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Nitric oxide activates glibenclamide-sensitive K^+ channels in urinary bladder myocytes through a c-GMP-dependent mechanism

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Abstract

In the present investigation, we used standard patch clamp techniques to test whether nitric oxide (NO) generation has any role to play with either activation or inhibition of ATP-sensitive (K_{ATP}) channels in guinea-pig urinary bladder. We found that NO generation leads to activation of K_{ATP} channels through a cyclic guanosine monophosphate (c-GMP)-dependent protein kinase. 3-Morpholinosydnonimine (SIN, 100 μM) potentiated activation of an inward current in whole cell patch clamp experiments. Glibenclamide (10 μM) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM) inhibited the SIN-activated current. Both in cell-attached and in inside out patches, SIN (200 μM) potentiated K_{ATP} channel activity, and the increased channel activity in inside out patches was suppressed by glibenclamide (50 μM), ATP (1 mM) and (9*s*,10*R*,12*R*)-2,3,9,10,11,12-Hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12,-epoxy-1*H*-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo[3,4-i][1,6] benzodiazocine-10-carboxylic acid, methyl ester (KT-5823, 10 nM). 8-Br-cGMP (100 μM) increased the K_{ATP} channel activity in cell-attached patches, and this was suppressed by glibenclamide (50 μM). These results suggest that the NO-c-GMP-PKG pathway contributes to activation of K_{ATP} channels in guinea-pig urinary bladder myocytes.

Keywords: Nitric oxide; K_{ATP}; c-GMP; PKG; Urinary bladder

1. Introduction

Over the last decade, nitric oxide (NO) has increasingly gained recognition as an important cell mediator with a broad range of functions in the lower urinary tract. Before the discovery of nitrergic transmission, non-adrenergic non-cholinergic (NANC)-nerve mediated relaxation was reported in the female rabbit (Andersson et al., 1983), porcine urethra (Klarskov et al., 1983) and human trigone (Speakman et al., 1998), and later shown to be mediated by NO (Bridgewater et al., 1993; Persson et al., 1993). NANC-mediated relaxation of the bladder neck and urethral smooth muscle is associated with increased c-GMP levels (Dokita et al., 1994).

Unlike bladder neck and urethra, there is as yet no convincing evidence for nerve-mediated relaxation of the detrusor muscle, or the involvement of NO as an inhibitory transmitter, and the effects of applied NO are variable. In the rat, photo-activated release of NO from a donor can relax the detrusor (Chung et al., 1996), whereas in the guinea pig, NO donors cause contraction (Moon, 2000), and in the mouse NO has been reported to function as an excitatory neuro-modulator (Fujiwara et al., 2000). Exogenous NO as well as endogenous NO produced by the smooth muscle cells have been reported to elicit detrusor smooth muscle relaxation in human (James et al., 1993) and more recently NO donors have been shown to evoke a complex response in pre-contracted human detrusor, i.e. relaxation, contraction or a transient relaxation followed by contraction (Moon, 2002). In contrast, K^+ channels play an important role in regulating membrane potential and cellular excitability in urinary tract smooth muscle cells (reviewed by Brading, 1992; Andersson, 1993). Cromakalim and pinacidil have been shown to hyperpolarize and relax guinea pig urinary bladder smooth muscle cells and these effects were prevented by glibenclamide, which suggests the existence of ATP-sensitive K^+ (K_{ATP}) channels in the urinary bladder (Foster et al., 1989; Fuji et al., 1990; Bonev and Nelson, 1993). Based on their findings, Bonev and Nelson have

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proposed that bladder smooth muscle function is influenced by modulation of K_{ATP} channels. NO has been reported to modulate the activation of ion channels in many tissues, including K_{ATP} channels. In the heart, for instance, NO activates K_{ATP} in both a c-GMP-dependent (Baker et al., 2001) and -independent mechanism (Shinbo and Iijima, 1997). Despite the fact that NO is involved in many tissues in regulating membrane potential, there is no available report as to the modulatory effects of NO on ionic currents in regulating membrane potential in urinary bladder. In the present investigation, therefore, we have studied whether there is any interaction between NO and K_{ATP} channel currents in guinea pig urinary bladder myocytes using patch clamp techniques. We propose that a NO/c-GMP/PKG-signalling pathway is involved in activation of K_{ATP} channels in guinea-pig urinary bladder.

2. Methods

Adult male guinea pigs weighing 250–300 g were killed by cervical dislocation in accordance with UK home office regulations and with *the Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The urinary bladder was removed into ice-cold physiological saline solution, the urothelium was removed and the smooth muscle cut into small pieces (about 1–2 mm cube) under a dissecting microscope. These tissue pieces were then incubated at 37 °C for 20 min in a Ca^{2+}-free dissolution solution of the following composition (mM): Na glutamate 80, NaCl 55, KCl 5, $MgCl_2$ 1.2, glucose 10, HEPES 10 (pH 7.4 with Tris base) complemented with 1 mg/ml of collagenase type 1 (Calbiochem), 0.2 mg/ml of pronase and 1 mg/ml of fatty acid free albumin (bovine serum albumin—BSA). Subsequently, digested tissue pieces were washed with Ca^{2+}-free solution and single smooth muscle cells were yielded by gentle agitation of the tissue pieces through a fire polished Pasteur pipette. Single cells thus obtained were stored in low Ca^{2+} (0.5 mM) solution with 0.5 % BSA at 4 °C for use within 3–4 h.

