Potent contractile activity of endothelin on the human isolated urinary bladder

¹Carlo Alberto Maggi, Sandro Giuliani, Riccardo Patacchini, Paolo Santicioli,
*Damiano Turini, *Gabriele Barbanti & Alberto Meli

Pharmacology Department, Smooth Muscle Division, Res. Labs., A. Menarini Pharmaceuticals, Via Sette Santi 3, Florence 50131 and *Department of Urology, University of Ferrara, Italy

Endothelin (1 nM–0.3 μ M) produced a concentration-related contraction of mucosa-free muscle strips excised from the dome of the human urinary bladder. The response to endothelin was unaffected by either atropine (1 μ M) or nifedipine (1 μ M) at concentrations that abolished the response to carbachol and KCl, respectively. These findings indicate that mechanisms other than activation of dihydropyridine- and voltage-sensitive calcium channels may be involved in the action of endothelin on smooth muscles.

Introduction Endothelin is an endothelium-derived 21-residue peptide recently discovered by Yanagisawa *et al.* (1988). It possesses a very potent vasoconstrictor activity on several isolated blood vessels (Yanagisawa *et al.*, 1988; Tomobe *et al.*, 1988). In vascular smooth muscle, the contractile action of endothelin seems critically dependent upon influx of calcium ions from the extracellular space through dihydropyridine-sensitive calcium channels. On the basis of structural analogies with certain toxins which act directly on membrane ion channels, the hypothesis was advanced that endothelin acts as an endogenous agonist of the dihydropyridine-sensitive calcium channel (Yanagisawa *et al.*, 1988). To date, little information is available about the effects of endothelin on non-vascular smooth muscles. Uchida *et al.* (1988) reported recently that endothelin exerts a potent contractile effect on isolated bronchial tissue from both guinea-pigs and man. Even in this case, the response to endothelin was reported to be markedly sensitive to dihydropyridine calcium blockers. In the course of a more extensive study about the action of endothelin on various non-vascular smooth muscles, we noticed that the contractile responses to this peptide are, in certain instances, markedly or absolutely nifedipine-resistant. Here we describe the potent contractile effect of endothelin on muscle strips from the dome of the human urinary bladder and its modification by atropine and nifedipine.

Methods Specimens from the dome of human urinary bladder were obtained from 8 (6 males and 2 females) patients (age range, 40–81 years) undergoing total cystectomy for carcinoma of the bladder base. The specimens were from patients showing no signs of urinary retention or detrusor instability. No patient received radio- or chemotherapy before intervention. Pre-anaesthetic medication, induction and maintenance of anaesthesia were as described previously (Maggi *et al.*, 1987; 1988). All specimens appeared macroscopically normal, with no signs of tumour or inflammation. Tissues were placed in ice-cold Krebs solution within 2–3 min of surgical removal. Each sample was freed from mucosa, 4 to 6 small strips of muscular tissue were excised and stored overnight in oxygenated (95% O₂ and 5% CO₂) Krebs solution (CaCl₂ 2.5 mM). The strips were mounted in 5 ml baths for isolated organs, maintained at 37°C and isometric tension was recorded by means of a force-displacement transducer connected to a Basile 7050 pen recorder. Concentration-response curves to endothelin or KCl were obtained in a cumulative manner. Each experiment was started after a 120 min equilibration period. At this stage, the strips were challenged three or more times with KCl (80 mM final concentration, added to the bath) at 15 min intervals until reproducible responses were observed. Each value given in the Results section is the mean $\pm$ s.e.mean. Statistical analysis of the data was made by Student's *t* test or analysis of variance, followed by Dunnett's test, when applicable.

Drugs used were: endothelin (Peninsula), atropine HCl and isoprenaline HCl (Serva), nifedipine (Sigma) and carbachol (Merck).

