Inhibition of hypoxia-induced relaxation of rabbit isolated coronary arteries by N^G-monomethyl-L-arginine but not glibenclamide

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1 The effects of N^G-monomethyl-L-arginine, tetrodotoxin and glibenclamide on hypoxia-induced coronary artery relaxation, induced by bubbling Krebs solution with 95% N₂ and 5% CO₂ instead of 95% O₂ and 5% CO₂, were assessed by measuring the changes in isometric tension in isolated epicardial coronary artery rings of the rabbit. In addition, the effects of glibenclamide on the relaxation induced by adenosine were investigated.

2 Hypoxia caused a transient relaxation of $38 \pm 3\%$ ($P < 0.01$) and $17 \pm 2\%$ ($P < 0.01$) in endothelium-intact or -denuded arteries respectively. N^G-monomethyl-L-arginine (30 and 100 μM) inhibited the relaxation in endothelium-intact rings to $31 \pm 2\%$ ($P < 0.05$) and $16 \pm 2\%$ ($P < 0.01$) respectively and slightly but significantly attenuated the relaxation in endothelium-denuded rings to $15 \pm 1\%$ and $13 \pm 1\%$ ($P < 0.05$) respectively.

3 Glibenclamide, a potassium channel inhibitor, did not significantly affect the hypoxia-induced relaxation.

4 Incubation with tetrodotoxin (3 and 10 μM) for 30 min reduced the relaxation to $31 \pm 3\%$ ($P < 0.05$) and $14 \pm 2\%$ ($P < 0.01$), and $14 \pm 2\%$ ($P < 0.05$) and $11 \pm 1\%$ ($P < 0.05$) in endothelium-intact and -denuded rings respectively. However, indomethacin (10 μM), atropine (1 μM), propranolol (10 μM) and phentolamine (10 μM) did not significantly affect the relaxation.

5 Adenosine (1, 10 and 100 μM) caused relaxation of $6 \pm 1\%$, $52 \pm 3\%$ and $97 \pm 2\%$ respectively in endothelium-denuded rings precontracted with prostaglandin F_{2 α} (PGF_{2 α} , 3 μM) and the relaxation was markedly inhibited by 8-phenyltheophylline. Furthermore, glibenclamide (1 and 10 μM) reduced the relaxation induced by adenosine (1, 10 and 100 μM) to $2 \pm 1\%$ ($P < 0.05$), $38 \pm 3\%$ ($P < 0.05$) and $85 \pm 2\%$ ($P < 0.05$), and $0.6 \pm 0.4\%$ ($P < 0.05$), $27 \pm 4\%$ ($P < 0.05$) and $72 \pm 4\%$ ($P < 0.01$) respectively, in these endothelium-denuded preparations.

6 These data suggest that hypoxia-induced relaxation is mediated by the release of nitric oxide rather than by the activation of glibenclamide-sensitive potassium channels in rabbit isolated coronary arteries. A neurogenic mechanism partially modulates the relaxation, possibly by activating non-adrenergic and non-cholinergic nerve endings. The inhibition by glibenclamide on adenosine-induced relaxation in isolated coronary arteries may help to explain the fact that glibenclamide inhibits hypoxic coronary relaxation in perfused hearts but not in isolated coronary preparations.

Keywords: Adenosine; hypoxia; relaxation; nitric oxide; neurogenic; ATP-sensitive potassium channels; coronary artery

Introduction

Hypoxia can cause either relaxation or contraction of vascular tissue depending on vessel and species and the degree of hypoxia (Rubanyi & Vanhoutte, 1985a; Wadsworth *et al.*, 1990; Vanhoutte & Lüscher, 1990). Various mechanisms underlying coronary artery relaxation induced by hypoxia have been proposed. The release of endothelium-derived relaxing factor (EDRF), recognised as nitric oxide or a closely related moiety (Palmer *et al.*, 1987; Ignarro *et al.*, 1987), and prostaglandin I₂, may be involved in the hypoxia-induced coronary artery relaxation (Busse *et al.*, 1984; Pohl & Busse, 1989). It is now known that an endothelium-derived hyperpolarization factor (EDHF), identified in certain vascular beds, may contribute to endothelium-dependent relaxation (Chen *et al.*, 1988; Edwards & Weston, 1990; Jiang *et al.*, 1991). Whether EDHF is involved in the mechanism of hypoxia-induced, endothelium-dependent relaxation is not known.