Normal bathing solution had the following composition (mM): NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1.2, glucose 10, HEPES 10 and the pH was adjusted to 7.4 with Tris base. Ca^{2+}-free high K^+ (140 mM) solution was made by replacing NaCl with equimolar concentration of KCl and omitting Ca^{2+} from the solution. Pipette solution for whole cell current recording contained (mM): KCl 140, $MgCl_2$ 1.2, uridine diphosphate (UDP) Na salt 1, adenosine triphosphate (ATP) Mg salt 0.1, HEPES 10, EGTA(ethyleneglycol-bis-(β-amino-ethyl-ether) *N-N′*-tetra-acetic-acid) 5 and the pH was adjusted with Tris base. For single channel recording, the pipette solution and bathing solution were the same and contained (mM) 140 KCl, 1.2 $MgCl_2$, glucose 10, HEPES 10.

Standard patch clamp techniques (Hamil et al., 1981) were used to record either whole cell membrane currents or single channel currents in cell attached and excised inside out patches. Membrane currents were recorded by use of a patch clamp amplifier (EPC-7, List, Germany) and operated through a Macintosh computer equipped with an AD/DA converter (ITC-16, INSTRUTECH, USA). Data were stored in digitized format on digital audiotapes with the use of a Sony DAT recorder for off-line analysis. Patch pipettes had a resistance of 3–5 MΩ for whole cell and 6–9 MΩ for single channel current recording. Both whole cell and single channel currents were filtered at 4 kHz. Single channel currents were digitized at 10 kHz. Channel open state probability (P_o) was determined from the following relation.

$$P_o = \sum_{J=1}^{N} t_j j/(TN)$$

Where t_j is the time spent at each current level corresponding to $j=1,2,—N$, T is the duration of the recording (usually 30 s) and N is the number of channels active in the patch.

SIN-chloride, ODQ and KT-5823 were obtained from Tocris and all other chemicals were procured from Sigma. Values in the text are shown as mean ± S.E.M. (n = sample size). The statistical significance of difference between values was assessed using Student's unpaired t-test with $P<0.05$, taken to indicate significance.

3. Results

3.1. Whole cell current recording

Guinea pig urinary bladder myocytes were held at − 50 mV. Three consecutive ramp depolarising pulses each at 15 s interval were applied from − 100 to + 100 mV over a period of 1 s. The pipette solution contained 1 mM UDP and 0.1 mM ATP in order to stimulate glibenclamide-sensitive channels and to prevent their rundown. In normal PSS (5 mM K^+), the current amplitude at − 100 mV was − 38 ± 2.7 pA ($n=6$). The cells were then superfused in Ca^{+}-free high K^+ (140 ± 2 mM TEA) PSS in order to shift the reversal potential to 0 mV and to suppress both the Ca^{2+} current and Ca^{2+}-activated K^+ current. In this high K^+ PSS, there was activation of an inward current of − 183 ± 28.9 pA (range − 122 to − 259 pA, $n=6$), which was inhibited in the presence of 10 μM glibenclamide (Fig. 1A). Therefore, this current was mediated by glibenclamide-sensitive K^+ channels. Following wash K_{ATP} completely recovered from glibenclamide block.

Fig. 1B shows typical currents elicited by voltage-ramps in a cell in K^+-high PSS and in 100 μM SIN in the absence and presence of 10 μM glibenclamide. SIN (100 μM) elicited a further activation of the inward current. The amplitude of

