

## Effects of NS1608 on MaxiK Channels in Smooth Muscle Cells from Urinary Bladder

C. Siemer<sup>1</sup>, M. Bushfield<sup>2</sup>, D. Newgreen<sup>3</sup>, S. Grissmer<sup>1</sup>

<sup>1</sup>Department of Applied Physiology, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

<sup>2</sup>Cambridge Drug Discovery Ltd., 3 Brookman Court, Cambridge CB4 2QH, UK

<sup>3</sup>Pfizer Ltd., Discovery Biology, Sandwich, Kent, UK

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**Abstract.** Using the patch-clamp technique, we have characterized membrane currents in single detrusor smooth muscle cells from rat and human urinary bladder. From the voltage- and  $\text{Ca}^{2+}$ -dependence of the current as well as the single channel conductance we conclude that rat and human urinary bladder smooth muscle cells express MaxiK channels. In smooth muscle cells from rat urinary bladder we tested the action of NS1608 on current through these MaxiK channels. Application of  $10\ \mu\text{M}$  NS1608 increased the amplitude of the current and this increase could be explained by a shift in the activation voltage of the MaxiK channels  $\sim 100\ \text{mV}$  towards more negative potentials. Charybdotoxin as well as paxilline, well known blockers of MaxiK channels, were able to reduce current through MaxiK channels in our cell preparation. In addition, application of  $10\ \mu\text{M}$  NS1608 hyperpolarized the membrane potential of the investigated cells. This hyperpolarization could be antagonized by the application of paxilline. We conclude that application of NS1608 results in the opening of MaxiK channels under physiological conditions that leads to a hyperpolarization of the cells. This hyperpolarization in turn could relax urinary bladder smooth muscle cells. MaxiK channels in these cells could therefore play a role in directly controlling muscle tone by regulating the membrane potential. This opens up the possibility of MaxiK channels being targets for the treatment of urge incontinence.

**Key words:** Bladder — Smooth muscle cells — MaxiK channels — NS1608 — Hyperpolarization — Muscle relaxation — Urge incontinence

## Introduction

MaxiK channels are ubiquitously distributed in a variety of tissues including bladder smooth muscle cells (for review *see* Siemer & Grissmer, 1999). In other smooth muscles, MaxiK channels determine the contractile status of the cells. Factors which promote the opening of MaxiK channels could cause hyperpolarization and relaxation of the cells (Nelson et al., 1995), whereas inhibition of the channels leads to depolarization and constriction of the smooth muscle (Anwer et al., 1993; Nelson et al., 1995).

Under physiological conditions the bladder detrusor muscle contracts to empty the bladder. If the bladder fills again the detrusor needs to relax for the bladder to extend. During urge incontinence, the detrusor muscle contracts spontaneously, leading to loss of urine. One mechanism that might cause these contractions could be depolarizations of the detrusor smooth muscle cells. These depolarizations in turn would open voltage-gated  $\text{Ca}^{2+}$  channels and this opening would be followed by an influx of  $\text{Ca}^{2+}$  into the cell and the smooth muscle would contract due to the increase in  $[\text{Ca}^{2+}]_i$ . Simultaneously, the increase in  $[\text{Ca}^{2+}]_i$  as well as the depolarizations would activate MaxiK channels and the cells would repolarize again. If MaxiK channels could be opened at rest or near rest this would stabilize the membrane potential,  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels would be limited and therefore would prevent or inhibit contraction.

Therefore the purpose of our investigation was to demonstrate the presence of functional MaxiK channels in single smooth muscle cells from urinary bladder, then to find out whether substances that are known to open  $\text{K}^+$  channels also open MaxiK channels in bladder smooth muscle cells and whether this opening will result in a hyperpolarization that would be required if this opener

should relax the smooth muscle cell. In this article we could show in voltage-clamp experiments that this channel type is present and can be modulated by the application of NS1608 (*N*-(3-trifluoromethyl)phenyl)-*N'*-(2-hydroxy-5-chlorophenyl)urea, a biaryl urea (Oleson, Moldt, & Pedersen, 1994; Hu, Kim & Fink, 1995; for review see also Starrett, Dworetzky & Gribkoff, 1996). NS1608 seemed to shift the voltage-dependence of channel opening towards more negative potentials. In addition, in current-clamp experiments we could unequivocally demonstrate that application of NS1608 resulted in a hyperpolarization of the cells. This information is essential and crucial and might open up the possibility to modulate the contractile status of the cell by opening of MaxiK channels. Some of the results have been reported in preliminary communications (Siemer & Grissmer, 1997, 1998).

## Materials and Methods

### ISOLATION OF SINGLE CELLS

Basis of our isolation procedure was the protocol reported by Klöckner and Isenberg (1985) for smooth muscle cells from guinea pig urinary bladder. Female WAG rats (~200 g) were stunned and killed by cervical dislocation. The bladder was dissected, cut open and then spread on a rubber-coated culture dish. After rinsing with NaCl solution (in mM: NaCl 100, KCl 10, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgCl<sub>2</sub> 5, glucose 20, taurine 50, MOPS 5 adjusted to pH 6.9 with NaOH) the urothelium was removed using forceps and fine scissors. The detrusor muscle was cut into small pieces (1 mm × 5 mm) which were digested in a collagenase-containing solution (in mM: KOH 130, taurine 20, pyruvate 5, creatine 5, HEPES 10 adjusted to pH 7.4 with methanesulfonic acid, and complemented with 1 mg/ml collagenase (Sigma C2139, Sigma-Aldrich Chemie, Deisenhofen, Germany), 0.2 mg/ml pronase E (Serva, Heidelberg, Germany) and 1 mg/ml fatty acid free albumin) at 37°C for 40 min. Single cells were obtained by trituration of the preparation with a wide-bore, smooth-tipped pipette in Na-Glu solution (in mM: Na glutamate 80, NaCl 55, KCl 6, MgCl<sub>2</sub> 2, glucose 11, HEPES 10 adjusted to pH 7.4 with NaOH and complemented with 1 mg/ml fatty acid free albumin). The cells were stored on ice in this solution for up to 2 days. Smooth muscle cells from human urinary bladder were dissociated similarly to the procedure described above for rat urinary bladder. Human bladder tissue was only obtained from patients if surgical removal of bladder tissue was clinically indicated.

