J. Pharm. Pharmacol. 1995, 47: 921–925
Received January 26, 1995
Accepted July 20, 1995

Effect of Selective Inhibition of Potassium Channels on Vasorelaxing Response to Cromakalim, Nitroglycerin and Nitric Oxide of Canine Coronary Arteries

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Abstract

A comparative study was performed on the sensitivity of in-vitro vasorelaxation by nitroglycerin and cromakalim to block glibenclamide, a blocker of ATP-sensitive potassium channels, and iberiotoxin, a selective inhibitor of large-conductance calcium-activated potassium channels.

In isolated canine coronary arteries preconstricted with 25 μM prostaglandin $F_{2\alpha}$, nitroglycerin (0·005–1·8 μM) and cromakalim (0·15–9·6 μM) produced dose-dependent vasodilations. Glibenclamide (30 μM) had no significant effect on relaxation of the dose-response curve to nitroglycerin and almost completely abolished the relaxation by cromakalim, a known opener of ATP-sensitive potassium channels. Iberiotoxin (90 nM) decreased the maximal response to nitroglycerin and had no effect on the vasodilation induced by cromakalim. The effect of iberiotoxin on the vasorelaxing action of nitric oxide, the active metabolite of nitroglycerin, was also examined. In a low potassium chloride (14·4–20·4 mM) medium, as a contractile stimulus, iberiotoxin inhibited relaxations by exogenous nitric oxide (100–200 nM). Enhancement of potassium concentrations to 35·4–40·4 mM significantly decreased relaxation by nitric oxide and under these conditions the inhibitory action of iberiotoxin disappeared.

The present study demonstrated that in canine coronary arteries, the functional role of two potassium channels can be separated by pharmacological means. Nitroglycerin-induced vasorelaxation may be mediated, at least in part, by its enzymatic breakdown product, nitric oxide that activates large-conductance calcium-activated potassium channels.

A change in potassium conductance of the smooth-muscle cell membrane that accompanies relaxation of blood vessels to pharmacological agents is currently the focus of several studies. The heterogeneous distribution of different potassium channels in different vascular preparations determines the subtypes of those channels involved in the mechanism of action of a particular drug (Atwal 1992). Agents that open ATP-sensitive potassium channels (K_{ATP}) such as cromakalim, pinacidil and nicorandil, hyperpolarize the vascular smooth muscle and cause relaxation (Weston 1989). Although the mechanism of vasodilating action of cromakalim, the known representative of this class of drugs, is generally considered specific for opening K_{ATP} (Weston 1989), evidence for the involvement of calcium-activated potassium channels has also been shown (Gelband et al 1989; Cowan & Cohen 1992).

Calcium-activated potassium channels of the large-conductance type (BK_{Ca}) have been identified in smooth-muscle cells from coronary arteries by using patch-clamp techniques (Fujino et al 1991; Taniguchi et al 1993). BK_{Ca} channels were found to be inhibited by scorpion toxins such as charybdotoxin and more selectively by iberiotoxin (Garcia et al 1991), a 37-amino acid peptide produced by the scorpion *Buthus tamulus* (Galvez et al 1990). In isolated canine coronary arteries, agents that elevate guanosine 3′,5′-cyclic monophosphate (cGMP) concentration have been suggested to activate BK_{Ca} channels (Taniguchi et al 1993). Previously, a high concentration (10 μM) of nitroglycerin was found to increase the open probability of a calcium-activated potassium channel with large conductance (300 picosiemens) in cultured smooth muscle of porcine coronary artery (Fujino et al 1991). In epicardial coronary arteries, however, the functional effect of a selective blocker of BK_{Ca} channels on nitroglycerin-induced relaxation has not been demonstrated.

The objective of this investigation was to characterize and compare the effect of the potent and selective blocker of BK_{Ca} channels, iberiotoxin, and that of a blocker of K_{ATP}, glibenclamide, on the in-vitro vasorelaxations induced by cromakalim and nitroglycerin in isolated canine coronary arteries. Further, because nitric oxide is thought to mediate vasodilation by nitroglycerin, the effect of iberiotoxin on the relaxation induced by nitric oxide was also investigated.

