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Short communication

Lack of endothelin ET_B receptor-mediated smooth muscle hyperpolarization in rat mesenteric resistance arteries

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Abstract

The presence of functional endothelin ET_B receptors was investigated in rat isolated mesenteric resistance arteries. Neither endothelin-3 (0.1–100 nM) nor the endothelin ET_B selective agonists sarafotoxin S6c and BQ 3020 (both 1–100 nM) induced any measurable hyperpolarization or relaxation in stimulated (α_1-adrenoceptor agonist; phenylephrine) or unstimulated arteries. In both cases, the subsequent addition of acetylcholine (1 μM) hyperpolarized the membrane potential by 10–20 mV and totally reversed any contraction which was present. These results indicate that the endothelin ET_B-mediated vasodilatation observed in the intact mesenteric bed does not reflect hyperpolarization of smooth muscle cells in resistance arteries arising from the mesenteric artery.

Keywords: Endothelium; Smooth muscle, vascular; Hyperpolarization; Endothelin

1. Introduction

In addition to causing a long lasting increase in blood pressure, intravenous bolus doses of endothelin can also stimulate an initial, transient reduction in pressure (Yanagisawa et al., 1988a). Based on the relative potencies of endothelin-1 and endothelin-3 in vivo, it has been suggested that endothelin ET_B receptors mediate this transient decrease in blood pressure (Gardiner et al., 1990a; Yanagisawa et al., 1988b).

Endothelin-induced vasodilatation has been demonstrated in the isolated perfused rat mesenteric bed (Douglas and Hiley, 1990; Warner et al., 1989) and in anaesthetized and pithed, but not conscious, rats (Gardiner et al., 1990a,b; Le Monnier de Gouville et al., 1990). In the perfused bed, the vasodilator response was only partially attenuated by inhibitors of cyclooxygenase and nitric oxide synthase, suggesting the involvement of an additional, non-nitric oxide factor contributing to smooth muscle relaxation (Douglas and Hiley, 1990; Fukuda et al., 1991; Warner et al., 1989). Endothelin-3 evoked a monophasic hyperpolarization of 8 mV in the main isolated superior mesenteric artery from Wistar rats, which was unaffected by either nitro-arginine or the endothelin ET_A antagonist BQ123, and was mimicked by the endothelin ET_B receptor-specific agonist IRL 1620, suggesting a role for endothelium-derived hyperpolarizing factor (EDHF) released by endothelin ET_B receptor stimulation (Nakashima and Vanhoutte, 1993).

We investigated whether similar hyperpolarization, which could lead to smooth muscle relaxation, also occurs in small resistance arteries from the rat mesenteric bed, and if endothelin ET_B receptors are functionally linked to the response. Previous experiments in the superior mesenteric artery were performed in unstimulated artery segments, making it impossible to determine if tension changes accompanied the increase in membrane potential and if the hyperpolarization occurred in stimulated cells (Nakashima and Vanhoutte, 1993).

2. Materials and methods

2.1. General

Segments (1–2 mm in length) of mesenteric artery from male Wistar or Sprague-Dawley rats (approximately 240–

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350 g) were mounted in a Mulvany-Halpern wire myograph for simultaneous recording of changes in smooth muscle membrane potential and tension (Garland and McPherson, 1992). Only segments in which acetylcholine reversed phenylephrine-induced contraction by over 80% were studied further. For intracellular recording, individual cells were impaled through the adventitial or intimal surface of the artery segment, in the latter through a small hole made by the removal of a side-branch. Records from endothelial cells, obtained before penetration of the internal elastic lamina, were not included.

2.2. Solutions and drugs

Experiments were performed in physiological salt solution (PSS) of the following composition (mM): NaCl, 119; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.2; $CaCl_2$ 2.5; disodium EDTA, 0.027 and glucose, 11. Drugs used were: acetylcholine chloride (BDH, Poole, UK); BQ123, sodium salt [cyclo D-Asp-Pro-D-Val-Leu-D-Trp] (SMA, Barnet, UK), BQ3020 [*n*-acetyl-Leu-Met-Asp-Lys-Glu-Ala-Val-Tyr-Phe-Ala-His-Leu-Asp-Ile-Ile-Trp], ET-1, ET-3 (Rhone-Poulenc Rorer, Dagenham, UK); indomethacin (Sigma, Poole, UK); (−)-phenylephrine hydrochloride (Aldrich, Milwaukee, WI, USA); sarafotoxin S6c (SMA).

All drugs were dissolved and diluted in PSS except for stock solutions of endothelin-3 and sarafotoxin S6c dissolved in 0.1% acetic acid; BQ3020 dissolved in 75% 0.1 M $NaHCO_3$ and indomethacin dissolved in 0.1 M $NaHCO_3$ and added to the PSS to a final concentration of 2.8 μM.

2.3. Analysis of data

Relaxation is expressed as a percentage decrease of the tone induced by phenylephrine (mean ± S.E.M.). The significance between mean values was calculated using an unpaired *t*-test, with rejection of the null hypothesis at the 5% level.

3. Results

Artery segments from third and fourth order branches of the mesenteric artery had diameters (D100) of 318 ± 11 μm ($n = 14$) and 230 ± 13 μm ($n = 11$). The respective resting membrane potentials of smooth muscle cells in the segments were −61 ± 2.2 mV ($n = 11$ cells) and −59 ± 1.6 mV ($n = 19$ cells).