Recent experiments suggest that activation of glibenclamide-sensitive potassium channels may mediate hypoxia-induced relaxation of coronary arteries in perfused heart preparations (Daut *et al.*, 1990; von Beckerath *et al.*, 1991). These authors have proposed that activation of the channel mediates the hypoxia-induced relaxation. However, it is well known that adenosine and other metabolites can be released from the hypoxic myocardium (Newby *et al.*, 1985; Deussen *et al.*, 1986). These vasoactive substances may affect coronary artery tone

(Wei *et al.*, 1989). Indeed, adenosine has been found to activate directly glibenclamide-sensitive potassium channels in cardiac myocytes (Kirsch *et al.*, 1990) and dog coronary artery *in vivo* (Belloni & Hintze, 1991). Conversely, endothelium-dependent relaxation induced by acetylcholine has been shown to be mediated partially by the activation of glibenclamide-sensitive potassium channels in some vessels, including rabbit coronary arteries (Jiang *et al.*, 1991).

In order to assess the possible interaction of the endothelium-dependent and adenosine triphosphate-sensitive potassium (K_{ATP}) channel mechanisms, we investigated the effects of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthase, and glibenclamide, which is an inhibitor of the K_{ATP} channels, on hypoxia-induced relaxation in isolated epicardial coronary artery preparations of the rabbit. The possible involvement of non-adrenergic, non-cholinergic and capsaicin nerve endings was assessed by use of tetrodotoxin and capsaicin. Furthermore, the effects of glibenclamide on adenosine-induced relaxation in similar preparations were investigated.

Methods

Animals and tissues

Adult male New Zealand white rabbits (2.5–3 kg) were anaesthetized by an injection of pentobarbitone (60 mg kg⁻¹) into the

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ear artery after an injection of heparin (150 units kg^{-1}). The hearts were removed and immediately immersed in ice-cold modified Krebs solution. The epicardial coronary artery was dissected free of connective tissue. Rings (2–3 mm length) were prepared and, in alternate rings, endothelium was removed by gently rubbing the intimal surface with a wooden probe. The presence or absence of endothelium was verified by the relaxation in response to substance P (0.3, 1, 3 and 10 nM).

Coronary artery rings with, or without, endothelium were suspended horizontally between two stainless steel hooks for measurement of isometric tension in individual organ baths containing 7 ml modified Krebs solution at 37°C, bubbled with 95% O_2 and 5% CO_2 . The millimolar composition of modified Krebs solution was as follows: NaCl 118.3, KCl 4.7, CaCl_2 2.5, MgSO_4 1.2, K_2PO_4 1.2 and glucose 11.1. Rings were equilibrated for 90 min under a resting tension of 1 g before drugs were added. All experiments were performed in rings with, or without, endothelium in the presence of indomethacin (10 μM).

Coronary artery relaxation induced by hypoxia

Concentration-dependent constrictor response curves to prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) were performed in endothelium-intact and -denuded coronary arteries. The EC_{70} concentration of $\text{PGF}_{2\alpha}$ was used to pre-contract individual rings. Hypoxia was induced by bubbling modified Krebs solution with 95% N_2 and 5% CO_2 instead of 95% O_2 and 5% CO_2 after which a stable plateau of contraction was achieved. PO_2 was measured by rapid anaerobic collection of the Krebs solution from the organ bath and its immediate analysis with a Corning 178 pH/blood gas analyser (Corning, Essex), at the time of maximal transient relaxation.

Effects of L-NMMA or glibenclamide on hypoxia-induced relaxation

In groups of endothelium-intact or -denuded rings, L-NMMA (30 and 100 μM), L-NMMA (30 and 100 μM) plus L-arginine (1 mM) or D-arginine (1 mM) or glibenclamide (1 and 3 μM , dissolved in ethanol) were added to coronary arteries precontracted with $\text{PGF}_{2\alpha}$. Hypoxia was induced 5 min after the addition of the agent. Because of possible impairment of nitric oxide-dependent relaxation by hypoxia and reoxygenation, these experiments were performed in different tissues. Time-matched controls were performed in the presence of the solvent, ethanol (1/2000, v/v).

Effects of tetrodotoxin (TTX) or capsaicin pretreatment on hypoxia-induced relaxation

A group of endothelium-intact or -denuded arteries was incubated with atropine (10 μM) plus propranolol (10 μM) and phentolamine (10 μM). In the presence of these three agents, another group of endothelium-intact or -denuded arteries was incubated with TTX (3 μM) or capsaicin (1 μM) for 20 min. Rings were then precontracted with $\text{PGF}_{2\alpha}$ 10 min after washout of TTX or capsaicin. Hypoxia was subsequently induced.

Effects of 8-phenyltheophylline or glibenclamide on adenosine-induced relaxation

In endothelium-denuded rings the dose-dependent relaxing responses to adenosine (1, 10 and 100 μM) were performed after the rings had been precontracted with $\text{PGF}_{2\alpha}$ (3 μM). In different rings precontracted with $\text{PGF}_{2\alpha}$ (3 μM), glibenclamide (1 and 10 μM), 8-phenyltheophylline (10 μM) or the solvent ethanol (1/2000, v/v) were added. Adenosine dose-dependent relaxant responses were then obtained 5 min later. In two endothelium-denuded rings, precontracted by $\text{PGF}_{2\alpha}$ (3 μM), hypoxia was induced 5 min after the addition of 8-phenyltheophylline (10 μM).