Materials and Methods

Measurement of isometric tension

Mongrel dogs of either sex, 9–15 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.) and heparinized (1000 int. units kg^{-1}). The heart was excised and placed into a Krebs-Henseleit solution of the following composition (mM): NaCl 120, KCl 4·2, $CaCl_2$ 1·5, $NaHCO_3$ 20, $MgCl_2$ 1·2, KH_2PO_4 1·2 and glucose 11. Rings (1·1–1·9 mm o.d., 5 mm widths) from the descending and circumflex branches of left coronary artery were isolated from the heart.

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Endothelium was removed by gently rubbing the endothelial surface with a stainless steel wire covered with a cotton swab. Preparations were then mounted in water-jacketed baths containing 2 mL Krebs-Henseleit solution bubbled with 95% O_2-5% CO_2 gas mixture at 37°C. The isometric tension was recorded with a force-displacement transducer (Hugo Sachs Elektronic, Type F30, Germany). Rings were stretched up to 10 mN and allowed to stabilize for 45 min. This tension was readjusted to 10 mN during equilibration. Following equilibration, contractions were induced by 25 μM prostaglandin (PG) $F_{2\alpha}$ and at the maximum amplitude of contraction 1 μM acetylcholine was applied. Only those arterial preparations were used for the experiments that responded with contraction after addition of 1 μM acetylcholine. This protocol served as evidence for functionally de-endothelialized arterial preparations. Indomethacin (10 μM) was used in all experiments to exclude the effect of endogenous prostaglandins on the arterial tone.

Effects of glibenclamide and iberiotoxin on nitroglycerin- and cromakalim-induced relaxation

Two parallel rings isolated from the same branch of a coronary artery were used for measurement of contraction and relaxation. After checking the functional denudation with acetylcholine, the rings were then washed with Krebs-Henseleit solution. One arterial ring was exposed to solvent and served as a control while the other was exposed to a potassium channel blocker (90 nM iberiotoxin or 30 μM glibenclamide) for 30 min. Contractions were induced again by the addition of 25 μM $PGF_{2\alpha}$ to both rings. At the steady state of contraction cromakalim or nitroglycerin was applied in cumulative fashion. The same arterial rings were used for further contractions.

Effect of iberiotoxin on nitric oxide-induced relaxation

In another series of experiments the contraction of the rings was induced with low (14·4–20·4 M) or higher (35·4–40·4 M) concentrations of depolarizing potassium chloride (KCl). Elevation of potassium concentration in the solution was made by substituting NaCl with equimolar KCl in the Krebs-Henseleit medium. Experiments were started with 14·4 mM KCl medium and, if it was necessary, replaced with higher concentrations of KCl until it was enough to induce contraction. Relaxation by exogenous nitric oxide in the presence and absence of 90 nM iberiotoxin was examined.

Preparation of nitric oxide solution

A saturated solution of nitric oxide (about 1·6 mM) in double-distilled water was prepared using a slight modification of a previously described method (Menon et al 1991). Water in a 10-mL Vacutainer tube was deoxygenated by purging with 100% nitrogen for 1 h and then bubbled with nitric oxide for 20 min. For diluting nitric oxide, 100 μL of this solution was transferred with a gas tight syringe (Hamilton, Bonaduz, Switzerland) to another tube containing 10 mL deoxygenated water and used for experiments within 1.5 h. Relaxation with nitric oxide (100–800 nM) was induced at the steady state of KCl contraction in control rings and in rings pretreated with iberiotoxin as described above.