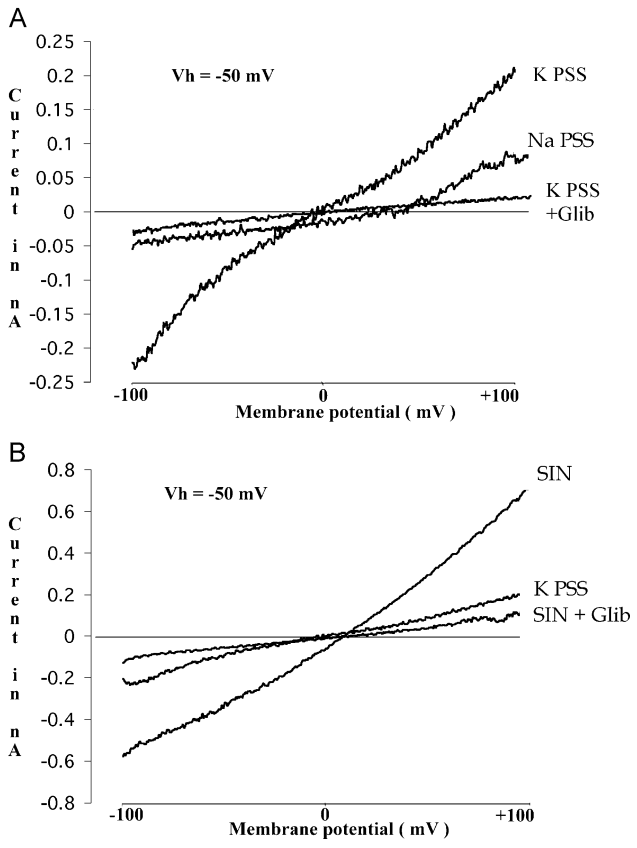

Fig. 1. Conventional whole cell recording of glibenclamide-sensitive K_{ATP} current. K^+ in the bath solution was either 5 mM or 140 mM (0 Ca^{2+} and 2 mM TEA) and in the pipette was 140 mM (with 1 mM UDP and 0.1 mM ATP). Voltage ramps of 1 s duration was applied from − 100 to 100 mV at a holding potential of − 50 mV. (A) shows current ramps in a cell bathed with 5 mM K^+ (Na PSS) and 140 mM K^+ in the absence (K PSS) and presence of 10 μM of glibenclamide (glib). As can be noted, the inward current measured at a ramp potential of − 100 mV was activated under symmetrical K^+ condition, which was inhibited by glibenclamide. (B) shows SIN (100 μM) further activated this inward current under symmetrical K^+ condition and this current inhibited by glibenclamide (10 μM).

the current activated by SIN was − 550.17 ± 21.2 pA (range − 403 to − 672 pA, $n = 12$). Addition of glibenclamide (10 μM) inhibited the SIN-activated current to − 113 ± 35.4 pA (range − 85 to − 117 pA, $n = 4$) and the inhibition was upto 79.57 ± 8.04% ($n = 4$).

To determine whether SIN-activated whole cell current in urinary bladder myocytes was mediated via a c-GMP-dependent mechanism, we used ODQ, a known guanylyl cyclase inhibitor. Fig. 2 shows the effect of ODQ (10 μM) on the SIN-activated current. The amplitude of the current was reduced to − 114.5 ± 16.71 pA ($n = 4$) when measured at − 100 mV and the inhibition was 80.9 ± 2.8%.

Fig. 3 illustrates the effect of SIN (100 μM) on K_{ATP} current under symmetrical K^+ conditions and the effects of glibenclamide (10 μM) and ODQ (10 μM). Current amplitude is expressed as current density: the cells had a mean capacitance of ~ 20 pF. Current amplitude was measured about 3 min after the cells were superfused with high K^+ (0

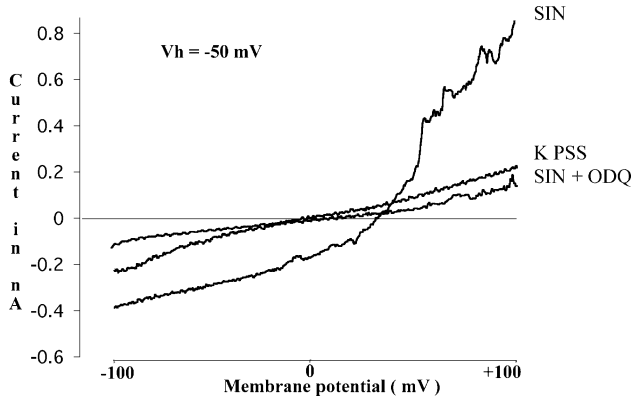

Fig. 2. Effect of ODQ (10 μM) on SIN (100 μM)-activated inward current in a cell under symmetrical K^+ condition in whole cell mode of current recording. Voltage ramps of 1 s duration from − 100 to 100 mV was applied under symmetrical K^+ (0 Ca^{2+} and 2 mM TEA in the bath) condition at a holding potential of − 50 mV. Pipette solution had 1 mM UDP and 0.1 mM ATP to stimulate K_{ATP} current. Inward current measured at − 100 mV of ramp potential. ODQ inhibited the SIN-activated current.

Ca^{2+}) bath solution containing 2 mM tetra-ethyl-ammonium (TEA).

