Dual modulation of swelling-activated chloride current by NO and NO donors in rabbit portal vein myocytes

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1. The effects of authentic NO and the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) on swelling-activated chloride currents (I_{swell}) were investigated in freshly dispersed rabbit portal vein smooth muscle cells. I_{swell} was recorded with the perforated patch configuration of the whole-cell patch clamp technique.

2. In approximately 50 % of cells NO and SNAP inhibited the amplitude of I_{swell} by about 45 % in a voltage-independent manner. I_{swell} was also inhibited by an inhibitor of NO-sensitive guanylate cyclase (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and by KT5823, an inhibitor of cGMP-dependent protein kinase.

3. In other cells both NO and SNAP enhanced I_{swell} by about 40 % in a voltage-independent manner. A similar increase was produced by application of the cell-permeable cGMP analogue 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-Br-cGMP). However, 8-Br-cGMP had no effect on current amplitude in cells pre-treated with KT5823. In contrast 8-Br-cGMP increased the amplitude of I_{swell} in cells which had been pre-treated with ODQ.

4. SNAP also modulated I_{swell} recorded in the conventional whole-cell configuration with internal solutions containing 10 mM EGTA to rule out any contribution from Ca^{2+}-activated Cl^- currents.

5. These data suggest that the amplitude of I_{swell} can be enhanced by NO via a cGMP-dependent phosphorylation and inhibited by NO in a cGMP-independent manner.

A membrane chloride current which is activated by cell swelling (I_{swell}) has been identified in many cell types and is implicated in cell volume regulation, solute transport and also, perhaps, in processes such as cell proliferation (see reviews by Strange *et al.* 1996; Okada, 1997). I_{swell} has also been observed in vascular (Yamazaki *et al.* 1998; Greenwood & Large, 1998) and non-vascular smooth muscle (Xu *et al.* 1997; Dick *et al.* 1998). It has been suggested that in vascular smooth muscle activation of I_{swell} produces depolarisation and contraction because the chloride equilibrium potential (E_{Cl}, about −20 to −30 mV) is substantially more positive than the resting membrane potential (Nelson, 1998; Greenwood & Large, 1998). This proposal was based on the observations that the myogenic response in rat cerebral arteries was inhibited by chloride channel blockers (Nelson *et al.* 1997) and that the sensitivity of this response and I_{swell} to 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acids (DIDS) was similar (Greenwood & Large, 1998).

Recently evidence has been provided to indicate that NO released from the endothelium regulates smooth muscle chloride conductance. In rat aorta it was shown that reduction of the chloride concentration of the bathing solution (and hence increasing the electrochemical gradient for Cl^- ion efflux to produce depolarisation) evoked contraction in de-endothelialised preparations and in aortic segments treated with the NO synthase inhibitor *N*-nitro-L-arginine but not in aorta with a functionally active endothelium (Lamb & Barna, 1998). It was proposed that the Cl^- conductance of smooth muscle cells in intact vessels is low but increases after reducing NO production by the endothelium (Lamb & Barna, 1998). In rat isolated small coronary arteries we demonstrated that inhibition of NO synthesis induces contraction and this response is inhibited by the blockers of I_{swell} DIDS and tamoxifen (Graves *et al.* 1998). These results also suggest that NO released spontaneously from the endothelium in coronary arteries may suppress a chloride conductance in the smooth muscle cells, possibly I_{swell}. In the present study we have investigated the effects of NO and NO donors on I_{swell} in rabbit portal vein smooth muscle cells, which has been characterised previously (Greenwood & Large, 1998). A preliminary account of this work has been presented to The Physiological Society (Ellershaw *et al.* 1999).

METHODS

Cell preparation

New Zealand White rabbits (2–3 kg) were killed by injection of a lethal dose of sodium pentobarbitone into the ear vein. Portal veins were excised, cleaned of fat and connective tissue and the exposed muscle sheet was cut into strips which were then immersed in physiological salt solution (PSS) containing 50 μM $CaCl_2$ at 37 °C. Single smooth muscle cells were isolated by treating the tissue with protease Type 1 crude or protease Type 14 (0·2–0·3 mg ml^{-1}) for 5 min. After washout of the protease the tissue was immersed in collagenase Type 1A (0·5–1 mg ml^{-1}) for 10 min. Cells were released from the digested tissue by gentle mechanical agitation using a wide-bore Pasteur pipette. Isolated cells were transferred to PSS containing 0·75 mM $CaCl_2$, placed on cover slips for storage at 4 °C and used within 6 h of isolation. All experiments were conducted at room temperature (21–23 °C).

Electrophysiological recording

Whole-cell membrane currents were recorded with a List LM PCA amplifier using the perforated patch configuration of the whole-cell patch clamp technique. The perforated patch was obtained by adding amphotericin B (200–250 μg ml^{-1}) to the pipette solution from a stock solution of amphotericin B dissolved in dimethyl sulphoxide (DMSO). The stock solution was stored at −10 °C and fresh pipette solution was prepared every 2 h. All voltage protocols were generated by the CED (Cambridge, UK) Voltage Clamp program and evoked currents were analysed using the corresponding CED analysis package after filtering at 3 kHz. Further analysis and graphics were produced using Microcal Origin (Northampton, MA, USA). Changes in junction potentials between pipette and bath solutions were minimised by the use of a KCl agar bridge. The voltage-dependent characteristics of the hypotonicity-activated current were investigated by applying voltage ramps every 5 s. This protocol involved stepping the voltage from the holding potential of −50 mV to −100 mV for 50 ms followed by continuously changing the voltage from −100 to +100 mV at a rate of 250 mV s^{-1} in normal PSS and hypotonic solutions.

