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Effects of metabolic inhibitors on endothelium-dependent and endothelium-independent vasodilatation of rat and rabbit aorta

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1 Basal release of endothelium-derived relaxing factor (EDRF) rendered endothelium-containing rings of rat aorta 4.7 fold less sensitive to the contractile actions of phenylephrine and depressed the maximum response when compared with endothelium-denuded rings. The responsiveness and maximum response to phenylephrine was, however, similar in rings of rabbit aorta with or without endothelium.

2 Rotenone (1 nM–0.1 μM), an inhibitor of oxidative phosphorylation, induced a profound, irreversible blockade of phenylephrine-induced tone in endothelium-containing and endothelium-denuded rings of rat aorta, but induced only slight inhibition of tone in rings of rabbit aorta.

3 2-Deoxy glucose (10 mM), an inhibitor of glycolysis, had no effect on phenylephrine-induced contraction in endothelium-denuded rings of rat aorta, but inhibited reversibly the endothelium-dependent depression of contraction in endothelium containing rings. 2-Deoxy glucose had no effect on phenylephrine-induced contraction in rings of rabbit aorta with or without endothelium.

4 Rotenone (0.1 μM) inhibited acetylcholine-induced, endothelium-dependent relaxation of phenylephrine-contracted rings or rat and rabbit aorta. In endothelium-denuded rings of rat aorta, relaxation induced by glyceryl trinitrate of isoprenaline was also inhibited, but relaxation induced by 8-bromo cyclic GMP or dibutyryl cyclic AMP was not. Relaxation induced by verapamil on KCl-contracted, endothelium-denuded rings of rat aorta was also unaffected.

5 2-Deoxy glucose (10 mM) inhibited acetylcholine-induced, endothelium-dependent relaxation of phenylephrine-contracted rings of rat and rabbit aorta. In endothelium-denuded rings of rat aorta, relaxation induced by glyceryl trinitrate and by isoprenaline was also inhibited, but relaxation induced by 8-bromo cyclic GMP or dibutyryl cyclic AMP was not. Relaxation induced by verapamil on KCl-contracted, endothelium-denuded rings of rat aorta was also unaffected.

6 These data suggest that in rabbit and in rat aorta, rotenone inhibits acetylcholine-induced relaxation by inhibiting EDRF production, and by depressing smooth muscle sensitivity to EDRF, respectively. They further suggest that 2-deoxy glucose inhibits acetylcholine-induced relaxation in both tissues by depressing the sensitivity to EDRF, probably as a result of reduced synthesis of cyclic GMP. The additional possibility that 2-deoxy glucose inhibits EDRF production warrants further investigation.

7 The blockade by 2-deoxy glucose of the endothelium-dependent depression of phenylephrine-induced tone in rat aorta probably reflects blockade of the actions of spontaneously released EDRF.

Introduction

The vascular endothelial cell produces a powerful vasodilator substance, endothelium-derived relaxing factor (EDRF; Furchgott & Zawadzki, 1980), which relaxes vascular smooth muscle by stimulating soluble guanylate cyclase (Forstermann *et al.*, 1986; Ignarro *et al.*, 1986) and elevating cellular gua nosine 3′:5′-cyclic monophosphate (cyclic GMP) content (Holzmann, 1982; Rapoport & Murad, 1983). It has recently been shown that EDRF is nitric oxide (Palmer *et al.*, 1987; Ignarro *et al.*, 1987; Furchgott, 1988) the precursor of which in the endothelium is L-arginine (Palmer *et al.*, 1988).

Little is known of how endothelial production of EDRF is controlled. Production occurs in the resting state (Griffith *et al.*, 1984; Martin *et al.*, 1985) and can be increased further following chemical (Furchgott, 1984) or physical (Holtz *et al.*, 1984) stimulation. Calcium is clearly involved in basal as well as stimulated production of EDRF (Singer & Peach, 1982; Furchgott, 1983; Long & Stone, 1985; Griffith *et al.*, 1986), and this probably relates to the calcium-sensitivity of the enzyme, nitric oxide synthase (Palacios *et al.*, 1989). It has been proposed that stimulated, but not basal, production of EDRF requires metabolic energy (Griffith *et al.*, 1986; 1987). This conclusion was drawn from the finding that metabolic inhibitors such as rotenone powerfully inhibit stimulated but not basal endothelium-dependent vasodilatation in rabbit aorta. The inhibitor of glycolysis, 2-deoxy glucose, was much less effective suggesting that the required metabolic energy was derived from oxidative rather than glycolytic metabolism.