Drugs

Prostaglandin $F_{2\alpha}$, indomethacin and glibenclamide were obtained from Sigma (St Louis, MO, USA). Nitric oxide gas (99·8% pure) was purchased from Messer Griesheim Co. (Düsseldorf, Germany) and nitroglycerin from G. Pohl-Boskamp Gmbh & Co. (Hohenlockstedt, Germany). Cromakalim (BRL 34915) was obtained from Beecham Pharmaceuticals (Harlow, UK). Iberiotoxin was synthesized by Gabor K. Toth (Department of Medicinal Chemistry, Szeged, Hungary). Prostaglandin $F_{2\alpha}$ was dissolved in 70% ethanol (stock solution 10·5 mM), indomethacin was dissolved in 96% ethanol at a concentration of 1 mM. Stock solution of glibenclamide was prepared in dimethylsulphoxide and cromakalim in 70% ethanol to give a concentration of 10 mM. Iberiotoxin was dissolved in double-distilled water to give a concentration of 3 μM. Nitroglycerine was dissolved in 99·8% ethanol (4·4 mM). All stock solutions, except nitroglycerin, were stored frozen at −20°C. From these stock solutions the appropriate concentrations were obtained by diluting with Krebs-Henseleit solution.

Statistical analysis

Enhancement or reduction of arterial tone was calculated as percent maximum increase or decrease of contractile force compared with the pre-drug values. Results are expressed as mean ± s.e.m. and n refers to the number of experiments. Student's *t*-test for paired data or analysis of variance was used to determine the significance of differences between mean values. $P < 0.05$ was taken as statistically significant. EC50 values were calculated by fitting the individual values to the logistic equation of $100*x / (x+b)$.

Results

In the first part of the experiments, the effect of cromakalim and nitroglycerin was studied in the absence and presence of glibenclamide. The activator of K_{ATP} channels, cromakalim, produced a dose-dependent relaxation of coronary arterial rings in a concentration range of 0·15–9·6 μM (Fig. 1A). The calculated EC50 of cromakalim was 0·41 μM in the absence of glibenclamide. Preincubation of coronary rings for 30 min with 30 μM glibenclamide did not affect the resting tone of the arteries (control = 0·8 ± 1·1%, glibenclamide = 1·7 ± 2·0%, n = 7, $P > 0.05$; percent increase of tone compared with the amplitude of steady-state contraction induced by $PGF_{2\alpha}$) nor was there an effect on the magnitude of contraction induced by $PGF_{2\alpha}$ (control = 49·4 ± 6·0 mN, glibenclamide = 53·8 ± 7·4 mN, n = 7, $P > 0.05$). However, glibenclamide almost completely inhibited the relaxation induced by cromakalim. Cumulative addition of nitroglycerin (0·005–1·8 μM) relaxed the coronary arteries with an EC50 of 0·15 μM (Fig. 1B). Preincubation of arterial preparations with 30 μM glibenclamide for 30 min did not significantly modify the relaxation induced by nitroglycerin. The EC50 value of nitroglycerin in the presence of glibenclamide was 0·17 μM.

In the second part of the experiments, the vasorelaxing effect of cromakalim and nitroglycerin was studied in the absence and presence of 90 nM iberiotoxin, the specific inhibitor of BK_{Ca} channels (Fig. 2). At the end of the 30-min incubation period with iberiotoxin, the resting tone of