### SOLUTIONS

All experiments were done at room temperature (21–25°C). The cells were superfused with a mammalian Na<sup>+</sup> Ringer solution (in mM: NaCl 160, KCl 4.5, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 1, HEPES 5 adjusted to pH 7.4 with NaOH). Internal solution for whole-cell experiments was K-aspartate solution (in mM: K-aspartate 135, MgCl<sub>2</sub> 2, EGTA 10, HEPES 10 adjusted to pH 7.4 with KOH) with 2.468 or 7.632 mM total added Ca<sup>2+</sup> yielding 50 nM and 500 nM free Ca<sup>2+</sup>, respectively. The computer program by Fabiato (1988) was used for calculating the required total Ca<sup>2+</sup> concentrations. Pipette solution for cell-attached experiments was a K<sup>+</sup> solution (in mM: K-aspartate 165, CaCl<sub>2</sub> 2, MgCl<sub>2</sub> 2, HEPES 10, adjusted to pH 7.2 with KOH).

## CHEMICALS

NS1608 was synthesized in the Research Department of Pfizer (Sandwich, Kent, UK) dissolved in DMSO (dimethylsulfoxide) as a 100 mM stock solution and diluted with mammalian Na<sup>+</sup> Ringer solution to the final concentration. Paxilline (Sigma, Deisenhofen, Germany) was dissolved in DMSO as a 10 mM stock solution and diluted to the final concentration of 1 μM with mammalian Na<sup>+</sup> Ringer. In all experiments the total DMSO concentration was lower than 0.02%.

Charybdotoxin was purchased from Latoxan (Rosans, France). A stock solution of 1 μM charybdotoxin in mammalian Na<sup>+</sup> Ringer solution was prepared and stored below –20°C. For experiments a small amount of this solution was diluted to the final concentration of 10 nM.

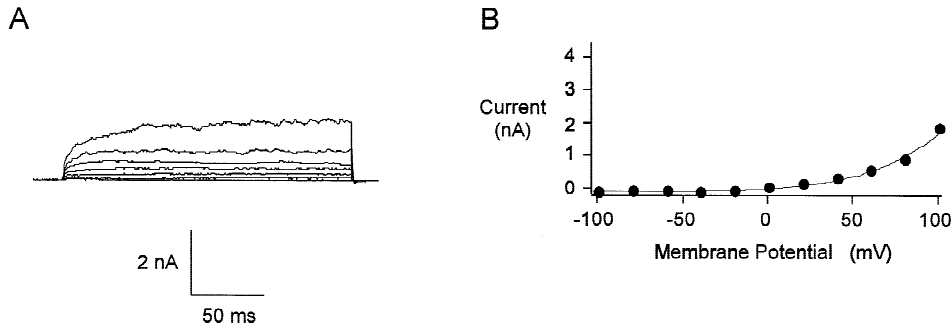
## ELECTROPHYSIOLOGY

Experiments were carried out using the whole-cell recording mode of the patch-clamp technique (Hamill et al., 1981) as described earlier (Grissmer et al., 1990, 1992a,b; Grissmer, Nguyen & Cahalan, 1993; Hanselmann & Grissmer, 1996; Rauer & Grissmer, 1996; Steinert & Grissmer, 1997; Jäger et al., 1998). Electrodes were pulled from borosilicate glass capillaries (Clark Electromedical Instruments, Reading, UK) in three stages and fire-polished to resistances of 2.5 to 5 MΩ. Membrane currents were recorded with an EPC-9 patch-clamp amplifier (HEKA elektronik, Lambrecht, Germany) interfaced to a Macintosh computer running acquisition and analysis software (Pulse/Pulsefit). The holding potential was either –80 or –120 mV. Capacitative and leak currents elicited through voltage steps were subtracted using the P/8 procedure. Ramp currents elicited by continuously ramping the voltage from –120 to +120 mV within 600 msec were not subtracted for leak currents. The cell potential was recorded using the current clamp mode of the patch-clamp amplifier. Amplitude histograms in single channel recordings were calculated using the TAC program (Bruxon Corporation, Seattle, WA).

## Results

### PRESENCE OF MAXIK CHANNELS IN RAT AND HUMAN URINARY BLADDER SMOOTH MUSCLE CELLS

Figure 1 demonstrates that smooth muscle cells from rat and human urinary bladder exhibit a voltage-dependent current, which activated with depolarizing potentials. The currents were elicited by 200 msec depolarizing voltage steps from the holding potential of –80 mV to potentials between –120 and +100 mV. The internal Ca<sup>2+</sup> concentration was 50 nM. Original current traces can be seen in Fig. 1A. At potentials more negative than +20 mV hardly any current could be detected. Above +20 mV a current can be elicited which seem to increase nonlinearly with more positive voltages. Figure 1B shows peak current amplitude from A plotted against the depolarizing voltage step. The fit of a Boltzmann equation to these peak current amplitudes yielded a voltage for half-maximal activation of the current ( $E_{1/2}$ ) of at least +110 mV. Averaging several experiments under similar measuring conditions, with depolarizing potentials up to +160 mV, we determined an  $E_{1/2}$  of  $124 \pm 7$