3.2. Single channel recording

To study further the activation of K_{ATP} by NO, single channel recordings were performed in cell-attached and inside out patches at a holding potential of + 50 mV under symmetrical K^+ conditions with 5 mM EGTA in the bath and 2 mM TEA in the pipette. Under this condition in cell-

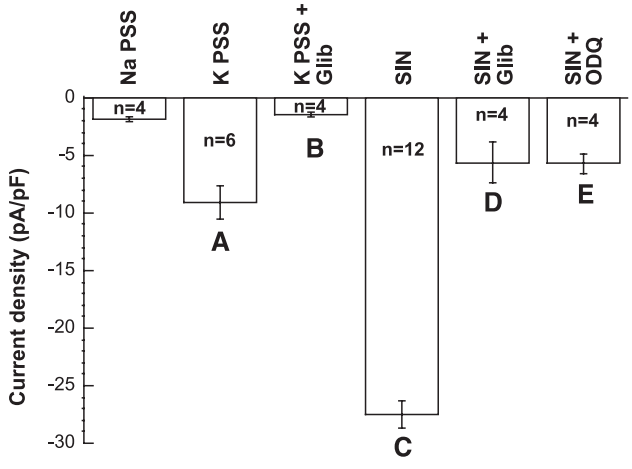

Fig. 3. Averages of peak current densities measured at a ramp potential of − 100 mV. Voltage ramps were applied first in presence of 5 mM K^+ (Na PSS) and then superfused in 140 mM K^+ (0 Ca^{2+} and 2 mM TEA). Pipette solution contained 1 mM UDP and 0.1 mM ATP. (A) Refers to significant ($P < 0.01$) potentiation of the inward current when compared to Na PSS. (B) Indicates significant ($P < 0.001$) inhibition of the inward current when compared to K PSS. (C) Represents significant ($P < 0.001$) potentiation when compared to K PSS. (D) Denotes significant ($P < 0.001$) when compared to SIN alone and (E) implies significant ($P < 0.001$) when compared to SIN alone.

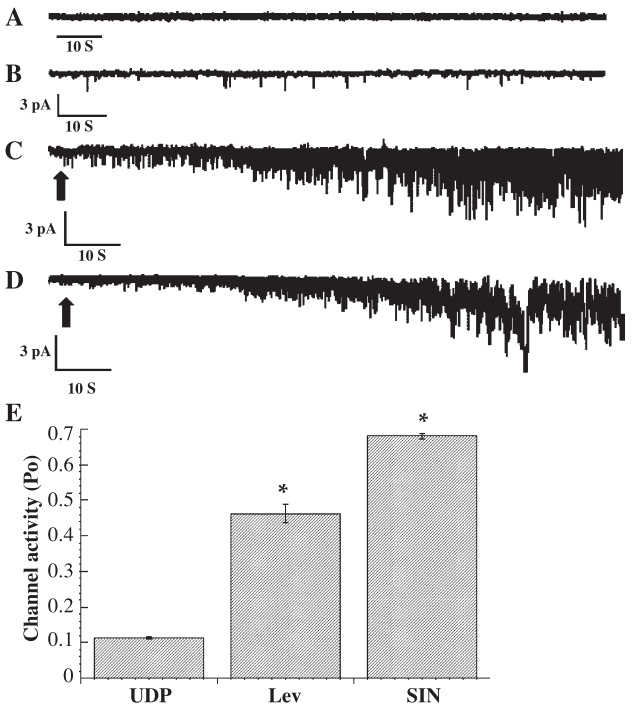

Fig. 4. Channel activity in excised membrane patch in inside out configuration under symmetrical K^+ condition (140 mM K^+ and 1.2 mM Mg^{2+}) with 5 mM EGTA in the bath and 2 mM TEA in the pipette and held at +50 mV. (A) Immediately after cell excision there was channel run down and no channel activity could be seen for 10 min. (B) Addition of 1 mM UDP to the bath reactivated the channel which can be noted as downward deflection. (C) 20 μM levcromakalim increased the channel activity in a patch after UDP reactivated the channel. (D) Increased channel activity seen in another patch added with 200 μM of SIN after UDP was added to reactivate channel after run down. The average P_o value in UDP ($n=4$), levcromakalim ($n=4$) and SIN ($n=12$) activated current have been summarised in Fig 3E. *Significantly ($P<0.001$) different when compared to UDP (i.e. Control).

attached patches, K_{ATP} had a slope conductance of ~50 pS. SIN (200 μM) when added alone induced channel activation in all the cell-attached patches in these conditions without the need of a K^+ channel opener.

In order to minimise the interference from cellular metabolic changes and regulatory enzymes, we also used inside out patches to study the effects of SIN on K_{ATP} channel activity. When an inside out patch was established no channel opening was observed for at least 10 minutes (Fig. 4A). Addition of 1 mM UDP in the bath solution containing 5 mM EGTA caused activation of the channels with occasional opening, which is evident as a downward deflection. 1 mM of UDP had earlier been shown to activate K_{ATP} channel after channel rundown in excised patches (Teramoto et al., 1997). The peak amplitude of this UDP-activated K^+ current was 3 pA (Fig. 4B). Addition of levcromakalim (20 μM) to the bath solution caused channel opening to increase. A similar experimental protocol was used with SIN (200 μM). SIN alone did not cause channel opening in inside out patch immediately after excision. However, SIN in a way similar to that of levcromakalim did increase the channel opening induced by UDP. Fig. 4C and D shows activation of current by levcromakalim and SIN in inside out patches in the presence of UDP. The mean P_o (Fig. 4E) of UDP, levcromakalim and SIN-activated current were 0.114 ± 0.003 ($n=4$), 0.463 ± 0.026 ($n=4$) and 0.681 ± 0.007 ($n=12$), respectively.

