Calcium-Activated Potassium Channels and Nitrate-Induced Vasodilation in Human Coronary Arteries

ROSTISLAV BYCHKOV, MAIK GOLLASCH, TOBIAS STEINKE, CHRISTIAN RIED, FRIEDRICH C. LUFT and HERMANN HALLER

Franz Volhard Clinic and the Max-Delbrück Center for Molecular Medicine (R.B., M.G., T.S., C.R., F.C.L., H.H.), Virchow University Hospitals, I. Medical Clinic (R.B., T.S.), Charité University Hospital, Humboldt University of Berlin, Berlin, Germany

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ABSTRACT

In some but not all arterial beds, smooth muscle cell calcium-activated K⁺ channels (KCa channels) play a central role in the mediation of the vasodilator response to nitric oxide (NO) and other nitrates. We investigated the effect of nitrates on KCa channels in the relaxation of human coronary arteries by means of isometric contraction experiments in arterial rings. We also measured whole-cell currents in freshly isolated human coronary artery vascular smooth muscle cells via the patch-clamp technique. Sodium nitroprusside, diethyamine-nitrile oxide complex sodium salt and isosorbide mononitrate completely relaxed rings preconstricted with 5 μM serotonin and produced dose-dependent relaxations of 5 μM serotonin-preconstricted human rings. The relaxations were inhibited by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-oxyl 3-oxide (10 μM)

which neutralizes nitric oxide. The KCa channel blockers iberiotoxin (100 nM) and tetraethylammonium ions (1 mM) significantly inhibited SNP-induced relaxations of human coronary arteries. Moreover, in the patch-clamp experiments, SNP (1 μM) stimulated KCa currents and spontaneous transient outward K⁺ currents carried by Ca spark activated KCa channels. The SNP-induced (1 μM) KCa current was strongly inhibited by iberiotoxin (100 nM). These data show that activation of KCa channels in smooth muscle cells contributes to the vasodilating actions of nitrates and nitric oxide in human coronary arteries. This finding may have unique clinical significance for the development of antianginal and antihypertensive drugs that selectively target K⁺ channels and Ca sparks.

Nitrovasodilators are frequently used for treatment of coronary heart disease and heart failure. By releasing NO either spontaneously (e.g., sodium nitroprusside) or after both enzymic and nonenzymic metabolism (e.g., isosorbide mononitrate, isosorbide dinitrate and molsidomine), these agents induce relaxation of coronaries and other arteries (Taniguchi et al., 1993; Khan et al., 1993; Archer et al., 1994). The precise mechanism by which NO and other nitrovasodilators cause relaxation of smooth muscle remains to be defined and probably involves multiple mechanisms. Membrane hyperpolarization has been invoked as an important mechanism for the relaxation produced by nitrates in some, but not all, arterial beds (Tare et al., 1990). K⁺ channel activity is the main determinant of membrane potential in smooth muscle cells, and K⁺ efflux resulting from K⁺ channel opening causes hyperpolarization, inhibits voltage-dependent Ca²⁺ channels and promotes relaxation (Nelson et al., 1990; Nelson and Quayle, 1995; Gollasch et al., 1992). Recent evidence suggests that NO as well as other nitrovasodilators can activate large-conductance KCa channels (Robertson et al., 1993; Taniguchi et al., 1993; Miyoshi and Nakaya, 1994), which may contribute to vessel relaxation (Williams et al., 1988; Taniguchi et al., 1993; Hecker et al., 1995).

KCa currents and STOCs have been identified in many types of smooth muscle, including human coronary artery vascular smooth muscle cells (Gollasch et al., 1996). These currents are carried by KCa channels. KCa currents are activated by submicromolar Ca²⁺ as well as by membrane depolarization and are blocked by external tetraethylammonium ions and iberiotoxin (Golasch et al., 1996). STOCs are generated by spontaneous Ca²⁺ (calcium sparks) released through ryanodine-sensitive Ca²⁺ channels of the sarcoplasmic reticulum (Nelson et al., 1995). Recently, we were able to show that STOCs are present in human coronary arteries and that Ca²⁺ entry into the cell through reverse mode

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Present address: Department of Pharmacology, University of Vermont Medical Research Facility, 55A South Park Drive, Colchester, VT 05446.