Solutions

Normal PSS used for dissection contained (mM): NaCl 126, KCl 6, $MgCl_2$ 1·2, $CaCl_2$ 1·5, Hepes 10 and glucose 11, and was adjusted to pH 7·2 with NaOH. Experiments were performed in K^+-free conditions to remove contaminating K^+ currents and the normal K^+-free extracellular solution had the following composition (mM): NaCl 126, $MgCl_2$ 1·2, $CaCl_2$ 1·5, Hepes 10 and glucose 11, and was adjusted to pH 7·2 with NaOH. In all experiments voltage-dependent Ca^{2+} currents were blocked by the inclusion of 5 μM nicardipine in the bathing solution. In all perforated patch experiments the K^+-free pipette solution contained (mM): CsCl 126, $MgCl_2$ 1·2, Hepes 10, glucose 11 and EGTA 0·1, and the pH was adjusted to 7·2 with CsOH. However, in a series of experiments I_{swell} was recorded using the conventional whole-cell configuration and the K^+-free pipette solution contained 10 mM EGTA to eliminate any contamination from Ca^{2+}-activated Cl^- currents. I_{swell} was evoked by bathing the cell in a hypotonic external solution in which the NaCl concentration was reduced to 60 mM (see Greenwood & Large, 1998, for further details).

Chemicals

NO solution was prepared by a method described by Trepakova *et al.* (1999). Briefly, NO gas (BDH, Poole, Dorset, UK) was bubbled through distilled water containing an anion exchange resin (Bio-Rad) to mop up possible nitrites and nitrates formed by NO reacting with oxygen. The resulting 3 mM solution was stored at 4 °C and used within 1 week. Dilutions were made by drawing off NO solution with a syringe and adding it directly to the external solution in the perfusion reservoir immediate to the recording chamber. With this method we estimated that the concentration of NO which reached the smooth muscle cells was about 1 μM. All enzymes, amphotericin B and DIDS were purchased from Sigma (Poole, Dorset, UK). *S*-nitroso-*N*-acetylpenicillamine (SNAP), 8-bromo-guanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) and KT5823 were purchased from Calbiochem (La Jolla, CA, USA). 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was purchased from Tocris (Avonmouth, Bristol, UK). Reagents were dissolved in either DMSO or ethanol, which at the highest concentration used (0·1 %) had no effect on I_{swell}. None of the agents investigated had any effect on the degree of cell swelling produced by bathing in hypotonic solutions. Maximum cell width was measured by a graticule located within the optical pathway and in isotonic conditions was 12 ± 2 μm ($n = 30$). Perfusion with hypotonic external solution produced cell swelling and increased cell width to 20 ± 3 μm. In the continued presence of hypotonic solution the maximum width in the presence of 100 μM 8-Br-cGMP, 10 μM SNAP and NO was 19 ± 3, 21 ± 3 and 20 ± 2 μm, respectively ($n = 6$–8).

Statistics

All data are presented as the mean ± S.E.M. of n cells. Student's t test was used to compare mean values and statistical significance was set at $P < 0{\cdot}05$.

RESULTS

Inhibition of I_{swell} by NO and SNAP

We have shown previously that application of hypotonic solutions to rabbit portal vein myocytes caused significant cell swelling and concomitant activation of a Cl^- current that was designated I_{swell} (Greenwood & Large, 1998). In the present study, changing the external solution from one containing 126 mM NaCl to one with 60 mM NaCl evoked a current that reversed at -5 ± 1 mV ($n = 50$), close to the predicted E_{Cl} as calculated by the Nernst equation. This current exhibited slight outward rectification and was inhibited by DIDS in a voltage-dependent manner consistent with I_{swell} in this cell type (Greenwood & Large, 1998). Figure 1*A* shows that after I_{swell} had reached a plateau the current was well maintained and persisted in the continued presence of the hypotonic solution as reported previously (Greenwood & Large, 1998). Similar well-sustained currents were observed in five other control cells bathed in hypotonic solution for 30 min. The addition of NO (approximate concentration was 1 μM) to the bathing solution reduced the amplitude of I_{swell} in five out of 11 cells. The maximal inhibition was produced after approximately 500 s exposure to NO and a typical record is shown in Fig. 1*B*. The effect of NO was not voltage dependent and the maximum inhibition at −50 and +100 mV was 49 ± 3 % ($n = 5$) and 42 ± 2 % ($n = 5$ cells from 4 different animals), respectively (Fig. 1*C*). Application of 10 μM SNAP to the bathing solution also reduced I_{swell} in 15 out of 30 cells isolated from 22 different animals and the maximal inhibition was observed after about 500 s (Fig. 2*A*). The SNAP-induced inhibition was also voltage independent and the maximum inhibition at

−50 and +100 mV was 50 ± 7 and 57 ± 7%, respectively ($n = 15$), which was qualitatively similar to that produced by NO (Fig. 2*B*). SNAP had no effect on the resting conductance recorded under isotonic conditions ($n = 9$, 3 different animals).

Enhancement of I_{swell} by NO and SNAP

However, in nine cells isolated from seven different animals SNAP produced an increase in the current amplitude. An example of the SNAP-induced increase in I_{swell} is illustrated in Fig. 3*A* and it can be seen that this effect was reversible. The enhancement had a slow time course similar to the inhibitory effect (cf. Fig. 3*B* and Fig. 2*A*). In these cells SNAP increased I_{swell} at −50 mV by 39 ± 4% and at +100 mV by 40 ± 6%. A similar increase in the amplitude of I_{swell} was observed in six cells when NO was added to the bathing solution. Thus, NO increased I_{swell} at −50 and +100 mV by 41 ± 5 and 43 ± 5%, respectively, which was not significantly different to the enhancement produced by 10 μM SNAP ($P < 0{\cdot}05$, Fig. 4*C*). Therefore, it is apparent that NO and SNAP can increase or decrease I_{swell} evoked in vascular myocytes but have no effect on the resting conductance recorded under isotonic conditions. In six cells (4 different animals) SNAP had no effect on I_{swell}.

