ELSEVIER

European Journal of Pharmacology 406 (2000) 79–84

ejp

www.elsevier.nl/locate/ejphar

Guanylate cyclase and not ATP-dependent K^+ channels seems temperature-dependent in smooth muscle relaxation of human umbilical arteries

Alberto Tiritilli *

Laboratoire de Physiologie et Pharmacologie Cardiovasculaire, Centre Hospitalier, 20, Rue Armagis 78104 Saint-Germain-en-Laye, France

Received 23 March 2000; received in revised form 18 July 2000; accepted 21 July 2000

Abstract

The effects of K^+ channel opener, nicorandil [*N*-(2-hydroxyethyl)-nicotinamide nitrate], on isolated human umbilical arteries were investigated at two temperatures: 37°C and 25°C. The purpose of this investigation was: (1) to confirm the relaxant effect of nicorandil, (2) to elucidate the influence of endothelium and temperature on nicorandil-induced relaxation, (3) to determine which of guanylate cyclase or ATP-sensitive K^+ channels was implicated in temperature-induced relaxation of smooth muscles. Rings, 3-mm-wide, were suspended in organ chambers for isometric force measurement. All solutions were aerated with 95% O_2–5% CO_2 and maintained at 37°C or 25°C (cooling), pH 7.4. The presence of an intact endothelium was confirmed by immunohistochemistry. During the first set of experiments after contraction with 5-hydroxytryptamine (5-HT 10^{-5} M), nicorandil (10^{-9}–10^{-4} M) was added to the organ chambers with controls and in with rings incubated with L-arginine, *N*-nitro-L-arginine (L-NNA) an inhibitor of nitric oxide (NO) synthase, [1-*H*-(1,2,4) oxadiazole (4,3-a) quinoxalin-1-one] (ODQ), a specific inhibitor of guanylate cyclase, or glibenclamide, an antagonist of nicorandil, all at 10^{-5} M. In another set of experiments, rings were contracted with 5-HT (10^{-5} M) and relaxed with 3-morpholino-sydnonimine [SIN-1 (10^{-9}–3×10^{-5} M) or cromakalim (10^{-9}–3×10^{-5} M)]. Our results showed that nicorandil induced concentration-dependent relaxation. At 37°C, in the control, the maximum relaxation was $90 \pm 5\%$, and $60 \pm 8\%$ at 25°C ($P < 0.01$). However, the relaxation at 37°C or 25°C remained unchanged after pretreatment with L-arginine, L-NNA, this suggests that the same concentration of drugs used in this type of vessel does not appear to modulate the relaxant effect of nicorandil. On the other hand, we observed that the relaxant effect of SIN-1 was $72 \pm 5\%$ at 37°C and only $28 \pm 7\%$ at 25°C ($P < 0.01$). However, relaxations with cromakalim were partly influenced by cooling. In the presence of ODQ, the nicorandil-induced relaxation observed at 37°C or 25°C was less than that in the control and in the rings incubated with glibenclamide. These results for human umbilical arteries indicate that cooling decreases the relaxation response of smooth muscles and that this is partly due to a decreased response to guanylate cyclase.

Keywords: Umbilical artery, human; 5-HT (5-hydroxytryptamine, serotonine); Nicorandil; ATP-sensitive, K^+ channels; SIN-1 (3-Morpholinosydnonimine); Guanylate cyclase; Glibenclamide; ODQ ([1-*H*-(1,2,4) Oxadiazole (4,3-a) quinoxalin-1-one]); Temperature

1. Introduction

K^+ channel openers, such as cromakalim, pinacidil, [(+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidin-l-yl)-6*H*-pyrano-(2,3-f)benz-2,1,3,-oxadiazole] (NIP-121), are known to activate adenosine triphosphate (ATP)-sensitive K^+ channels in a variety of tissues including cardiac and smooth muscle (Henry and Escande, 1994; Lawson, 1996; Saito et al., 1997). Nicorandil is a nicotinamide derivative and has a pharmacological profile characterised by two mechanisms of action (Kinoscita and Sakai, 1990; Kukovez et al., 1991, 1992). In the first place, activation of cytoplasmic guanylate cyclase leads to a reduction in the cytosolic Ca^{2+} concentration and thus to relaxation of the vascular smooth muscle. In addition, nicorandil causes hyperpolarisation of the cell membrane by activating ATP-dependent channels. A number of studies have since focused on quantifying the relative contribu-

* Tel.: +33-1-3950-6567; fax: +33-1-3951-9054.
E-mail address: tiritillialb@aol.com (A. Tiritilli).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00529-X

tion of each of these vasodilator mechanisms. Glibenclamide and methylene blue have been used as suitable pharmacological tools to differentiate between hyperpolarisation and c-GMP-mediated actions (Eltze, 1989; Borg et al., 1991; Kreye et al., 1991).