ABBREVIATIONS: EGTA, ethyleneglycol bis(oxyethylendinitril)tetra-acetic acid; DMSO, dimethylsulfoxide; KCa channel, calcium-activated K⁺ current; Kdp, delayed rectifier K⁺ channel; Ktp, channel, ATP-dependent K⁺ channel; PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazolino-oxyl 3-oxide; DEA-NO, diethyamine-nitric oxide complex sodium salt; SNP, sodium nitroprusside; STOC, spontaneous transient outward K⁺ current; IMN, isosorbide mononitrate; NO, nitric oxide.
Na\(^+\)/Ca\(^++\) exchanger determines calcium store refilling, which in turn regulates the generation of STOCs in human coronary vascular smooth muscle cells (Bychkov et al., 1997). Whether or not nitrosodilators affect K_{ca} current and STOCs in human coronary smooth muscle is unclear. We present the first direct evidence that nitrosodilators can activate K_{ca} currents and STOCs in human coronary arteries. We show that activation of K_{ca} channels contributes to the vasorelaxing action of these drugs.

Materials and Methods

Coronary preparations. Human coronary arteries were obtained from patients with dilatative cardiomyopathy, but without significant atherosclerosis, after orthotopic heart transplantation. The tissue was immediately placed in cold (8°C) Hanks’ solution (119 NaCl, 4.7 KCl, 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 1.2 MgSO\(_4\), 11.1 glucose, 0.026 EDTA, 2.5 CaCl\(_2\) mM, 5% CO\(_2\)-95% O\(_2\)) during transportation. The tissue was immediately placed in cold (8°C) Hanks’ solution (119 NaCl, 4.7 KCl, 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 1.2 MgSO\(_4\), 11.1 glucose, 0.026 EDTA, 2.5 CaCl\(_2\) mM, 5% CO\(_2\)-95% O\(_2\)) during transportation. In some experiments, the endothelium was removed by gentle scrubbing of the lumen with a steel wire (diameter 0.6 mm) and washed with Hanks’ solution (119 NaCl, 4.7 KCl, 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 1.2 MgSO\(_4\), 11.1 glucose, 0.026 EDTA, 2.5 CaCl\(_2\) mM, 5% CO\(_2\)-95% O\(_2\)). The tissue was immediately placed in cold (8°C) Hanks’ solution (119 NaCl, 4.7 KCl, 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 1.2 MgSO\(_4\), 11.1 glucose, 0.026 EDTA, 2.5 CaCl\(_2\) mM, 5% CO\(_2\)-95% O\(_2\)). One of the two wires was connected to a F-30 force transducer (Hugo Sachs, Freiburg, FRG) for isometric tension recordings. The other output from the transducer was displayed on a strip chart recorder. The arterial segments were stretched in a stepwise manner to preload of approximately 2 g. The organ baths were continuously bubbled with carbogen (5% CO\(_2\)-95% O\(_2\)) to provide oxygenation and pH of 7.4. The temperature was maintained at 37°C. After equilibration for 1 h, the isometric contraction was measured. The contractile capacity of the arterial segments was assessed by changing the bath solution to an isotonic 50 mM K\(^+\) solution with the following composition (in mM): 75.0 NaCl, 48.8 KCl, 1.2 KH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), 1.2 MgSO\(_4\), 11.1 glucose, 0.026 EDTA, 2.5 CaCl\(_2\) (5% CO\(_2\)-95% O\(_2\)). In some experiments, the endothelium was removed by gentle scrubbing of the lumen with a stainless steel rod (Gollasch et al., 1995).