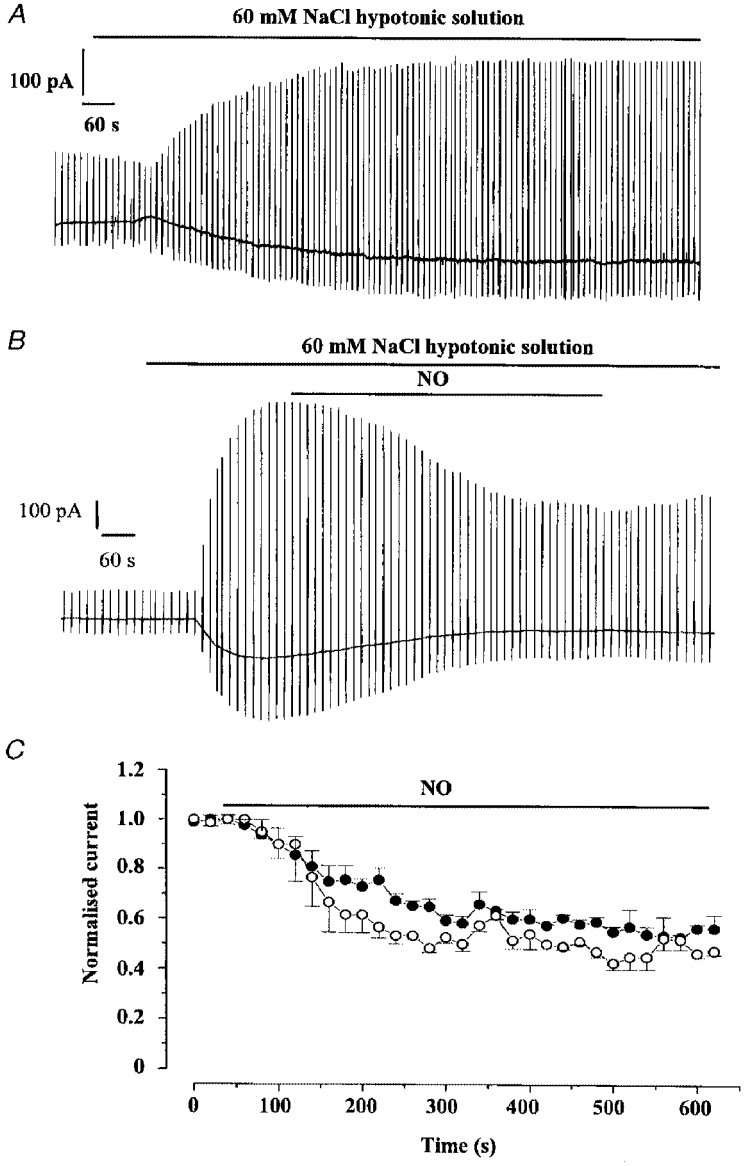

Figure 1. Inhibitory effect of NO on I_{swell}

A, representative trace showing a control cell where I_{swell} developed following exposure of a single portal vein smooth muscle cell to hypotonic solution. Note that the current is sustained throughout the application of hypotonic solution. *B*, the inhibitory effect of NO on I_{swell}. The concentration of NO was about 1 μM (see Methods) and in *A* and *B* large deflections represent the ramp protocol from a holding potential of −50 mV. *C*, the mean time dependence of the NO-induced inhibition shown at −50 mV (○) and +100 mV (●). Currents were normalised to peak I_{swell} prior to application of NO. Each point is the mean ± S.E.M. of 5 cells.

Effect of SNAP on I_{swell} recorded from cells with the whole-cell configuration using pipette solutions containing 10 mM EGTA

Since Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) are readily recorded with the perforated patch configuration it is possible that the variable effects of SNAP and NO may be due to an effect on $I_{Cl(Ca)}$ as well as I_{swell}. Consequently, we performed experiments using the conventional whole-cell configuration with a pipette solution containing 10 mM EGTA to chelate any increase in $[Ca^{2+}]_i$ evoked by cell swelling and therefore prevent activation of $I_{Cl(Ca)}$. Under these conditions bathing the cell in hypotonic solution evoked I_{swell} that was indistinguishable from I_{swell} recorded with the perforated patch technique. The current exhibited outward rectification, reversed at $+5 \pm 2$ mV (close to the theoretical E_{Cl}, $n = 12$) and was well maintained in the continued presence of the hypotonic solution. Application of SNAP had similar variable effects on I_{swell} recorded under these conditions to those described above. Thus in five cells from four different rabbits SNAP inhibited I_{swell} at -50 and $+100$ mV by 50 ± 8 and 51 ± 8%, respectively. However, in five other cells (5 animals) SNAP enhanced I_{swell} at -50 and $+100$ mV by 60 ± 4 and 55 ± 7%, respectively. SNAP had no effect on I_{swell} in four cells isolated from four different animals. The magnitudes of the changes produced by SNAP under these conditions were similar to those observed for I_{swell} recorded with the perforated patch technique. These data show that the effects of NO and SNAP are due to an action on I_{swell} and not on $I_{Cl(Ca)}$.

Reversal potential of I_{swell} when Na^+ was replaced by *N*-methyl-D-glucamine

We were concerned that a cation conductance may contribute to I_{swell} which may give rise to the variability of the data with NO and SNAP. To test this possibility we replaced the external Na^+ ions with the less permeant cation *N*-methyl-D-glucamine (NMDG). The reversal potential of I_{swell} with 75 mM NaCl was $+5 \pm 0{\cdot}6$ mV ($n = 7$, 6 rabbits) and with 75 mM NMDG-Cl^- it was $+7 \pm 0{\cdot}7$ mV ($n = 8$, 6 rabbits).