Drugs

The following drugs were used: adenosine, N^G monomethyl-L-arginine, tetrodotoxin, indomethacin, atropine sulphate, propranolol hydrochloride, phentolamine hydrochloride, (all supplied by Sigma chemical company and of analytical grade); glibenclamide (gift from Hoechst, UK.).

Statistics

Coronary artery relaxation was expressed as a percentage of the contraction induced by $\text{PGF}_{2\alpha}$. Data are shown as mean $\pm$ s.e.mean. Student's unpaired *t* tests were used to analyse the data. In some experiments, the values between groups were compared by one-way analysis of variance, differences between groups being confirmed by the use of an unpaired *t* test with Bonferroni's modification. A probability of less than 0.05 ($P < 0.05$) was considered significantly different.

Results

Coronary artery relaxation induced by hypoxia

The EC_{70} concentration of $\text{PGF}_{2\alpha}$ induced comparable contractions of 0.9 ± 0.1 and 0.9 ± 0.2 g respectively ($n = 8$) in endothelium-intact or -denuded rings ($P > 0.05$). Hypoxia (PO_2 20–30 torr) caused a transient relaxation in endothelium-intact and -denuded coronary arteries pre-contracted by $\text{PGF}_{2\alpha}$ followed by contraction. The relaxation occurred 2–7 min after hypoxia was induced. However, the relaxation was greater in endothelium-intact than -denuded rings ($P < 0.05$, Figure 1). In time-matched controls, the contraction induced by $\text{PGF}_{2\alpha}$ was no different.

Effects of L-NMMA, L-NMMA plus L-arginine, or L-NMMA plus D-arginine, on hypoxia-induced relaxation

L-NMMA (100 μM) caused a small further contraction (0.06 ± 0.04 g) in endothelium-intact rings precontracted by $\text{PGF}_{2\alpha}$, but none in endothelium-denuded arteries. L-NMMA (30 and 100 μM) significantly reduced hypoxia-induced relaxation in endothelium-intact and -denuded arteries in the presence of

Figure 1 The effects of N^G -monomethyl-L-arginine (L-NMMA, 30 and 100 μM , left slide- and right slide-hatched columns respectively), L-NMMA (100 μM) plus L-arginine (1 mM, cross-hatched columns) or L-NMMA (100 μM) plus D-arginine (1 mM, solid columns) on the relaxation induced by hypoxia (open columns) in rabbit coronary arteries with, or without endothelium. Data (mean $\pm$ s.e.mean, $n \geq 6$) are expressed as percentage relaxation of contraction induced by prostaglandin $\text{F}_{2\alpha}$ (EC_{70} concentration). * $P < 0.01$ compared to time-matched non-hypoxic controls; ** $P < 0.01$ between endothelium-intact and -denuded arteries; # $P < 0.05$ and ### $P < 0.01$ compared with corresponding hypoxia alone groups.

Table 1 The effects of glibenclamide (1 and 3 μ M), atropine plus propranolol plus phentolamine (10 μ M, Atr + Prop + Phent) or capsaicin (1 μ M) on hypoxia-induced relaxation in endothelium-intact or -denuded rabbit coronary arteries, precontracted by an EC₇₀ dose of prostaglandin F_{2 α}

	Endothelium-intact	Endothelium-denuded
Hypoxia control	38 $\pm$ 3%	17 $\pm$ 2%
Glibenclamide (1 μ M)	38 $\pm$ 4%	15 $\pm$ 2%
Glibenclamide (3 μ M)	36 $\pm$ 2%	15 $\pm$ 3%
Capsaicin	34 $\pm$ 2%	14 $\pm$ 3%
Atr + Prop + Phent	39 $\pm$ 4%	19 $\pm$ 2%

Data are expressed as percentage relaxation of contraction to prostaglandin F_{2 α} (mean $\pm$ s.e.mean, $n \geq 6$).

Figure 2 The effects of tetrodotoxin (TTX, 3 and 10 μ M) on hypoxia-induced relaxation of rabbit coronary arteries with, or without endothelium (open and cross-hatched columns respectively). Data (mean $\pm$ s.e.mean, $n \geq 6$) are expressed as percentage relaxation of contraction induced by prostaglandin F_{2 α} (EC₇₀ concentration). * $P < 0.05$ and ** $P < 0.01$ compared with corresponding hypoxia alone groups.

atropine (10 μ M), propranolol (10 μ M) and phentolamine (10 μ M). Neither L-arginine nor D-arginine had an effect on contraction induced by PGF_{2 α} . L-Arginine (1 mM) partially reversed this reduction in relaxation, whilst D-arginine (1 mM) had no effect (Figure 1).