**Fig. 1.** Voltage dependence of the MaxiK current. (A) Original current traces elicited by 200 msec depolarizing voltage steps from the holding potential of  $-80$  mV to potentials between  $-120$  and  $+160$  mV in  $20$  mV increments. The free internal  $\text{Ca}^{2+}$ -concentration was  $50$  nM. (B) Maximum peak currents from A plotted against the test potential. The line through the data points represents a fit of a modified Boltzmann equation to the data from A and yielded voltages for half-maximal activation of the current,  $E_{1/2}$  of  $+141$  mV.

mV ( $n = 28$ ) and a slope of  $39 \pm 4$  mV with  $50$  nM internal  $\text{Ca}^{2+}$ . In additional experiments we determined an  $E_{1/2}$  of  $96 \pm 11$  mV ( $n = 15$ ) with a slope of  $42 \pm 4$  mV with  $100$  nM  $\text{Ca}^{2+}$  and an  $E_{1/2}$  of  $40 \pm 7$  mV ( $n = 28$ ) with a slope of  $26 \pm 2$  mV with  $500$  nM internal  $\text{Ca}^{2+}$ . These results are in agreement with the finding by Strøbæk et al. (1996) as well as Cui et al. (1997) on *hsl*o and *msl*o channels, respectively, who reported that the activation curve of the channels was steeper if  $[\text{Ca}^{2+}]_i$  was in the micromolar range compared to when it was in the low nanomolar range. They interpreted their finding by assuming a  $\text{Ca}^{2+}$  independent gating mechanism with an intrinsic gating charge when  $[\text{Ca}^{2+}]_i$  was low and that this gating charge was increased by an increase in  $[\text{Ca}^{2+}]_i$ .

#### SINGLE CHANNEL RECORDINGS OF MAXIK CHANNELS IN RAT AND HUMAN URINARY BLADDER SMOOTH MUSCLE CELLS

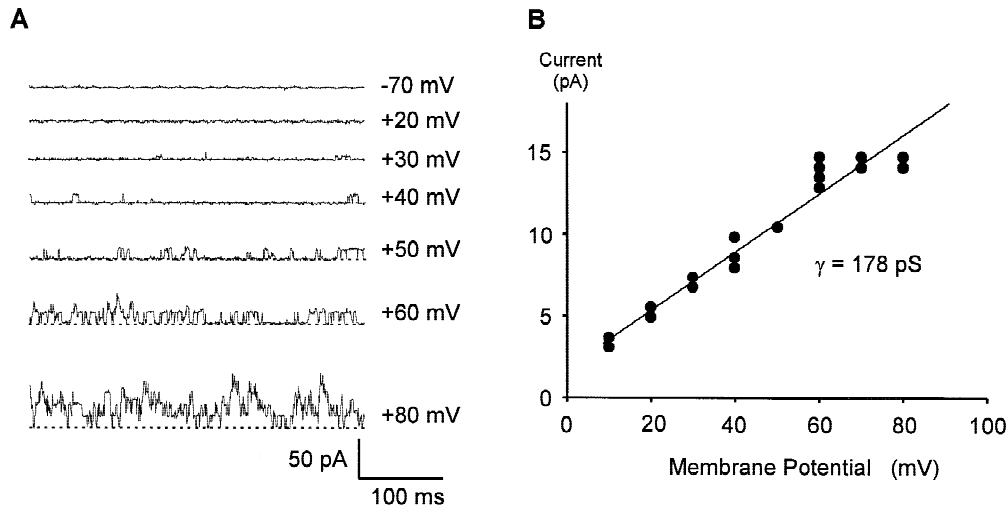
Single MaxiK channels with a single channel conductance of  $170$ – $200$  pS were recorded in the cell-attached and the inside-out configuration. Figure 2A shows original current traces from a cell-attached experiment. The pipette solution was a  $\text{K}^+$  aspartate solution containing  $2$  mM free  $\text{Ca}^{2+}$ . At negative voltages no channel openings were observed. At  $+30$  mV only a few openings could be seen. With increasing depolarizing potentials channel activity was enhanced and more channels were activated. In this experiment at least three channels were active when the holding potential was  $+80$  mV. The amplitude of single channel openings at different holding potentials was determined and plotted against the holding potential (Fig. 2B). The data were fitted by linear regression and the single channel conductance was determined as the slope of the regression line. In this case the single channel conductance was  $178$  pS.