The effects of K^+ channel openers are reported to be temperature-sensitive and much more potent at 35–37°C than at lower temperatures to open ATP-sensitive K^+ channels (Henry and Escande, 1994).

Nitric oxide (NO) is a major factor in the cardiovascular system. Its multiple roles include regulation of vasomotor tone and cell adhesion to the endothelium, inhibition of platelet aggregation and vascular smooth cell proliferation (Andrew and Mayer, 1999). This suggests that NO plays a crucial role in the prevention of cardiovascular damage such as is seen in hypertension, atherosclerosis and other diseases (Kojda and Harrison, 1999).

Although efforts are currently being made to understand the regulation, production and function of endothelium, its role in the effects of cooling on vascular reactivity has been little studied (Karaki and Nagase, 1987; Bedelsson et al., 1989). In canine and simian femoral arteries, Kawaki and Chiba (1989) have demonstrated that cooling inhibits norepinephrine and KCl-induced contraction; the same result was obtained with rat tail arteries (Harker et al., 1991). Conversely, Flavahan and Vanhoutte (1986) have shown that cooling enhanced norepinephrine-induced contraction in the canine saphenous vein. Recently, it was shown that, in rabbits, cooling increases the relaxation of ear but not of femoral arteries to cholinergic stimulation (Garcia-Villalon et al., 1995).

Thus, despite current research to understand the effects of cooling on vascular reactivity of different animals species, studies with human tissues remain incomplete and very few have examined the effect of temperature on the smooth muscle of the human umbilical artery. In the present study, only rings with endothelium were investigated.

The purpose of this investigation on human umbilical arteries was: (1) to confirm the relaxant effect of nicorandil, (2) to elucidate the influence of endothelium and temperature on nicorandil-induced relaxation, (3) to determine which of guanylate cyclase or ATP-sensitive K^+ channels is implicated in temperature-induced relaxation of smooth muscles.

2. Materials and methods

2.1. Preparation of rings and recording of muscle tension

Human umbilical cords obtained from normal, spontaneous and full-term transvaginal deliveries were used in this study. Cords from mothers with eclampsia, hypertension, diabetes or other overt diseases were not included. Those from mothers on medication, such as uterotonic agents during labour, were also excluded. Human umbilical cords were collected immediately after delivery. Segments (about 15 cm in length) were cut from the cord midway between placenta and infant and placed in oxygenated glucose (5%) at 4°C. All specimens were immediately transported to the laboratory. All experiments were performed within 5 h of delivery.

2.2. Mounting rings and recording of smooth muscle tension

The umbilical arteries, carefully dissected from the cord which was bathed continuously in Krebs–Henseleït solution, were prepared for mechanical studies. Care was taken to avoid stretching. The arteries used had an outside diameter of 1.5–2.0 mm and were cut into rings, 3-mm-long. Rings were mounted on nichrome wires in jacketed, 20 ml capacity, drop-away bath chambers containing Krebs–Henseleit solution (37°C); the preparations were continuously gassed with 95% O_2 and 5% CO_2 at pH 7.4. The upper nichrome wire of each ring was attached to a force-displacement transducer (UF1 Pioden Controls) coupled with an SGA 92201-01 Bionic Instrument amplifier. Changes in isometric force were recorded on a polygraph (Linseis L 6512 B). Arterial rings were equilibrated in Krebs–Henseleit solution, aerated with 95% O_2–5% CO_2 for 2 h, with frequent adjustments of baseline tension during stress contraction obtained by depolarization with KCl 40 mM. After optimal tension was achieved, the umbilical artery rings were washed and allowed to equilibrate for 45 min before initiation of any given protocol. The presence of an intact endothelium was confirmed by immunohistochemistry. The rings were fixed in buffered formalin (10%) and embedded in paraffin. Four micron sections were cut onto silane-coated slides. For each ring, a section was stained with hematoxylin and eosin. Sequential sections, blocked in 3% nitrogen peroxide and pretreated with protease for antigen retrieval were stained immunohistochemically using two monoclonal antibodies: anti CD_{31} Mab (DAKO, dilution 1/30), and anti CD_{34} Mab (IMMUNOTHECH, dilution 1/2). For staining, the strepto-biotin-peroxydase complex with diaminobenzidine as chromogen was used in a VENTANA autoprocessor. Each slide was counterstained with Mayer's hematoxylin; only rings with an intact endothelium were selected.