Effects of glibenclamide on hypoxia-induced relaxation

Glibenclamide (1 and 3 μ M) did not cause significant constrictor responses in endothelium-intact or -denuded arteries precontracted with PGF_{2 α} . Hypoxia-induced relaxation was slightly but not significantly decreased in the presence of glibenclamide ($n = 7$, $P \geq 0.05$, Table 1). The solvent ethanol (1/2000, v/v) did not affect hypoxia-induced relaxation in time-matched controls (data not shown).

Effects of TTX or capsaicin pretreatment on hypoxia-induced relaxation

Incubation with capsaicin for 30 min slightly, but non-significantly, reduced hypoxia-induced relaxation in endothelium-intact and -denuded coronary arteries (Table 1). However, pretreatment with TTX significantly reduced hypoxia-induced relaxation ($P < 0.05$) though the relaxation was not completely abolished. The reduction was greater in endothelium-intact than -denuded arteries (Figure 2). In a group of endothelium-intact or -denuded arteries, atropine, propranolol and phentolamine (10 μ M) did not affect hypoxia-induced relaxation ($n = 6$, $P < 0.05$, Table 1).

Figure 3 The effects of glibenclamide (1 μ M (●) and 10 μ M (▲)) or 8-phenyltheophylline (10 μ M, ◆) on adenosine-induced relaxation (controls, ■) in endothelium-denuded rabbit coronary arteries, precontracted with prostaglandin F_{2 α} (3 μ M). Data (mean $\pm$ s.e.mean, $n \geq 8$) are expressed as percentage relaxation of contraction. * $P < 0.05$ and ** $P < 0.01$ compared to controls.

Effects of glibenclamide and 8-phenyltheophylline on adenosine-induced relaxation

Adenosine caused relaxation of endothelium-denuded rabbit coronary artery rings precontracted with PGF_{2 α} . 8-Phenyltheophylline (10 μ M) had no effect on PGF_{2 α} -induced contraction. The relaxation was reduced by the adenosine-receptor antagonist, 8-phenyltheophylline (10 μ M). Glibenclamide (1 and 3 μ M) inhibited the adenosine-induced relaxation (Figure 3), whilst the solvent ethanol (1/2000, v/v) had no effect. In two endothelium-denuded rings, 8-phenyltheophylline (10 μ M) did not alter the relaxation induced by hypoxia.

Discussion

We have demonstrated that hypoxia causes a transient relaxation in isolated epicardial coronary arteries of the rabbit. This hypoxia-induced relaxation was reduced significantly by L-NMMA whilst glibenclamide and atropine plus propranolol plus phentolamine did not significantly affect the relaxation. The relaxation was also significantly reduced by pretreatment with TTX but not capsaicin.

It has been suggested that anoxia inhibits the release of nitric oxide from the endothelium (De Mey & Vanhoutte, 1983), and endothelium-dependent contraction is induced by the release of endothelium-dependent constricting factor(s) (Rubanyi & Paul, 1984; Rubanyi & Vanhoutte, 1985a). It has also been proposed that hypoxia may promote a more rapid inactivation of the released nitric oxide, possibly due to the enhanced production of superoxide anions (Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986). However, it has been

demonstrated that hypoxia ($PO_2 < 300$ mmHg) stimulates release of EDRF in rabbit aorta or femoral arteries (Bassenge & Busse, 1988; Pohl & Busse, 1989). In the present study, we observed that transient hypoxia-induced relaxation occurred within 2–3 min in both endothelium-intact and -denuded arteries. The relaxation was greater in endothelium-intact than -denuded rings ($P < 0.05$). L-NMMA, a specific inhibitor of nitric oxide biosynthesis, significantly attenuated hypoxia-induced relaxation in endothelium-intact coronary arterial rings. L-Arginine, but not D-arginine, partially reversed this effect. This may be evidence that nitric oxide partially mediates hypoxia-induced relaxation in rabbit coronary arteries. The mechanism by which hypoxia stimulates the release of nitric oxide is unknown. However, endothelial cells may release substance P and other metabolites such as ATP and ADP under hypoxic conditions (Burnstock *et al.*, 1988; Milner *et al.*, 1989; Burnstock, 1989). The release of nitric oxide may therefore be secondary to the release of receptor- or non-receptor-mediated stimuli from endothelial cells in endothelium-intact tissues. Prostaglandin I_2 has been implicated in the mechanism of hypoxia-induced coronary relaxation (Busse *et al.*, 1984; Pohl & Busse, 1989). In the present study, experiments were performed in the presence of indomethacin, therefore precluding the effects of these vasodilator prostanooids.