The voltage dependence of the MaxiK channels was

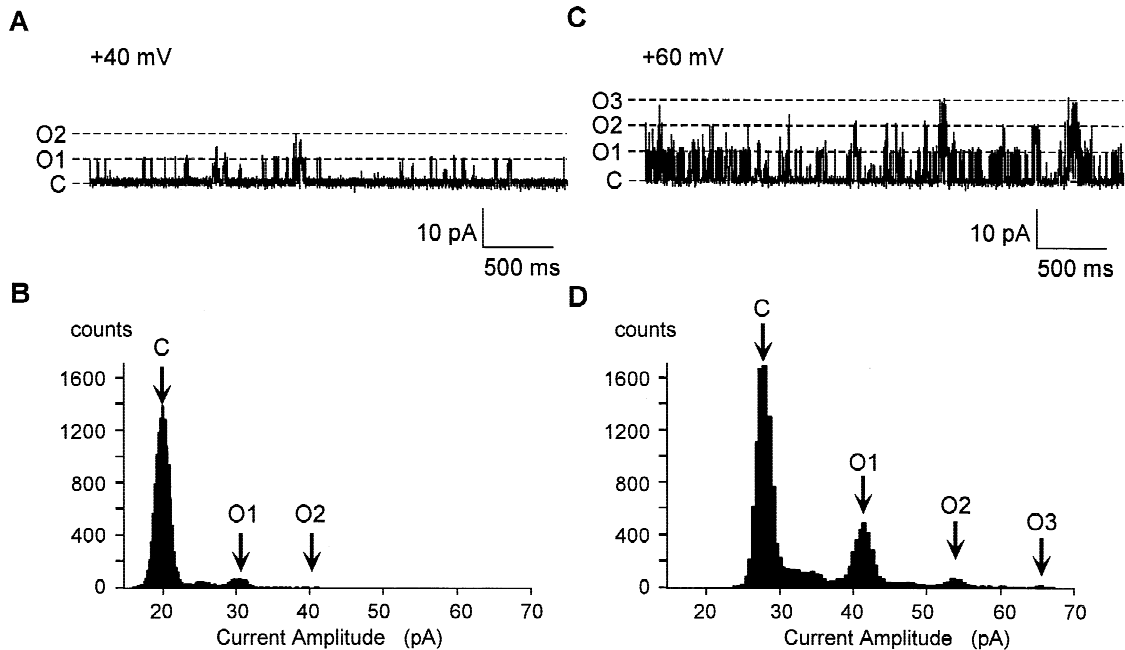
also shown by calculating current amplitude histograms of single channel recordings at different voltages. Figure 3 shows original current traces at  $+40$  mV (Fig. 3A) and  $+60$  mV (Fig. 3C) and the corresponding current amplitude histograms of a single channel recording in the cell-attached configuration at  $+40$  and  $+60$  mV (Fig. 3B and D, respectively). The letter C in the graph marks the baseline current and thus the closed level. O1, O2, O3 mark the current amplitude when one, two or three channels are open simultaneously. There are at least three active MaxiK channels in this patch. It is obvious that there are more counts at the open levels at  $+60$  than at  $+40$  mV. The probability of channel openings increased at positive potentials.

#### EFFECT OF NS1608 ON THE WHOLE-CELL CURRENT USING VOLTAGE STEPS

To initially characterize the effect of NS1608 on MaxiK channels in rat urinary bladder smooth muscle cells, whole-cell currents were elicited by applying a family of depolarizing voltage steps from the holding potential to potentials from  $-100$  to  $+120$  mV in  $20$  mV increments. The free internal  $\text{Ca}^{2+}$  concentration was  $50$  nM. Figure 4A and B shows the original current traces before (A) and after application of  $10 \mu\text{M}$  NS1608 (B). Under control conditions, in the absence of NS1608, hardly any current can be elicited at potentials more negative than  $0$  mV. This can be visualized in Fig. 4C, where the maximal peak current from A was plotted against the absolute membrane potential. Only at potentials more positive than  $50$  mV a significant current could be activated. After application of NS1608 less positive potentials were needed for activation of the current as can be seen from Fig. 4B where the original current traces are shown after the application of NS1608. It is obvious that the current amplitudes under these conditions were larger compared with the control. In addition, current through



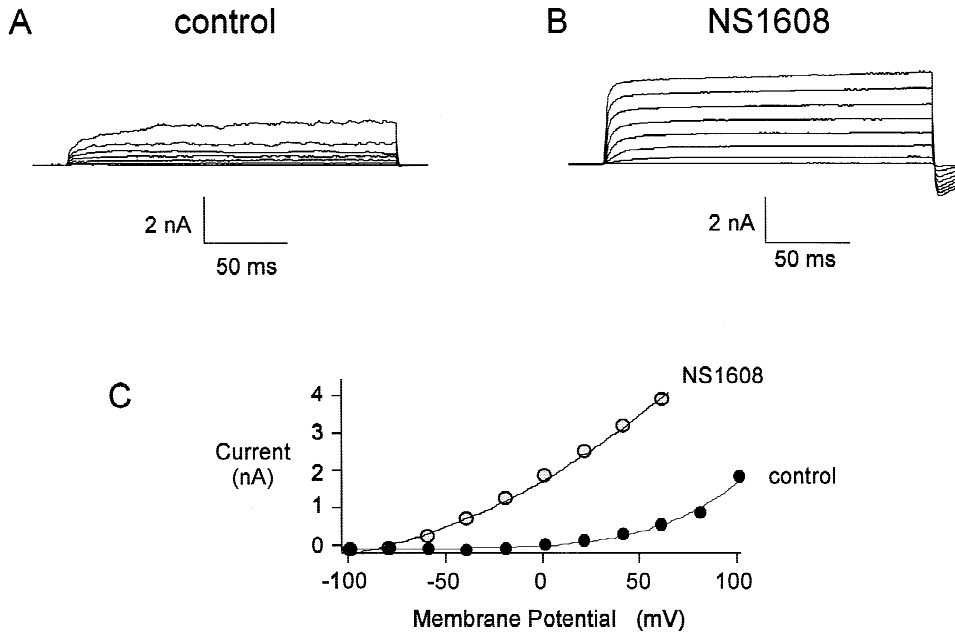
**Fig. 2.** Current through single MaxiK channels. (A) Original current traces from a recording in the cell-attached configuration at different potentials. The pipette solution contained 165 mM  $\text{K}^+$ -aspartate and 2 mM  $\text{Ca}^{2+}$ . No activity could be observed at negative potentials. At +30 mV only few openings of the channel can be seen. With further increase of the holding potential more channels are activated. Extracellular solution was normal mammalian  $\text{Na}^+$ Ringer. (B) Current-voltage relationship for a single channel. Single channel amplitudes (from A) were plotted against the applied membrane potential. The line through the data points is a fit by linear regression and the single channel conductance was determined as the slope points of the regression line. In this case the single channel conductance was 178 pS.



