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ATP-sensitive K^+ channels in smooth muscle cells of guinea-pig mesenteric lymphatics: role in nitric oxide and β-adrenoceptor agonist-induced hyperpolarizations

[1]Pierre-Yves von der Weid

[1]Vascular Cell Physiology, Department of Zoology and Animal Biology, University of Geneva, Switzerland

1 Intracellular microelectrode recordings were performed to investigate the membrane K^+ conductances involved in smooth muscle hyperpolarization of lymphatic vessels in the guinea-pig mesentery.

2 Nitric oxide (NO), released either by the endothelium after acetylcholine (ACh; 10 μM) stimulation or by sodium nitroprusside (SNP; 50–100 μM), hyperpolarized lymphatic smooth muscle. These responses were inhibited with the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazole [4,3-a]quinoxalin-1-one (ODQ, 10 μM).

3 ACh and SNP-induced hyperpolarizations were inhibited (by about 90%) upon application of the ATP-sensitive K^+(K_{ATP}) channel blocker, glibenclamide (10 μM), or with 4-aminopyridine (2.5 mM), but were not affected by the Ca^{2+}-activated K^+ channels blocker, penitrem A (100 nM).

4 Hyperpolarization caused by the K^+ channel opener, cromakalim (0.1–10 μM), isoprenaline (0.1 μM) or forskolin (0.5 μM) were all significantly blocked by glibenclamide.

5 Hyperpolarization evoked by ACh and SNP were inhibited with N-[2-(p-bromociannamylamino)-ethyl]-5-isoquinolinesulfonamide-dichloride (H89, 10 μM), suggesting the involvement of cyclic AMP dependent protein kinase (PKA).

6 These results suggest that K_{ATP} channels play a central role in lymphatic smooth muscle hyperpolarization evoked by a NO-induced increase in cyclic GMP synthesis, as well as by β-adrenoceptor-mediated production of cyclic AMP. Interestingly, both pathways lead to K_{ATP} channels opening through the activation of PKA.

Keywords: Acetylcholine; electrophysiology; endothelium-dependent hyperpolarization; glibenclamide; isoprenaline; K_{ATP} channel; lymphatic vessel; nitric oxide; smooth muscle

Introduction

Many collecting lymphatic vessels actively propel lymph. This is achieved by the spontaneous constrictions of tubular chambers present along the vessel. Spontaneous constrictions are intrinsic to the lymphatic chambers and are triggered by action potentials generated in the smooth muscle within the vessel wall (Kirkpatrick & McHale, 1977; Allen *et al*., 1983; Van Helden, 1993). Lymphatic pumping can be significantly affected by various factors, which influence the rhythm and amplitude of these spontaneous constrictions. Substances like nitric oxide (NO), released by the endothelium which separates the smooth muscle from the vessel lumen, or the β-adrenoceptor agonist, isoprenaline, have been shown to decrease the frequency of the spontaneous constrictions and to hyperpolarize membrane potential of the lymphatic smooth muscle *via* the activation of K^+ conductances (Allen *et al*., 1986; von der Weid *et al*., 1996; von der Weid & Van Helden, 1996). Hyperpolarizations evoked by NO and isoprenaline were shown to be mediated by an increase in the intracellular concentration of cyclic monophosphate nucleotides. The NO-dependent hyperpolarizations were induced *via* the accumulation of cyclic GMP, an effect blocked by methylene blue (von der Weid *et al*., 1996). In contrast, isoprenaline-induced hyperpolarization was mimicked by forskolin and blocked by H89, a cyclic AMP-dependent protein kinase (PKA) inhibitor (von der Weid & Van Helden, 1996), indicating that the response was dependent on an increase in cyclic AMP synthesis and a consequent activation of PKA.

The aim of the present study was to determine the nature and the intracellular pathways of activation of the K^+ channels involved in these hyperpolarization responses. The results show that NO-mediated hyperpolarizations were blocked by glibenclamide, an inhibitor of ATP-sensitive K^+ (K_{ATP}) channels. Interestingly, the smooth muscle hyperpolarizations induced by isoprenaline and forskolin, were also blocked by glibenclamide. This suggests that K_{ATP} channel activation can be achieved by at least two different intracellular pathways and that these channels might play an important role in the modulation of lymphatic pumping.