2.3. Experimental procedure

All experiments were performed at 37°C or 25°C. During the first set of experiments after contraction with 5-hydroxytryptamine (5-HT) (10^{-5} M), nicorandil (10^{-9}–10^{-4} M)-induced relaxation was compared in umbilical artery segments incubated in either a standard Krebs–Henseleït solution or after incubation (30 min) with L-arginine, *N*-nitro-L-arginine (L-NNA), [1-*H*-(1,2,4) oxadiazole (4,3-a) quinoxalin-1-one] (ODQ) or glibenclamide, all

Table 1
Values for the maximum (E max %) and pD_2 of the relaxation in response to nicorandil (10^{-9}–10^{-4} M) in control and in presence of L-arginine, L-NNA, ODQ and glibenclamide all at 10^{-5} M, at 37°C and 25°C

Nicorandil (10^{-9}–10^{-4} M)	Temperature			
	37°C		25°C	
	E max (%)	pD_2 ($-\log EC_{50}$)	E max (%)	pD_2 ($-\log EC_{50}$)
Control	90 ± 5	7.2 ± 0.1	$60 \pm 8^{a,d}$	6.3 ± 0.1
+ L-Arginine (10^{-5} M)	91 ± 7	7.4 ± 0.1	$58 \pm 7^{a,d}$	7.4 ± 0.1
+ L-NNA (10^{-5} M)	92 ± 3	7.4 ± 0.2	$57 \pm 9^{a,d}$	6.3 ± 0.1
+ ODQ (10^{-5} M)	42 ± 3^{b}	7.0 ± 0.1	$19 \pm 3^{a,c}$	$6.0 \pm 0.1^{a,c}$
+ Glibenclamide (10^{-5} M)	66 ± 4^{b}	8.3 ± 0.3	$39 \pm 3^{a,c}$	7.4 ± 0.1

Data are means ± S.E.M.
[a] $P < 0.05$; significantly different from its control at 37°C.
[b] $P < 0.01$; significantly different from its control at 37°C.
[c] $P < 0.05$; significantly different from 37°C.
[d] $P < 0.01$; significantly different from 37°C.

at 10^{-5} M. In another set of experiments, rings were contracted with 5-HT (10^{-5} M) and relaxed with 3-morpholinosydnonimine (SIN-1) (10^{-9}–3×10^{-5} M) or cromakalim (10^{-9}–3×10^{-5} M). These drug concentrations were chosen on the basis of previous publications.

2.4. Chemicals and solutions

The following drugs were used: 5-HT, KCl, L-NNA, cromakalim glibenclamide, chloranphenicol, papaverine, and were obtained from Sigma, St. Louis MO, USA. Nicorandil was kindly donated by Merck (Darmstadt, Germany). ODQ was purchased from Alexis (Paris, France). SIN-1 was kindly given by Hoechst (Frankfurt, Germany). Solutions of these drugs were freshly prepared each day in distilled water except for ODQ, which was dissolved in Krebs–Henseleit solution and dimethylsulfoxide, which had no effect on the parameter measured at the concentration used. Nicorandil, was dissolved in ethanol, then diluted in Krebs–Henseleit solution. The final concentration of ethanol (0.03% v/v) did not alter the effect of nicorandil. The composition of the Krebs–Henseleït solution was (in mM) NaCl, 114; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; Glucose, 11.7.

