Br. J. Pharmac (1987), **92**, 483–485

Differential selectivity of endothelium-derived relaxing factor and nitric oxide in smooth muscle

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The selectivity of endothelium-derived relaxing factor (EDRF) and nitric oxide (NO) on smooth muscle relaxation was examined and compared. EDRF released from bovine pulmonary arterial endothelium (BPAE) in culture and NO were superfused over vascular, tracheal, gastrointestinal and uterine smooth muscle. EDRF relaxed vascular smooth muscle but not tracheal, gastrointestinal or uterine smooth muscle. NO relaxed vascular and gastrointestinal smooth muscle but not tracheal or uterine smooth muscle. There was a differential selectivity between the relaxant effect of EDRF and NO on smooth muscle

Introduction Endothelium-derived relaxing factor (EDRF), a novel vascular relaxing factor, was first described by Furchgott & Zawadzki (1980). Although identification of EDRF has been attempted by many investigators, the chemical structure is still unknown. It has been observed recently that the profile of vasodilator activity between EDRF and NO is similar and it has thus been proposed that EDRF is NO (Ignarro *et al.*, 1987; Furchgott *et al.*, 1987; Palmer *et al.*, 1987). The present study examined this hypothesis by comparing the relaxant effects of EDRF and NO in various smooth muscle tissues.

Methods Rings of thoracic aorta (4 mm long) were obtained from male rabbits (New Zealand White, 3–4 kg) and guinea-pigs (Albino Hartley strain, 400–600 g) killed by cervical fracture and exsanguinated. Tracheal strips (3-cartilages wide) were obtained from male guinea-pigs and taenia coli strips (5 mm long) from male rabbits. Uterine rings were obtained from the uterine horns of female guinea-pigs which had been treated with diethylstilboestrol (0.1 mg kg^{-1}, i.p.) 24 h before the test. Tissues were maintained at 37°C and superfused with the effluent of the column containing BPAE cells (CCL-209, American Type Culture Collection) grown on microcarrier beads (Shikano & Berkowitz, 1987). The column was perfused with Krebs solution containing indomethacin (10^{-5} M), bubbled with 95% O_2: 5% CO_2 at a rate of 3 ml min^{-1} by a peristaltic pump. After equilibration (2 h), tissues were precontracted to approximately 75% of maximal contraction, and superfused with EDRF released from BPAE cells by A23187 (10^{-10}–10^{-5} M). Tissues were also superfused with the effluent of the column without cells, and 50 µl of NO-containing buffer was injected into the perfusate at the distal end of the column. Tissues were precontracted with the following drugs; rabbit aorta: phenylephrine, 3×10^{-7} M; guinea-pig aorta: histamine, 6×10^{-6} M; trachea: histamine, 6×10^{-6} M; taenia coli: histamine, 1×10^{-5} M; uterus: oxytocin, 1×10^{-6} M.

Solutions of NO (99.99%, Matheson Gas Co.) were prepared immediately before use. One ml of 50 mM Tris-HCl, pH 7.4, was placed in a sealed glass tube and evacuated under vacuum for 20 min at 25°C and flushed with O_2-free N_2 for 20 min on ice. NO (50 ml) was flushed through the tube in order to saturate the atmosphere, then vortexed and placed on ice. After 20 min the atmospheric NO was removed by flushing with N_2 for 20 min. Actual concentrations of NO in the buffer are unknown, however, an estimation of the maximal concentrations is possible. The solubility of NO in H_2O at 0°C is 7.34 cm^3/100 ml and assuming saturation, the maximal concentration of NO in solution is approximately 3.3 mM. Thus, when 50 µl of saturated NO-buffer is administered to the bioassay, this represents an upper limit of 165 nmol of NO. ED_{50} values for NO have been calculated on this basis.

Results Superfusion of BPAE cells (3×10^6 cells/column) with A23187 (10^{-10}–10^{-5} M) caused release of EDRF and a dose-dependent relaxation of rabbit and guinea-pig aortic rings. The ED_{50} values were 14 nM and 18 nM, respectively, and the maximal relaxation was obtained at a dose of 10^{-6} M (88 ± 4%, $n = 6$ and 84 ± 6%, $n = 6$, respectively). Trachea, taenia coli and uterus did not relax to EDRF released by A23187. NO solution relaxed rabbit and guinea-pig aorta and rabbit taenia coli. The ED_{50} values were 2.6 nmol ($n = 4$), 3.8 nmol ($n = 4$), and 2.6 nmol ($n = 4$), respectively, and maximal relaxation was obtained with 50 µl of saturated NO solution (78 ± 5%, $n = 4$, 77 ± 4%, $n = 5$ and 94 ± 4%, $n = 4$, respectively). Guinea-pig