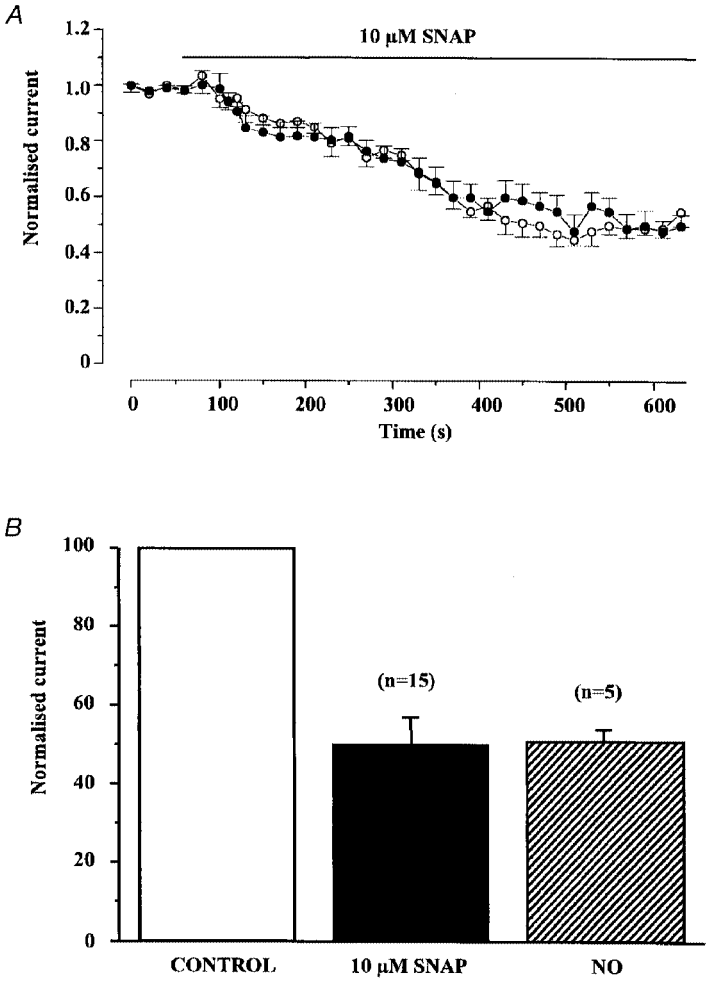

Figure 2. Inhibitory effect of SNAP on I_{swell}

A, the mean time dependence of the SNAP-induced inhibition of I_{swell} shown at -50 mV (○) and $+100$ mV (●). Each point is the mean ± S.E.M. of 15 cells. *B*, comparison of the maximum SNAP- and NO-induced inhibition of I_{swell} recorded at -50 mV. Currents were normalised to peak I_{swell} prior to application of SNAP or NO.

Since there was no change in the reversal potential it is concluded that in this preparation I_{swell} is purely an anion current with no contribution from a cation conductance.

Modulation of I_{swell} by cGMP and cGMP-dependent protein kinase

Many of the effects of NO are due to the activation of guanylate cyclase and subsequent increase in cGMP (McDonald & Murad, 1996) and therefore we investigated the effect of cGMP on I_{swell} to delineate the effects of NO on this conductance. In six cells isolated from five different animals application of the cell-permeable cGMP analogue 8-Br-cGMP (100 μM) to the bathing solution during activation of I_{swell} produced a reversible increase in the amplitude of the current (Fig. 4*A*), although in two cells 8-Br-cGMP had no effect on I_{swell}. The enhancement of I_{swell} by 8-Br-cGMP reached a peak after about 300 s (Fig. 4*B*) and the mean increase at −50 and +100 mV was 52 ± 14 and 50 ± 8% ($n = 6$ cells, 5 animals), respectively. The magnitude of the enhancement produced by 8-Br-cGMP was similar to that produced by NO or SNAP (Fig. 4*C*). A further series of experiments was performed to investigate the effect of a selective inhibitor of NO-sensitive guanylate cyclase, ODQ, on I_{swell}. In five cells (4 animals) application of 10 μM ODQ to the bath solution caused inhibition of I_{swell}. A typical cell is shown in Fig. 5*A*. The mean reduction of I_{swell} by ODQ was 34 ± 5 and 22 ± 5% at −50 and +100 mV, respectively ($n = 5$ cells). Application of either ODQ or 8-Br-cGMP for 8 min had no effect on the resting conductance recorded under isotonic conditions ($n = 4$ cells from 4 different animals for each agent). These data suggest that an increase in cGMP levels is insufficient to activate I_{swell} but cGMP augments I_{swell} when it has been activated by hypotonic solution. Consequently increases in cGMP levels in portal vein myocytes appear to enhance the activation of I_{swell}.

Most of the cellular effects of cGMP are mediated by a cGMP-dependent protein kinase (McDonald & Murad, 1996). Consequently we studied the effect of the selective cGMP-dependent protein kinase inhibitor KT5823 on evoked I_{swell}. This agent has a K_i for inhibition of protein kinase G of 234 nM compared to K_i values against protein kinase C and