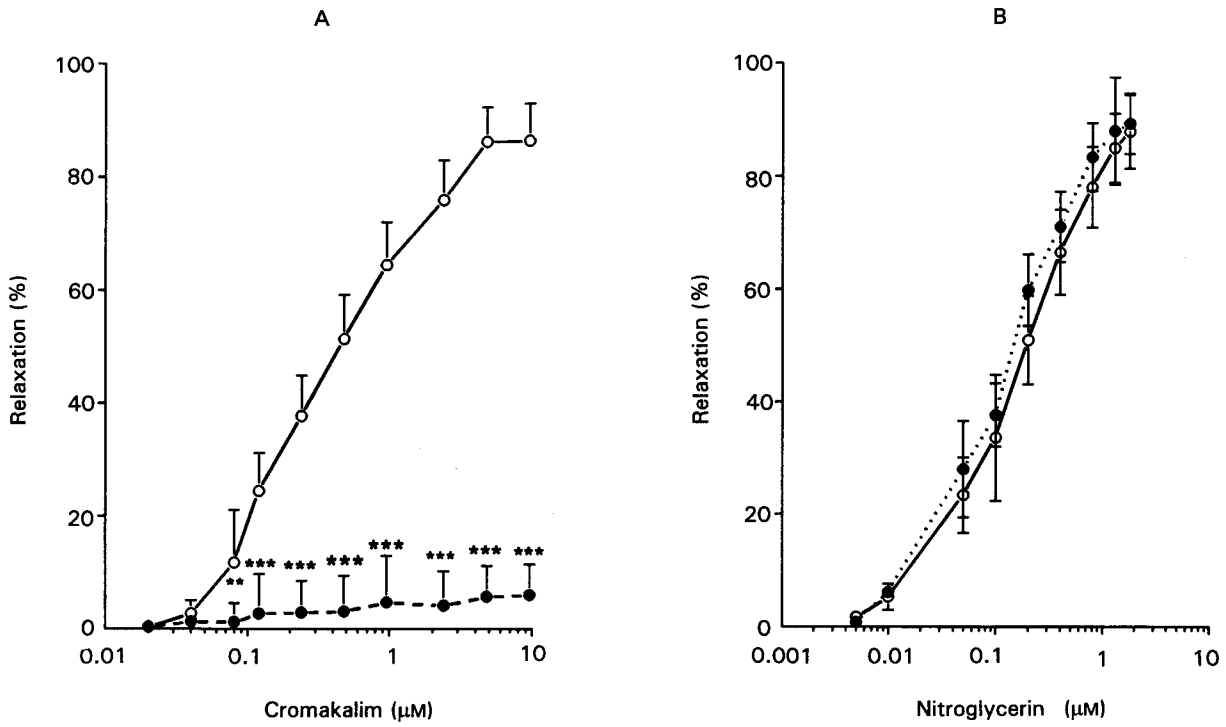

FIG. 1. Effect of glibenclamide on the relaxant responses to cromakalim (A) and nitroglycerin (B) in isolated rings of canine coronary arteries. Paired arterial rings prepared from the same heart were pretreated with either 30 μM glibenclamide (●) or the corresponding volume of vehicle (○) 30 min before addition of 25 μM $PGF_{2\alpha}$. At the steady-state contraction induced by $PGF_{2\alpha}$ cromakalim or, in two different rings from the same heart, nitroglycerin was applied cumulatively. Each value represents the mean of percent relaxation obtained in seven coronary rings from different dogs. Vertical lines show the s.e.m. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with vehicle-treated group.

the arteries was slightly but significantly increased compared with control (control = 1·2 ± 1·2%, iberiotoxin-treated = 10·4 ± 2·5%, n = 7, $P < 0.05$). The maximum contraction amplitude produced by $PGF_{2\alpha}$ did not differ between the iberiotoxin-treated group and the control (control = 55·6 ± 5·3 mN, iberiotoxin-treated = 53·3 ± 7·8 mN, n = 7, $P > 0.05$). Preincubation of coronary arteries with iberiotoxin for 30 min did not change the vasodilating potency of cromakalim (EC50 cromakalim = 0·43 μM, EC50 cromakalim + iberiotoxin = 0·42 μM, Fig. 2A). When the isolated rings were preincubated with iberiotoxin, a significant decrease of maximum relaxation by nitroglycerin at the doses of 1·3 and 1·8 μM was seen (Fig. 2B). Because only the maximum relaxation of the drug was affected by iberiotoxin, the EC50 value for nitroglycerin was not calculated.