We found that hypoxia induced a small but significant transient relaxation in endothelium-denuded rings. We tested the hypothesis that activation of glibenclamide-sensitive potassium channels may mediate the hypoxia-induced relaxation, since hypoxia-induced coronary relaxation in perfused guinea-pig hearts is inhibited by glibenclamide and mimicked by the potassium channel opener, cromakalim (Daut *et al.*, 1990; von Beckerath *et al.*, 1991). Glibenclamide significantly inhibits endothelium-independent relaxation induced by lemakalim, a potassium channel opener, in similar preparations of the rabbit epicardial coronary artery (Jiang *et al.*, 1991). However, the same concentration of glibenclamide did not significantly affect hypoxia-induced relaxation in our experiments. These results are consistent with the findings that inhibition of the glibenclamide-sensitive potassium channel does not affect hypoxia-induced relaxation in rat isolated aortic preparations (Rodman *et al.*, 1990). This would argue against the activation of glibenclamide-sensitive potassium channels being responsible for the relaxation demonstrated in these experiments. Furthermore, hypoxia may stimulate the release of endothelium-independent vasoactive substances. It is also possible that hypoxia directly affects intracellular pathways which modulate smooth muscle relaxation.

Adenosine may be the substance that is involved in this relaxation. It is a potent coronary arterial relaxing agent (Kusachi *et al.*, 1984) and an important cardiac metabolite in the regulation of coronary arterial tone (Olsson & Bünger, 1987). EDRF mediates adenosine-induced relaxation in certain arteries, including canine coronary arteries (Rubanyi & Vanhoutte, 1985b). However, in other arteries, including rabbit coronary arteries (De Mey & Vanhoutte, 1981; Furchgott, 1983; Corr, 1992), adenosine causes endothelium-independent relaxation. It has been shown that the relaxation induced by adenosine is mediated by a stimulation of the A_2 receptor (Mustafa, 1980; Cushing *et al.*, 1991; Mathie *et al.*, 1991) and a receptor-linked activation of adenylate cyclase (Kukovetz *et al.*, 1981; Cassis *et al.*, 1987). The release of adenosine may be responsible for the observed coronary arterial relaxation, since adenosine has been shown to be released from hypoxic myocardium (Newby *et al.*, 1985; Deussen *et al.*, 1986; Headrick & Willis, 1988). Adenosine has been shown directly to activate K_{ATP} channels in cardiac myocytes (Kirsch *et al.*, 1990) and the coronary artery *in vivo* (Belloni & Hintze, 1991). It can mediate hypoxic coronary dilatation (Merrill *et al.*, 1986, 1988; Wei *et al.*, 1988, 1989) and has also been shown to hyperpolarize coronary arterial smooth muscle (Sabouni *et al.*, 1989). In the present study, we have demonstrated that adenosine induces relaxation which is inhibited

by the adenosine-receptor antagonist, 8-phenyltheophylline. Glibenclamide significantly reduced this relaxation. Thus, adenosine may be responsible for the glibenclamide-sensitive hypoxia-induced relaxation in guinea-pig coronary arteries in perfused hearts. It is possible that mechanisms may vary between different preparations with differing degrees of hypoxia. It should also be noted that there may be differences between the epicardial coronary arteries used in our experiments and the resistance vessels in perfused hearts, and that the hypoxia-induced relaxation may vary from one species to the other. K_{ATP} channel activity has been demonstrated in vascular smooth muscle (Standen *et al.*, 1989; Nelson *et al.*, 1990). This is inhibited by glibenclamide and opened by potassium-channel openers such as cromakalim (Standen *et al.*, 1989). Some authors propose that glibenclamide-antagonized relaxation may be due to the activation of K_{ATP} channels (Nelson *et al.*, 1990; Kirsch *et al.*, 1990; Belloni & Hintze, 1991). Thus, it is a possibility that adenosine may act on K_{ATP} channels. However, further investigation is required to clarify the precise mechanisms of smooth muscle relaxation by adenosine.

Glibenclamide did not significantly inhibit the hypoxia-induced relaxation in endothelium-intact arteries. However, the same concentration of glibenclamide has been shown to inhibit significantly the endothelium-dependent relaxation induced by acetylcholine in similar preparations (Edwards & Weston, 1990; Jiang *et al.*, 1991). It is well established that nitric oxide induces relaxation by increasing the levels of cyclic GMP in smooth muscle, while EDHF has been proposed to activate K_{ATP} channels (Chen *et al.*, 1989). EDHF can be released by acetylcholine and contributes to a small part of the endothelium-dependent relaxation in certain vascular beds (Edwards & Weston, 1990; Jiang *et al.*, 1991). Either EDHF is not released during hypoxia, or, if it is, not in sufficient amounts to make a significant contribution to relaxation.