Methods

Tissue preparation

Guinea pigs (4–15 days of age) of either sex were killed by overexposure to the inhalation anaesthetic, halothane (5–10%), followed by decapitation. Small collecting lymphatic vessels (diameter <230 μM) supplying the jejunum and ileum were dissected together with their associated artery and vein and left intact within the surrounding mesentery. The mesentery was used to pin out the tissues on the Sylgard-coated base of a small organ bath (volume 100 μl) mounted on

[1]Author for correspondence at: Vascular Cell Physiology, Department of Zoology and Animal Biology, University of Geneva, Sciences III, quai Ernest Ansermet 30, CH-1211 Geneva 4, Switzerland.

the stage of an inverted microscope (Nikon TMS). The tissue was continuously superfused at a flow rate of 3 ml min^{-1} with a physiological saline, heated to 34–36°C, of the following composition (mM): $CaCl_2$, 2.5; KCl, 5; $MgCl_2$, 2; NaCl, 120; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose, 11. The pH was maintained at 7.4 by constant bubbling with 95% O_2/5% CO_2.

Electrophysiology

Resting membrane potential (RMP) was measured using conventional glass intracellular microelectrodes with resistances of 150–250 MΩ when filled with 0.5 M KCl. Electrodes were connected to an amplifier (Intra 767, WPI, Germany) through an Ag-AgCl half-cell. RMP was monitored on a digital oscilloscope (420, Gould, U.S.A.) and simultaneously recorded on a computer (Power Macintosh 7600/120) *via* an analog-digital convertor (MacLab/8S, ADI, Australia). Impalements of smooth muscle cells were obtained from the adventitial side of a lymphatic vessel which was cut into short segments (125–400 μM) with fine dissecting scissors. Short segments were made in order to ensure simplified electrical properties of the smooth muscle so that electrical activity, even though generated at localized foci within the smooth muscle, produces a similar potential change in all the smooth muscle cells of the segment (Van Helden, 1993).

Chemicals and drugs

Acetylcholine (ACh), 4-aminopyridine (4-AP), 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP), cromakalim, forskolin, glibenclamide, halothane, isoprenaline (±isoproterenol hydrochloride), sodium nitroprusside (SNP) were all purchased from Sigma. ODQ (1H-[1,2,4]oxadiazole [4,3-a]quinoxalin-1-one), H89 (N-[2-(p-bromociannamylamino)-ethyl]-5-isoquinolinesulfonamide-dichloride) and penitrem A were from Alexis Corp. (Laüfelfingen, Switzerland). 8-pCPT-cGMP (8-(4-chlorophenylthio)-guanosine-3′,5′-monophosphate) was from Biolog (Bremen, Germany). 8-Br-cGMP was directly added to the superfusion solution to give a 1 mM final concentration. Forskolin and penitrem A were prepared as a 1 mM stock solution in ethanol and methanol, respectively. H89, ODQ, glibenclamide and cromakalim were diluted in dimethylsulfoxide to give 10 mM stock solutions. After dilution of the drugs to their final concentrations in physiological saline, the diluted vehicles had no effect on the tested responses. All other compounds were prepared as stock solutions in distilled water and appropriately diluted in physiological saline.

Data analysis

The effects of agonists and inhibitors were analysed only when the membrane potential at the begining of the recording period was more negative than −45 mV. In experiments where inhibitors were studied, the agonist to be tested was applied for 1 min first as a control and a second time, about 15 min later while tissues were superfused for at least 10 min with the solution containing the blocker alone at the required concentration. This protocol was usually performed during the same impalement. No significant difference in the amplitude of two successive hyperpolarizations induced by a given agonist applied 15 min apart in the absence of a blocker was observed (see also von der Weid & Van Helden, 1996; von der Weid *et al*., 1996). When the effects of various agonists were tested during the same experiment, the order of application was varied. Experimental data have been expressed as means ± one standard error of the mean (s.e.m.). Statistical significance was assessed using a one-tailed paired Student's *t*-test, with $P<0.05$ being considered significant.