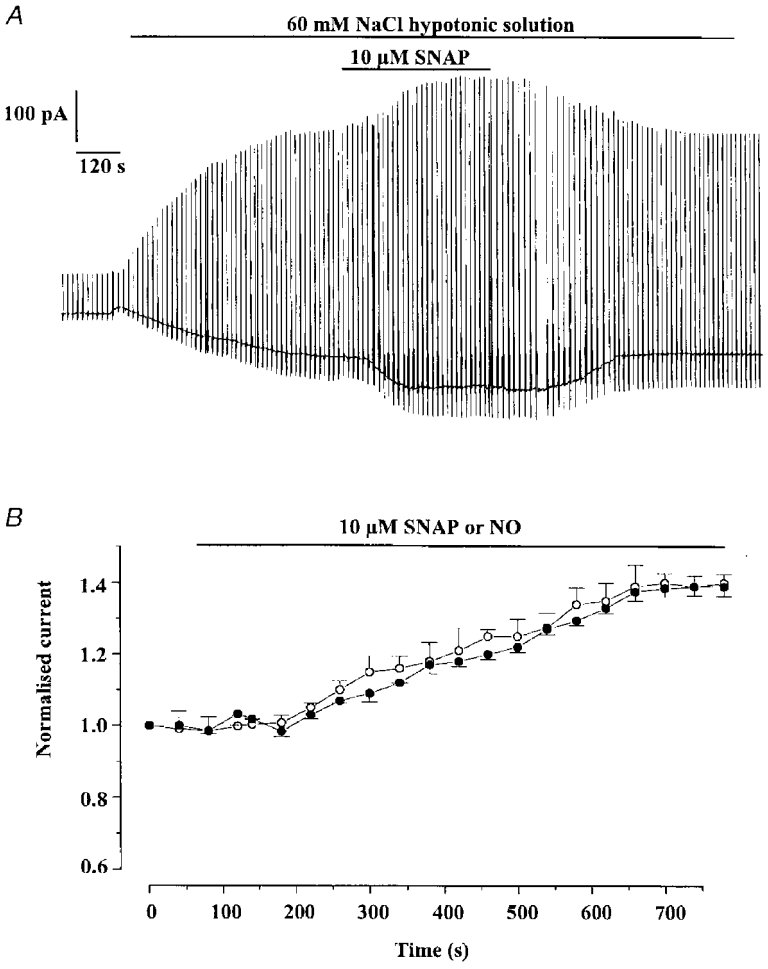

Figure 3. Cells in which SNAP and NO increased I_{swell}

A, typical cell showing the increase of I_{swell} by 10 μM SNAP. *B*, time dependence of the potentiating effect of SNAP (○) and NO (●) on I_{swell} recorded at −50 mV. Each point is the mean ± S.E.M. of 9 cells for SNAP and 6 cells for NO.

protein kinase A of 4 and 10 μM, respectively (Ito & Karachot, 1990). Consequently we used KT5823 at a concentration of 1 μM where it is relatively selective for cGMP-dependent protein kinase. In nine cells isolated from eight animals addition of 1 μM KT5823 to the bathing solution produced a slow, voltage-independent inhibition of I_{swell}. A typical cell is shown in Fig. 5*B*. The degree of inhibition was 60 ± 3 and 69 ± 7% at −50 and +100 mV, respectively ($n = 9$, Fig. 5*C*), which was significantly greater than the inhibition produced by ODQ ($P < 0{\cdot}05$). Removal of KT5823 from the bathing solution caused a partial reversal of the inhibition (Fig. 5*B*). In four cells (4 animals) KT5823 was without effect, but it is worth noting that neither KT5823 nor ODQ increased I_{swell} in any cell tested. Consequently, when I_{swell} has been activated the intracellular concentration of cGMP is capable of stimulating a cGMP-dependent protein kinase that in turn enhances I_{swell}. When 8-Br-cGMP was applied in the presence of KT5823 there was no increase in I_{swell} (Fig. 6*A*); similar results were observed in six cells from four different rabbits.