We wished to establish whether the dependence of EDRF production on oxidative metabolism was a generalised phenomenon by comparing the actions of rotenone and 2-deoxy glucose on endothelium-dependent relaxation in rabbit and rat aorta. We also examined the effects of these metabolic inhibitors on the sensitivity of the vascular smooth muscle of these preparations to the constrictor effects of phenylephrine and to the dilator effects of endothelium-independent relaxants.

Methods

Preparation of aortic rings and tension recording

The preparation of aortic rings was similar to that originally described by Furchgott & Zawadzki (1980). Briefly, male Wistar rats weighing 300–400 g and male New Zealand white rabbits weighing 2–3 kg were killed by stunning and exsanguination. The aorta was removed, cleared of adhering fat and connective tissue and cut into 2.5 mm wide transverse rings with a razor blade slicing device. Endothelial cells were removed from some rings by gently rubbing the intimal surface with a moist wooden stick for 30–60 s. Successful

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removal of endothelial cells from aortic rings was confirmed later by the inability of acetylcholine (1 μM) to induce relaxation and in some experiments histological examination of endothelial integrity was performed by use of a silver staining technique (Poole *et al.*, 1958). Rat and rabbit aortic rings were then mounted under 1 g and 2 g resting tension, respectively, on stainless steel hooks in 12 ml organ baths, and bathed at 37°C in Krebs solution containing (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, glucose 11 and disodiumedetate 0.03, and gassed with 95% O_2 and 5% CO_2. In experiments in which 2-deoxy glucose was used, the Krebs solution was identical to that described above except that glucose was omitted and replaced with 2-deoxy glucose (10 mM). Tension was recorded isometrically with Grass FTO3 transducers and displayed on Linseis chart recorders. Tissues were allowed to equilibrate for 90 min before experiments were begun, during which time the resting tension was maintained at the pre-set level.

For relaxation studies, submaximal (30–70%) tone was first induced with phenylephrine or KCl (60 mM). Relaxations were then expressed as percentage relaxation of phenylephrine- or KCl-induced tone.

Drugs

Acetylcholine chloride, 8-bromo cyclic GMP, 2-deoxy-D-glucose, dibutyryladenosine 3':5'-cyclic monophosphase (db cyclic AMP), (±)-isoprenaline hydrochloride, phenylephrine hydrochloride, rotenone and verapamil hydrochloride were obtained from Sigma. Glyceryl trinitrate was obtained from Napp Laboratories. All drugs were dissolved in twice distilled water except for rotenone which was dissolved in ethanol to give a stock solution of 1 mM.

Statistical analysis

Results are expressed as the mean ± s.e.mean and comparisons were made by means of Student's *t* test. A probability of 0.05 or less was considered significant.

Results

Phenylephrine-induced tone

Following contraction with phenylephrine (0.3 μM), rotenone (1 nM–0.1 μM) produced similar concentration-dependent relaxations in rings of rat aorta with or without endothelium (Figure 1). At concentrations above 0.1 μM, rotenone produced contractions that were mimicked by the solvent (ethanol) alone. The ability of rotenone to inhibit phenylephrine-induced tone in rat aorta was not reversed even with extensive washing. In endothelium-containing and endothelium-denuded rings of rabbit aorta, rotenone (1 nM–0.1 μM) produced only small relaxations of phenylephrine (0.3 μM)-induced tone (Figure 1).

Rings of rat aorta without endothelium were 4.7 fold more sensitive to the contractile actions of phenylephrine than endothelium-containing rings and displayed a significantly greater maximum contraction (Figure 2.). Following incubation for 20 min in glucose-free Krebs containing 2-deoxy glucose (10 mM), the maximum phenylephrine-induced contraction in endothelium-containing rings increased significantly with no significant change in the EC_{50} concentration (Figure 2). This augmentation of contraction was reversed when tissues were returned to normal glucose-containing Krebs and washed extensively. In endothelium-denuded rings of rat aorta the maximum phenylephrine-induced contraction and EC_{50} concentration were not significantly different from those obtained in normal glucose-containing Krebs (Figure 2).