Isolation of smooth muscle cells. Vascular smooth muscle cells were isolated as previously described (Gollasch et al., 1995, 1996). The vessels were cut into small segments (about 3 mm in length) and placed in a Ca\(^+\)\(^+\)-free Hanks’ solution containing (in mM) 137 NaCl, 5.4 KCl, 0.44 KH\(_2\)PO\(_4\), 0.42 NaH\(_2\)PO\(_4\), 2 MgCl\(_2\), 0.05 Ca\(^++\), 11.11 glucose, 10 HEPEs, pH adjusted to 7.4 with NaOH) for 2 to 10 min at room temperature (20°C–24°C). The segments were then placed in the Ca\(^+\)\(^+\)-free solution containing 2 mg/ml collagenase (Sigma type IA; Sigma, Deisenhofen, FRG), 10 mg/ml bovine serum albumin (BSA) and 0.5 mg/ml elastase (Sigma type IIA; Sigma, Deisenhofen, FRG) and were incubated for 40 min with gentle agitation at 36°C. After the digestion was complete, single cells were dispersed by gentle agitation in the Ca\(^+\)\(^+\)-free Hanks’ solution.

K\(^+\) current recordings. Whole-cell K\(^+\) currents were measured according to the conventional patch-clamp method of Hamill et al. (1981) (for details see Gollasch et al., 1991, 1993) or using the perforated patch method with nystatin (Gollasch et al., 1996). Cells were held at –80 mV, and linear voltage-ramp pulses at 0.67 V/s from −100 mV to +100 mV or 500-ms depolarizing step pulses to different voltages were applied (stimulation frequency, 0.5 Hz). The membrane capacity was 37 ± 3.8 pF (mean ± S.E.M., n = 16). The external solution E1 contained (in mM) 140 NaCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5.4 KCl, 0.1 CaCl\(_2\), 10 glucose and 10 Na-HEPES (pH 7.4). The patch pipette (resistance, 4–8 M\(_{\Omega}\)) was filled with a solution containing (in mM) 80 K-aspartate, 50 KCl, 1 MgCl\(_2\), 3 Mg-ATP, 10 EGTA, 5 K-HEPES (pH 7.4). If not otherwise indicated, experiments were done at room temperature (20°C–24°C). Nystatin (Sigma, Deisenhofen, FRG) was dissolved in DMSO and diluted into the pipette solution to give a final concentration ranging from 50 to 100 µg/ml. Whole-cell access was achieved by nystatin within 10 to 20 min of seal formation. Whole-cell currents were recorded using a List EPC-7 or an Axopatch 200A amplifier, digitized at 10 kHz using a CED1401 interface (Cambridge Electronic Design Limited, Cambridge, UK) and analyzed using CED Patch and Voltage Clamp Software Version 6.08.

Materials. Iberiotoxin and DEA-NO were obtained from RBI (Natick, MA). PTIO was purchased from (Sigma, FRG). Sodium nitroprusside was obtained from Sigma (Deisenhofen, FRG). Isosorbide mononitrate was a gift from Astra GmbH (Wedel, FRG). Stock (10 mM) solutions of PTIO were made using DMSO as the solvent.

Statistical analysis. All values are given as mean ± S.E.M.; n represents the number of arterial rings or cells tested. The Wilcoxon rank sum test or the Mann-Whitney-Wilcoxon test was used to determine significant differences. Comparisons of dependent samples were done using one-way analysis of variance and Bonferroni’s inequality (Wallenstein et al., 1980). A value of P < 0.05 was considered significant. The terms increase and decrease are employed only when the results were statistically significant. All contraction experiments examining the effects of iberiotoxin and PTIO on nitrovasodilator relaxation were conducted on coronary arteries from different patients.