In the third part of the experiments, exogenous nitric oxide was added to the coronary rings partially depolarized with KCl. Threshold concentration of KCl necessary to induce contraction varied between 14·4 and 20·4 mM. When the KCl concentration was 14·4–20·4 mM, iberiotoxin significantly enhanced the amplitude of contraction (control = 11·6 ± 4·3 mN, KCl = 25·3 ± 3·7 mN, n = 7, $P < 0.05$). The results obtained with nitric oxide are summarized in Table 1. Relaxation by 100 and 200 nM nitric oxide was significantly inhibited by iberiotoxin. The effect of higher concentrations (400–800 nM) of nitric oxide was not influenced by the toxin. Depolarization of the artery with 35·4–40·4 mM KCl resulted in a contraction amplitude of 35·7 ± 7·0 mN (control) that did not differ from that of the iberiotoxin pretreated group (38·4 ± 4·7 mN, n = 6, $P > 0.05$ compared with control). Amplitudes of relaxation by nitric oxide were significantly smaller in higher KCl concentrations and the inhibitory effect of iberiotoxin on nitric oxide-induced relaxation disappeared at higher concentrations of depolarizing KCl.

Discussion

This study provides a comparative analysis in coronary arterial smooth muscle of the sensitivity of vasorelaxation to cromakalim and nitroglycerin as well as to the specific antagonists of potassium channels, K_{ATP} and BK_{Ca} in-vitro. Glibenclamide almost completely inhibited the relaxation response to cromakalim and did not influence the cumulative dose-response curve induced by nitroglycerin in canine coronary vessels. In conscious dogs, a similar functional distinction in coronary dilations by pinacidil, another K_{ATP} opener, and sodium nitroprusside, another cGMP-elevating substance, has been performed (Duncker et al 1993). Iberiotoxin, the most selective inhibitor of BK_{Ca} channels (Garcia et al 1991), did not modify vasorelaxation to cromakalim. Some observations support (Strong et al 1989; Green et al 1991) while others contradict (Gelband et al 1989; Okabe et al 1990) this finding, suggesting the possibility that K_{ATP} and BK_{Ca} channels are not functionally distinct entities in some blood vessels. In our study, the basal tone was also differentially affected by glibenclamide and iberiotoxin, supporting the hypothesis that the two potassium channels can be separated by pharmacological means in epicardial coronary arteries.