The modulation by autonomic nerves of coronary vasomotor tone is becoming increasingly recognised. Autonomic nerve endings, which contain neuropeptides, have been demonstrated by functional and immunocytochemical studies in coronary artery tissue (Maggi & Meli, 1988; Gaw *et al.*, 1991). The activation of these nerve endings in response to chemical or electrical stimulation induces endothelium-dependent or -independent relaxation in the presence of adrenergic and cholinergic antagonists (Hynes *et al.*, 1986; Kawasaki *et al.*, 1988; Han *et al.*, 1990). Non-adrenergic and non-cholinergic (NANC) nerves may release endothelium-dependent or -independent vasodilators such as substance P (Hökfelt *et al.*, 1980; Ellis & Burnstock, 1989) and ATP (MacKenzie *et al.*, 1988). It has been proposed that NO itself may be a neurotransmitter in non-adrenergic and non-cholinergic nerves in certain vascular beds (for review see Moncada *et al.*, 1991). It is not known whether a neurogenic mechanism is involved in hypoxia-induced relaxation. However, the neuropeptides, ATP and ADP, may be released from NANC nerve endings by electrical and chemical stimulation as well as hypoxia in some tissues (Hökfelt *et al.*, 1980). In the present study, we have observed that pretreatment with TTX significantly reduced the hypoxia-induced relaxation. Atropine and propranolol did not affect the relaxation. These results suggest that the hypoxia-induced relaxation is partially mediated via a neurogenic mechanism, possibly by stimulating NANC nerve endings. This possible mechanism might explain why L-NMMA attenuates hypoxia-induced relaxation in endothelium-denuded tissues by inhibiting the release of NO from NANC in response to hypoxia. Recent reports suggest that L-NMMA partially inhibits endothelium-independent relaxation induced by vasoactive intestinal polypeptide (VIP) (Gaw *et al.*, 1991). Therefore, we cannot exclude the possibility that L-NMMA may directly inhibit the action of VIP, possibly released from NANC nerve endings. In some tissues, capsaicin-sensitive sensory nerves are responsible for the autonomic nerve-mediated actions (Fujimori *et*

al., 1990). In this study, capsaicin incubation *in vitro* did not significantly affect the hypoxia-induced relaxation. We suggest that capsaicin-sensitive fibres are not involved in the mechanism underlying hypoxia-induced relaxation in rabbit epicardial coronary artery preparations.

In summary, these results suggest that hypoxia-induced relaxation is mediated by the release of endothelium-derived

relaxing factor rather than by the activation of K_{ATP} in rabbit isolated coronary arteries. A neurogenic mechanism partially modulates the relaxation, possibly by activating non-adrenergic and non-cholinergic nerve endings. Adenosine-induced relaxation may explain the different effects of glibenclamide on hypoxic coronary artery relaxation in perfused hearts and isolated coronary arterial preparations.