3.2.1. Effect of intracellular ATP and glibenclamide on SIN-activated current

To confirm that the observed increased channel activity was due to K^+ flowing through K_{ATP} channels, ATP (1 mM) was added to the inner surface of the patch. ATP suppressed channel activity when added to the bath solution in a reversible manner (Fig. 5A). The P_o was reduced by ~75% in presence of ATP (P_o in presence of SIN was 0.689 ± 0.024 ($n=4$) and after the addition of ATP was 0.17 ± 0.022 ($n=4$)).

Further, to study the glibenclamide sensitivity of this SIN-activated K^+ current, we applied 50 μM of glibenclamide during inside out patch recording under symmetrical K^+ conditions. The SIN-induced increased channel opening was suppressed by glibenclamide (Fig. 5B). However, unlike after ATP, channel activity did not completely recover upon washing out of glibenclamide. The P_o value was reduced by ~97% in presence of glibenclamide (P_o in

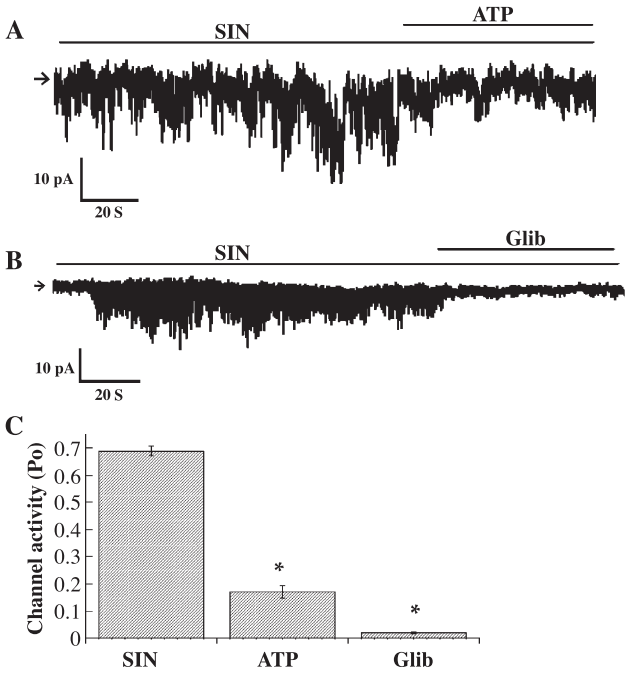

Fig. 5. (A) Effect of ATP (1 mM) on SIN-activated K_{ATP} in an inside out patch under symmetrical K^+ condition (140 mM K^+ and 1.2 mM Mg^{2+}) with 5 mM EGTA in the bath and 2 mM TEA in the pipette. Holding potential was +50 mV. ATP reversibly suppressed the SIN (200 μM)-activated current. (B) Effect of 50 μM of glibenclamide on SIN-activated current in another patch under identical condition. However, P_o did not completely recover upon wash out of glibenclamide up to 25 min. The average P_o value in presence of SIN, ATP and glibenclamide have been summarised in C. *Significantly ($P<0.001$) different when compared to SIN.

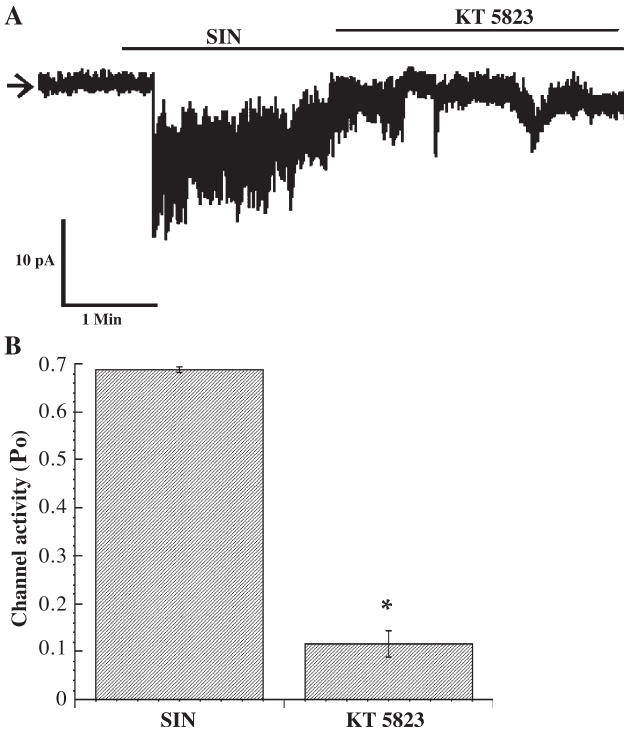

Fig. 6. (A) Effect of KT 5823 (10 nM) on SIN activated K_{ATP} current in an inside out patch under symmetrical K^+ condition (140 mM K^+ and 1.2 mM Mg^{2+}) with 5 mM EGTA in the bath and 2 mM TEA in the pipette and holding potential was +50 mV. KT 5823 reversibly suppressed the SIN-activated K^+ current. (B) Comparative P_o value in SIN and in presence of KT 5823. *Significantly different at $P<0.001$.

presence of SIN was 0.665 ± 0.014 ($n=4$) and after addition of glibenclamide was 0.02 ± 0.002 ($n=4$)).