Phenylephrine (1 nM–10 μM) induced similar concentration-dependent contractions in rings of rabbit aorta with or without endothelium. Incubation of endothelium containing or endothelium-denuded rings in glucose-free Krebs containing 2-deoxy glucose (10 mM) had no significant effect on phenylephrine-induced contractions (Figure 2).

A combination of rotenone (0.1 μM) and 2-deoxy glucose (10 mM) completely blocked, irreversibly, the ability of phenylephrine to contract both rabbit and rat aorta.

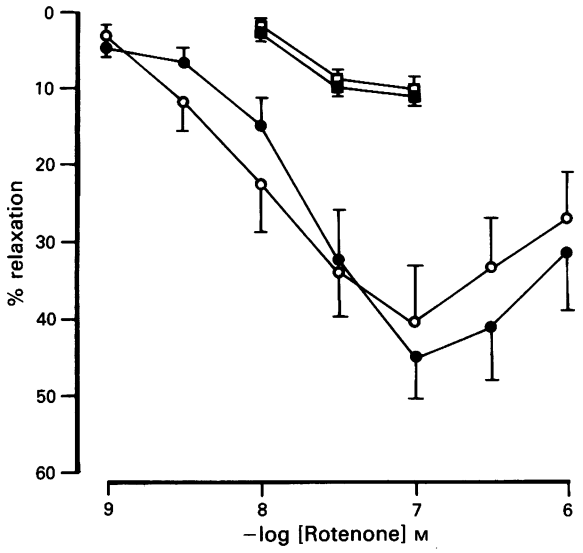

Figure 1 Concentration-response curves showing the ability of rotenone to inhibit phenylephrine (0.3 μM)-induced tone in endothelium-containing (●) and endothelium-denuded (○) rings of rat aorta and endothelium-containing (■) and endothelium-denuded (□) rings of rabbit aorta. Each point is the mean and vertical bars indicate the s.e.mean of 5–9 observations.

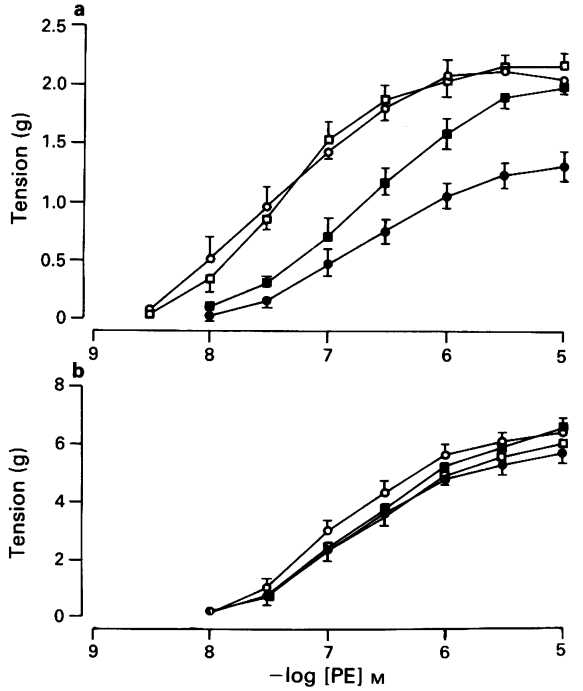

Figure 2 Concentration-response curves showing the contractile effects of phenylephrine (PE) on untreated endothelium-containing (●) and endothelium-denuded (○) rings of rat (a) and rabbit (b) aorta and responses obtained following treatment with 2-deoxy glucose (10 mM) on endothelium-containing (■) and endothelium-denuded (□) rings. Each point is the mean and vertical bars indicate the s.e.mean of 5–12 observations. * $P < 0.05$, indicates a significant difference in the maximum response from untreated, endothelium-containing rings.

Acetylcholine-induced relaxation

Following contraction with phenylephrine (0.3 μM), acetyl choline (3 nM–3 μM) induced concentration-dependent relaxation of endothelium-containing but not endothelium-denuded rings of rat and rabbit aorta (Figure 3).

Following treatment with rotenone (0.1 μM) for 20 min, acetylcholine-induced relaxation was inhibited in rings of rat and rabbit aorta (Figure 3). The ability of rotenone to depress acetylcholine-induced relaxation was only partially reversed following extensive washing. Following incubation for 20 min in glucose-free Krebs containing 2-deoxy glucose (10 mM), acetylcholine-induced relaxation was inhibited in endothelium-containing rings of rat and rabbit aorta (Figure 3). The ability of 2-deoxy glucose to depress acetylcholine-induced relaxation was reversed when tissues were returned to normal glucose-containing Krebs and washed extensively.