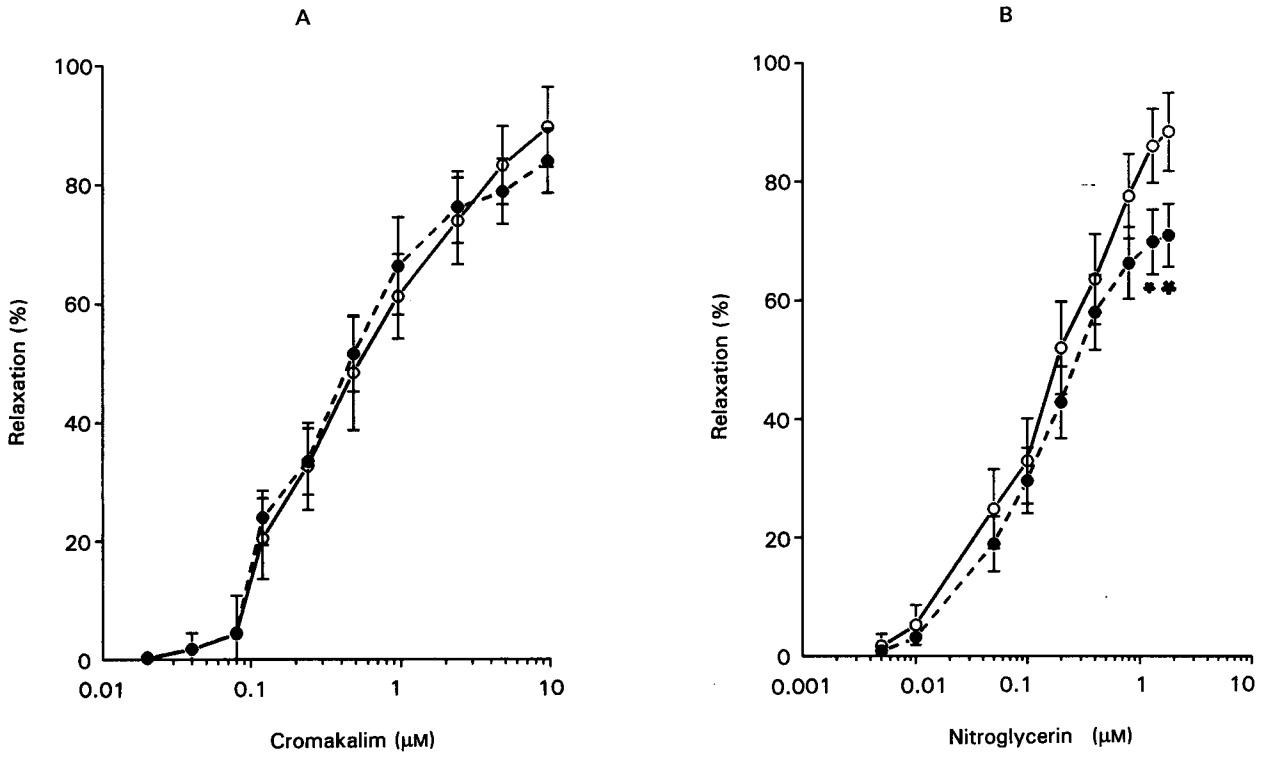

FIG. 2. Effect of iberiotoxin on the relaxation induced by cromakalim (A) and by nitroglycerin (B) in isolated rings of canine coronary arteries. One of two arterial rings was pretreated with 90 nM iberiotoxin (●) and the other was exposed to the solvent of iberiotoxin (○). After 30 min preincubation with iberiotoxin, contraction was induced with 25 μM $PGF_{2\alpha}$. At the steady-state of contraction, cromakalim was added in a cumulative fashion. Experiments were repeated with nitroglycerin in two other arteries prepared from the same heart. Values are mean ± s.e.m. each representing seven coronary preparations obtained from different dogs. $^{*}P < 0.05$ compared with vehicle-treated group.

It is generally accepted that nitroglycerin increases cGMP in the smooth-muscle cell which is associated with relaxation (Ignarro & Kadowitz 1985). In coronary arteries, the open probability of large-conductance calcium-activated potassium channels was increased by nitroglycerin, 8-bromo-cGMP (Fujino et al 1991) as well as by cGMP-dependent protein kinase (Taniguchi et al 1993), suggesting a connection between BK_{Ca} channels and the cGMP-messenger system. Although a large density of this potassium channel was measured in the isolated smooth muscle cells of canine coronary arteries (Wilde & Lee 1989; Taniguchi et al 1993), the functional effect of nitroglycerin, as a major coronary dilator, on BK_{Ca} channels has not yet been investigated. In the current study, iberiotoxin, the most selective inhibitor of BK_{Ca} channels, significantly inhibited the maximum relaxation by nitroglycerin. This is in agreement with the observation that 1 μM nitroglycerin decreased the amplitude of the action potential evoked by tetraethylammonium (Harder et al 1979), a nonselective inhibitor of BK_{Ca} channels (Nelson et al 1990). However, the vascular action of submicromolar concentrations of nitroglycerin was not sensitive to the inhibitory effect of the toxin. Many proposals have been made concerning other mechanisms. These include the inhibition of phospholipase C and the subsequent decrease of inositol 3′,5′-triphosphate formation, the inhibition of Ca^{2+} release by the sarcoplasmic reticulum, the activation of

Table 1. Effect of iberiotoxin on nitric oxide-induced relaxation in depolarizing potassium chloride (KCl) solution.