3.2.2. Effect of KT 5823 and 8-Br-cGMP on SIN-activated current

To study whether SIN-activated increased channel activity was mediated via a c-GMP/PKG pathway in guinea pig urinary bladder myocytes, we applied KT-5823, a selective inhibitor of PKG to inside out patches (Fig. 6A,B). KT 5823 (10 nM) suppressed the potentiating effect of SIN on K_{ATP} channel activity in a reversible manner. Channel activity declined rapidly on application of KT 5823. The average P_o was 0.688 ± 0.005 ($n=4$) before and 0.117 ± 0.028 ($n=4$) during application of KT 5823 and the suppression was ~ 82%. In two of the patches, it was found that KT 5823 did not have any effect on levcromakalim (20 μM)-induced channel current.

To investigate more directly whether a c-GMP/PKG-dependent mechanism produces effects similar to those of the application of SIN, we examined the effects of 8-Br-cGMP, a potent membrane permeant PKG activator on the single channel activity of detrusor myocytes under symmetrical K^+ conditions in cell-attached patches. Interestingly, 8-Br-cGMP (100 μM) facilitated the activation of single channel activity in cell-attached patches with an average P_o of 0.395 ± 0.012 ($n=4$). Subsequent application of glibenclamide (50 μM) inhibited the channel activity ($P_o = 0.007 \pm 0.001$, $n=4$) (Fig. 7A,B).

4. Discussion

The results in the present study are the first to directly demonstrate through patch clamp studies that the NO donor SIN activates ATP-sensitive K^+ channels in guinea-pig urinary bladder smooth muscle cells. The activation of K_{ATP} in the guinea pig urinary bladder is mediated through a c-GMP/PKG dependent mechanism. These conclusions are based on the following observations:

(1) SIN potentiated the whole cell inward current in symmetrical K+ condition.
(2) This SIN-activated current was inhibited by glibenclamide.
(3) The potentiating effect was also suppressed by ODQ, a known guanylyl cyclase inhibitor.
(4) In single channel recording, SIN increased the open channel probability. The increased channel opening was suppressed by glibenclamide and ATP.
(5) KT-5823, a potent and selective PKG inhibitor reduced the ability of SIN to increase the open probability of KATP channels.
(6) 8-Br-cGMP mimicked the effect of SIN and this effect was suppressed by glibenclamide.

Our results are very similar to the situation in rabbit ventricular myocytes in which a NO-PKG signal transduction mechanism has been reported to activate K_{ATP} channels

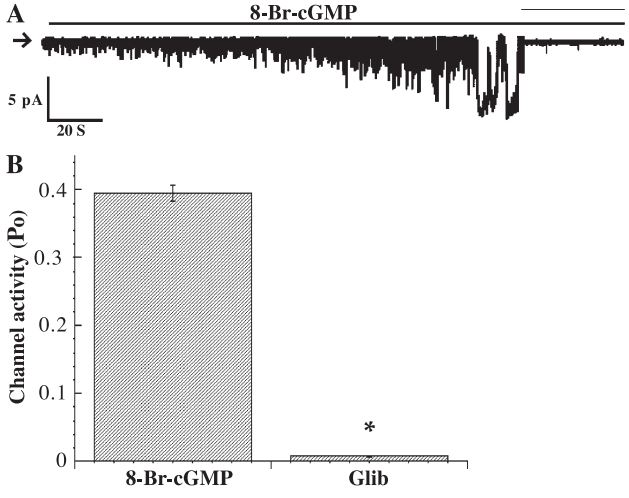

Fig. 7. A channel activity in a cell-attached patch under symmetrical K^+ condition (140 mM K^+ and 1.2 mM Mg^{2+}) with 5 mM EGTA in the bath and 2 mM TEA in the pipette and held at +50 mV. 8-Br-cGMP (100 μM) increased channel opening, which was suppressed by 50 μM of glibenclamide. *Significantly different at $P<0.001$. In this series of experiment also P_o did not completely recover upon wash out of glibenclamide up to 20-min period time.