Results

Relaxant effects of SNP, DEA-NO and IMN on human coronary arteries. The effects of the nitrosodilators SNP, DEA-NO and IMN on human coronary artery rings are shown in figure 1. Serotonin 5 µM was given in a sustained fashion over 15 min. The characteristic sustained contractions of human vessels by serotonin were observed. The same serotonin concentrations were found to constrict intact human coronary arteries (McFadden et al., 1991). In addition, we have previously shown that 5 µM serotonin induced sustained contractions, mediated primarily by Ca\(^+\)\(^+\) influx through voltage-dependent Ca\(^+\)\(^+\) channels. These contractions did not decrease within 30 to 40 min (Gollasch et al., 1995) in all investigated arteries. SNP, DEA-NO and IMN were added at concentrations ranging from 10 nM to 100 µM. All three nitrosodilators induced a dose-dependent decrease in vascular tone. Figure 1A shows a preconstricted human coronary artery exposed to increasing doses of SNP. The stepwise relaxation is apparent. Half-maximal relaxation obtained by fit was about 0.72 ± 0.09 µM SNP (IC50 = n = 8; fig. 1B). The Hill coefficient (nH) was 0.95 ± 0.05. The SNP effect was completely reversed with washout. DEA-NO and IMN induced half-maximal relaxation of human coronary arteries at 35.1 ± 5.0 µM (n = 5) and 17.9 ± 3.0 µM (n = 5), respectively (fig. 1C). The Hill coefficients of DEA-NO and IMN dependent relaxation were 1.52 ± 0.23 and 0.80 ± 0.11, respectively. We then repeated these experiments (n = 5) with the endothelium removed from human coronary arteries. Half-maximal relaxation was observed at 0.67 ± 0.08 µM SNP, which was not different from when the endothelium was present. The Hill coefficient was 0.90 ± 0.06. We next studied the effects of NO neutralization. PTIO is known to neutralize NO in biological systems specifically and directly via a unique radical-radical reaction with NO (Miyoshi and...
Dose-dependent relaxation of human coronary arteries with SNP, DEA-NO and IMN in vessels precontracted with serotonin. Effects of PTIO, iberiotoxin and tetraethylammonium ions on SNP relaxation. A) Dose-dependent relaxation of human coronary arteries with SNP in vessels without (upper trace) and with pretreatment with iberiotoxin (lower trace). The presence of serotonin (5-HT, 5 μM), iberiotoxin (IBTX, 100 nM) and SNP (cumulative doses) in the bath is indicated by horizontal bars. Doses of half-maximal relaxation (IC_{50}) and Hill coefficients (n_{H}) were calculated by fitting the data with the equation:

\[ T = (B_0 - B_\infty)/(1 + [(D)/IC_{50}^H]^n_H) + B_\infty \]

where \( T \) is tension in response to SNP, DEA-NO or IMN; \( B_0 \) is maximum response induced by SNP, DEA-NO or IMN; \( B_\infty \) is a constant; \( IC_{50} \) is the dose of SNP, DEA-NO or IMN that elicits a half-maximal response and \( D \) is the dose of SNP, DEA-NO or IMN. The symbols represent means ± S.E.M. Tension is expressed as a percentage of the steady-state tension (100%) obtained with 5 μM serotonin before administration of the drugs. Panel B) Cumulative dose-response curve for relaxation induced by SNP and by SNP plus PTIO (SNP + PTIO). IC_{50} and n_{H} were obtained by fit and were IC_{50} = 0.72 ± 0.09 μM, n_{H} = 0.95 ± 0.05 (n = 8) and IC_{50} = 4.04 ± 0.20 μM, n_{H} = 0.95 ± 0.03 (n = 4) for SNP and SNP + PTIO, respectively. Cumulative dose-response curve for relaxation induced by SNP in the presence of 100 nM iberiotoxin (SNP-IBTX) or 1 mM tetraethylammonium chloride (SNP-TEA). Tension is expressed as a percentage of the steady-state tension (100%) obtained with 5 μM serotonin before administration of SNP. In arteries pretreated with IBTX or TEA, SNP was added cumulatively. IC_{50} and n_{H} were obtained by fit and were IC_{50} = 17.9 ± 3.0 μM, n_{H} = 0.80 ± 0.09 (n = 5) for DEA-NO and IMN, respectively.