**Fig. 3.** Voltage dependence of current amplitude histograms of single channel recordings at different voltages. The histograms were determined from a single channel recording in the cell-attached configuration. A and C show original current traces at +40 and +60 mV, respectively. B and D show the corresponding amplitude histograms from A and C. The letter C in the graph marks the baseline current and thus the closed level. O1, O2, O3 mark the current amplitude when one, two or three channels are open simultaneously. There are at least three active MaxiK channels in this patch.

MaxiK channels were activated at more negative potentials compared to control as can be seen in Fig. 4C. To quantify the change in voltage-dependence of activation of MaxiK channels under NS1608, the  $I/V$  curves of the

data in A and B were fitted in PulseFit (see Materials and Methods) and we obtained voltages for half-maximal activation of the current ( $E_{1/2}$ ) of +110 mV and  $-2.7$  mV before and after application of NS1608, respectively.



**Fig. 4.** Effect of extracellularly applied NS1608 on the whole-cell current of a single smooth muscle cell from rat urinary bladder. A family of depolarizing voltage steps from the holding potential to potentials from  $-100$  to  $+120$  mV in  $20$  mV increments was applied to the cells. The free internal  $\text{Ca}^{2+}$  concentration was  $50$  nM. Original current traces before (A) and after application of  $10$   $\mu\text{M}$  NS1608 (B). (C) Maximum peak currents from A and B plotted against the test potential. The lines through the data points are fits of a modified Boltzmann equation to the data from A and B and yielded voltages for half-maximal activation of the current  $E_{1/2}$  of  $+141$  mV and  $-2.7$  mV before and after application of NS1608, respectively. The fit also yielded a reversal potential of the current of  $-88$  mV, close to the calculated reversal potential for a  $\text{K}^{+}$ -selective pore.

Therefore activation of the current through MaxiK channels was shifted by  $\sim 100$  mV towards more negative potentials by the application of NS1608.

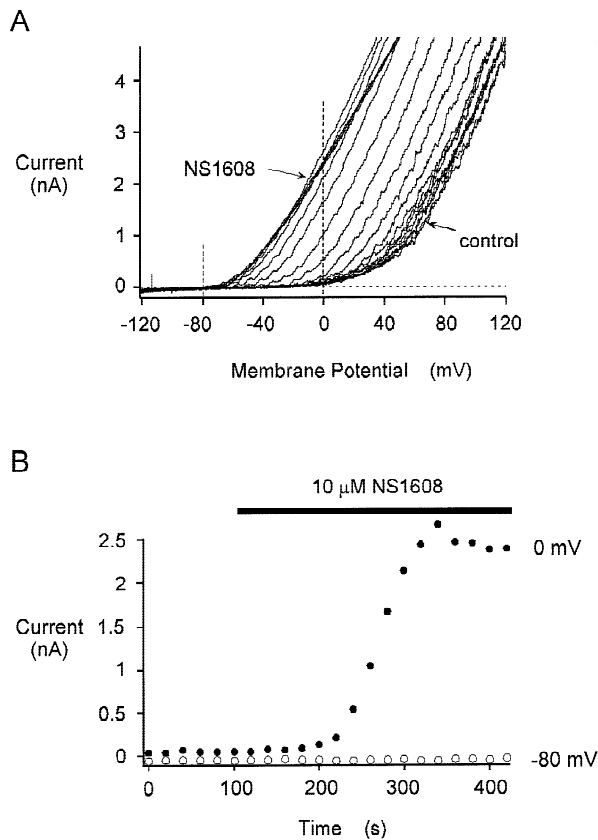
#### EFFECT OF NS1608 ON THE WHOLE CELL CURRENT USING VOLTAGE RAMPS

To visualize the time course of the NS1608 action, we performed experiments by continuously changing the membrane potential from  $-120$  to  $+120$  mV within  $600$  msec every  $20$  sec. The free internal  $\text{Ca}^{2+}$ -concentration was  $500$  nM. Figure 5 shows such an experiment. It shows in A original ramp currents before and during application of  $10$   $\mu\text{M}$  NS1608. Before the application of NS1608 the ramp current was flat at potentials below  $0$  mV and started to increase at potential more positive than  $0$  mV. Application of  $10$   $\mu\text{M}$  NS1608 resulted in an apparent shift of the current traces towards more negative voltages without changing the overall time course of the ramp current indicating that NS1608 activated the current through a shift in the activation potential towards more negative potentials. Comparing the ramp current traces before and after complete activation through NS1608, assuming that the difference in the ramp current was only due to a shift in the activation voltage, this shift amounted to  $\sim 100$  mV, similar to the shift observed with voltage steps (Fig. 4).

The time course of the NS1608 action can be seen in Fig. 5B. This figure shows the amplitude of the ramp currents shown in A at  $-80$  mV (open symbols) and at  $0$  mV (closed symbols). Within  $100$  sec after the application of the NS1608 the current at  $0$  mV started to increase to reach a plateau value of  $\sim 2$  nA within another  $100$  sec. Current at  $-80$  mV did not change during the application indicating that NS1608 did not induce any unspecific changes in leak current.