Results

Intracellular microelectrode recordings presented in this study were made in smooth muscle cells from lymphatic segments, 298 ± 15 μm in length and 152 ± 5 μm in diameter ($n=31$; 36 tissues from 30 animals). The mean resting membrane potential (RMP) obtained from 50 recordings in these segments was −51 ± 1 mV. Membrane potential recordings from guinea-pig mesenteric lymphatic segments often display spontaneous transient depolarizations (STDs, Van Helden, 1993) which are believed to be the pacemaker of action potentials causing vessel constriction. STDs were observed in 40 out of the 50 recordings made in this study.

Presence of ATP-sensitive K^+ (K_{ATP}) channels in lymphatic smooth muscle and role in RMP

In order to demonstrate the existence of functional K_{ATP} channels in lymphatic smooth muscle, the effects of cromakalim and glibenclamide were tested. As illustrated in Figure 1a, cromakalim (5 μM) clearly hyperpolarized the smooth muscle. The membrane potential changed from a control value of −56 ± 2 mV to −73 ± 2 mV at the peak of the cromakalim (0.1–10 μM)-induced hyperpolarization ($P<0.001$, $n=12$). In three cases where this was tested, the hyperpolarization was inhibited by glibenclamide (10 μM), with cromakalim hyperpolarizing the smooth muscle membrane potential from −55 ± 1 mV to −75 ± 3 mV in control and from −51 ± 1 mV to −54 ± 4 mV during the application of glibenclamide ($P=0.04$). Comparison of RMP values

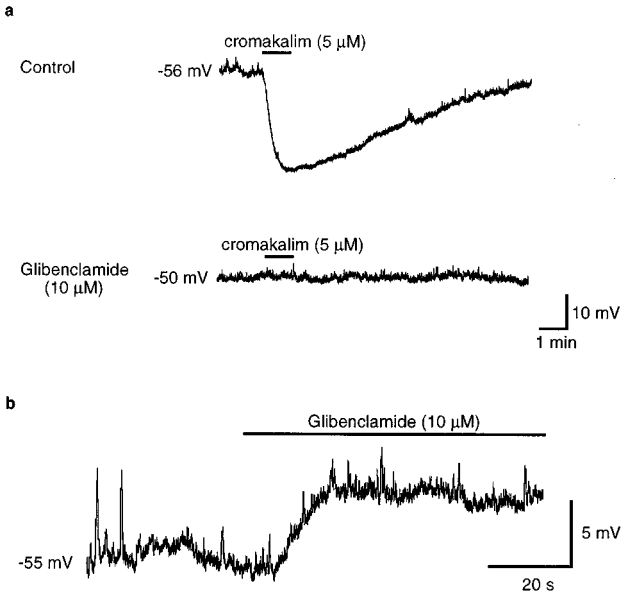

Figure 1 Effect of a K_{ATP} channel opener and blocker on resting membrane potential of smooth muscle in lymphatic vessel. (a) Response to a 1 min application of cromakalim (5 μM) in control conditions (top trace) and in the presence of glibenclamide (10 μM) superfused for 10 min (lower trace). (b) Recording of the change in membrane potential caused by application of glibenclamide (10 μM) in a different cell. Upward deflections appearing in this and the following figure traces are spontaneous transient depolarizations (STDs) which occur in the lymphatic smooth muscle.

shown above revealed that on its own glibenclamide produced a significant depolarization. Overall, glibenclamide depolarized the smooth muscle membrane potential from -51 ± 1 mV to -46 ± 1 mV ($P<0.001$, $n=12$). This is illustrated in Figure 1b (see also Figure 2a,b and 5). The glibenclamide-induced depolarization was associated with a reduction of the amplitude of STDs in seven out of eight experiments where these events were recorded. This is probably due to the membrane potential now closer to the inversion potential for STDs (close to -35 mV, Van Helden, 1993).