Fig. 1. A) Dose-dependent relaxation of human coronary arteries with SNP, DEA-NO and IMN in vessels precontracted with serotonin. Effects of PTIO, iberiotoxin and tetraethylammonium ions on SNP relaxation. B) Cumulative dose-response curve for relaxation induced by SNP and by SNP plus PTIO (SNP + PTIO). C) Cumulative dose-response curve for relaxation induced by DEA-NO and IMN in vessels precontracted with serotonin. Effects of PTIO, iberiotoxin and tetraethylammonium ions on SNP relaxation.

Effect of \( K_{Ca} \) channel blockers on relaxation. Figure 1 shows the effect of the \( K_{Ca} \) channel blocker iberiotoxin on SNP-dependent relaxation in 5 μM serotonin-preconstricted human coronary arteries with intact endothelium. Iberiotoxin 100 nM is known to block \( K_{Ca} \) channels completely in human coronary artery vascular smooth muscle cells (Gollasch et al., 1996; Bychkov et al., 1997). Iberiotoxin elevated the sustained phase of serotonin-induced contraction. After pretreatment with 100 nM iberiotoxin, SNP relaxed rings preconstricted with 5 μM serotonin but produced half-maximal relaxation of arteries at significantly higher doses than without the presence of the \( K_{Ca} \) channel blocker. Half-maximal relaxation at 7.20 ± 0.30 μM SNP (n = 8) was observed in the presence of iberiotoxin (100 nM) in human rings. We next administered tetraethylammonium ions, which block \( K_{Ca} \) channels in human coronary artery smooth muscle cells (concentration of half-block \( K_{Ca} \), 0.2 mM; Gollasch et al., 1996). Tetraethylammonium decreased the cumulative relaxation to SNP. In the presence of 1 mM tetraethylammonium, half-maximal relaxation of human coronary arteries was observed at 7.09 ± 0.21 μM SNP (n = 4; fig. 1B).

SNP-induced stimulation of \( K_{Ca} \) current in coronary myocytes. To provide direct evidence that nitrovasodilators open \( K^+ \) channels and hyperpolarize human coronary arterial myocytes, we measured transmembrane \( K^+ \) currents with the patch-clamp technique on single smooth muscle cells from human coronary arteries. The currents were recorded using a high-\( K^+ \) dialyzing pipette solution (11). Interfering currents through voltage-dependent \( Ca^{2+} \) channels were blocked by 100 μM \( Ca^{2+} \). The current-voltage relationships (I-V curve) of outward currents were investigated using step depolarizations in the whole-cell (wc) configuration or perforated patch (pp) configuration with nystatin. Depolarizing step pulses of voltage (duration, 400 ms) were applied from a holding potential of −80 mV as shown in figure 2. The first detectable outward current was observed when the voltage ramp reached approximately −40 mV. For voltages positive to this value, the magnitude of the outward current increased, and at very positive potentials (> +40 mV), the current became very noisy. We have previously shown that the total outward currents were due to \( K^+ \) currents through both \( K_{in} \) channels and \( K_{Ca} \) channels (Gollasch et al., 1996).

In 19 of 24 human coronary vascular smooth muscle cells, SNP (1 μM) increased noisy outward currents elicited by step depolarization to voltages between −40 mV and +80 mV (n_{pp} = 10, n_{wc} = 9; fig. 2A). The SNP-induced current was reversible after removal of the drug from the bath and was not inactivating during 400-ms step pulses (fig. 2, A and B). In the presence of SNP, the outward current was increased by 99 ± 7% at +50 mV (n = 19). We used another protocol to demonstrate the activation of potassium currents at the range of −50 mV to 0 mV that corresponds to membrane potentials observed in coronary myocytes (Bychkov et al., 1997). When the voltage ramp was changed linearly from −100 mV to 0 mV, induced potassium currents were recorded under higher gains than with the previous protocol (fig. 2C).
The digitized points of the recorded current were plotted against the corresponding voltage. The threshold of SNP-induced potassium current was $-37 \pm 5$ mV. The mean values of the control current were subtracted from the mean values of the SNP-induced current (fig. 2D). The difference showed strong voltage dependence, and the threshold of activation was about $-35$ mV.