Glyceryl trinitrate and 8-bromo cyclic GMP

Treatment with rotenone (0.1 μM), inhibited glyceryl trinitrate (1 nM–1 μM)-induced relaxation in endothelium-denuded rings of rat but not rabbit aorta (Figure 4): in rat aorta the EC_{50} concentration was increased 4.3 fold, ($n = 4$), but the maximum relaxation was not affected (Figure 4). Rotenone (0.1 μM) had no effect, however, on the relaxation of endothelium-denuded rings of rat aorta induced by 8-bromo cyclic GMP (1–100 μM, Figure 4). The rotenone-induced depression of sensitivity to glyceryl trinitrate was reversed following extensive washing. Following incubation for 20 min in glucose-free Krebs solution containing 2-deoxy glucose (10 mM), glyceryl trinitrate-induced relaxation was inhibited in endothelium-denuded rings of rat and rabbit aorta (Figure 4): The EC_{50} concentration was increased 5.6 fold ($n = 8$), and 2.0 fold ($n = 6$), in rat and rabbit aorta, respectively, but only in rat aorta was there a significant reduction in the maximum relaxation (Figure 4). Treatment with 2-deoxy glucose (10 mM) had no significant blocking effect on relaxation of rat aortic rings induced by 8-bromo cyclic GMP except at a concentration of 30 μM (Figure 4).

In both rat and rabbit aorta the ability of 2-deoxy glucose to depress glyceryl trinitrate-induced relaxation was completely reversed when tissues were returned to normal glucose-containing Krebs and washed extensively.

Isoprenaline and dibutyryl cyclic AMP

Treatment with rotenone (0.1 μM), inhibited isoprenaline (10 nM–10 μM)-induced relaxation of endothelium-denuded

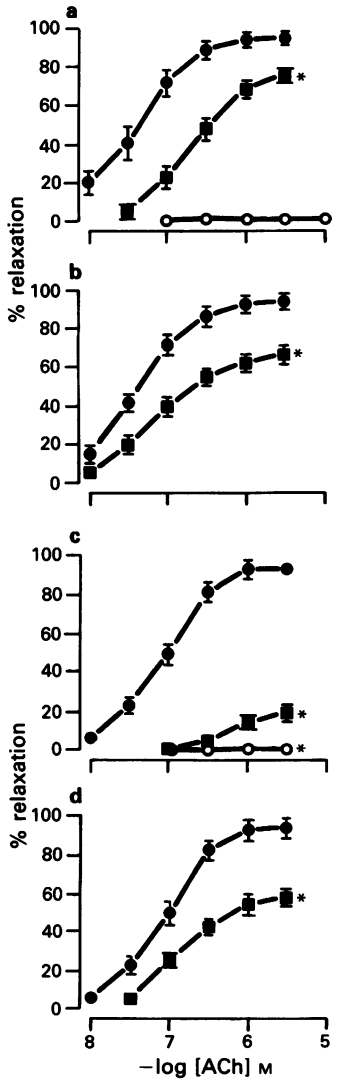

Figure 3 Concentration-response curves showing the relaxant effects of acetylcholine (ACh) on endothelium-containing (●) and on endothelium-denuded (○) rings of rat (a, b) and rabbit (c, d) aorta and on endothelium-containing rings following treatment (■) with rotenone (0.1 μM, a, c) or 2-deoxy glucose (10 mM, b, d). Each point is the mean and vertical bars indicate the s.e.mean of 4–8 observations. *$P < 0.05$ indicates a significant difference from the maximum relaxation obtained on untreated, endothelium-containing rings.