Agonist	Relaxation (%)			
Nitric oxide (nM)	100	200	400	800
14·4–20·4 mM KCl	30·0 ± 5·2	50·8 ± 4·6	71·3 ± 3·6	89·6 ± 4·1
+ iberiotoxin	12·0 ± 4·9**	28·3 ± 6·0**	67·8 ± 5·5	92·5 ± 3·3
35·4–40·4 mM KCl	3·2 ± 2·0++	13·4 ± 4·1++	41·3 ± 6·90+	67·4 ± 6·6+
+ iberiotoxin	5·3 ± 3·3	17·2 ± 5·2	46·0 ± 4·7	59·1 ± 5·7

Data are mean ± s.e.m. Number of experiments was six in the low-potassium medium and seven in the high-potassium medium. $^{**}P < 0.01$ compared with the corresponding 14·4–20·4 mM KCl, $^{+}P < 0.05$, $^{++}P < 0.01$ compared with values obtained in 14·4–20·4 mM KCl.

the Ca^{2+} pump ATPase in cellular and subcellular membranes and dephosphorylation of the myosin light chain. These mechanisms are all known to be mediated by cGMP (Rapoport 1986; Ahlner et al 1991; Ignarro 1991). Thus, opening of an iberiotoxin-sensitive potassium channel by nitroglycerin represents only a part of the complex mechanism by which the drug decreases coronary tone. Because the relaxation by cromakalim was not affected by iberiotoxin at all and cromakalim did not influence cyclic nucleotides (Taylor et al 1988), we hypothesized a functional connection between BK_{Ca} channels and nitric oxide, the active metabolite of nitroglycerin.

Nitroglycerine is converted into nitric oxide in coronary arteries (Chung & Fung 1993) and nitric oxide has also been found to open BK_{Ca} channels (Bolotina et al 1994). In our experiments, the effect of 100 and 200 nM but not 400 and 800 nM nitric oxide was decreased by iberiotoxin in a medium of low potassium (< 20·4 mM KCl). Elevation of potassium concentration to 35·4–40·4 mM significantly depressed the relaxation by nitric oxide and, under this condition, the iberiotoxin-sensitive component disappeared. These findings support the importance of the membrane potential in the vasorelaxing mechanism of nitric oxide (Tare et al 1990) and also show that most of the relaxations, similar to nitroglycerin, are not sensitive to the high concentration of iberiotoxin.

In conclusion, there are at least two potassium channels, K_{ATP} and BK_{Ca}, that can be activated or inhibited in epicardial coronary arteries of dogs. We have demonstrated for the first time that nitroglycerin and its active metabolite, nitric oxide possess an iberiotoxin-sensitive mechanism in their acute vasodilator action in-vitro. This mechanism involves a large-conductance potassium channel (BK_{Ca}) that mediates a part of the relaxation induced by nitroglycerin and nitric oxide. Further studies are necessary for exploring the significance of this hyperpolarizing potassium channel, known to be a protective mechanism against depolarization overload in the smooth muscle (Brayden & Nelson 1992).

Acknowledgements

This study was supported by the Hungarian National Scientific Foundation (OTKA T 12848). The authors are grateful to Maria Feher for her valuable technical assistance.

References

Ahlner, J., Andersson, R. G. G., Torfgard, K., Axelsson, K. L. (1991) Organic nitrate esters: clinical use and mechanisms of actions. Pharmacol. Rev. 43: 351–423

Atwal, K. S. (1992) Modulation of potassium channels by organic molecules. Med. Res. Rev. 12: 569–591

Bolotina, V. M., Najibi, S., Palacino, J. J., Pagano, P. J., Cohen, R. A. (1994) Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 368: 850–853

Brayden, J. F., Nelson, M. T. (1992) Regulation of arterial tone by activation of calcium-dependent potassium channels. Science 256: 532–535

Chung, S.-J., Fung, H.-L. (1993) Relationship between nitroglycerin-induced vascular relaxation and nitric oxide production. Biochem. Pharmacol. 45: 157–163

Cowan, C. L., Cohen, R. A. (1992) Different mechanisms of relaxation of pig coronary artery to bradykinin and cromakalim are distinguished by potassium channel blockers. J. Pharmacol. Exp. Ther. 260: 248–253

Duncker, D. J., Van Zon, N. S., Altman, J. D., Pavek, T. J., Bache, R. J. (1993) Role of K^+_{ATP} channels in coronary vasodilation during excercise. Circulation 88: 1245–1253

