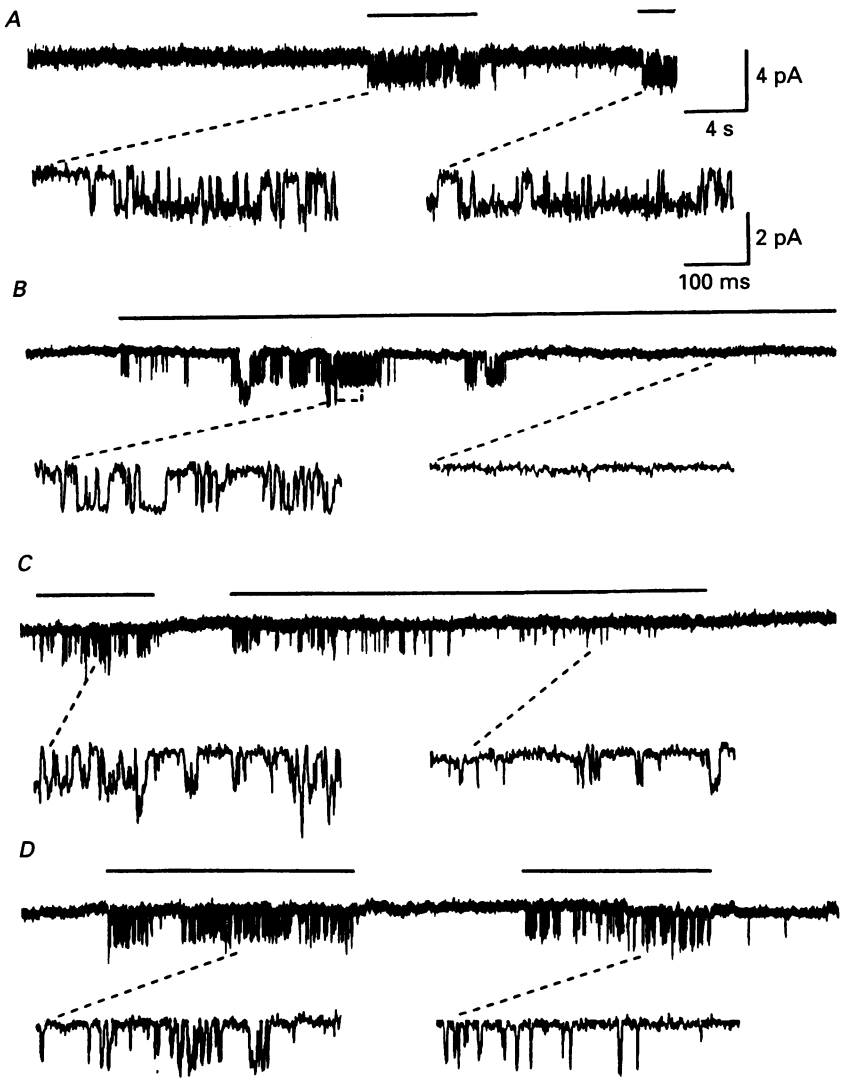

Fig. 7. Block of SACs by Gd^{3+}. Original pen-recordings (suction marked by bars) and computer playbacks (periods indicated by the dashed lines). *A*, control, −4 kPa, no Gd^{3+}; *B*, −5 kPa, 200 μM Gd^{3+}; *C*, −5 kPa, 20 μM Gd^{3+}; *D*, −2·4 kPa, 5 μM Gd^{3+}. Holding potential, −50 mV for all sweeps.

was quantified with histograms (Fig. 6). The data could be separated according to short and long openings (> 12 ms). Since long openings have not been described for SACs in smooth muscle cells, we counted the percentage they contributed to the total

number of openings. When the electrode was filled with 145 mM KCl but no Mg^{2+}, 19% of the total openings lasted longer than 12 ms. In the presence of 2 mM $[Mg^{2+}]_p$, 8% of the openings were longer than 12 ms. With 2 mM $[Ca^{2+}]_p$, 4·4% of the openings were longer than 12 ms. The change to the high 110 mM $[Ca^{2+}]_p$ or $[Ba^{2+}]_p$ nearly abolished the long openings (1·5% in both solutions) as indicated by the comparison of the computer playbacks of Figs 2*A* (KCl) and 4*A* ($BaCl_2$). In summary, SACs of urinary bladder myocytes have a long-lived open state which is suppressed by the presence of divalent cations.

The open time histograms were fitted with two exponentials; with Cs^+ as charge carrier (Fig. 6*A*), the fast exponential had a time constant τ_1 of 2 ms and an amplitude A_1 of 52%, the slow exponential had a τ_2 of 18·2 ms and an A_2 of 48%. For 110 mM Ca^{2+} as charge carrier, the fit yielded a fast exponential with τ_1 of 1·1 ms and an A_1 of 70% whereas the slow time exponential had a τ_2 of 6·3 ms and an A_2 of 30%. Thus, the histograms support the conclusion that extracellular divalent cations abbreviate the mean open channel time.