#### EFFECT OF MAXIK CHANNEL BLOCKERS ON THE NS1608 INDUCED WHOLE-CELL CURRENT

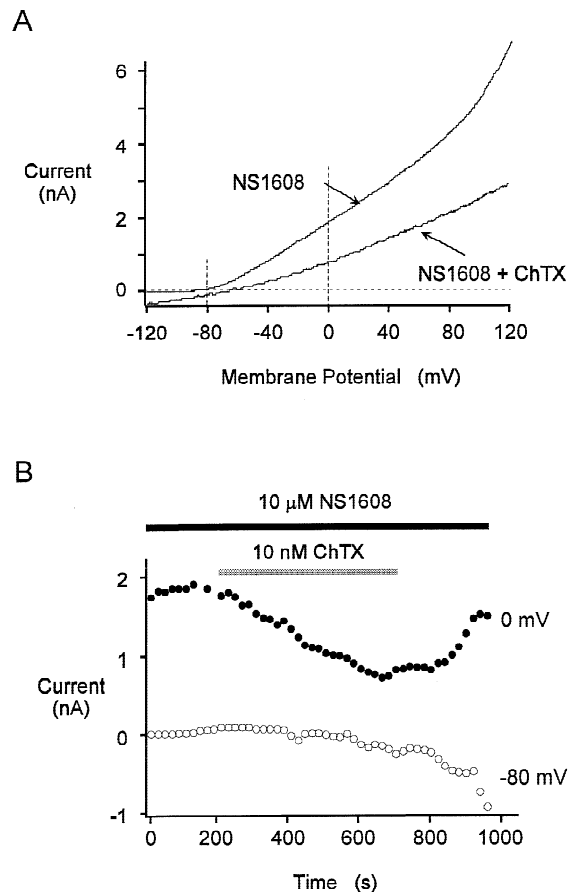
To substantiate that the induced current was flowing through MaxiK channels we pharmacologically characterized the NS1608 induced current. Charybdotoxin (ChTX) as well as paxilline are well known blockers of MaxiK channels in other preparations (Miller et al., 1985; Knaus et al., 1994). Therefore, we tried these two blockers on the NS1608 induced current. Figure 6 shows the effect of charybdotoxin on the NS1608 induced current in rat urinary bladder smooth muscle cells using voltage ramps. The free internal  $\text{Ca}^{2+}$  concentration was  $500$  nM. Figure 6A shows original current traces after full activation of the current by application of  $10$   $\mu\text{M}$  NS1608 and after additional application of  $10$  nM charybdotoxin. Charybdotoxin reduced the slope of the



**Fig. 5.** The Maxi K current in smooth muscle cells from rat urinary bladder is activated by application of NS1608 ( $10\ \mu\text{M}$ ). The membrane potential was continuously changed from  $-120$  to  $+120$  mV within 600 msec. The time interval between two voltage ramps was 20 sec, the holding potential was  $-80$  mV and the free internal  $\text{Ca}^{2+}$  concentration was 500 nM. (A) Original ramps currents before and during application of  $10\ \mu\text{M}$  NS1608. (B) Time course of the NS1608 action. The amplitude of the whole-cell current in the ramps was measured at  $-80$  mV (open symbols) and at 0 mV (closed symbols) and plotted against the absolute time during the experiment.

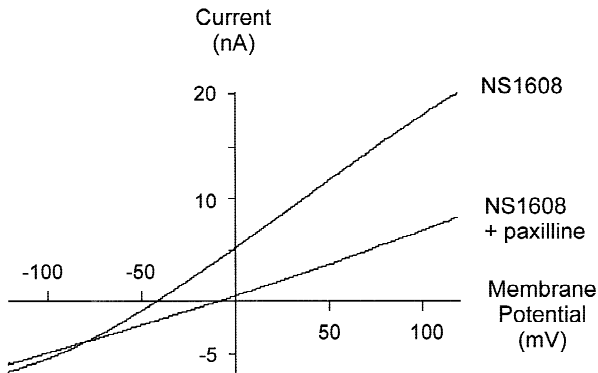
ramp current, a measure of the activation of the MaxiK channels, to  $\sim 50\%$  of the slope obtained after  $10\ \mu\text{M}$  NS1608 application alone. The time course of the charybdotoxin action on the NS1608 induced current is shown in Fig. 6B. This figure shows, similar to Fig. 5B, the current amplitudes of the ramp currents at  $-80$  mV (open symbols) and at 0 mV (closed symbols) plotted against the absolute time during the experiment. One can see that charybdotoxin reduced the current at 0 mV without affecting current at  $-80$  mV suggesting that charybdotoxin blocked current through the NS1608 activated MaxiK channels.

Although charybdotoxin was first described as a specific blocker for MaxiK channels (Miller et al., 1985) it became soon apparent that charybdotoxin also blocks current through other  $\text{K}^+$  channels, i.e., “pure” voltage-gated channels (Grissmer et al., 1994) as well as “pure”



**Fig. 6.** Effect of charybdotoxin (ChTX) on the whole-cell current of a smooth muscle cell from rat urinary bladder induced by NS1608. The membrane potential was continuously changed from  $-120$  to  $+120$  mV within 600 msec. The time interval between two voltage ramps was 20 sec, the holding potential was  $-80$  mV and the free internal  $\text{Ca}^{2+}$  concentration was 500 nM. (A) Original ramp currents after application of  $10\ \mu\text{M}$  NS1608 in the absence and presence of 10 nM charybdotoxin. (B) Time course of the charybdotoxin action. The amplitude of the whole-cell current in the ramps was measured at 0 mV and plotted against the absolute time during the experiment.

$\text{Ca}^{2+}$ -activated (Grissmer et al., 1993; Ishii et al., 1997; Jäger & Grissmer, 1997; Joiner et al., 1997; Logsdon et al., 1997). A more specific blocker is paxilline (Knaus et al., 1994). Therefore to test our hypothesis that NS1608 activated current through MaxiK channels in rat urinary bladder smooth muscle cells we tried to modulate the NS1608 induced current by application of paxilline. The result of such an experiment is illustrated in Fig. 7. Again, Fig. 7 shows ramp current traces after full induction of the current by application of  $10\ \mu\text{M}$  NS1608 and in the presence of  $1\ \mu\text{M}$  paxilline. Application of paxilline clearly reduced the NS1608 induced current indicating that MaxiK channels in rat urinary bladder smooth muscle cells were opened by the application of NS1608.