Role of K_{ATP} channels in NO-mediated smooth muscle hyperpolarizations

ACh has been shown previously to induce a hyperpolarization in lymphatic smooth muscle which was mediated through the endothelial release of NO and a subsequent increase in the membrane K^+-conductance (von der Weid *et al.*, 1996). To determine whether K_{ATP} channels were involved in this hyperpolarization, the ACh response was tested before and during superfusion with glibenclamide (10 μM). In control conditions, lymphatic smooth muscle membrane potential was hyperpolarized by a 1 min application of ACh (10 μM) from -52 ± 1 mV to a peak value of -62 ± 1 mV. This effect was inhibited by glibenclamide as the membrane potential was changed from -47 ± 2 mV to -48 ± 2 mV in the presence of ACh. This corresponds to an inhibition of $92\pm4\%$ ($P=0.002$, $n=6$; Figure 2a and c). When used at a lower concentration (100 nM), glibenclamide inhibited the ACh-induced hyperpolarization by $56\pm4\%$ ($P=0.006$, $n=5$). Similarly SNP (50-100 μM) which hyperpolarized the smooth muscle from -50 ± 2 mV to -58 ± 1 mV in control conditions, changed the membrane potential from -45 ± 2 mV to -46 ± 2 mV in the presence of 10 μM glibenclamide ($88\pm7\%$ inhibition, $P=0.001$, $n=5$; Figure 2b). The blocking action of glibenclamide against ACh and SNP-induced hyperpolarizations was slowly reversed and hyperpolarizations reached values similar to control after the compound was washed out for up to 1 h.

The ACh-induced hyperpolarization was also inhibited by 4 aminopyridine (4-AP, 2.5 mM) with control response of -12 ± 2 mV decreasing to -1 ± 1 mV in 4-AP, ($97\pm2\%$ inhibition, $P=0.004$, $n=5$; Figure 2c). Similarly, 4-AP blocked the response to SNP from -11 ± 2 mV in control to -2 ± 1 mV in 4-AP ($84\pm13\%$ inhibition, $P=0.03$, $n=4$). 4-AP by itself did not consistently change the RMP (see also von der Weid & Van Helden, 1996).

To test a possible involvement of high conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels in the NO-induced hyperpolarization, Penitrem A, which was recently shown to block BK_{Ca} channels (Knaus *et al.*, 1994) in lymphatic smooth muscle (Cotton *et al.*, 1997) was used. The ACh-induced hyperpolarization was not significantly affected by penitrem A (100–200 nM), with mean values of -10 ± 2 mV in control conditions and -9 ± 3 mV in the presence of penitrem A ($n=4$; Figure 2c). However on its own, penitrem A significantly depolarized the smooth muscle membrane potential from a control value of -46 ± 2 mV to -41 ± 1 mV ($P=0.02$, $n=4$).

Involvement of the soluble guanylyl cyclase-cyclic GMP pathway in NO-induced hyperpolarization

Responses to ACh (10 μM) and SNP (100 μM) were tested in the presence of the soluble guanylyl cyclase inhibitor ODQ (10 μM). As illustrated in Figure 3, both hyperpolarizations were consistently inhibited with ODQ and sometimes converted into a membrane depolarization. The overall ACh-evoked hyperpolarizations reached amplitudes of -9 ± 1 mV and 2 ± 3 mV before and during the application of ODQ ($P=0.02$, $n=4$), respectively and SNP-induced hyperpolarizations were -8 ± 1 mV before and 4 ± 2 mV during application of ODQ ($P=0.007$, $n=4$). The cause of the depolarization to SNP with ODQ sometimes observed was not further investigated here. ODQ applied alone caused a depolarization from -50 ± 2 mV to -42 ± 2 mV ($P=0.002$, $n=7$) and increased STD activity. This was observed each time ODQ was tested (see Figure 3b).