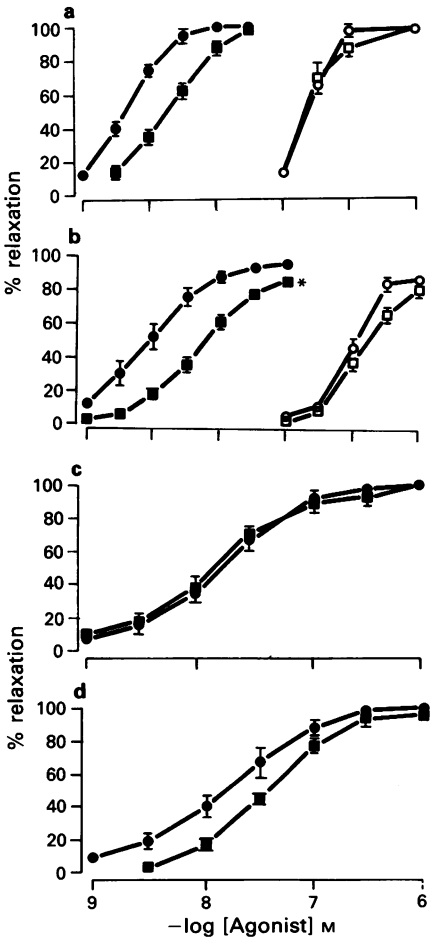

Figure 4 Concentration-response curves obtained on endothelium-denuded rings of rat (a,b) and rabbit (c,d) aorta showing the relaxant effects of glyceryl trinitrate (●) and 8-bromo cyclic GMP (○) before, and of glyceryl trinitrate (■) and 8 bromo cyclic GMP (□) after treatment with rotenone (0.1 μM, a,c) or 2-deoxy glucose (10 mM, b,d). Each point is the mean and vertical bars indicate the s.e.mean of 4–8 observations. *$P < 0.05$ indicates a significant difference from the maximum relaxation obtained on untreated rings.

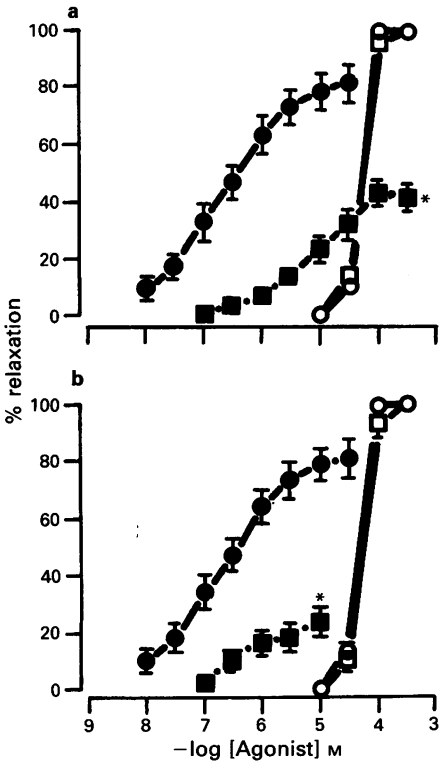

Figure 5 Concentration-response curves obtained on endothelium-denuded rings of rat aorta showing the relaxant effects of isoprenaline (●) and dibutyryl cyclic AMP (○) before, and of isoprenaline (■) and dibutyryl cyclic AMP (□) after treatment with rotenone (0.1 μM, a) or 2-deoxy glucose (10 mM, b). Each point is the mean and vertical bars indicate the s.e.mean of 4–9 observations. * $P < 0.05$ indicates a significant difference from the maximum relaxation obtained on untreated rings.

rings of rat aorta, but had no effect on relaxation induced by db cyclic AMP (10–300 μM, Figure 5). Incubation for 20 min in glucose-free Krebs solution containing 2-deoxy glucose (10 mM) inhibited isoprenaline-induced relaxation, but had no effect on relaxation induced by db cyclic AMP (Figure 5).

Verapamil

Treatment with rotenone (0.1 μM), or with 2-deoxy glucose (10 mM) in glucose-free Krebs solution, had no effect on the ability of verapamil (1–100 nM) to relax KCl (60 mM)-contracted, endothelium-denuded rings of rat aorta (data not shown).