References

- BASSENGE, E. & BUSSE, R. (1988). Endothelial modulation of coronary tone. *Prog. Cardiovasc. Dis.*, **30**, 349–380.
- BELLONI, F.L. & HINTZE, T.H. (1991). Glibenclamide attenuates adenosine-induced bradycardia and coronary vasodilatation. *Am. J. Physiol.*, **261**, H720–H727.
- BURNSTOCK, G. (1989). Vascular control by purines with emphasis on the coronary system. *Eur. Heart J.*, **10**, Suppl F, 15–21.
- BURNSTOCK, G., LINCOLN, J., FEHÉR, E., HOPWOOD, A.M., KIRKPATRICK, K., MILNER, P. & RALEVIC, V. (1988). Serotonin is localized in endothelial cells of coronary arteries and released during hypoxia: a possible new mechanism for hypoxia-induced vasodilatation of the rat heart. *Experientia*, **44**, 705–707.
- BUSSE, R., FÖRSTERMANN, U., MATSUDA, H. & POHL, U. (1984). The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia. *Pflügers Arch.*, **401**, 77–83.
- CASSIS, L.A., LOEB, A.L. & PEACH, M.J. (1987). Mechanisms of adenosine- and ATP-induced relaxation in rabbit femoral artery: role of the endothelium and cyclic nucleotides. In *Topics and Perspectives in Adenosine Research*, ed. Gerlach, E. & Becker, B.F. pp. 486–496. Berlin: Springer-Verlag.
- CHEN, G., HASHITANI, H. & SUZUKI, H. (1989). Endothelium-dependent relaxation and hyperpolarization of canine coronary artery smooth muscles in relation to the electrogenic Na-K pump. *Br. J. Pharmacol.*, **98**, 950–956.
- CHEN, G., SUZUKI, H. & WESTON, A.H. (1988). Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br. J. Pharmacol.*, **95**, 1165–1174.
- CORR, L.A. (1992). Peptidergic, purinergic and adrenergic neurotransmitters in the control of the coronary artery. *PhD Thesis, University of London*.
- CUSHING, D.J., BROWN, G.L., SABOUNI, M.H. & MUSTAFA, S.J. (1991). Adenosine receptor-mediated coronary artery relaxation and cyclic nucleotide production. *Am. J. Physiol.*, **261**, H343–H348.
- DAUT, J., MAIER-RUDOLPH, W., BECKERATH, N., MEHRKE, G., GÜNTHER, K. & GOEDEL-MEINEN, L. (1990). Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science*, **247**, 1341–1344.
- DE MEY, J.G. & VANHOUTTE, P.M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.*, **316**, 347–355.
- DE MEY, J.G. & VANHOUTTE, P.M. (1983). Anoxia and endothelium-dependent reactivity of the canine femoral artery. *J. Physiol.*, **335**, 65–74.
- DEUSSEN, A., MÖSER, G. & SCHRADER, J. (1986). Contribution of coronary endothelial cells to cardiac adenosine production. *Pflügers Arch.*, **406**, 608–614.
- EDWARDS, G. & WESTON, A.H. (1990). Potassium channel openers and vascular smooth muscle relaxation. *Pharmacol. Ther.*, **48**, 237–258.
- ELLIS, J.L. & BURNSTOCK, G. (1989). Modulation of neurotransmission in the guinea-pig vas deferens by capsaicin: involvement of calcitonin gene-related peptide and substance P. *Br. J. Pharmacol.*, **98**, 707–713.
- FUJIMORI, A., SAITO, A., KIMURA, S. & GOTO, K. (1990). Release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive vasodilator nerves in the rat mesenteric artery. *Neurosci. Lett.*, **112**, 173–178.
- FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.
- GAW, A.J., ABERDEEN, J., HUMPHREY, P.P.A., WADSWORTH, R.M. & BURNSTOCK, G. (1991). Relaxation of sheep cerebral arteries by vasoactive intestinal polypeptide and neurogenic stimulation: inhibition by L-N^G-monomethyl arginine in endothelium-denuded vessels. *Br. J. Pharmacol.*, **102**, 567–572.
- GRYGLEWSKI, R.J., MONCADA, S. & PALMER, R.M. (1986). Bioassay of prostacyclin and endothelium-derived relaxing factor (EDRF) from porcine aortic endothelial cells. *Br. J. Pharmacol.*, **87**, 685–694.
- HAN, S.P., NAES, L. & WESTFALL, T.C. (1990). Calcitonin gene-related peptide is the endogenous mediator of nonadrenergic-noncholinergic vasodilation in rat mesentery. *J. Pharmacol. Exp. Ther.*, **255**, 423–428.
- HEADRICK, J. & WILLIS, R.J. (1988). Mediation by adenosine and bradycardia in rat heart during graded global ischaemia. *Pflügers Arch.*, **412**, 618–623.
- HÖKFELT, T., JOHANSSON, O., LJUNGAHL, A., LUNDBERG, J.M. & SCHULTZBERG, M. (1980). Peptidergic neurones. *Nature*, **284**, 515–521.
- HYNES, M.R., BANNER, W.Jr., YAMAMURA, H.I. & DUCKLES, S.P. (1986). Characterization of muscarinic receptors of the rabbit ear artery smooth muscle and endothelium. *J. Pharmacol. Exp. Ther.*, **238**, 100–105.
- IGNARRO, L.J., BYRNS, R.E., BUGA, G.M. & WOOD, K.S. (1987). Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ. Res.*, **61**, 866–879.
- JIANG, C., POOLE-WILSON, P.A. & COLLINS, P. (1991). Comparison of rabbit coronary artery relaxation induced by acetylcholine and lemakalim: activation of ATP sensitive potassium channels. *Cardiovasc. Res.*, **25**, 930–935.
- KAWASAKI, H., TAKASAKI, K., SAITO, A. & GOTO, K. (1988). Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat. *Nature*, **335**, 164–167.
- KIRSCH, G.E., CODINA, J., BIRNBAUMER, L. & BROWN, A.M. (1990). Coupling of ATP-sensitive K⁺ channels to A₁ receptors by G proteins in rat ventricular myocytes. *Am. J. Physiol.*, **259**, H820–H826.
- KUKOVETZ, W.R., POCH, G. & HOLZMANN, S. (1981). Cyclic nucleotides and relaxation of vascular smooth muscle. In *Vasodilatation*, ed. Vanhoutte, P.M. & Leusen, I. pp. 339–353. New York: Raven Press.
- KUSACHI, S., BUGNI, W.J. & OLSSON, R.A. (1984). Forskolin potentiates the coronary vasoactivity of adenosine in the open-chest dog. *Circ. Res.*, **55**, 116–119.
- MACKENZIE, I., KIRKPATRICK, K.A. & BURNSTOCK, G. (1988). Comparative study of the actions of AP5A and α , β -methylene ATP on nonadrenergic, noncholinergic neurogenic excitation in the guinea-pig vas deferens. *Br. J. Pharmacol.*, **94**, 699–706.
- MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmacol.*, **19**, 1–43.
- MATHIE, R.T., ALEXANDER, B., RALEVIC, V. & BURNSTOCK, G. (1991). Adenosine-induced dilation of the rabbit hepatic arterial bed is mediated by A₂-purinoceptors. *Br. J. Pharmacol.*, **103**, 1103–1107.
- MERRILL, G.F., DOWNEY, H.F. & JONES, C.E. (1986). Adenosine deaminase attenuates canine coronary vasodilation during systemic hypoxia. *Am. J. Physiol.*, **250**, H579–H583.
- MERRILL, G.F., DOWNEY, H.F., YONEKURA, S., WATANABE, N. & JONES, C.E. (1988). Adenosine deaminase attenuates canine coronary vasodilation during regional non-ischaemic myocardial hypoxia. *Cardiovasc. Res.*, **22**, 345–350.
- MILNER, P., RALEVIC, V., HOPWOOD, A.M., FEHÉR, E., LINCOLN, J., KIRKPATRICK, K.A. & BURNSTOCK, G. (1989). Ultrastructural localisation of substance P and choline acetyltransferase in endothelial cells of rat coronary artery and release of substance P and acetylcholine during hypoxia. *Experientia*, **45**, 121–125.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
- MUSTAFA, S.J. (1980). Cellular and molecular mechanism(s) of coronary flow regulation by adenosine. *Mol. Cell. Biochem.*, **31**, 67–87.
- NELSON, M.T., HUANG, Y., BRAYDEN, J.E., HESCHELER, J. & STANDEN, N.B. (1990). Arterial dilations in response to calcitonin gene-related peptide involve activation of K⁺ channels. *Nature*, **344**, 770–773.

