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BRADYKININ-INDUCED RELAXATION OF RENAL AND PULMONARY ARTERIES IS DEPENDENT UPON INTACT ENDOTHELIAL CELLS

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When most arteries are removed from mammals and man, the *in vitro* response to kinins, particularly of helically-cut vascular strips, is usually one of contraction; and often no response is observed. This is in sharp contrast to the *in situ* arterial vasodilator action of kinins. The reason(s) for this transformation is not known. The present *in vitro* experiments demonstrate that bradykinin can produce potent relaxation of canine isolated intrapulmonary arteries (threshold concentration = $7.5 \pm 2.7 \times 10^{-10}$ M) and renal arteries (threshold concentration = $3.2 \pm 1.6 \times 10^{-10}$ M) contracted by phenylephrine, provided the endothelium is left intact. Selective, mechanical destruction of the endothelium transforms the vasodilator activity of bradykinin to either contraction or to no response at all. Our results probably explain why previous investigators have found that bradykinin usually induced contraction, rather than relaxation, of excised peripheral arteries.

Introduction It has long been known that the peptide bradykinin induces potent peripheral arteriolar and arterial dilatation *in vivo* (Weiner & Altura, 1967; Haddy, Emerson, Scott & Daugherty, 1970; Altura, Hershey & Altura, 1970; Altura, 1978; Johnson, 1979). However, when most arteries are removed from mammals and man, the *in vitro* response, particularly of helically-cut vascular strips, is usually one of contraction, and often no response is observed at all (Johnson, 1979). The reason(s) for the latter is, at present, unknown.

Recently, it has been demonstrated that rabbit aortic rings prepared for *in vitro* studies with their endothelial cells left intact, and contracted with various stimulants, will relax upon addition of acetylcholine (Furchgott & Zawadzki, 1980). With this in mind, we wondered whether selective removal of the endothelium from intrapulmonary and renal arteries would result in a loss of ability of these vessels to relax in response to bradykinin.

We have found that bradykinin produces potent, and concentration-dependent, relaxation of canine intrapulmonary and renal arteries, provided the endothelium is left intact. Selective removal of the endothelium results in a transformation to either a contractile action or no effect at all.

Methods Mongrel dogs of either sex, weighing 15 or 20 kg, were anaesthetized with pentobarbitone sodium, 30 mg/kg. After thoracotomy, intrapulmonary and renal arteries (2–4 mm o.d.) were carefully excised. Helical strips (carefully prepared, so as not to injure the endothelium), cut from segments of these vessels, were 30–40 mm long by 3–4 mm. These were then suspended isometrically under tension of 1 and 2 g for the renal and pulmonary arteries, respectively, in 10 ml muscle chambers containing normal Krebs-Ringer bicarbonate solution (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and $NaHCO_3$ 25) at 37°C through which a mixture of O_2 (95%) and CO_2 (5%) was bubbled (Altura, 1970). Force of contraction was measured with Grass FT-03C force-displacement transducers and recorded on a Grass Model 7 polygraph. Two hours after the preparations were incubated, under tension, the contractile effect of 80 mM KCl was determined in order to ascertain the maximal response. Subsequently, tissues were exposed to phenylephrine (5×10^{-7} to 1.2×10^{-6} M) in order to produce sustained, submaximal contractions. The agonists to be tested (i.e., bradykinin and papaverine) were then added to the latter sustained contractions in a cumulative dose manner to establish control relaxant responses. Using paired tissues (cut from same segment), the endothelium was selectively removed by rubbing the endothelial surface of each vessel on filter paper (Whatman number 4) for 30 to 60 s. Electron microscopy performed on such-treated tissues clearly showed that only the endothelial cells are removed by this technique; the underlying structures including the vascular smooth muscle cells are not damaged. Control vessels (with endothelium left intact) were always run in parallel with the endothelial-denuded vessels, so as to make certain that tachyphylaxis (or time of incubation) did not play any role in the observed responses. Where appropriate, mean (±s.e.mean) threshold, EC_{50} and maximal relaxations (mg) were calculated.

Results Addition of bradykinin to the physiological salt solution, bathing the precontracted blood vessels, resulted in rapid and potent concentration-dependent relaxation in the intrapulmonary ($n = 17$) and renal arteries ($n = 15$) with intact endothelium

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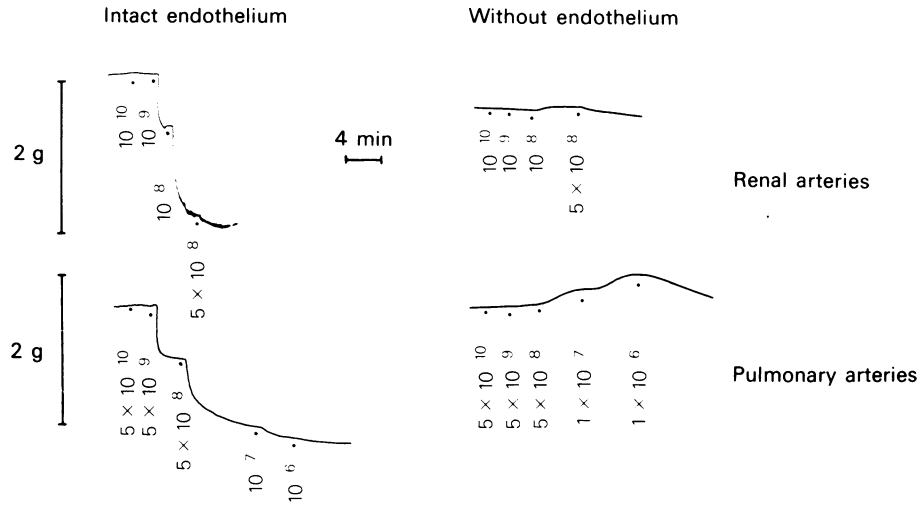

Figure 1 Bradykinin induces concentration-dependent relaxation of canine renal arteries and pulmonary arteries, provided the endothelium is left intact (left two panels). Removal of the endothelium (see methods) results, often, in a transformation to concentration-dependent contractile responses (right two panels). Vertical bars on extreme left represents tension. Horizontal bar = time marker.

(Figure 1). The relative threshold, EC_{50}s and maximal relaxation observed for the renal arteries with intact endothelium were: $3.2 \pm 1.6 \times 10^{-10}$ M, $4.3 \pm 1.5 \times 10^{-9}$ M and 2300 ± 350 mg; and for intrapulmonary arteries with intact endothelium were: $7.5 \pm 2.7 \times 10^{-10}$ M, $9.1 \pm 1.6 \times 10^{-9}$ M and 1370 ± 250 mg.

Removal of the endothelium by the above method resulted in a complete loss of relaxation responses to bradykinin, and often a transformation (at the same concentrations which formerly produced relaxation) into contractile responses was noted (Figure 1). Papaverine (10^{-4} to 5×10^{-4} M) produced equivalent and potent relaxation (1500 to 2500 mg), irrespective of whether or not the endothelium was present.

Discussion Irrespective of the exact mechanism (e.g., release of mediator(s) from endothelial cells), the results presented here clearly demonstrate that endothelial cells are required for the expression of renal and pulmonary arterial relaxant responses to bradykinin. Our results probably explain why previous investigators have found that bradykinin usually induced contraction, rather than relaxation, of excised peripheral arteries. In view of such findings, it is tempting to speculate that *in vivo* damage to vascular endothelial cells, resulting in bradykinin-induced vasoconstriction, might be responsible for some of the elevations in vascular resistance noted in the lungs and kidneys in certain disease states.

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