(Han et al., 2002). In their study, Han et al., have shown that a PKG inhibitor suppressed the potentiating effect of SNP on channel activity in single channel recording. Further, 8-(4-chlorophenylthio)-c-GMP, a membrane permeable analog of c-GMP increased channel activity in cell-attached patches. One difference between the two studies is that in detrusor myocytes we found that the nitric oxide donor SIN on its own could activate K_{ATP} in cell-attached patches whereas in the ventricular myocytes previous activation of K_{ATP} by a K^+-channel opener was necessary to demonstrate the enhancing effects of NO donors. In both tissues the NO donors were ineffective on inside out patches unless K_{ATP} activity had been preserved. Nitric oxide donors have also been proposed to activate K_{ATP} channels in vascular smooth muscle through an increase in intracellular c-GMP (Kubo et al., 1994; Murphy and Brayden, 1995), and have been shown to activate several types of K^+ channel in colonic smooth muscle (Koh et al., 1995). These effects are all associated with inhibition of the smooth muscles and hyperpolarization.

In spite of the similarity with the work of Han et al., the ability of SIN to activate K_{ATP} in guinea-pig bladder smooth muscle is unexpected, since NO donors in this species have been reported to enhance spontaneous and evoked activity on strips of detrusor (Moon, 2000). Guinea pig bladder has been shown to hyperpolarize and relax in response to activation of K_{ATP} by cromakalim (Foster et al., 1989), and thus if the predominant effect of NO donors is to enhance K_{ATP}, an inhibitory response would be expected. In the mouse the enhancement of the nerve evoked activity is mediated by a c-GMP dependent mechanism, but in both mouse and guinea-pig bladder immunohistochemical localization of c-GMP after application of NO donors shows little if any increase in c-GMP-immuno-reactivity in the smooth muscle cells and an increase in the stromal cells as well as in the nerves running in the smooth muscle bundles (Smet et al., 1996; Fujiwara et al., 2000).

The potentiality of NO to evoke inhibitory as well as excitatory responses is seen in human detrusor pre-contracted through muscarinic receptor stimulation (Moon, 2002). In this tissue, inhibition of soluble guanylyl cyclase significantly attenuated NO-mediated enhancement of the contraction, indicating the involvement of c-GMP, but this inhibition did not significantly affect the relaxant response.

In spite of the questionable role of NO in evoking relaxation of the detrusor, there is considerable interest in the role of NO in human detrusor, and the possibility that alterations in nitrergic pathways could underlie the development of detrusor over-activity. Nitric oxide synthase immunoreactivity can be demonstrated in nerves in the bladder wall (Smet et al., 1996), and may co-localize with acetylcholinesterase activity (Zhou and Ling, 1999). Alteration in the distribution of these nerves and their immunoreactivity is seen after bladder outflow obstruction (Zhou and Ling, 1999). Moon also compared normal human detrusor with specimens from patients with overactive bladders, and found significant differences. Persson et al. (1999) have demonstrated that inactivation of the c-GMP-dependent protein kinase gene (cGK1) in mice as expected impairs NO/c-GMP-dependent relaxation of urethral smooth muscle, but these animals also developed bladder hyperactivity which suggested that bladder instability might be associated with impaired NO/cGK1 signalling. In ventricular myocytes Han et al. (2002), suggested that the c-GMP/PKG signalling pathway may have a protective role against ischaemia by activating K_{ATP}. If this is the case, a rationale also exists for a protective role in the bladder. The bladder wall is susceptible to ischaemia, and there are clear links between bladder wall ischaemia and the development of bladder instability (Greenland et al., 2000; Mills et al., 2000). Enhanced production of NO might protect the bladder under these conditions.

In conclusion, although our results clearly show that in the isolated detrusor myocytes NO donors can activate K_{ATP} through a c-GMP/PKG pathway, the role of this in the intact bladder may be minimal. However, such a mechanism might have a protective role under conditions of ischaemia. Further studies on the effects of NO on detrusor are clearly necessary.

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References

Andersson, K.-E., 1993. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol. Rev. 45, 253–308.

Andersson, K.-E., Mattiasson, A., Sjogren, C., 1983. Electrically induced relaxation of the nor-adrenaline contracted isolated urethra from rabbit and man. J. Urol. 129, 210–213.

Baker, J.E., Contney, S.J., Singh, R., Kalyanaraman, B., Gross, G.J., Bosnjak, Z.J., 2001. Nitric oxide activates the sarcolemmal K_{ATP} channel in normoxic and chronically hypoxic hearts by a cyclic GMP-dependent mechanism. J. Mol. Cell. Cardiol. 33, 331–341.

Bonev, A.D., Nelson, M.T., 1993. ATP- sensitive channels in smooth muscle cells from guinea pig urinary bladder. Am. J. Physiol. 264, C1190–C1200.

Brading, A.F., 1992. Ion channels and control of contractile activity in urinary bladder smooth muscle. Jpn. J. Pharmacol. 58 (Suppl. 2), 120P–127P.

Bridgewater, M., Macneil, H.F., Brading, A.F., 1993. Regulation of tone in pig urethral smooth muscle. J. Urol. 150, 223–228.

Chung, B.H., Seung, K.C., Ki, C.C., 1996. Effects of nitric oxide on detrusor relaxation. J. Urol. 155, 2090–2093.