Results Endothelin (1 nM–0.3 μ M) produced a concentration-dependent, slowly developing contraction of the human isolated bladder (Figure 1b). Concentrations higher than 0.3 μ M were not tested

¹ Author for correspondence.

because of the limited availability of the peptide. At $0.3 \mu\text{M}$, the contraction reached a plateau and then declined slowly to baseline. Washing with endothelin-free Krebs solution had little influence on this decline but prompt relaxation was observed upon addition of isoprenaline ($1\text{--}3 \mu\text{M}$, $n = 4$). At $0.3 \mu\text{M}$, the response to endothelin averaged $90 \pm 9\%$ of that to KCl (80 mM). The action of endothelin was not significantly prevented either by atropine ($3 \mu\text{M}$) or nifedipine ($1 \mu\text{M}$) which were added to the bath 30 min before the start of the concentration-response curve (Figure 1c). By contrast, $1 \mu\text{M}$ nifedipine had a marked inhibitory action on the contractile response produced by addition of increasing concentrations of KCl (10–80 mM) to the bath (Figure 1d). Atropine ($1 \mu\text{M}$) abolished the contractile response to carbachol ($0.3 \mu\text{M}$, $n = 3$) which produced a response averaging 75–100% of that to KCl (80 mM).

Discussion Present findings indicate that endothelin exerts a potent contractile effect on the mucosa-free smooth muscle from the human urinary bladder. To date, expression of the pre-pro-endothelin messenger RNA has been reported only in the vascular endothelium and the potential physiological meaning of these findings is only a matter of speculation. The action of endothelin was substantially atropine-resistant which excludes a significant participation of the cholinergic nerves, the major excitatory innervation of the normal human bladder (Sjogren *et al.*, 1982; Sibley, 1984; Maggi *et al.*, 1988). Further, we found that the action of endothelin is totally resistant to nifedipine at a concentration that abolished the KCl-induced contractions. Therefore the action of endothelin on the human bladder is markedly different from that on isolated blood vessels, this latter being strictly dependent upon extracellular calcium influx through dihydropyridine-sensitive channels (Yanagisawa *et al.*, 1988). The present findings do not necessarily contradict the hypothesis (Yanagisawa *et al.*, 1988) that endothelin might be an endogenous agonist of the

Figure 1 (a and b) Typical tracings showing the contractile response of muscle strips of the human isolated urinary bladder to endothelin as compared to KCl (80 mM). (c) Effect of atropine or nifedipine ($1 \mu\text{M}$ each, 30 min beforehand) on the concentration-response curve to endothelin: (○) control; (□) nifedipine; (△) atropine. (d) Effect of nifedipine ($1 \mu\text{M}$, 30 min beforehand) on the response to KCl: symbols as in (c). Each point is the mean of at least 4 experiments; s.e. mean shown by vertical bars. All the points of the KCl curve in presence of nifedipine were significantly different from controls: $P < 0.05$.

dihydropyridine-sensitive calcium channels in vascular smooth muscle but indicate that other mechanisms should also be considered in the action of this peptide. Further studies are needed to determine the calcium sources utilized by endothelin in its action on the human bladder muscle.

References

- MAGGI, C.A., SANTICIOLI, P., PATACCHINI, R., TURINI, D., BARBANTI, G., BENEFORTI, P., GIULIANI, S. & MELI, A. (1987). Galanin: a potent modulator of excitatory neurotransmission in the human urinary bladder. *Eur. J. Pharmacol.*, **143**, 135–137.
- MAGGI, C.A., SANTICIOLI, P., PATACCHINI, R., CELLERINI, M., TURINI, D., BARBANTI, G., BENEFORTI, D., ROVERO, P. & MELI, A. (1988). Contractile response of the human isolated urinary bladder to neurokinins: involvement of NK-2 receptors. *Eur. J. Pharmacol.*, **145**, 335–340.
- SIBLEY, G.N.A. (1984). A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. *J. Physiol.*, **354**, 431–443.
- SJOGREN, C., ANDERSSON, K.E., HUSTED, S., MATTIASO, A. & MOLLERMAEDSEN, B. (1982). Atropine resistance of transmurally-stimulated human bladder muscle. *J. Urol.*, **128**, 1368–1371.

- TOMOBE, Y., MIYAUCHI, T., SAITO, A., YANAGISAWA, M., KIMURA, S., GOTO, K. & MASAKI, T. (1988). Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar-Kyoto rats. *Eur. J. Pharmacol.*, **152**, 373–374.
- UCHIDA, Y., NINOMIYA, H., SAOTOME, M., NOMURA, A., OHTSUKA, M., YANAGISAWA, M., GOTO, K., MASAKI, T. & HASEGAWA, S. (1988). Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor. *Eur. J. Pharmacol.*, **154**, 227–228.
- YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

(Received November 14, 1988

Accepted December 9, 1988)