Discussion

Our results confirm those of previous reports (Griffith *et al.*, 1986; 1987) that rotenone, an inhibitor of oxidative phosphorylation, rapidly and almost irreversibly, blocks endothelium-dependent relaxation in rabbit aorta. This blockade is probably due to inhibition of stimulated EDRF production since cascade bioassay experiments show that responsiveness of the vascular smooth muscle in rabbit aorta to EDRF is unaffected (Griffith *et al.*, 1986). Our finding that glyceryl trinitrate-induced relaxation, which occurs by the same cyclic GMP-dependent mechanism as EDRF-induced relaxation (Rapoport *et al.*, 1983), was unaffected by rotenone supports this conclusion. The possibility that rotenone inhibits EDRF production by lower levels of NADPH, a co-factor for the enzyme nitric oxide synthase (Palacios *et al.*, 1989), warrants further study. In rat aorta, however, rotenone inhibits acetylcholine-induced, endothelium-dependent relaxation to a lesser degree, and this is associated with a depression of the relaxant effects of glyceryl trinitrate. In contrast to rabbit aorta, therefore, rotenone-induced inhibition of endothelium-dependent relaxation involved a depression in sensitivity of the vascular smooth muscle. A possible additional inhibition of EDRF production by rotenone in rat aorta is suggested by the reversibility of blockade of glyceryl trinitrate-induced, but not acetylcholine-induced relaxation, and this warrants further investigation.

We confirmed also, the finding of Griffith *et al.*, (1986) that 2-deoxy glucose, an inhibitor of glycolysis, induces a slight inhibition of acetylcholine-induced relaxation in rabbit aorta, and found a similar inhibition in rat aorta. This inhibition is seen only in the absence of glucose and can be reversed following the addition of glucose (Griffith *et al.*, 1986; Richards *et al.*, 1990). Rather than being due to inhibition of EDRF production (Griffith *et al.*, 1986), the blockade probably results from a reduction in sensitivity of the vascular smooth muscle to EDRF since glyceryl trinitrate-induced relaxation was also inhibited in both tissues. Whether 2-deoxy glucose had an additional effect of blocking EDRF production could not be determined in our experiments.

The mechanisms by which rotenone and 2-deoxy glucose inhibit responsiveness of vascular smooth muscle to EDRF and glyceryl trinitrate might be due to lowered levels of the high energy phosphates, adenosine 5'-triphosphate (ATP) and guanosine 5'-triphosphate (GTP). ATP is required for smooth muscle contraction and relaxation, and a fall in cellular content may prevent relaxation by, for example, preventing the phosphorylation of myosin light chain kinase (Hathaway *et al.*, 1985). A more likely explanation, however, is that lowered levels of ATP and GTP result in a reduced capacity to form the second messengers cyclic AMP and cyclic GMP, respectively. Consistent with this explanation are our findings that metabolic inhibitors block relaxations induced by isoprenaline and glyceryl trinitrate, but not those induced by membrane permeant analogues of the second messengers that mediate their respective relaxations, i.e., db cyclic AMP and 8-bromo GMP. This ability of metabolic inhibitors to block relaxations mediated via cyclic nucleotides appears to be selective, since relaxation induced by the calcium channel blocking agent, verapamil, which acts independently of cyclic nucleotides, was completely unaffected.

The effects of metabolic inhibitors on phenylephrine-induced tone were complex. In rabbit aorta rotenone had only a slight depressant action and 2-deoxy glucose had none, but a combination of the two led to a complete and irreversible reduction of tone. This would suggest the smooth muscle of rabbit aorta derives energy from both oxidative and glycolytic metabolism. The profound depressant action of rotenone on tone in rat aorta suggests here the smooth muscle derives most of its energy from oxidative metabolism, and the lack of effect of 2-deoxy glucose on tone in endothelium-denuded rings is consistent with this. In endothelium-containing rings of rat aorta vasoconstrictor responses are depressed by the tonic vasodilator actions of spontaneously released EDRF (Egleme *et al.*, 1984; Martin *et al.*, 1986). The ability of 2-deoxy glucose to augment phenylephrine-induced tone in endothelium-containing rings of rat aorta is likely to be due to inhibition of this depressant action resulting from the reduced sensitivity of the smooth muscle to EDRF discussed above.

In conclusion, in rat aorta rotenone and 2-deoxy glucose each block acetylcholine-induced, endothelium-dependent relaxation by reducing the sensitivity of the vascular smooth muscle to EDRF. In rabbit aorta inhibition of acetylcholine-induced relaxation by rotenone and 2-deoxy glucose results from an inhibition of EDRF production and reduction in smooth muscle sensitivity to EDRF, respectively. Thus, depending on the tissue studied, metabolic inhibitors can block endothelium-dependent vasodilatation by affecting endothelial production of EDRF, smooth muscle sensitivity to EDRF, or both.