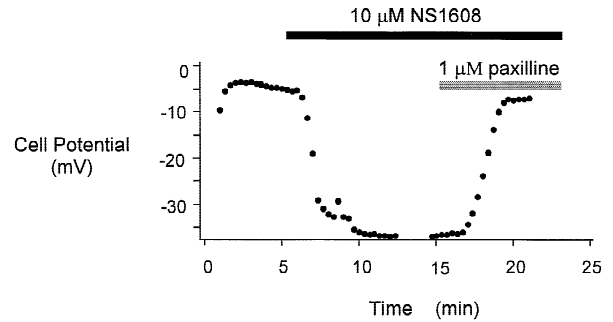
**Fig. 7.** Effect of paxilline on the whole-cell current of a smooth muscle cell from rat urinary bladder induced by NS1608. The membrane potential was continuously changed from  $-120$  to  $+120$  mV within 600 msec. The time interval between two voltage ramps was 20 sec, the holding potential was  $-80$  mV and the free internal  $\text{Ca}^{2+}$ -concentration was  $50$  nM. Original ramp currents after full activation of the MaxiK channels by application of  $10 \mu\text{M}$  NS1608 in the absence and presence of  $1 \mu\text{M}$  paxilline.

#### EFFECT OF NS1608 ON THE CELL'S MEMBRANE POTENTIAL

To find out whether the opening of MaxiK channels by NS1608, as shown above, can be used under physiological conditions to relax the smooth muscle cells, the application of NS1608 should influence the membrane potential of the investigated cells. Therefore we measured the cell potential directly using the current-clamp mode of the patch-clamp amplifier. The current was clamped to zero and the cell potential was monitored every 20 sec. An example of such an experiment is shown in Fig. 8. With a free internal  $\text{Ca}^{2+}$  concentration of  $50$  nM (applied through the patch pipette) the cell's membrane potential was  $\sim 5$  mV. This value seemed very depolarized and was probably due to the isolation procedure. Application of  $10 \mu\text{M}$  NS1608 resulted in an increase in the membrane potential towards  $-35$  mV. This effect could be reversed by the application of  $1 \mu\text{M}$  paxilline. In additional experiments we obtained values for the membrane potential of urinary bladder smooth muscle cells before the application of NS1608 of  $-9 \pm 4$  mV (mean  $\pm$  SD;  $n = 15$ ). After application of  $10 \mu\text{M}$  NS1608 the cells were hyperpolarized to  $-33 \pm 8$  mV (mean  $\pm$  SD;  $n = 11$ ). These results indicated that MaxiK channels in bladder smooth muscle cells exist, that they can be opened by NS1608 and that this opening results in a hyperpolarization of the cells.

#### Discussion

To find out whether MaxiK channels were expressed in urinary bladder detrusor smooth muscle cells and wheth-



**Fig. 8.** Effect of NS1608 on the cell potential of a smooth muscle cell from rat urinary bladder. The cell potential was recorded in the current clamp mode of the patch-clamp amplifier. The current was clamped to zero and the potential was determined every 20 sec before and during application of  $10 \mu\text{M}$  NS1608 in the absence and presence of  $1 \mu\text{M}$  paxilline. The free internal  $\text{Ca}^{2+}$  concentration was  $50$  nM.

er opening of these channels would result in a hyperpolarization of the cells, we isolated single cells of the detrusor and characterized electrophysiologically and pharmacologically membrane currents in the whole-cell configuration of the patch-clamp technique. We observed voltage-dependent MaxiK current in these cells and the voltage-dependence of this current was shifted toward more hyperpolarized potentials by the application of a known  $\text{K}^+$  channel opener, NS1608 (Strøbæk et al., 1996). In addition, using current-clamp measurements we could demonstrate that the opening of these MaxiK channels resulted in a hyperpolarization of the membrane potential in these cells.

#### COMPARISON WITH OTHER MAXIK CHANNELS

MaxiK channels have been implicated to play an important role for the relaxation of blood vessels (Nelson et al., 1995) as well as airways (Kume et al., 1989; Jones et al., 1990). A recent study (Strøbæk et al., 1996) took advantage of the progress in molecular biology and conducted electrophysiological experiments on cloned human MaxiK channels, *hsl* $\alpha$ . They expressed the  $\alpha$ -subunit of *hsl* $\alpha$  in a mammalian cell line, human embryonic kidney cells (HEK), and characterized the current recorded in these cells in respect to the modulation of the channels by  $[\text{Ca}^{2+}]_i$ , membrane potential, and substances that either block or presumably open MaxiK channels (Strøbæk et al., 1996). They identified a current that was very sensitive to changes in  $[\text{Ca}^{2+}]_i$ , although  $\text{Ca}^{2+}$ -independent openings were seen at  $[\text{Ca}^{2+}]_i$  of  $15$  nM. Application of NS1608 shifted the voltage dependence of activation towards more negative potentials and the NS1608-induced current was sensitive to block by paxilline. They concluded that MaxiK channels were modulated by a  $\text{Ca}^{2+}$ -independent interaction with the  $\alpha$ -subunit of the channel. Our observations are in total agree-

ment with their reported results indicating that in bladder smooth muscle cells MaxiK channels are expressed with electrophysiological and pharmacological properties similar to those channels encoded by *hsl**o*. In addition, these observations on MaxiK channel properties including the action of NS1608 are also in agreement with experiments done on cells from guinea pig urinary bladder (Hu & Kim, 1996).