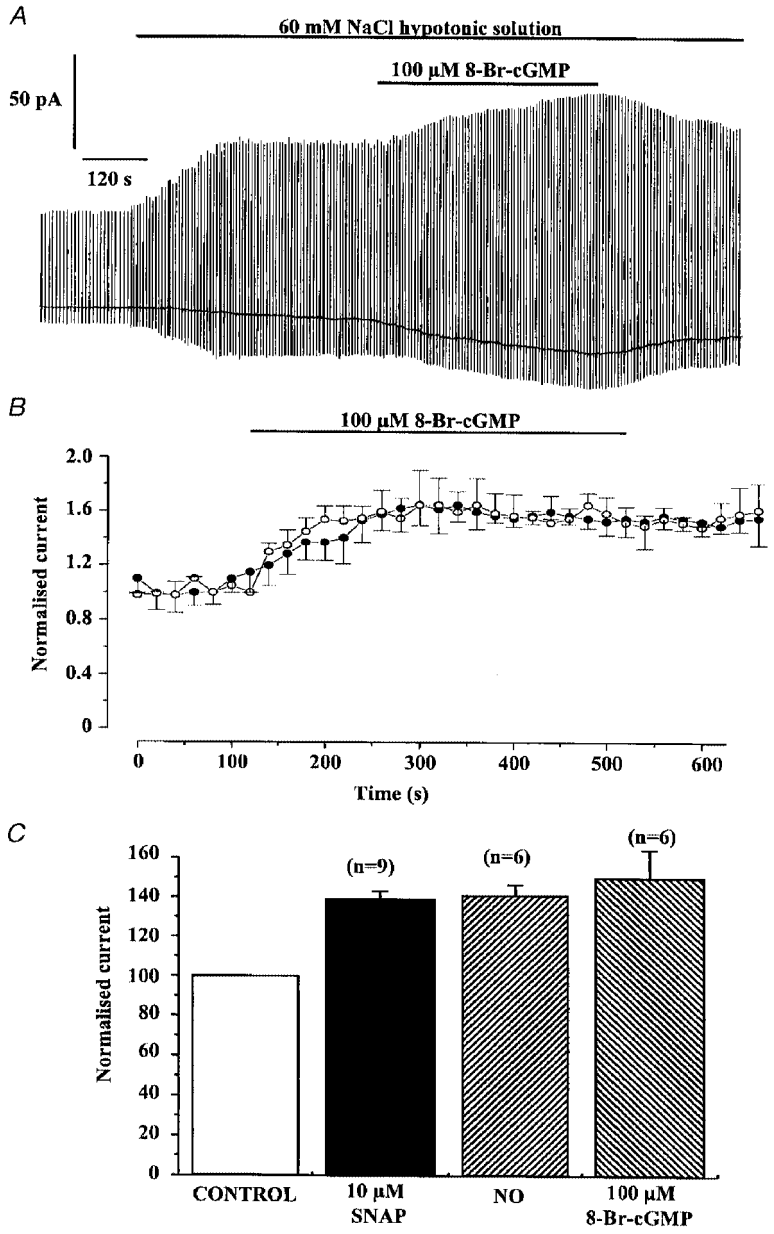

Figure 4. Effect of 8-Br-cGMP on I_{swell}

A, record illustrating the excitatory effect of 100 μM 8-Br-cGMP on I_{swell}. *B*, time dependence of the 100 μM 8-Br-cGMP-induced increase shown at −50 mV (○) and +100 mV (●). Each time point is the mean ± S.E.M. of 6 cells. *C*, comparison of the potentiating effect of SNAP, NO and 8-Br-cGMP on I_{swell} recorded at −50 mV. Currents were normalised to peak I_{swell} prior to application of SNAP, NO or 8-Br-cGMP and the number of cells is shown in parentheses.

Consequently, the stimulatory effect of 8-Br-cGMP appears to be mediated by a cGMP-dependent protein kinase. In comparison, application of 8-Br-cGMP in the continued presence of ODQ caused an increase in the amplitude of I_{swell}. Thus, application of 10 μM ODQ inhibited I_{swell} by $37 \pm 4\%$ at -50 mV (similar to that reported above, $n = 3$) and after application of 8-Br-cGMP in the presence of ODQ the current was increased by $40 \pm 10\%$ ($n = 3$), which is similar to the increase of I_{swell} by 8-Br-cGMP in the absence of ODQ (about 50%, see above).

Since NO can either decrease or increase the amplitude of I_{swell}, we investigated whether these actions occurred simultaneously in the same cell. For these experiments SNAP was applied simultaneously with ODQ, which would be expected to inhibit the NO-sensitive guanylate cyclase-mediated increase in I_{swell} in response to SNAP. A typical experiment is shown in Fig. 6*B* where 10 μM SNAP applied with 10 μM ODQ decreased I_{swell} by about 60%. In five cells (3 animals) the mean reduction of I_{swell} produced by SNAP and ODQ was $52 \pm 5\%$ at -50 mV. This value is not significantly different to the decrease in I_{swell} produced by SNAP in the absence of ODQ (see Fig. 2*B*). If SNAP was producing simultaneous excitatory and inhibitory effects on I_{swell}, it might have been expected that after the removal of the facilitatory action with ODQ SNAP would produce a larger inhibitory effect than observed with SNAP alone.

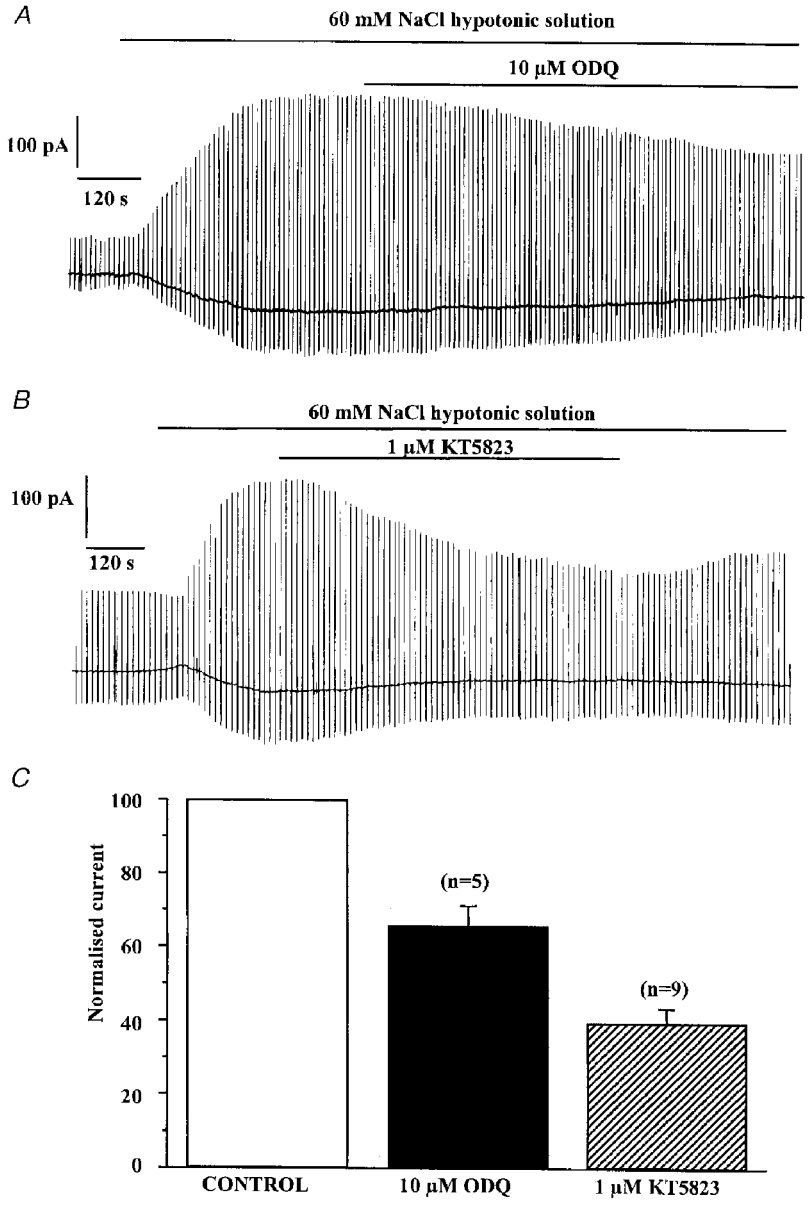

Figure 5. Effect of ODQ and KT5823 on I_{swell}

A, the inhibitory effect of 10 μM ODQ on I_{swell}. *B*, the inhibitory effect of 1 μM KT5823 on I_{swell}. *C*, comparison of ODQ- and KT5823-induced inhibition of I_{swell} recorded at -50 mV. Currents were normalised to peak I_{swell} prior to application of ODQ or KT5823.