2.5. Calculations and statistical analysis

Statistical analysis was done with a polystat computer program (Cricket Software, Philadelphia, PA). The contractile response to 5-HT (10^{-5} M) was estimated in grams. The vascular relaxation produced by nicorandil, SIN-1 and cromakalim was expressed as a percentage (%) of the maximum relaxation obtained with papaverine (10^{-3} M). The concentration producing a half-maximum response (EC_{50}), was determined directly from the dose–effect curve in each experiment; EC_{50} values were expressed as $pD_2 = -\log (EC_{50})$. Results are presented as means ± S.E.M. Multiple comparisons were made using a one-way analysis of variance (ANOVA) followed by a Scheffé *F*-test (Wallenstain et al., 1980). The level of significance was $P < 0.05$.

3. Results

Our results are summarized in Table 1. No difference was noted concerning the contraction obtained with 5-HT; we found 5.0 ± 0.5 g at 37°C and 4.9 ± 0.9 g at 25°C. (P = NS). As shown in Fig. 1, human umbilical rings gave a cumulative concentration–response curve to nicorandil (10^{-9}–10^{-4} M). In control rings, we found a pD_2 value of 7.2 ± 0.1 and maximum relaxation of $90 \pm 5\%$ of the response to papaverine (10^{-3} M). This relaxation was not modified by L-arginine or L-NNA; the pD_2 value was 7.4 ± 0.1 and maximum relaxation of $91 \pm 7\%$ for L-arginine. In the presence of L-NNA we found a pD_2 7.4 ± 0.2 and maximum relaxation of $92 \pm 3\%$ (P = NS vs. control).

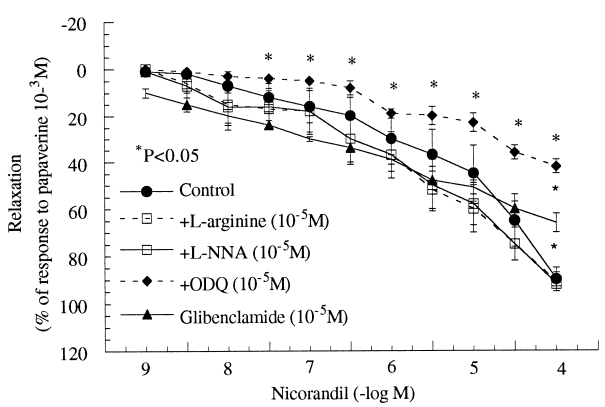

Fig. 1. At 37°C. Cumulative concentration–reponse curves for nicorandil (10^{-9}–10^{-4} M) with human umbilical arteries; control (black circles) and in presence of L-arginine (white circles), L-NNA (squares), ODQ (diamonds) and glibenclamide (triangles). The relaxations are expressed as percent (%) of papaverine-induced relaxation and are given as means ± S.E.M. for five to seven vessels. * $P < 0.05$ vs. control, Scheffé *F*-test.

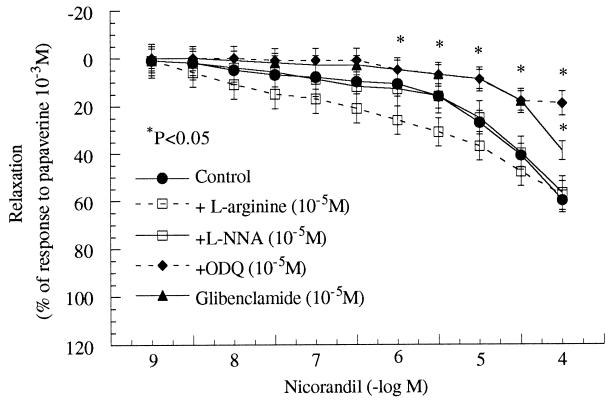

Fig. 2. At 25°C. Cumulative concentration–reponse curves for nicorandil (10^{-9}–10^{-4} M) with human umbilical arteries; control (black circles) and in presence of L-arginine (white circles), L-NNA (squares), ODQ (diamonds) and glibenclamide (triangles). The relaxations are expressed as percent of papaverine-induced relaxation and are given as means ± S.E.M. of seven vessels. * $P < 0.05$ vs. control by the Scheffé F-test.