Gadolinium-block of SACs

Gd^{3+} has been introduced as a specific blocker of SACs (cf. Sachs, 1990). In the present experiments, the Gd^{3+} block of SACs was tested by adding $GdCl_3$ to the electrode solution containing 140 mM CsCl plus 2 mM Mg^{2+}. A short control run before the Cd^{3+} block was possible by using tips that were filled with $GdCl_3$-free CsCl solution to a height of approximately 2 mm whereas the $GdCl_3$-containing Cs^+ solution was back-filled; diffusion of Gd^{3+} to the SAC blocked the activity within 1–2 min. $GdCl_3$ (200 μM) completely blocked the SACs, i.e. the activity disappeared during the continuous suction and could not be restored (−5 kPa in Fig. 7*B*). Also with 20 μM $GdCl_3$, SACs activity disappeared within approximately 1 min, however, there was a transient 20 s period (Fig. 7*C*) during which Gd^{3+} reduced the single channel currents in regard to their amplitude and open time. In the presence of 5 μM $GdCl_3$, SAC activity could be recorded for a period up to 10 min; the openings were shorter and reached only rarely the full amplitude (Fig. 7*D*). In the open time histogram (Fig. 6*C*) 5 μM $CdCl_3$ suppressed the occurrence of openings longer than 12 ms to 1·4%. The comparison between panel *A* and *C* of Fig. 7 suggests that the lifetime of short openings was not significantly changed.

DISCUSSION

In this paper we have shown that application of a negative pressure to the patch electrode activates single channel currents in the membrane of guinea-pig urinary bladder myocytes. The hypothesis that these currents flow through 'stretch-activated channels' or SACs is based upon the following general properties (compare Sachs, 1990): (1) the channel activity NP_0 increased with negative pressure; (2) the channel carried cations with a selectivity $K^+ > Na^+ \geqslant Cs^+ \gg Ba^{2+} > Ca^{2+}$; (3) micromolar concentrations of Gd^+ blocked the channel; (4) single channel conductance was reduced by divalent cations. With physiological concentrations of Na^+, Ca^{2+} and Mg^{2+}, the conductance was approximately 34 pS. This value is close to the 33 pS in smooth muscle cells from toad stomach (Kirber *et al.* 1988), to the

35 pS in embryonic skeletal muscle (Guhary & Sachs, 1984), to the 38 pS in liver cells (Bear, 1990), to the 38 pS in oocytes (Taglietti & Toselli, 1988), or the 28 pS in lens epithelia (Cooper, Tang, Rae & Eisenberg, 1986) but is somewhat higher than the 22 pS found in the kidney epithelia (Ubl, Murer & Kolb, 1988).

In the absence of divalent cations, SACs had a more than twofold higher conductance than in their presence. Reduction of channel conductance by divalent cations could be attributed to a reduction of the charge carrier concentration, locally at the outer mouth of the channel; Ca^{2+}, and to a smaller extent Mg^{2+} cations, are thought to reduce the effective concentrations of K^+ or Na^+ ions. On the other hand, the reduction of single channel conductance could be the result of binding to the intrachannel pore. Permeation with binding is suggested by the concentration dependence of the conductance. Whereas the conductance for K^+ increased with $[K^+]_p$ up to 145 mM, the dependence of single channel conductance on $[Ca^{2+}]_p$ was very steep (Hill coefficient of 3) and saturated at 10 mM Ca^{2+} (half-maximal conductance at 4 mM). Generally, the conductance saturates when the binding–unbinding steps of permeation become limiting. The comparison between K^+ and Ca^{2+} currents suggests that Ca^{2+} but not K^+ ions strongly bind to the inner side of the pore. In this regard, the SACs resemble the L-type Ca^{2+} channels (Hess *et al.* 1986). Intrachannel Ca^{2+} or Mg^{2+} binding is also suggested to limit the permeation of the monovalent cations, as the rate of ion entry approaches the maximum rate of Ca^{2+} unbinding (see Hess *et al.* 1986; Hille, 1992). Block by intracellular Mg^{2+} is probably responsible for the inward going rectification that was seen with Ca^{2+}- and Mg^{2+}-free pipette solutions. Addition of $[Mg^{2+}]_p$ reduced the inward currents in such a way that the *I–V* curves became linear; an effect that can be interpreted as a block of both inward and outward currents through SACs if the divalent cations can enter the pore from both intra- and extracellular space.

Extracellular divalent cations also determine the ability of the channel in discriminating the charge carrier (Hess *et al.* 1986). Our measurements of current reversal potentials suggested that, in the non-physiological absence of divalent cations, large $Tris^+$ cations can permeate through SACs. Permeation of $Tris^+$ would suggest that the selectivity filter has a diameter of at least 0·6 nm. Permeation of $Tris^+$ through SACs has been suggested for the rabbit sino-atrial node (Irisawa & Hagiwara, 1991; whole cell currents). Also, choline was reported to permeate through SACs in opossum kidney (0·1 μM Ca^{2+}; Ubl *et al.* 1988).