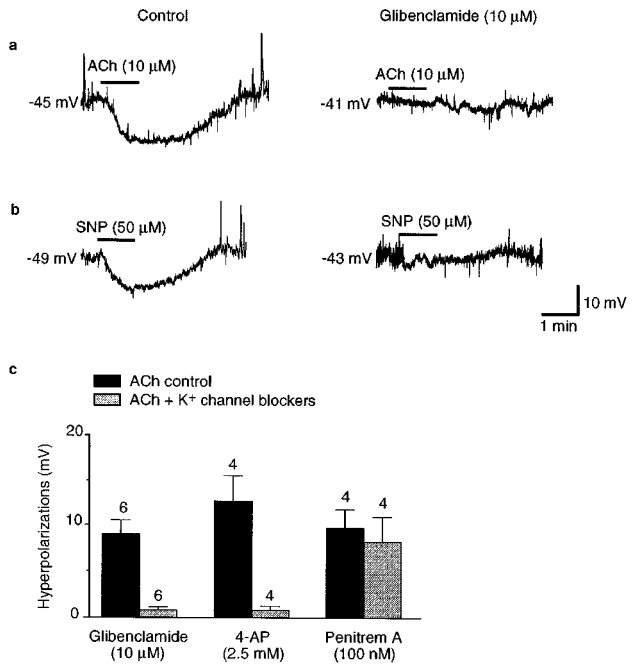

Figure 2 Effect of glibenclamide and other K^+ channel blockers on electrical responses induced by NO. Intracellular recording of the hyperpolarizations induced by ACh (10 μM, a) and SNP (100 μM, b) in control conditions (left traces) and in the presence of glibenclamide (10 μM, right traces). (c) Responses of ACh in control conditions (solid columns) and in the presence (open columns) of glibenclamide (10 μM), 4-AP (2.5 mM) and penitrem A (100 nM). Columns are means$\pm$s.e.mean, with the number of experiments indicated for each column.

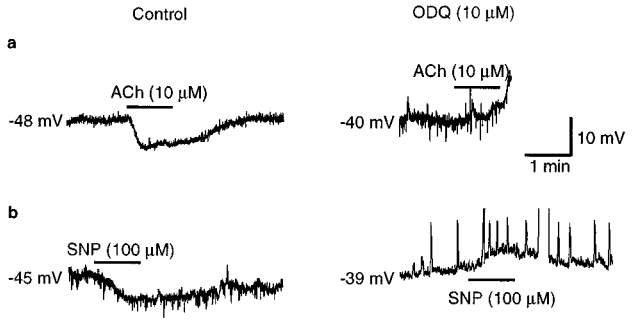

Figure 3 Role of guanylyl cyclase in NO-induced electrical response of lymphatic smooth muscle. Responses induced by ACh (10 μM, a) and SNP (100 μM, b) before (left traces) and in the presence of ODQ (10 μM, right traces). Recordings in (a) and (b) were obtained from two different cells. ODQ in addition to causing depolarization, increased the frequency of occurrence of STDs (right traces). This caused the lost of the impalement shortly after the end of ACh application (a) and the temporary lost of the impalement after application of SNP (b). Scale bars apply to all recordings.

The ability of cyclic GMP to hyperpolarize the lymphatic smooth muscle membrane potential was directly tested by adding membrane permeant analogues of cyclic GMP to the superfusion solution. 8-Br-cGMP, applied for 1–2 min up to a concentration of 1 mM, did not induce any consistant change in the smooth muscle membrane potential and a mean value of -48 ± 3 mV was measured before as well as in the presence of 8-Br-cGMP ($P=0.84$, $n=5$). Similarly, no change in membrane potential was observed with 100 μM 8-pCPT-cGMP, a more lipophilic cyclic GMP derivative. The membrane potential was -51 ± 1 mV before and -52 ± 2 mV during the application of 8-pCPT-cGMP ($P=0.1$, $n=4$). However, when 8-pCPT-cGMP was used at 500 μM, it caused a significant hyperpolarization of 3 ± 1 mV, with membrane potential values of -49 ± 2 mV before and -51 ± 2 mV at the peak of pCPT-cGMP-induced hyperpolarization ($P=0.03$, $n=5$).