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References

EGLEME, C., GODFRAIND, T. & MILLER, R.C. (1984). Enhanced responsiveness of rat isolated aorta to clonidine after removal of the endothelial cells. *Br. J. Pharmacol.*, **81**, 16–18.

FORSTERMANN, U., MULSCH, A., BOHME, E. & BUSSE, R. (1986). Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from rabbit and canine arteries. *Circ. Res.*, **58**, 531–538.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.

FURCHGOTT, R.F. (1984). The role of endothelium in the responses of vascular smooth muscle to drugs. *Ann. Rev. Pharmacol. Toxicol.*, **24**, 175–197.

FURCHGOTT, R.F. (1988). Studies on the relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that the acid-activatable inhibitory factory from bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide. In *Mechanisms of Vasodilatation.* ed. Vanhoutte, P.M., pp. 31–36. New York: Raven Press.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

GRIFFITH, T.M., EDWARDS, D.H., LEWIS, M.J., NEWBY, A.C. & HENDERSON, A.H. (1984). The nature of endothelium-derived vascular relaxant factor. *Nature*, **308**, 645–647.

GRIFFITH, T.M., EDWARDS, D.H., NEWBY, A.C., LEWIS, M.J. & HENDERSON, A.H. (1986). Production of endothelium-derived relaxant factor is dependent on oxidative phosphorylation and extracellular calcium. *Cardiovasc. Res.*, **20**, 7–12.

GRIFFITH, T.M., EDWARDS, D.H. & HENDERSON, A.H. (1987). Unstimulated release of endothelium derived relaxing factor is independent of mitochondrial ATP generation. *Cardiovasc. Res.*, **21**, 565–568.

HATHAWAY, D.R., KONICKI, M.V. & COOLICAN, S.A. (1985). Phosphorylation of myosin light chain kinase from vascular smooth muscle by cAMP- and cGMP-dependent protein kinases. *J. Mol. Cell. Cardiol.*, **17**, 841–850.

HOLTZ, J., FORSTERMANN, U., POHL, U., GIESLER, M. & BASSENGE, E. (1984). Flow-dependent, endothelium-mediated dilatation of epicardial coronary arteries in conscious dogs: effects of cyclooxygenase inhibition. *J. Cardiovasc. Pharmacol.*, **6**, 1161–1169.

HOLZMANN, 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.

IGNARRO, L.J., HARBISON, R.G., WOOD, K.S. & KADOWITZ, P.J. (1986). Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid. *J. Pharmacol. Exp. Ther.*, **237**, 893–900.

IGNARRO, L.J., BUGA, G.M., WOOD, K.S. & BYRNES, R.E. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 9265–9269.

LONG, C.J. & STONE, T.W. (1985). The release of endothelium-derived relaxant factor is calcium-dependent. *Blood Vessels*, **22**, 205–208.

MARTIN, W., VILLANI, G.M., JOTHIANANDAN, D. & FURCHGOTT, R.F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.*, **232**, 708–716.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOTHIANANDAN, D. (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor (EDRF). *J. Pharmacol. Exp. Ther.*, **237**, 529–538.

PALACIOS, M., KNOWLES, R.G., PALMER, R.M.J. & MONCADA, S. (1989). Nitric oxide from L-arginine stimulates the soluble guanylate cyclase in adrenal glands. *Biochem. Biophys. Res. Commun.*, **165**, 802–809.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524–526.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **33**, 664–666.

POOLE, J.C.F., SANDERS, A.G. & FLOREY, H.W. (1958). The regeneration of aortic endothelium. *J. Pathol. Bacteriol.*, **75**, 133–143.

RAPOPORT, R.M., DRAZNIN, M.B. & MURAD, F. (1983). Endothelium-dependent vasodilator- and nitrovasodilator-induced relaxation may be mediated through cyclic GMP-dependent protein phosphorylation. *Trans. Assoc. Am. Physicians*, **96**, 19–30.

RAPOPORT, R.M. & MURAD, F. (1983). Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.*, **52**, 352–357.

RICHARDS, J.M., GIBSON, I.F. & MARTIN, W. (1991). Effects of hypoxia and metabolic inhibitors on production of prostacyclin and endothelium-derived relaxing factor by pig aortic endothelial cells. *Br. J. Pharmacol.*, **102**, 203–209.

SINGER, H.A. & PEACH, M.J. (1982). Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. *Hypertension*, **4**, (Suppl. II), 19–25.

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