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The local modulation of vascular permeability by endothelial cell derived products

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Abstract—Endothelin has been shown to suppress increased vascular permeability in the rat at doses of 0·01 pmol. The agonists used were nitric oxide and nitroprusside, which have the same activity as endothelial-derived relaxing factor. Histamine, 5-hydroxytryptamine, platelet activating factor and carrageenan were the other agonists used. It is proposed that endothelin and EDRF act as local hormones produced by endothelial cells to control local vascular permeability.

Yanagisawa et al (1988) isolated a peptide from cultured porcine aortic endothelial cells, which displayed potent vasoconstrictor activity in numerous arterial strips. This peptide, which they named endothelin, has been cloned and sequenced. Furthermore the expression of the endothelin gene was shown to be regulated by several vasoactive agents. In view of its potent vasoconstrictor activity, we have investigated its ability to influence increased vascular permeability induced by an endothelial cell product endothelial-derived relaxing factor, EDRF (Furchgott & Zawadzki 1980), and several other mediators. It has recently been shown that nitric oxide (NO) accounts for the activity of EDRF (Palmer et al 1987). The relaxation effect of EDRF on vascular smooth muscle is mediated by activation of guanyl cyclase, leading to increased intracellular cyclic guanosine monophosphate (cGMP) (Griffith et al 1985).

Materials and methods

Vascular permeability. Male Hooded Lister rats (250 ± 10 g; n = 6) were anaesthetized with ether and their abdominal surfaces shaved. Evans blue (0·5 mL of 0·5% solution in sterile saline) was administered intravenously into the tail. Intradermal injections were given in a volume of 0·1 mL sterile saline according to a Latin square design. After 30 min the animals were killed, the injection sites excised, and the dye was extracted with formamide and assayed spectrophotometrically according to Lykke & Cummings (1969). The permeability enhancing factors were used in amounts to give a leakage of protein-bound dye, expressed in μg Evans blue, at between 5·0 and 7·0. In absolute doses these were histamine 10 μg; 5-hydroxytryptamine (5-HT) 120 ng; bradykinin 2 μg; platelet activating factor (PAF) 40 ng; NO 0·05 mL of a saturated solution in saline mixed with an equal volume of saline; nitroprusside 100 ng; carrageenan 1·0 μg; endothelin 0·01–1 pmol. To study the inhibitory effect of endothelin, it was used at 0·5 pmol injected together with each mediator.

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Results and discussion

Injections of endothelin caused an intense vasoconstriction at all doses tested. In contrast, the permeability inducing agents histamine, 5-HT, bradykinin and PAF all gave a maximal leakage of protein-bound Evans blue. Carrageenan, a commonly used inflammatory irritant, also caused a leakage of Evans blue. Nitric oxide and nitroprusside, both reflecting EDRF activity, also provoked an increase in vascular permeability (see Table 1).

Table 1. Permeability effects of various factors in the presence or absence of endothelin, as judged by leakage of protein bound dye.

	Leakage of protein-bound dye (μg Evans blue	
Substance	Control	With Endothelin
Histamine	6·2 ± 0·7	1·8 ± 0·2
5-HT	7·2 ± 0·6	1·7 ± 0·2
Bradykinin	6·3 ± 0·5	1·2 ± 0·1
PAF	5·9 ± 0·4	1·3 ± 0·1
NO	4·9 ± 0·3	1·8 ± 0·2
Nitroprusside	7·1 ± 0·9	1·6 ± 0·1
Carrageenan	7·4 ± 1·0	1·3 ± 0·1

When the permeability-inducing agents were mixed with 0·5 pmol of endothelin, a lower level of Evans blue was extracted compared with saline alone, thus reflecting the intense vasoconstrictor activity.

Subsequently, the effect of endothelin was tested on carrageenan-induced pleurisy, by adding endothelin to the carrageenan. This was without effect possibly because it has recently been found by de Nucci et al (1988, unpublished data) that endothelin is rapidly removed by the lungs.

We have shown that endothelin in low doses is capable of suppressing the increased vascular permeability of rat skin to a variety of agents causing an increase in vascular permeability including EDRF. Both products are derived from endothelial cells and have contrasting actions, endothelin being a potent pressor substance and EDRF having vasodilator activity. We think these two substances could be exerting a local hormonal control or modulation of vascular permeability. These actions may be either part of a normal physiological function or involved in inflammatory reactions. It is known that catecholamines are released at sites of acute inflammation and suppress increased vascular permeability (Willoughby & Spector 1964). It is also recognized that catecholamines induce preproendothelin mRNA, thus leading to the release of endothelin (Yanagisawa et al 1988) which is a potent and longer-lasting vasoconstrictor agent than the catecholamines. This would seem to be a more efficient method of control of the microvasculature than that previously postulated for acting catecholamines.

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Letter to the Editor

Non-dopaminergic actions of quinpirole hydrochloride (LY 171555), a selective D_2-agonist, in the guinea-pig isolated ileum

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Quinpirole hydrochloride (LY 171555), a selective dopamine (D_2)-receptor agonist, has recently been shown to possess central dopminergic activities in a variety of animal models and is proposed to have potential use in the therapy of Parkinson's disease (Koller et al 1987). Other actions of LY 171555 include an inhibition of intracellular calcium mobilization leading to a reduction in the release of acetylcholine (ACh) from guinea-pig neostriatal slices, mediated through presynaptic D_2-receptors (Fujiwara et al 1987). Such presynaptic depression of transmitter release is also seen with baclofen, a $GABA_B$-receptor agonist (Ong & Kerr 1983), adenosine (Dowdle & Maske 1980) and noradrenaline (Paton & Vizi 1969) in the guinea-pig isolated ileum. We have therefore investigated the interaction of LY 171555 with these presynaptic effects, and now report that LY 171555 antagonized the depression of cholinergic ileal twitch contractions induced by baclofen, adenosine and noradrenaline, an action apparently unrelated to its D_2-agonist properties.

Guinea-pigs of either sex, 200–400 g, were killed by cervical dislocation and bled. Segments of the distal ileum, 3–4 cm in length, were quickly removed and mounted vertically in a 20 mL organ bath containing modified Krebs solution to record

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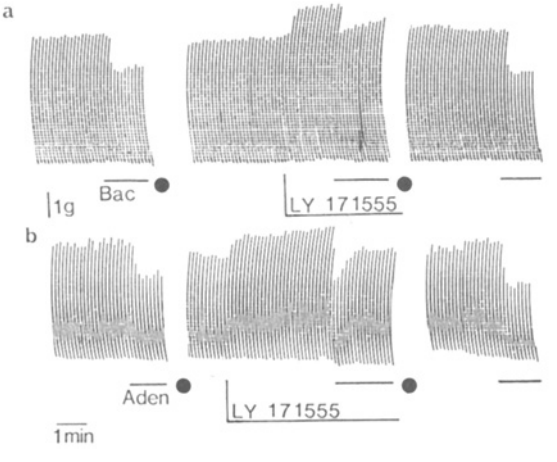

FIG. 1. Depressive responses of repetitive cholinergic twitch contractions in the guinea-pig isolated ileum to exogenously applied a) baclofen (Bac; 10 μM, n = 8) and b) adenosine (Aden; 0·6 μM, n = 8), and antagonism of the depression by baclofen and adenosine with LY 171555 (10 μM). LY 171555 itself induced an enhancement of the twitch contractions. Vertical bar indicates 1 g tension, and the horizontal bar indicates 1 min time interval. ● tissue wash-out.