DISCUSSION

The major finding of this study is that authentic NO and the NO donor SNAP modulate I_{swell} recorded in rabbit portal vein smooth muscle cells. In approximately half of the cells these agents produced marked voltage-independent inhibition of I_{swell}. Both the magnitude and the time course of the inhibition produced by NO and SNAP were similar suggesting a common mechanism. Furthermore, the rate of inhibition was markedly slower than that of voltage-dependent inhibition produced by blockers such as DIDS (see Greenwood & Large, 1998) suggesting that NO and SNAP were not acting as direct channel blockers but were probably affecting regulatory mechanisms of the conductance. In other cells, NO and SNAP produced a significant increase of I_{swell} similar to that produced by the application of a cell-permeable analogue of cGMP, 8-Br-cGMP. Similar effects were observed when I_{swell} was recorded with pipette solutions containing 10 mM EGTA to suppress Ca^{2+}-activated Cl^- currents. These results indicate that neither the increase nor the decrease of I_{swell} produced by NO was due to an effect on contaminating $I_{Cl(Ca)}$ recorded simultaneously with I_{swell}. In support of this conclusion, SNAP and NO at concentrations up to 1 mM and 1 μM, respectively, had no effect on whole-cell $I_{Cl(Ca)}$ in tracheal smooth muscle (Waniishi *et al.* 1998) or $I_{Cl(Ca)}$ in excised patches from aortic smooth muscle (Hirakawa *et al.* 1999). Consequently NO regulates I_{swell} without a direct effect on $I_{Cl(Ca)}$.

Mechanisms involved in the increase in I_{swell} by NO and NO donors

In some portal vein myocytes NO, SNAP and 8-Br-cGMP enhanced I_{swell}. Furthermore, inhibition of both NO-

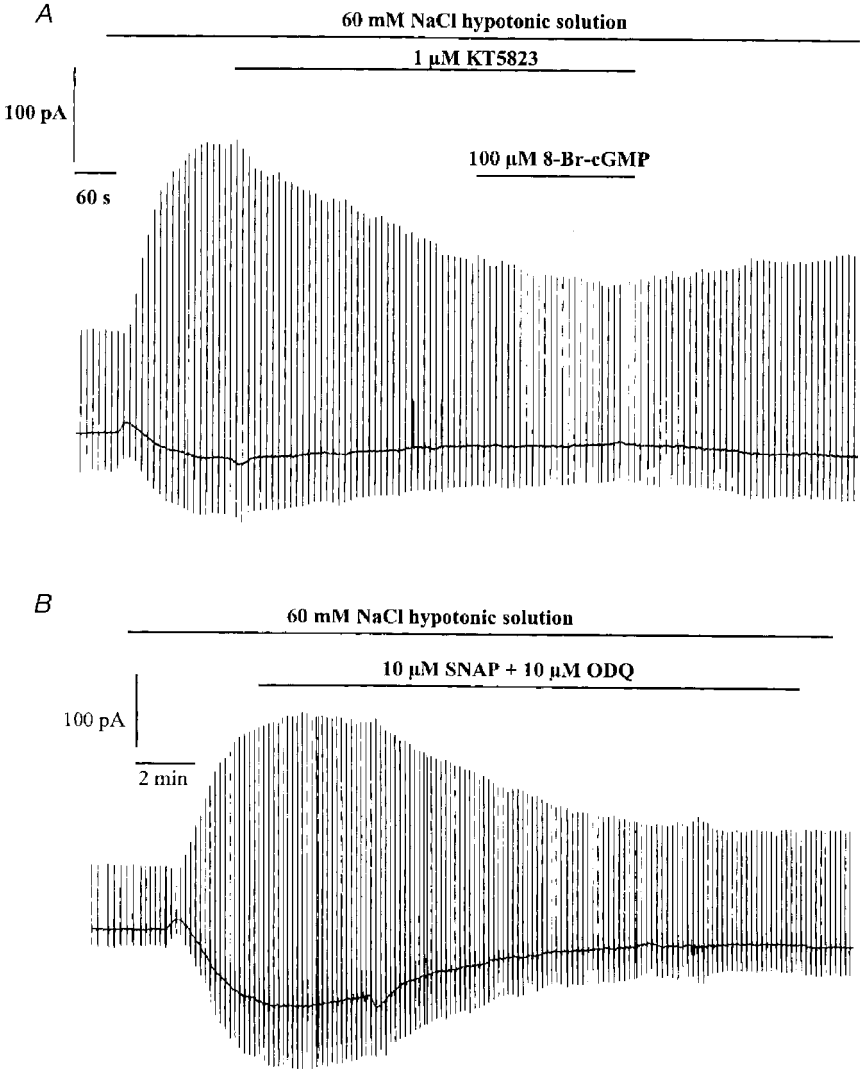

Figure 6. Effect of KT5823 on the modulation of I_{swell} by 8-Br-cGMP and effect of ODQ on the action of SNAP