Fujino, K., Nakaya, S., Wakatsuki, T., Miyoshi, Y., Nakaya, Y., Mori, H., Inoue, I. (1991) Effects of nitroglycerin on ATP-induced Ca^{++}-mobilization, Ca^{++}-activated K channels and contraction of cultured smooth muscle cells of porcine coronary artery. J. Pharmacol. Exp. Ther. 256: 371–377

Galvez, A., Gimenez-Gallego, G., Reuben, J. P., Contancin, L., Fiegenbaum, P., Kaczorowski, G. J., Garcia, M. L. (1990) Purification and characterization of a unique, potent, peptidyl probe for high-conductance calcium activated potassium channel from venom of the scorpion *Buthus tamulus*. J. Biol. Chem. 265: 11083–11090

Garcia, M. L., Galvez, A., Garcia-Calvo, M., King, V. F., Vazque, J., Kaczorowski, G. J. (1991) Use of toxins to study potassium channels. J. Bioenerg. Biomembr. 23: 615–646

Gelband, C. H., Lodge, N. J., van Breemen, C. (1989) A Ca^{2+}-activated K^+ channel from rabbit aorta. Eur. J. Pharmacol. 167: 201–210

Green, K. A., Foster, R. W., Small, R. C. (1991) A patch clamp study of K^+-channel activity in bovine isolated tracheal smooth muscle cells. Br. J. Pharmacol. 102: 871–878

Harder, D., Belardinelli, L., Sperelakis, N., Rubio, R., Berne, R. M. (1979) Differential effects of adenosine and nitroglycerin on the action potentials of large and small coronary arteries. Circ. Res. 44: 176–182

Ignarro, L. J. (1991) Signal transduction mechanisms involving nitric oxide. Biochem. Pharmacol. 41: 485–490

Ignarro, L. J., Kadowitz, P. J. (1985) The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. Annu. Rev. Pharmacol. Toxicol. 25: 171–191

Menon, N. K., Pataricza, J., Binder, T., Bing, R. J. (1991) Reduction of biological effluents in purge and trap micro reaction vessels and detection of endothelium-derived nitric oxide (Edno) by chemiluminescence. J. Mol. Cell. Cardiol. 23: 389–393

Nelson, M. T., Patlak, J. B., Worley, J. F., Standen, N. B. (1990) Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am. J. Physiol. 259: C3–C18

Okabe, K., Kajioka, S., Nakao, K., Kitamura, K., Kuriyama, H., Weston, A. H. (1990) Actions of cromakalim on ionic currents recorded from single smooth muscle cells of the rat portal vein. J. Pharmacol. Exp. Ther. 252: 832–839

Rapoport, R. M. (1986) Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidylinositol hydrolysis in rat aorta. Circ. Res. 58: 407–410

Strong, P. N., Weir, S. W., Beech, D. J., Hiestand, P., Kocher, H. (1989) Effects of potassium channel toxins from *Leiurus quinquestriatus hebraeus* venom on responses to cromakalim in rabbit blood vessels. Br. J. Pharmacol. 98: 817–826

Taniguchi, J., Furukawa, K.-I., Shigekawa, M. (1993) Maxi K^+ channels are stimulated by cyclic guanosine monophosphate-dependent protein kinase in canine coronary artery smooth muscle cells. Pflugers Arch. 423: 167–172

Tare, M., Parkington, H. C., Coleman, H. A., Neild, T. O., Dusting, G. J. (1990) Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. Nature 346: 69–71

Taylor, S. G., Southerton, J. S., Weston, A. H., Baker, J. R. J. (1988) Endothelium-dependent effects of acetylcholine in rat aorta: a comparison with sodium nitroprusside and cromakalim. Br. J. Pharmacol. 94: 853–863

Weston, A. H. (1989) Smooth muscle K^+ channel openers, their pharmacology and clinical potential. Pflugers Arch. 414: S99–S105

Wilde, D. W., Lee, K. S. (1989) Outward potassium currents in freshly isolated smooth muscle cell of dog coronary arteries. Circ. Res. 65: 1718–1734