Inactivation of the SNP current was studied using a double-pulse protocol. The degree of inactivation was assessed by examining the peak outward current at a test potential of +50 mV after holding the membrane (preconditioning) potential at voltages between $-80$ and $+80$ mV for 15 s. The peak outward current should be proportional to the degree of inactivation that occurred during the preconditioning potential. As shown in figure 3 (filled symbols), membrane depolarization increased inactivation as the availability of the current for activation decreased. Half-maximal inactivation ($V_{0.5}$) was at $-28.7 \pm 2.9$ mV and increased as much as $e$-fold per $8.0 \pm 1.7$ mV (steepness factor, $k$). These parameters are characteristic for the $K_{\text{Ca}}$ current contributing to the total outward $K^+$ current (Gollasch et al., 1996). The SNP-induced current showed no or very little inactivation. In the presence of 1 $\mu$M SNP, the outward $K^+$ current (open symbols) showed half-maximal inactivation at $-29.0 \pm 1.7$ mV and steepness factor $8.2 \pm 1.2$ mV. Iberiotoxin 100 nM inhibited the SNP-stimulated outward $K^+$ current and had no effect on $V_{0.5}$ and $k$ ($n = 4$). This finding indicated that Ca$^{2+}$-dependent potassium channels mediated mainly the nonactivating part of the current evoked by SNP.

The $K_{\text{Ca}}$ current was inactivated by a double pulse protocol in following experiments. The membrane potential was first held at 0 mV to inactivate $K_{\text{Ca}}$. The potassium current induced by the second pulse, applied immediately at 50 mV, was highly sensitive to iberiotoxin, as shown in figure 4A. SNP (1 $\mu$M) induced a large, noisy outward current. The increase in current was $96 \pm 9\%$ ($n = 12$). This current and the current before SNP administration were completely blocked by 100 nM iberiotoxin (current decrease to $7 \pm 2\%$ of control values, $n = 5$). In contrast, glibenclamide (3 $\mu$M), which blocks ATP-dependent K$^+$ channels in human coronary artery smooth muscle cells (Gollasch et al., 1996), had no effect on SNP-induced current (fig. 4C, $n = 5$). The voltage sensitivity, properties of inactivation, iberiotoxin sensitivity and glibenclamide insensitivity of the SNP-induced current suggest activation of $K_{\text{Ca}}$ channels in human coronary artery smooth muscle cells.

We next tested the effects of SNP on STOCs. Because STOCs are induced by spontaneous Ca$^{2+}$ release events (Ca sparks) through ryanodine-sensitive Ca$^{2+}$ release channels of the sarcoplasmic reticulum (Nelson et al., 1995), we tested the effects of SNP on STOCs using the perforated patch configuration. The intracellular Ca$^{2+}$ milieu remained unchanged under these conditions. STOCs were recorded under steady-state conditions and under step-pulse protocol from a holding potential of $-50$ mV to $-20$ mV with pulse duration of 5 s. Single STOCs had an asymmetrical bell shape with a fast upstroke and a decay phase that declined two- or three-exponentially. STOCs had different amplitudes, which indicates that single STOCs could represent the result of multiple elementary events. Several STOCs of the same amplitude or different amplitudes were observed and formed complex STOCs with different shapes, as shown in figure 5A. SNP (1 $\mu$M; $n = 7$) had a large stimulatory effect, increasing mainly the frequency of STOCs (fig. 5B). Statistical analysis of the shape and duration of the STOCs in the presence of SNP was limited by the finding that the number of complex STOCs increased under administration of SNP. STOCs, recorded within 2 min under control conditions and after SNP application, are shown in figure 5B. STOCs and SNP-stimulated STOCs were completely blocked by 100 nM iberiotoxin ($n = 5$).
We provide the first direct evidence that both KCa currents and STOCs in human coronary artery smooth muscle cells can be opened by nitrovasodilators. Furthermore, we demonstrate that opening of these channels is essential to relaxation by nitrovasodilators. We therefore propose the following cascade of events leading to nitrate-induced relaxation of human coronary arteries: 1) activation of STOCs and KCa currents in smooth muscle cells, 2) increase in K efflux, 3) membrane hyperpolarization, 4) closure of voltage-dependent Ca channels and 5) decrease in Ca entry and vasorelaxation.