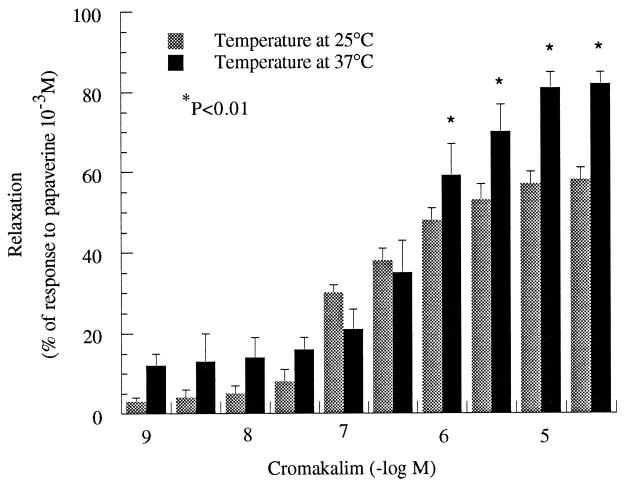

Fig. 4. Cumulative concentration–reponse curves for cromakalim (10^{-9}–3×10^{-5} M) with human umbilical arteries at 37°C (black columns) and at 25°C (grey columns). The relaxations are expressed as percent of papaverine-induced relaxation and are given as means ± S.E.M. for five to seven vessels. * $P < 0.05$ vs. control by the Scheffé F-test.

The effects of pretreatment with ODQ (10^{-5} M) and glibenclamide (10^{-5} M) were tested on responses to nicorandil (10^{-9}–10^{-4} M). In all rings, nicorandil induced a concentration-dependent relaxation response. ODQ and glibenclamide significantly shifted the concentration–response curves to the right and depressed the maximum response. The maximum relaxation was $42 \pm 3\%$ in the presence of ODQ and 66 ± 4 in the presence of glibenclamide. We found pD_2 of 7.0 ± 0.1 and 8.3 ± 0.2, respectively ($P < 0.05$ vs. control). SIN-1 in the range 10^{-9}–3×10^{-5} M induced a concentration-dependent relaxation. The magnitude of relaxation was $72 \pm 2\%$ and pD_2, 7.0 ± 0.1. In the same manner, cromakalim at concentration from 10^{-9} to 3×10^{-5} M, produced a concentration-dependent relaxation. The maximum for this relaxation was $82 \pm 4\%$ and pD_2, 8.6 ± 0.1.

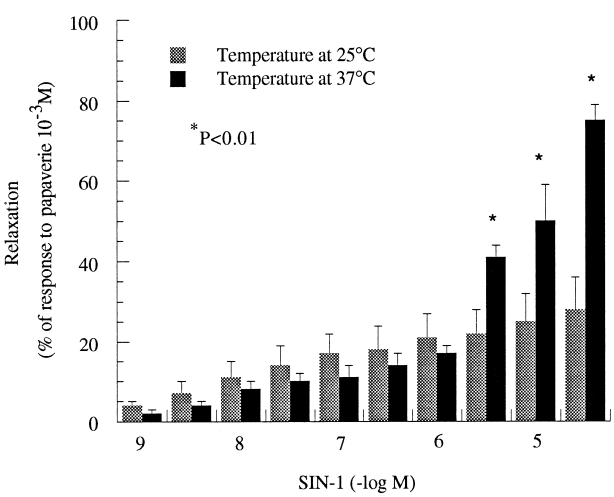

Fig. 3. Cumulative concentration–reponse curves for SIN-1 (10^{-9}–3×10^{-5} M) with human umbilical arteries at 37°C (black columns) and at 25°C (grey columns). The relaxations are expressed as percent of papaverine-induced relaxation and are given as means ± S.E.M. for five to seven vessels. * $P < 0.05$ vs. control by the Scheffé F-test.

At 25°C, nicorandil (10^{-9}–10^{-4} M) also produced relaxation in umbilical rings. However, at this temperature, the maximum relaxation was decreased by 30%. We found $60 \pm 8\%$ in the control ($P < 0.01$ vs. 37°C). Conversely, the pD_2 was not significantly different from the control at 37°C (Table 1). In subsequent experiments, following incubation of segments with L-arginine, L-NNA, ODQ or glibenclamide, in all cases, the cooler temperature (25°C) decreased the maximum relaxation (Table 1, Fig. 2). On the other hand, the pD_2 were not significantly different from those observed at 37°C, apart from ODQ. Concerning SIN-1, we found that the maximum relaxation was only $28 \pm 7\%$ ($P < 0.01$ vs. 37°C). In the same way, the pD_2 value was significantly affected (Table 1, Fig. 3). Similarly, cooling decreased the maximum relaxation and pD_2 of cromakalin; both were reduced significantly from 37°C (Fig. 4).