#### MEMBRANE POTENTIAL MEASUREMENTS

Our current-clamp measurements of the membrane potential revealed that the cells had a pretty depolarized membrane potential around 0 mV. One question arises on why the cells are so depolarized. Could this be a preparation artifact? This seems unlikely since Klöckner and Isenberg (1985) using a similar cell isolation procedure on urinary bladder smooth muscle cells from guinea pigs showed a resting membrane potential after cell isolation of  $\sim -50$  mV. This suggested to us the existence of another current component that would determine the membrane potential under resting conditions. This idea was supported by the observation that application of NS1608 resulted in a hyperpolarization of the cell's membrane potential of  $\sim 35$  mV. This value is somewhat smaller than reported by Strøbæk et al. (1996). Two reasons might account for this discrepancy. The first being the current component that determines the membrane potential under resting conditions might be larger in rat urinary bladder smooth muscle cells after the isolation procedure compared to HEK cells and second, the expression of MaxiK channels through the transfection procedure in HEK cells might result in more functional channels in the HEK cell's membrane compared to the smooth muscle cell. This hypothesis is also supported by the much larger current amplitude through MaxiK channels that can be elicited in HEK cells compared to our current measurements.

Our results also suggested that the hyperpolarization observed by the application of NS1608 was solely due to the opening of MaxiK channels since the effect could be totally antagonized by the application of paxilline. This observation excluded the possibility that the effect of NS1608 could be the result of a blockade on  $\text{Cl}^-$  or nonspecific currents or L-type  $\text{Ca}^{2+}$  channels as shown for NS1619 by Holland et al. (1996). Those currents could have dominated the membrane potential under resting conditions and the block of those conductances could have hyperpolarized the cells due to the fact that now, under these conditions, another  $\text{K}^+$  conductance could dominate the membrane potential. In that specific case we would have expected that the effects of NS1608 were not being antagonized by the application of paxilline.

#### MODE OF ACTION

The effect of NS1608 on MaxiK channels seems to be a shift of the voltage dependence of channel opening towards more negative potentials similar to what has been described under physiological conditions for an increase in  $[\text{Ca}^{2+}]_i$ , the interaction of a  $\beta$ -subunit with the  $\alpha$ -subunit or both (Meera et al., 1996, 1997; Wallner et al., 1996; Cox et al., 1997; Cui, Cox, & Aldrich, 1997). From the similarity of the  $\alpha$ -subunits of MaxiK channels with those of "pure" voltage-gated Kv channels it was concluded that the intrinsic voltage dependence of MaxiK channels presumably originates from the S4 segment of the  $\alpha$ -subunit and that either  $[\text{Ca}^{2+}]_i$  or the  $\beta$ -subunit or both can influence this voltage-dependence. The effect of  $[\text{Ca}^{2+}]_i$  on this voltage dependence alone seems to be  $\text{Ca}^{2+}$  binding to the C-terminal cytoplasmic domain of the  $\alpha$ -subunit leading to conformational changes that would allow the channel to get activated by less membrane depolarization. This scenario could be amplified even further by the interaction of the  $\beta$ -subunit with the extracellular N-terminus and the S0 segment of the MaxiK's  $\alpha$ -subunit. Therefore several possible site of actions could explain the effect of NS1608 on MaxiK channels (i) modulating the  $\text{Ca}^{2+}$  binding site at the C-terminal end of the  $\alpha$ -subunit, (ii) strengthen the interaction between  $\beta$ - and  $\alpha$ -subunit and (iii) mimicking the interaction of  $\beta$ - to the  $\alpha$ -subunit. From the high lipophilicity of NS1608 it seems unlikely that the action would be on the cytoplasmic C-terminal end of the  $\alpha$ -subunit since that part of the channel protein seems to be water exposed. In addition, the action of NS1608 seemed independent of  $[\text{Ca}^{2+}]_i$  as demonstrated by Strøbæk et al. (1996) using *hsl**o*. It also seems unlikely that NS1608 could strengthen the interaction between the  $\alpha$ - and the  $\beta$ -subunit since the action of NS1608 was also observed in the apparent absence of a  $\beta$ -subunit (Strøbæk et al., 1996). Therefore we favor the idea that NS1608 mimics a  $\beta$ -subunit and is probably interacting with the S0 segment of the  $\alpha$ -subunit. This is also consistent with earlier hypotheses that the carboxylic ring with the trifluoromethyl group of the NS1608 molecule lies within the membrane on the site of action (Hu & Kim, 1996). Experiments using site-directed mutagenesis in the S0 segment of the cloned *hsl**o* might be able to identify amino acids in the channel interacting with the drug.

#### Conclusion

MaxiK channels are expressed in a variety of tissues, exist in several alternative splice variants, and may or may not associate with a  $\beta$ -subunit. Through those combinations of splice variants with or without the  $\beta$ -subunit, specific MaxiK channels with different physiological



functions and different pharmacology may exist in each tissue as suggested by the influence of the  $\beta$ -subunit to the binding of charybdotoxin to the channel (Garcia et al., 1997; Hanner et al., 1997). In bladder smooth muscle cells we have demonstrated the presence of functional MaxiK channels that may associate with  $\beta$ -subunits to form functional  $K^+$  channels. These channels could be opened by the application of NS1608 and this opening resulted in a hyperpolarization of the cells. This hyperpolarization in turn could directly relax bladder smooth muscle by reducing  $Ca^{2+}$  influx through voltage-gated  $Ca^{2+}$  channels. These findings confirm and extend the results of a variety of investigators (Hu & Kim, 1996; Holland et al., 1996; Heppner et al., 1997) and pave the way for the use of specific MaxiK channel openers being attractive and probably tissue specific targets for therapeutic interventions for the treatment of specific forms of incontinence.

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