SNP, DEA-NO and IMN are thought to cause relaxation by liberating NO in smooth muscle cells. However, SNP was found to be the most potent nitrodilator for human coronary strip muscle rings. We observed that PTIO, which neutralizes NO (Miyoshi and Nakaya, 1994), significantly inhibited the human coronary vasorelaxations induced by SNP. The vasodilatory response to SNP was not influenced by removal of the endothelium. These studies demonstrate the functional significance of the NO-signaling pathway in dilating human coronary arteries.

In patch-clamp experiments on freshly isolated smooth muscle cells, we observed stimulation of iberiotoxin-sensitive KCa currents by SNP at concentrations that induce coronary vasorelaxation. Furthermore, we observed that iberiotoxin and tetraethylammonium ions inhibited the dose-dependent relaxations of human coronary arteries by SNP. The vasodilatory response to SNP was not influenced by removal of the endothelium. These studies demonstrate the functional significance of the NO-signaling pathway in dilating human coronary arteries.

**Discussion**

We provide the first direct evidence that both KCa channels and STOCs in human coronary artery smooth muscle cells can be opened by nitrovasodilators. Furthermore, we demonstrate that opening of these channels is essential to relaxation by nitrovasodilators. We therefore propose the following cascade of events leading to nitrate-induced relaxation of human coronary arteries: 1) activation of STOCs and KCa currents in smooth muscle cells, 2) increase in K efflux, 3) membrane hyperpolarization, 4) closure of voltage-dependent Ca channels and 5) decrease in Ca entry and vasorelaxation.

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nals to dilate coronary arteries through membrane hyperpolarization in coronary artery smooth muscle cells. We have previously shown that opening of K_{ATP} channels by exogenous or endogenous agonists, e.g., by pinacidil or by putative adenylyl cyclase peptides, leads to vasorelaxation of human coronary arteries (Gollasch et al., 1995; Bruch et al., 1997; Gollasch et al., 1996). Furthermore, the data from the present study provide an important support for the hypothesis, first presented by Williams et al. (1988), that nitrovasodilators are potent activators of vascular smooth muscle K_{Ca} channels. Recent studies have reported two pathways of K_{Ca} channel activation by NO and nitrovasodilators in smooth muscle. Whereas Bolotina et al. (1994) suggested that this class of channels can be activated directly in vascular smooth muscle, other investigators provided patch-clamp data showing that NO can stimulate K_{Ca} via cyclic GMP-dependent protein kinase (Taniguchi et al., 1994; Robertson et al., 1993; Archer et al., 1994; Koh et al., 1995). NO-induced stimulation of STOCs has been reported in a previous study using SNP and pulmonary arterial smooth muscle cells (Clapp and Gurney, 1991) suggesting activation of Ca sparks.

In conclusion, these are the first results showing that nitrovasodilators have an effect on K_{Ca} channels in human vascular smooth muscle cells. Furthermore, the data provide evidence for the modulation of this channel by NO and suggest that these channels play an important role in mediating the therapeutic responses of nitrovasodilators. We suggest that just as the K_{ATP} channels have been shown to play an important role in modulating human coronary artery relaxation during hypoxia and in response to drugs such as pinacidil (Gollasch et al., 1995), the present study shows that K_{Ca} channels may play a similar role in the regulation of vascular tone by nitrates. These findings may have clinical significance for the development of antiangiinal and antihypertensive drugs that selectively target K^+ channels and calcium sparks.

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References


Send reprint requests to: Hermann Haller, M.D., Franz Volhard Clinic, Virchow-Klinikum, Wilhelmsstrasse 50, 13125 Berlin, Germany.