A, representative trace showing the inhibitory effect of 1 μM KT5823. Application of 8-Br-cGMP had no effect on I_{swell} in the continued presence of KT5823. *B*, representative trace showing the combined inhibitory effect of 10 μM ODQ and 10 μM SNAP.

dependent guanylate cyclase and cGMP-dependent phosphorylation by ODQ and KT5823, respectively, inhibited I_{swell}. These data suggest that NO and SNAP stimulate guanylate cyclase to generate sufficient cGMP to activate cGMP-dependent protein kinase, which enhances I_{swell}. However, 8-Br-cGMP did not activate I_{swell} under isotonic conditions but enhanced I_{swell} once it had been activated. This result suggests that 8-Br-cGMP does not directly activate the Cl^- channel but accentuates channel activity once opened. In addition, the stimulatory effect of 8-Br-cGMP on I_{swell} was not apparent when the cell had been pre-treated with the selective cGMP-dependent protein kinase inhibitor KT5823, and the NO-sensitive guanylate cyclase inhibitor ODQ also reduced I_{swell}. These data suggest that during activation of I_{swell} there is a tonic generation of cGMP that activates sufficient KT5823-sensitive kinase to enhance the amplitude of I_{swell}. Since the inhibitory effect of KT5823 was greater than that of ODQ it is possible that during activation of I_{swell} cGMP is produced by both NO-sensitive and NO-insensitive guanylate cyclase. Interestingly, in contraction studies NO and sodium nitroprusside contracted the opossum oesophagus by a cGMP-dependent mechanism (Saha *et al.* 1993). Overall the data of the present study suggest that in vascular smooth muscle cells isolated from rabbit portal vein the activity of I_{swell} is enhanced by a cGMP-dependent protein kinase.

Mechanisms involved in the decrease of I_{swell} by NO and NO donors

Our experiments did not reveal the mechanism by which NO inhibits I_{swell}. The present study indicates that the inhibitory effect of NO on I_{swell} is not likely to be mediated via the well-known action of NO to stimulate NO-sensitive guanylate cyclase to produce cGMP. As discussed above, this mechanism enhances I_{swell}. It is worth re-iterating that NO did not reduce I_{swell} by an action of cGMP *independent* of cGMP-dependent protein kinase since cGMP had no effect on I_{swell} when the cells were pre-treated with the kinase inhibitor KT5823. Thus, NO may inhibit I_{swell} by an unidentified transduction mechanism. Alternatively, NO has been shown to activate directly Ca^{2+}-sensitive K^+ channels in vascular and tracheal smooth muscle (Bolotina *et al.* 1994; Abderrahmane *et al.* 1998) via modulation of redox-sensitive amino acid residues (Beckman & Koppenol, 1996). Consequently it is possible that the NO-induced inhibition of I_{swell} may be due to NO, either directly or via a reactive intermediate such as peroxynitrite, modifying the chloride channel protein.

Variable effects of NO and SNAP on I_{swell}

The data of the present study show that I_{swell} is regulated by NO via a cGMP-dependent pathway to increase I_{swell} and by a cGMP-independent mechanism to decrease I_{swell}. However, the net effect of NO and SNAP differed between populations of cells and in some cases NO, SNAP and 8-Br-cGMP had no effect. Similar variability has been observed in contraction studies on opossum oesophageal smooth muscle (Saha *et al.* 1993) and human detrusor smooth muscle (A. Moon, personal communication) where authentic NO and NO donors elicit both contraction and relaxation responses over the same concentration ranges. In the present study SNAP also produced similar effects on I_{swell} recorded with pipette solutions containing 10 mM EGTA to prevent any rise in $[Ca^{2+}]_i$ that would activate $I_{Cl(Ca)}$. Consequently, the different effects of SNAP and NO are not due to variable modulation of $I_{Cl(Ca)}$. The present work indicates that cGMP-dependent and -independent mechanisms modulate I_{swell} but there is also strong evidence that other kinases modulate I_{swell} in other cell types. Thus, activation of protein kinase C by phorbol esters inhibits I_{swell} in NIH3T3 cells transfected with ClC-3 isolated from canine atrial muscle (Duan *et al.* 1997, 1999) and native I_{swell} in canine colonic myocytes (Dick *et al.* 1998). However, protein kinase C and tyrosine kinases have been implicated in the activation of I_{swell} in other cell types (Lepple-Wienhues *et al.* 1998; Du & Sorota, 1999). Consequently, the observed variable responses in portal vein myocytes may reflect the balance of various regulatory mechanisms in the cells used. In summary, the data of the present study suggest that NO regulates I_{swell} in rabbit portal vein myocytes by a cGMP-dependent pathway to increase I_{swell} and by a cGMP-independent mechanism to decrease I_{swell}.

Physiological implications

If the inhibitory effect of NO on I_{swell} was to prevail in physiological conditions this interaction may have a profound effect on blood flow. Since opening of Cl^- channels in smooth muscle produces depolarisation and contraction, the inhibitory effect of NO on I_{swell} may contribute to the vasorelaxant effect of NO. In both vascular and non-vascular smooth muscle it has been shown that I_{swell} is active in isotonic conditions (Greenwood & Large, 1998; Dick *et al.* 1998). Moreover it has been proposed that I_{swell} may be involved in the myogenic response (Nelson, 1998). Therefore tonic release of NO from the endothelium may suppress I_{swell} in vascular smooth muscle cells. Reduction of NO release, for example by damage to the endothelium, may lead to depolarisation and increased contractility of the vascular smooth muscle cell. This mechanism would explain the results of both Lamb & Barna (1998) and Graves *et al.* (1998), which were outlined in the Introduction. Hence, NO-induced modulation of I_{swell} in vascular smooth muscle may play an important role in controlling blood flow in physiological and pathophysiological conditions.

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