Dokita, S., Smith, S.D., Nishimoto, T., Wheeler, M.A., Weiss, R.M., 1994. Involvement of nitric oxide and c-GMP in rabbit urethral relaxation. Eur. J. Pharmacol. 269, 269–275.

Foster, C.D., Fuji, K., Kingdon, J., Brading, A.F., 1989. The effect of cromakalim on the smooth muscle of the guinea-pig urinary bladder. Br. J. Pharmacol. 97 (1), 281–291.

Fuji, K., Foster, C.D., Brading, A.F., Parekh, A.B., 1990. K^+ channel blockers and the effects of cromakalim on the smooth muscle of the guinea-pig bladder. Br. J. Pharmacol. 99 (4), 779–785.

Fujiwara, M., Andersson, K.-E., Persson, K., 2000. Nitric oxide-induced c-GMP accummulation in the mouse bladder is not related to smooth muscle relaxation. Eur. J. Pharmacol. 401, 241–250.

Greenland, J.E., Hvistendahl, J.J., Andersen, H., Jorgensen, T.M., McMurray, G., Cortina-Borja, M., Brading, A.F., Frokiaer, J., 2000. The effect of bladder outlet obstruction on tissue oxygen tension and blood flow in the pig bladder. Br. J. Urol. Int. 85, 1109–1114.

Han, J., Kim, N., Joo, H., Kim, E., Earm, Y.E., 2002. ATP-sensitive K^+ channel activation by nitric oxide and protein kinase G in rabbit ventricular myocytes. Am. J. Physiol. 283, H1545–H1554.

James, M.J., Birmingham, A.T., Hill, S.J., 1993. Relaxation of human isolated detrusor strips in response to electrical field stimulation: a possible role for nitric oxide in human bladder. Br. J. Clin. Pharmacol. 35, 366–372.

Klarskov, P., Gernstenberg, T., Ramirez, D., Hald, T., 1983. Non-cholinergic, non-adrenergic nerve mediated relaxation of trigone, bladder neck and urethral smooth muscle in vitro. J. Urol. 129, 848–850.

Koh, S.D., Campbell, J.D., Carl, A., Sanders, K.M., 1995. Nitric oxide activates multiple potassium channels in canine colonic smooth muscle. J. Physiol. 489, 735–774.

Kubo, M., Nakaya, Y., Matsuoka, S., Saito, K., Kuroda, Y., 1994. Atrial natriuretic factor and isosorbide dinitrate modulate the gating of ATP-sensitive K^+ channels in cultured vascular smooth muscle cells. Circ. Res. 74, 471–476.

Mills, I.W., Greenland, J.E., McMurray, G., McCoy, R., Ho, K.M., Noble, J.G., Brading, A.F., 2000. Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. J. Urol. 163, 646–651.

Moon, A., 2000. Effect of nitric oxide on detrusor contractility. PhD Thesis. University of Newcastle.

Moon, A., 2002. Influence of nitric oxide signalling pathways on pre-contracted human detrusor smooth muscle in vitro. Br. J. Urol. Int. 89, 942–949.

Murphy, M.E., Brayden, J.E., 1995. Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. J. Physiol. 486, 47–58.

Persson, K., Alm, P., Johansson, K., Larsson, B., Andersson, K.-E., 1993. Nitric oxide synthase in pig lower urinary tract: immunohistochemistry, NADPH diaphorase histochemistry and functional effects. Br. J. Pharmacol. 110, 521–530.

Persson, K., Pandita, R.K., Aszodi, A., Fassler, R., Andersson, K.-E., 1999. The absence of cyclic GMP-dependent kinase 1 causes hyperactive voiding and impaired urethral relaxation (Abstract). J. Urol. 161, 143A.

Shinbo, A., Iijima, T., 1997. Potentiation by nitric oxide of the ATP-sensitive K^+ current induced by K+ channel openers in guinea-pig ventricular cells. Br. J. Pharmacol. 120, 1568–1574.

Smet, P.J., Jonavicius, J., Marshal, V.R., Vente, J. De., 1996. Distribution of nitric oxide synthase-immunoreactive nerves and identification of the cellular targets of nitric oxide in guinea pig and human urinary bladder by c-GMP immunohistochemistry. Neuroscience 71 (2), 337–348.

Speakman, M.J., Walmsley, D., Brading, A.F., 1998. An in vitro pharmacological study of the human trigone—a site of non-adrenergic, non-cholinergic neurotransmission. Br. J. Urol. 61, 304–349.

Teramoto, N., Mcmurray, G., Brading, A.F., 1997. Effects of levcromakalim and nucleoside diphosphates on glibenclamide-sensitive K^+ channels in pig urethral myocytes. Br. J. Pharmacol. 120, 1229–1240.

Zhou, Y., Ling, E.A., 1999. Nitric oxide synthase-its distribution and alteration in the intramural ganglia of the urinary bladder in normal and urethra-obstructed guinea pigs. Ann. Acad. Med. Singap. 28 (1), 49–61.