Effect of H89 on NO-induced hyperpolarization

In order to determine whether the cyclic GMP increase activates protein kinase G (PKG) or protein kinase A (PKA) to cause K_{ATP} mediated hyperpolarization, ACh and SNP were tested in the presence of H89, a PKA inhibitor (Figure 4). Control hyperpolarizations, which were -10 ± 2 mV with ACh (10 μM) and -6 ± 1 mV with SNP (100 μM), were blocked by $86\pm5\%$ (-1 ± 3 mV, $P=0.001$, $n=4$) and $93\pm7\%$ (1 ± 1 mV, $P=0.006$, $n=4$), respectively, in the presence of H89 (10 μM). The lymphatic smooth muscle membrane potential was also significantly depolarized by H89 from a control value of -51 ± 2 mV to -45 ± 2 mV ($P=0.003$, $n=5$).

Role of K_{ATP} channels on smooth muscle hyperpolarizations caused by isoprenaline and forskolin

Isoprenaline and forskolin have been shown to act directly on lymphatic smooth muscle cells where they induced consistent hyperpolarizations through the activation of PKA (von der Weid & Van Helden, 1996). In the present experiments, isoprenaline hyperpolarized the smooth muscle from a membrane potential of -52 ± 2 mV to -62 ± 1 mV. In the presence of glibenclamide (10 μM), isoprenaline-induced hyperpolarization was inhibited by $89\pm5\%$ ($P=0.02$, $n=4$), with the membrane potential changing from -46 ± 2 mV to -47 ± 2 mV in the presence of isoprenaline (Figure 5a). Similarly, forskolin, which hyperpolarized the smooth muscle from -53 ± 2 mV to -64 ± 1 mV under control conditions, changed the membrane potential from -46 ± 2 mV to -47 ± 2 mV in the presence of glibenclamide ($95\pm3\%$ inhibition, $P=0.01$, $n=5$; Figure 5b).

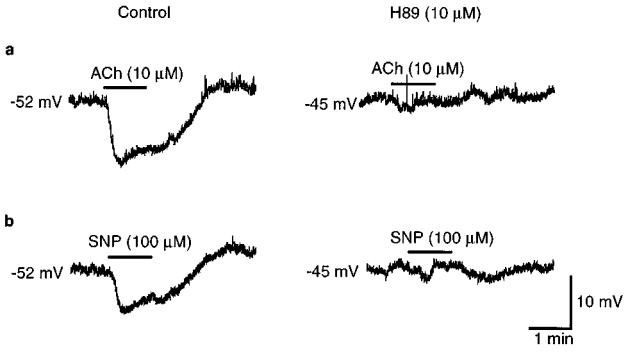

Figure 4 Effect of H89 on hyperpolarizations induced by NO in lymphatic smooth muscle. ACh (10 μM, a) and SNP (100 μM, b) were added before (left traces) and in the presence of H89 (10 μM, right traces) superfused for at least 10 min. Intracellular recordings were obtained from a single impalement.

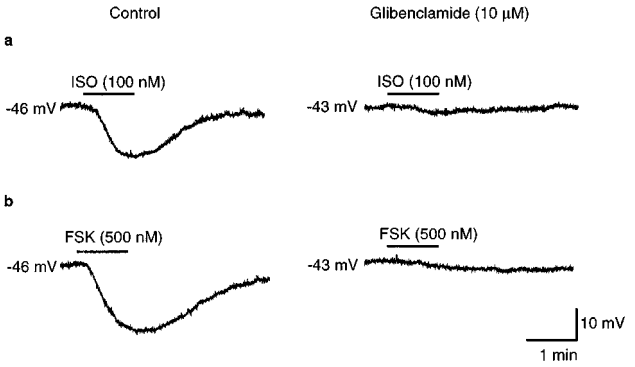

Figure 5 Effect of glibenclamide on hyperpolarizations induced by isoprenaline (ISO, a) and forskolin (FSK, b) in lymphatic smooth muscle. Intracellular recordings obtained from a single impalement show that responses to isoprenaline and forskolin applied for 1 min (left traces) are inhibited by glibenclamide (10 μM, right traces).

Discussion

The current study demonstrates the existence of functional K_{ATP} channels in lymphatic smooth muscle cells from the guinea-pig mesentery and shows that these channels are responsible for the hyperpolarization induced by nitric oxide and for the main part of the hyperpolarization caused by β-adrenoceptor agonists.