4. Discussion

This study using in vitro techniques attempted to analyze the effects of nicorandil at physiological (37°C) and lower (25°C) temperatures. The results showed that nicorandil has a powerful relaxant effect on human umbilical arteries. These results are in agreement with those previously published (Lawson, 1996). We have now demonstrated that cooling decreases nicorandil relaxation in umbilical arteries. Conversely, our results are slightly different from those reported by Garcia-Villalon et al. (1995), who have shown that cooling increases the relaxation of ear, but not of femoral arteries to cholinergic stimulation

partly by increasing the effect of potassium channel-mediated hyperpolarisation.

Recently, Chang et al. (1992) suggested that the activation of ATP-sensitive K^+ channels produces increase in pulmonary blood flow which can be partially attenuated by an enzymatic inhibitor of NO formation. In the present study, the NO pathway appears relatively unimportant, as pretreatment with L-arginine or the inhibition of NO-synthase by L-NNA did not modify the relaxation in response to nicorandil. Our results agree with those of Mayhan (1993) showing that N^G-monomethyl-L-arginine (L-NMMA) did not inhibit dilatation of arterioles in response to BRL 38227 (active enantiomer of cromakalim) and RP 52891 (aprikalim). The discrepancy between studies on responses to activation of ATP-sensitive K^+ channels may be related to differences in species, vascular beds or size of blood vessels studied.

Several studies have shown that substances known to release endothelium-derived relaxing factors can also cause hyperpolarisation of smooth muscle preparations. In the present study, guanylate cyclase, more than K^+ ATP-dependent channels, seems to be implicated in the temperature-decreased relaxation. In brief, nicorandil combines nitrate-like properties, (i.e., stimulation of guanylate cyclase), thereby increasing intracellular c-GMP concentration and membrane hyperpolarizing properties via K^+ channel opening; both actions contribute to vasorelaxation. Many studies have focused on quantifying the relative contribution of each of these vasodilator mechanisms. Glibenclamide and methylene blue have been used as pharmacological tools to differentiate between hyperpolarisation and c-GMP-mediated actions, respectively. Kreye et al. (1991) have shown that, in isolated rabbit aorta, nicorandil, like cromakalim, was able to enhance ^{86}Rb efflux which was antagonized by glibenclamide, suggesting that the opening of ATP-dependent K^+ channels was operative. In contrast to that of cromakalim, the relaxant effect of nicorandil in large arteries (e.g., the rabbit aorta) was resistant to glibenclamide. Thus, in large vessels studied in an organ bath, the nitrate-like rather than the hyperpolarising action, may be responsible for the relaxant effects of nicorandil. However, in coronary resistance arteries, nicorandil-induced vasodilation appears to be largely dependent on the activation of ATP-sensitive channels (Kukovez et al., 1992).