The divalent cations modulated the gating of the SACs. In general, the lifetime of the open state was shorter in the presence of 2 mM Mg^{2+} or 2 mM Ca^{2+} than in their absence. Ca^{2+}-induced reduction of the channel open time from 3 to 0·5 ms has been described and modelled in the literature (Yang & Sachs, 1989). Our histograms demonstrated that SACs can enter into an additional open state with a long lifetime (longer than 12 ms) and that 110 mM Ca^{2+} as well as 5 μM Gd^{3+} suppressed these long openings. Gating schemes including the Ca^{2+} effects on those long openings have not been modelled up to now.

The reversal potentials, recorded with different $[Ca^{2+}]_p$, gave a permeability ratio of approximately 1:1 for $P_K:P_{Na}$ and of 1:1–1:3 for $P_K:P_{Ca}$ (compare Yang & Sachs, 1990). With the assumptions of the constant field equations (cf. Hille, 1992) one estimates that, for a physiological solution containing 2 mM Ca^{2+}, about 5% of the

total charge current is carried by Ca^{2+} ions. From this number the Ca^{2+} influx through SACs can be extrapolated. The density of approximately one channel per 2 μm^2 gives for a surface area of 2231 ± 179 μm^2 (Klöckner & Isenberg, 1985*a*) approximately 1000 SACs per cell. On the assumption that the stretch activates SACs with open probability of 5%, one can expect at -50 mV a whole cell inward current of approximately -100 pA of which -5 pA may be carried by Ca^{2+} influx. Since this Ca^{2+} influx does not inactivate, it could increase the total calcium concentration in the cell (volume 6281 μm^3) by 3 μM (compare Yang & Sachs, 1989, 1990); 99·9% of this small increment will be bound (Isenberg *et al.* 1992). The increment due to Ca^{2+} influx through SACs is small in comparison with the one through L-type Ca^{2+} channels (60 μM/100 ms, Klöckner & Isenberg, 1985*b*). Thus, one wonders whether activation of SACs induces myogenic contraction indirectly, i.e. by depolarization and activation of L-type Ca^{2+} channels. However, it is quite possible that we underestimated the Ca^{2+} influx through SACs; the estimation of the permeability ratio $P_{Ca}:P_K$ assumed that Ca^{2+} and K^+ ions permeate independently of each other whereas our results suggest that binding of Ca^{2+} to the channel modifies the permeation of K^+ ions.

If the myocyte is not voltage clamped, the -100 pA current through SACs depolarizes the whole cell (input resistance $4 \pm 0{\cdot}4$ GΩ). At -35 mV, the threshold for L-type Ca^{2+} channel activation (Klöckner & Isenberg, 1985*b*), a Ca^{2+} action potential will be generated. Hence, stretch is thought to activate an inward background current suitable for turning the cell from electrical stability (isolated cells without mechanical load) into one with spontaneous activity (compare Davis, Donovitz & Hood, 1992). Each action potential goes along with Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels that is large in comparison with the Ca^{2+} influx through SACs.

Hyperpolarization increased the open probability of the SACs (compare Kirber *et al.* 1988). An inward current increasing with more negative potential is important for the generation of the pacemaker depolarization and exists in some smooth muscle cells in the absence of stretch, as in stomach (Hisada, Ordway, Kirber, Singer & Walsh, 1991) or ileum myocytes (Benham, Bolton, Denbigh & Lang, 1987). This is not the case in the guinea-pig urinary bladder myocytes, where the appearance of hyperpolarization-activated currents requires the mechanical stress.

Recently, SACs were recorded from myocytes isolated from porcine coronary arteries (Davies *et al.* 1992). The authors demonstrated that suction can activate SACs with a cation selectivity similar to that described above. Most importantly, they were able to stretch the whole cell to 5–30% greater than its slack length while recording membrane potential or whole cell current. Under whole cell voltage clamp, with the cells in physiological salt solution, stretch induced an inward current of -50 pA. Ca^{2+} entry during cell stretch seemed to be sufficient to produce a significant increase in intracellular calcium and to trigger cell contraction, even after the L-type Ca^{2+} channels had been blocked by 5 μM nifedipine (Davis, Hester, Donovitz, Montgomery & Meininger, 1990).

The function of SACs is integrated in cellular feedback systems. A positive feedback would result if the small Ca^{2+} influx through SACs could trigger release of Ca^{2+} from intracellular stores; the Ca^{2+} release would amplify the cytosolic $[Ca^{2+}]$

signal and could activate contraction even in the presence of 5 μM nifedipine. An example of negative feedback could be seen in the Ca^{2+} activation of BK-channels that re- or hyperpolarize the membrane and terminate the Ca^{2+} influx through L-type Ca^{2+} channels but not the Ca^{2+} influx through SACs. Forthcoming experiments that measure simultaneously cytosolic [Ca^{2+}] and whole cell currents from stretched myocytes should test these predictions.

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