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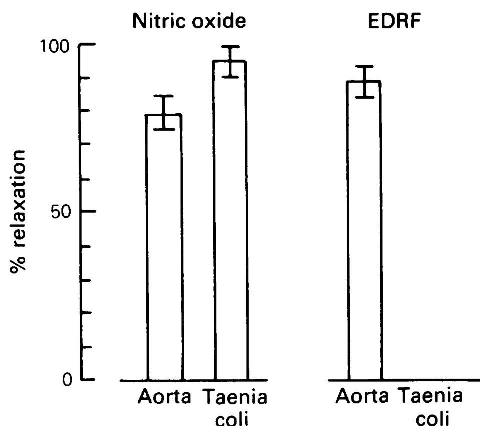

Figure 1 Comparison of the relaxant abilities of nitricoxide (NO) and endothelium-derived relaxing factor (EDRF) in the rabbit aorta and taenia coli: 50 μl of NO-containing solution or EDRF was superfused onto phenylephrine-contracted (3×10^{-7} M) aorta and histamine-contracted (1×10^{-5} M) taenia coli. EDRF was released with A23187 (10^{-6} M) from BPAE cells (3×10^6 cells). NO relaxed both aorta and taenia coli, whereas EDRF relaxed aorta but not taenia coli.

trachea (less than 20% relaxation) and uterus did not relax to NO solution. Figure 1 compares the relaxation in rabbit aortic rings and taenia coli strips caused by EDRF and by NO solution. NO relaxed both aorta and taenia coli, however EDRF relaxed rabbit aorta but not taenia coli. When A23187 was included in the perfusion buffer and superfused through the column without endothelial cells, the relaxation produced by NO in taenia coli and aorta was unaffected. Furthermore, taenia coli did not relax to EDRF released from 3×10^7 cells per column (10 times as many cells as used routinely) or EDRF potentiated with superoxide dismutase (150 u ml^{-1}) added to the superfusing solution.

Discussion Substantial differences have been demonstrated between EDRF and nitrovasodilators. Blood vessels tolerant to nitroglycerin were not cross-tolerent to endothelium-dependent vasodilators (Winquist *et al.*, 1987) and in addition, nitroglycerin preferentially relaxes veins whereas EDRF is less effective in veins than arteries. Recently, however, a resemblance between EDRF and NO has been noted (Ignarro *et al.*, 1987; Furchgott *et al.*, 1987). Both agents increase vascular smooth muscle cyclic GMP levels, are inhibited by haemoglobin and methylene blue, have short half-lives and are potentiated by superoxide dismutase. Whilst these behaviours of EDRF and NO are indeed similar, these criteria remain very indirect evidence upon which to assign an identity to EDRF. Further, Palmer *et al.* (1987) provided evidence for this hypothesis by measuring NO released from bradykinin-stimulated endothelial cells in culture, however, the chemical detection method used to measure NO was indirect and non-selective.

Our data demonstrate that both NO and EDRF exhibit selectivity in the relaxation of smooth muscle. Whilst aorta and taenia coli were relaxed potently by NO, trachea and uterus were not. EDRF relaxed aorta but not trachea, taenia coli or uterus, suggesting that the EDRF is selective for vascular smooth muscle (Shikano & Berkowitz, 1987). In this study, we confirmed the selectivity of EDRF for vascular smooth muscle at higher concentrations of the factor than used formerly by using more endothelial cells and superoxide dismutase (Rubanyi & Vanhoutte, 1986; Gryglewski *et al.*, 1986; Moncada *et al.*, 1986). The taenia coli remained unresponsive to EDRF even under these conditions. Clearly NO has a qualitatively different selectivity from EDRF for smooth muscle and this evidence appears to distinguish the relaxant character of NO from that of EDRF.

In summary we have shown a differential selectivity between NO and EDRF in the relaxation of taenia coli. We therefore conclude that whilst EDRF and NO are remarkably similar they may nevertheless be discriminated by bioassay selectivity. Thus further studies on EDRF identify seem appropriate.

We thank Dr Clive J. Long for helpful discussion.

References

FURCHGOTT, R.F., KHAN, M.T. & JOTHIANANDAN, D. (1987). Comparison of endothelium-dependent relaxation and nitric oxide-induced relaxation in rabbit aorta. *Fed Proc.*, **46**, 385.

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

GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature*, **320**, 454–456.

IGNARRO, L.J., BYRNS, R.E., BUGA, G.M. & WOOD, K.S. (1987). Endothelium-derived relaxing factor (EDRF) released from artery and vein appears to be nitric oxide (NO) or a closely related radical species. *Fed. Proc.*, **46**, 644.

MONCADA, S., PALMER, R.M.J. & GRYGLEWSKI, R.J. (1986). Mechanism of action of some inhibitors of

endothelium-derived relaxing factor. *Proc. Natn. Acad. Sci. U.S.A.*, **83**, 9164–9168.

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.

RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822–H827.

SHIKANO, K. & BERKOWITZ, B.A. (1987). Endothelium-derived relaxing factor is a selective relaxant of vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, (in press).

WINQUIST, R., WALLACE, A. & FAISONS, E. (1987). The effects of nitroglycerin-induced tolerance on the relaxation of endothelium-independent and dependent vasodilators in rabbit aortic segments. *Fed. Proc.*, **46**, 386.

(Received June 16, 1987.
Accepted August 5, 1987.)