In our study, we have shown that glibenclamide at a concentration of 10^{-5} M acts as partial antagonist; at 37°C it was 24% inhibition and at 25°C, 21%. This confirms that glibenclamide is probably not the best antagonist of nicorandil in human umbilical arteries. Our results are in agreement with those of many recent studies. In pig coronary arteries of different diameters, the nicorandil-provoked relaxation was completely inhibited by oxy haemoglobin but only at the epicardial level. Moreover, oxyhaemoglobin and glibenclamide inhibited relaxation equally in the intramyocardial coronary arteries, while only cromakalim relaxation was completely inhibited by glibenclamide. In another study performed in vivo and in vitro with dog mesenteric arteries, the nicorandil curves were displaced to the right by glibenclamide. Furthermore, during in vivo experiments, glibenclamide slightly decreased the vasodilating action of nicorandil but remained without effect on the relaxation induced by nifedipine. Moreover, several recent studies have confirmed the diversity of the effects of glibenclamide on arterial vessels. Indeed, Ishiyama et al. (1994) have shown in vivo the vasorelaxant effect of nicorandil on dog coronary arteries. These authors showed, after local application of nicorandil, that vasodilation is completely inhibited by methylene blue, but not by glibenclamide. However, glyceryl trinitrate and cromakalim are also powerful vasodilators and this effect is completely blocked by methylene blue and glibenclamide, respectively. In the same way, Abe et al. (1994) have shown that in the rabbit femoral artery, glibenclamide completely antagonized cromakalim-induced relaxation, partially antagonized relaxation induced by nicorandil, and had no antagonizing effect at all on relaxation induced by glyceryl trinitrate. It has been suggested that NO interacts with the heme moiety of soluble guanylate cyclase (Ohlstein et al., 1982), and this hypothesis was supported by results obtained with methylene blue, which interacts directly with protein-bound heme (McCord and Fridovich, 1970). The dye was found to inhibit NO-induced stimulation of soluble guanylate cyclase in cell-free systems (Miki et al., 1977) as well as to block smooth muscle relaxation induced by vasodilators or acethylcholine (Holtzmann, 1982). Thus, if methylene blue inhibits NO-synthase by oxidizing the protein-bound ferrous heme iron, methylene blue is only a poor inhibitor of soluble guanylate cyclase (Mayer et al., 1993). In this study, we thus used ODQ, a specific inhibitor of guanylate cyclase. At 37°C, in the presence of ODQ, the inhibition of nicorandil relaxation was 50%, and 40% at 25°C, significantly different from that found in the presence of glibenclamide under the same conditions. Furthermore, the relaxation after SIN-1, a direct donor of NO, was not different from the control at 37°C, but was weaker at 25°C. On the other hand, the relaxation with cromakalim at 35°C was not different from that found with nicorandil at these two different temperatures. In summary and conclusion, this study showed that cooling decreases the relaxant response of human umbilical arteries to nicorandil. Further investigation is needed to measure the levels of ATP/ADP to support these findings. In our study, the NO pathway appeared relatively unimportant, and in fact, the presence of L-arginine or the inhibition of NO-synthase by L-NNA did not modify the relaxation induced by nicorandil. It appears that glibenclamide is probably not the best antagonist of nicorandil on human umbilical arteries at 37°C or at 25°C. Conversely, ODQ strongly attenuates the vasodilation response to nicorandil, particularly so at 25°C. This was confirmed by the fact that relaxation by SIN-1 (direct donor of NO) was strongly

decreased at 25°C when compared to the relaxation observed after cromakalim under the same conditions. Thus, the effect of guanylate cyclase, more than ATP channels, seems to be temperature-dependent for relaxation of smooth muscle in human umbilical arteries.

Acknowledgements

I would like to thank the staff of the Department of Obstetrics (Professor Y. Villa) for kindly supplying us with umbilical cords. I also thank Dr G. Périé who provided immunohistochemistry of tissue samples and P. Vallée for technical assistance. Marie-Louise Kane is also acknowledged for critical reading of this text.

References

Abe, S., Nishimura, J., Nakamura, M., Kanaide, H., 1994. Effects of nicorandil on cytosolic calcium concentrations and on tension development in the rabbit femoral artery. J. Pharmacol. Exp. Ther. 2, 762–771.

Andrew, J.P., Mayer, B., 1999. Enzymatic function of nitric oxide synthase. Cardiovasc. Res. 43, 521–531.

Bedelsson, M., Arneklo-Nobin, B., Törnebrand, K., 1989. Cooling augments contractile response to 5-hydroxytryptamine via an endothelium-dependent mechanism. Blood Vessels 26, 347–359.

Borg, C., Mondot, S., Mestre, M., Cavero, I., 1991. Nicorandil: differential contribution of K^+ channel opening and guanylate cyclase stimulation to its vasorelaxant effects on various endothelin-1-contracted arterial preparations; comparison to aprikalim (RP 52891) and nitroglycerin. J. Pharmacol. Exp. Ther. 259, 526–534.

Chang, J.K., Moore, P., Fineman, J.R., Soifer, J.S., Heymann, M.A., 1992. K^+ channel pulmonary vasodilation in fetal lambs: role of endothelium-derived nitric oxide. J. Appl. Physiol. 73, 188–191.

Eltze, M., 1989. Glibenclamide is a competitive antagonist of cromakalim, pinacidil and RP 49356 in guinea pig pulmonary artery. Eur. J. Pharmacol. 165, 231–239.

Flavahan, N.A., Vanhoutte, P.M., 1986. Effect of cooling on $alpha_1$- and $alpha_2$-adrenergic responses in canines saphenous and femoral veins. J. Pharmacol. Exp. Ther. 238, 139–147.