Role of K_{ATP} channels in modulation of RMP

In the present experiments, the lymphatic smooth muscle RMP was depolarized by about 5 mV during the application of glibenclamide. This finding confirms previous observations made in the same tissue that K_{ATP} channels together with inward rectifier K^+ channels contribute to lymphatic smooth muscle RMP (von der Weid & Van Helden, 1997). K_{ATP} channels have also been shown to contribute to the RMP of other smooth muscles (see review by Quayle *et al.*, 1997).

Role of K_{ATP} channels in NO-induced hyperpolarization

Spontaneous constrictions of the lymphatic smooth muscle have been shown to be modulated by the lymphatic endothelium (Yokoyama & Ohhashi, 1993; Reeder *et al.*, 1994; von der Weid *et al.*, 1996). In particular, stimulation of guinea-pig mesenteric lymphatic vessels with ACh causes the endothelial release of NO which slows down lymphatic pumping. This effect is accompanied by an ACh/NO-induced hyperpolarization of the smooth muscle due to an increase in cyclic GMP synthesis (von der Weid *et al.*, 1996). The involvement of cyclic GMP was confirmed in the present study by experiments in which the soluble guanylyl cyclase inhibitor ODQ (Garthwaite *et al.*, 1995) blocked the hyperpolarization induced by NO, released either by the endothelium after ACh stimulation or by SNP.

NO and nitrovasodilators have been shown to diversely affect membrane potential in vascular smooth muscle with no

changes observed in the majority of the preparations studied. However, in some vascular smooth muscle, a hyperpolarization to NO and nitrovasodilators was described (see reviews by Bény & von der Weid, 1993; Nagano & Vanhoutte, 1993; Garland *et al*., 1995). To account for this hyperpolarization, NO was thought to activate different types of K^+ channels. Among them, BK_{Ca} channels were shown to be activated by NO either directly (Bolotina *et al*., 1994) or indirectly *via* a cyclic GMP-dependent protein kinase (Khan *et al*., 1993; Robertson *et al*., 1993). K_{ATP} channels often have been proposed to underlie the hyperpolarization to NO and nitrovasodilators. This proposal was based on the observations that NO-induced hyperpolarizations were inhibited by glibenclamide in mesenteric arteries of the rat (Garland & McPherson; 1992) and the rabbit (Murphy & Brayden, 1995) and in pig coronary smooth muscle cells in culture (Miyoshi *et al*., 1994).

The ACh/NO-induced hyperpolarization of lymphatic smooth muscle has been shown to be due to increased membrane K^+ conductance (von der Weid & Van Helden, 1996). In the present study, the ACh/NO-induced hyperpolarization was inhibited by glibenclamide. This finding strongly suggests the involvement of K_{ATP} channels. Moreover, Penitrem A, a selective inhibitor of BK_{Ca} channels (Knaus *et al*., 1994) which significantly depolarized lymphatic smooth muscle (present result), was without effect in reducing the ACh/NO-induced hyperpolarization, suggesting that BK_{Ca} channels played little role in this response. However, penitrem A has been shown to block BK_{Ca} channels in sheep mesenteric lymphatic smooth muscle (Cotton *et al*., 1997) and inhibit the BK_{Ca} channel-mediated transient hyperpolarization induced by ACh in endothelium of the lymphatic preparation used in the present study (unpublished result). The ACh/NO-induced hyperpolarization was also shown here to be partially blocked by 4-AP. This effect may be explained by a blocking action of 4-AP on K_{ATP} channels as has been reported in isolated smooth muscle cells from rabbit cerebral arteries (Kleppish & Nelson, 1995). However in smooth muscle, 4-AP is known to mainly block delayed rectifier K^+ channels. Glibenclamide has also been shown to non-selectively block other K^+ channels and in particular delayed rectifier K^+ channels, however mostly at concentrations higher than those used in the present study (Beech *et al*., 1993).