- NEWBY, A.C., WORKU, Y. & HOLMQUIST, C.A. (1985). Adenosine formation. Evidence for a direct biochemical link with energy metabolism. *Adv. Myocardiol.*, **6**, 273–284.
- OLSSON, R.A. & BÜNGER, R. (1987). Metabolic control of coronary blood flow. *Prog. Cardiovasc. Dis.*, **29**, 369–387.
- PALMER, R.M., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.
- POHL, U. & BUSSE, R. (1989). Hypoxia stimulates release of endothelium-derived relaxant factor. *Am. J. Physiol.*, **256**, H1595–H1600.
- RODMAN, D.M., HASUNUMA, K., PEACH, J.L. & MCMURTRY, I.F. (1990). Inhibitor of ATP-sensitive K^+ channel alters neither hypoxic contraction nor relaxation of rat aorta. *Blood vessels*, **27**, 365–368.
- RUBANYI, G. & PAUL, R.J. (1984). O_2 -sensitivity of beta adrenergic responsiveness in isolated bovine and porcine coronary arteries. *J. Pharmacol. Exp. Ther.*, **230**, 692–698.
- RUBANYI, G. & VANHOUTTE, P.M. (1985a). Endothelium-removal decreases relaxations of canine coronary arteries caused by beta-adrenergic agonists and adenosine. *J. Cardiovasc. Pharmacol.*, **7**, 139–144.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1985b). Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J. Physiol.*, **364**, 45–56.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822–H827.
- SABOUNI, M.H., HARGITTAI, P.T., LIEBERMAN, E.M. & MUSTAFA, S.J. (1989). Evidence for adenosine receptor-mediated hyperpolarization in coronary smooth muscle. *Am. J. Physiol.*, **257**, H1750–H1752.
- STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245**, 177–180.
- VANHOUTTE, P.M. & LÜSCHER, T.F. (1990). Endothelium-dependent vasoconstriction. In *Endothelium-Derived Contracting Factors*. ed. Rubanyi, G.M. & Vanhoutte, P.M. pp. 1–7. Basel: Karger.
- VON BECKERATH, N., CYRYS, S., DISCHNER, A. & DAUT, J. (1991). Hypoxic vasodilatation in isolated, perfused guinea-pig heart: an analysis of the underlying mechanisms. *J. Physiol.*, **442**, 297–319.
- WADSWORTH, R.M., AMATYA, M.P., KWAN, Y.W., KANE, K.A. & ZEITLIN, I.J. (1990). Modulation by hypoxia of the release of endothelium-derived mediators from sheep coronary artery rings. In *Endothelium-derived Contracting Factors*. ed. Rubanyi, G.M. & Vanhoutte, P.M. pp. 32–38. Basel: Karger.
- WEI, H.M., KANG, Y.H. & MERRILL, G.F. (1988). Coronary vasodilatation during global myocardial hypoxia: effects of adenosine deaminase. *Am. J. Physiol.*, **254**, H1004–H1009.
- WEI, H.M., KANG, Y.H. & MERRILL, G.F. (1989). Canine coronary vasodepressor responses to hypoxia are abolished by 8-phenyltheophylline. *Am. J. Physiol.*, **257**, H1043–H1048.

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