Garcia-Villalon, A.L., Fernandez, N., Garcia, J.L., Gomez, B., Dieguez, G., 1995. Role of nitric oxide and potassium channels in cholinergic relaxation of rabbit ear and femoral arteries: effects of cooling. J. Vasc. Res. 32, 387–397.

Harker, C.T., Ousley, P.J., Bowman, C.J., Porter, J.M., 1991. Cooling augment $alpha_2$-adrenoceptor-mediated contractions in rat tail artery. Am. J. Physiol. 260, H1166–H1171.

Henry, P., Escande, D., 1994. Do potassium channel openers compete with ATP to activate ATP-sensitive potassium channels? Cardiovasc. Res. 28, 754–759.

Holtzmann, S., 1982. Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. J. Cyclic Nucleotide Res. 8, 409–419.

Ishiyama, T., Dohi, S., Iida, H., Akamatsu, S., Ohta, S., Shimonaka, H., 1994. Mechanisms of vasodilatation of cerebral vessels induced by the potassium channel opener nicorandil in canine in vivo experiments. Stroke 8, 1644–1650.

Karaki, H., Nagase, H., 1987. Low temperature augment the endothelium-dependent relaxation in isolated rat aorta. Eur. J. Pharmacol. 142, 129–132.

Kawaki, K., Chiba, S., 1989. Different sensitivity changes of canine and simian femoral arterial and venous constrictions by cooling. Jpn. J. Pharmacol. 49, 544–548.

Kinoscita, M., Sakai, K., 1990. Pharmacoloy and therapeutic effects of nicorandil. Cardiovasc. Drugs Ther. 4, 1075–1988.

Kojda, G., Harrison, D., 1999. Interaction between NO and reactive oxygen species: pathophysiological importance in atherosclerosis, hypertension, diabetes and heart failure. Cardiovasc. Res. 43, 562–571.

Kreye, W.A.W., Lenz, T., Theiss, U., 1991. The dualistic mode of action of the vasodilator drug, nicorandil, differentiated by glibenclamide in ^{86}Rb flux studies in rabbit isolated vascular smooth muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 70–75.

Kukovez, W.R., Holtzman, S., Braide, C., Pöch, G., 1991. Dual mechanism of relaxing effect of nicorandil by stimulation of cyclic GMP formation and hyperpolarisation. J. Cardiovasc. Pharmacol. 17, 627–633.

Kukovez, W.R., Holtzman, S., Braide, C., Pöch, G., 1992. Molecular mechanism of action of nicorandil. J. Cardiovasc. Pharmacol. 20 (Suppl. 3), 1–7.

Lawson, K., 1996. Potassium channel activation: a potent therapeutic approach? Pharmacol. Ther. 70, 39–73.

Mayer, B., Brunner, F., Schmidt, K., 1993. Novel action of methylene blue. Eur. Heart J. 14 (Suppl. 1), 22–26.

Mayhan, W.G., 1993. In vivo reactivity of resistence arterioles to activation of ATP-sensitive K^+ channels. Eur. J. Pharmacol. 242, 109–112.

McCord, J.M., Fridovich, I., 1970. The utility of superoxide dismutase in studyng free radical reaction: II. The mechanism of the mediation of cytochrome *c* reduction by a variety of electron carriers. J. Biol. Chem. 245, 1374–1377.

Miki, N., Kawabe, Y., Kuriyama, K., 1977. Activation of cerebral guanylate cyclase by nitric oxide. Biochem. Biophys. Res. Commun. 75, 851–856.

Ohlstein, E.H., Wopod, K.S., Ignarro, L.J., 1982. Purification and properties of heme and other factors on enzyme activation by NO, NO-heme, and protoporphyrin IX. Arch. Biochem. Biophys. 281, 187–192.

Saito, W., Noguchi, K., Okazati, K., Matsuda, T., Kato, Y., Tanaka, H., Shigenobu, K., 1997. Temperature-sensitive effects of potassium channel openers on isolated guinea pig myocardium and aorta. J. Cardiovasc. Pharmacol. 31, 327–329.

Wallenstain, S., Zuckler, L.C., Fleiss, J.L., 1980. Some statistical methods useful in circulation research. Circ. Res. 208, 748–753.