Role of protein kinase A in NO-induced hyperpolarization

The cyclic GMP-PKG pathway was thought to be involved in the NO-induced hyperpolarization. Although, a role for PKG has been clearly demonstrated in the NO-activation of BK_{Ca} channels (Robertson *et al*., 1993; Taniguchi *et al*., 1993), specific investigation to assess an involvement of PKG in K_{ATP} channels activation has not been made. On the other hand, it is well known from studies using various vasodilators that K_{ATP} channels may be activated *via* PKA stimulation (see review by Quayle *et al*., 1997). The cyclic nucleotide binding sites regulating PKA and PKG are not perfectly selective for their respective nucleotides. This may lead to cross-activation of PKG by cyclic AMP or PKA by cyclic GMP if the cyclic nucleotide reaches a high enough intracellular concentration (see Lincoln & Cornwell, 1991; Schmidt *et al*., 1993). Such a cross-activation mechanism would explain the present result where NO-induced increase in cyclic GMP and activation of K_{ATP} channels was blocked with H89. H89 is considered a relatively specific blocker of PKA as its inhibition constant is ten times lower for PKA than PKG (Hidaka & Kobayashi, 1992). It was also shown to selectively inhibit PKA activity without affecting PKG activity up to a concentration of 10 μM in gastric smooth muscle cells from rabbit and guinea-pig (Murthy & Makhlouf, 1995). Moreover, H89 was shown to block PKA-induced activation of $^{86}Rb^+$ efflux through K_{ATP} channels opened by P1075 in rat aorta (Linde & Quast, 1995), adenosine activation of K_{ATP} currents in rabbit mesenteric artery (Kleppisch & Nelson, 1995) and K_{ATP} plus BK_{Ca} channels after stimulation with iloprost in pressurized rat tail small artery (Schubert *et al*., 1997). The current hypothesis is that ACh/NO hyperpolarizes lymphatic smooth muscle through activation of guanylyl cyclase which produces enough cyclic GMP to stimulate PKA and the subsequent opening of K_{ATP} channels. The suggestion that high concentration of cyclic GMP is needed to stimulate the PKA-induced hyperpolarization is further supported by the observation that only a concentration of 500 μM of 8-pCPT-cGMP was able to induce a response, whereas 100 μM 8-pCPT-cGMP or 8-Br-cGMP up to a concentration of 1 mM were without effect. By comparison, hyperpolarizations were usually observed when 100 μM of either 8-Br-cAMP or 8-CPT-cAMP were applied (von der Weid & Van Helden, 1996).

Role of K_{ATP} channels in β-agonist-induced hyperpolarization

H89 has previously been used in guinea-pig mesenteric lymphatic vessel to demonstrate that activation of β-adrenoceptors by isoprenaline produces a hyperpolarization of the lymphatic smooth muscle through a PKA-mediated activation of a K^+ conductance (von der Weid & Van Helden, 1996). Moreover, PKA was shown to be directly activated by cyclic AMP, as forskolin also induced a hyperpolarization which was blocked by H89. BK_{Ca} channels were suggested to have a small involvement as 15% of the response to isoprenaline was significantly blocked by iberiotoxin. Hyperpolarizations were clearly inhibited (56%) in the presence of 4-AP (2.5 mM), supporting the hypothesis that K^+ conductances other than BK_{Ca} played a role (von der Weid & Van Helden, 1996). One striking observation made in the present study is that, in addition to blockade of the ACh/NO-induced hyperpolarization, glibenclamide inhibited the response to isoprenaline by 85%, suggesting that K_{ATP} channels also played a major role in β-agonist-induced hyperpolarization. This result is consistent with recent observations made in vascular smooth muscle of the canine saphenous vein (Nakashima & Vanhoutte, 1995) and of the rat isolated mesenteric arterial bed (Randall & McCulloch, 1995) and in pig coronary artery (Wellman *et al*., 1998).

In conclusion, K_{ATP} channels appear to play a major role in lymphatic smooth muscle of the guinea-pig mesentery. They are involved in the establishment of the resting membrane potential and in hyperpolarizations caused by NO and isoprenaline. Although both agonists act through the production of different cyclic nucleotides, they both open K_{ATP} channels by activating PKA. It is suggested that PKA can be stimulated either by cyclic AMP or by high concentrations of cyclic GMP.

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