Application of either endothelin-3 (0.1–100 nM) or the specific endothelin ET_B receptor agonists sarafotoxin S6c (1–100 nM) and BQ3020 (10 pM–100 nM) failed to stimulate hyperpolarization of the resting potential, although with concentrations > 1 nM, endothelin-3 induced a depolarization and contraction which was blocked with the endothelin ET_A receptor antagonist, BQ123 (10 μM).

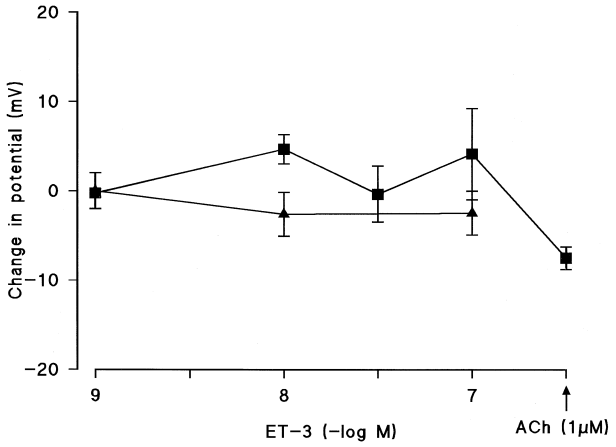

Fig. 1. Lack of effect of endothelin 3 (ET-3) on smooth muscle membrane potential in unstimulated segments of mesenteric artery. Endothelin-3 (ET-3; ●) failed to alter the resting membrane potential in mesenteric smooth muscle cells from fourth order arterial branches. However, the subsequent addition of acetylcholine (ACh; 1 μM) stimulated marked hyperpolarization. ▲ indicates the addition of vehicle (0.1% acetic acid) to the bathing solution did not stimulate any change in membrane potential ($n = 3$–5 separate arterial segments).

However, the subsequent application of 1 μM acetylcholine hyperpolarized the membrane potential by 8 ± 1.1 mV ($n = 8$; Fig. 1).

In the presence of BQ123 (10 μM), phenylephrine (0.5–5 μM) depolarized and contracted the tissues to −36 ± 3.2 mV and 2.9 ± 0.1 mN mm^{-1} (both $n = 6$). During this stimulation, endothelin-3 (0.1–30 nM) applied either cumulatively or as a bolus dose, failed to induce relaxation or repolarization, but at 30 nM induced a depolarization and increased tension (to −27 ± 1.3 mV and 128 ± 23% of tone induced by phenylephrine; $n = 3$ and 4, respectively). The subsequent application of 1 μM acetylcholine stimulated a marked hyperpolarization, to −48 ± 7.5 mV, and a concomitant relaxation of 89 ± 7.1% of the induced tone ($n = 3$ and 4, respectively), Fig. 2. In similar experiments, the endothelin ET_B receptor specific agonist, sarafotoxin S6c (0.1–30 nM), was associated with only a slight relaxation (14 ± 7.8%, $n = 5$) and no repolarization in phenylephrine (0.4–1 μM) depolarized and constricted the segments (to −34 ± 3.8 mV and 1.9 ± 0.19 mN mm^{-1}; $n = 6$ and 11, respectively). Again, the subsequent addition of acetylcholine (1 μM) to these tissues stimulated both relaxation (95 ± 0.7%; $n = 10$) and repolarization (to −65 ± 7.9 mV, $n = 3$).

Endothelin-3 also failed to hyperpolarize smooth muscle cells in which the resting tension was raised, or in cells close to the intima. Under a tension set at twice normal (2.5 mN mm^{-1}), the smooth muscle resting membrane potential was −54 ± 4.1 ($n = 3$). Endothelin-3 (1–100 nM) still failed to stimulate any increase in membrane potential, in spite of the fact that acetylcholine (1–10 μM) did increase the potential in these cells. The resting potential of smooth muscle cells impaled through the intima was

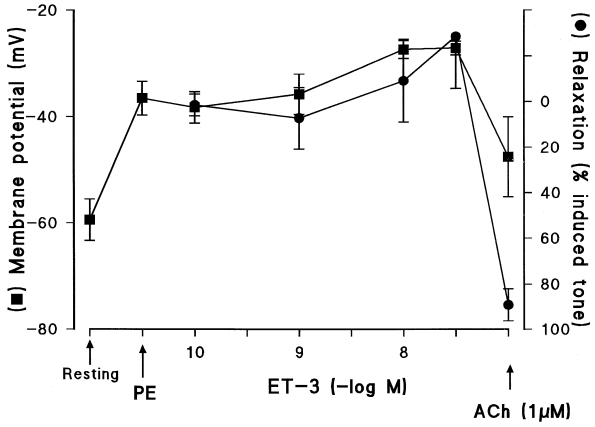

Fig. 2. Lack of hyperpolarization and relaxation to endothelin 3 (ET-3) in mesenteric artery smooth muscle cells depolarized and contracted in the presence of phenylephrine. The only response to endothelin-3 was additional depolarization and contraction with concentrations above 1 nM. The subsequent addition of acetylcholine (ACh; 1 μM) hyperpolarized (■) and relaxed (●) the smooth muscle cells ($n = 6$ arteries).

-66 ± 3.7 mV ($n = 4$) and not significantly different from cells impaled through the adventitial surface ($P > 0.05$). As in the latter, endothelin-3 (1–100 nM), in the presence of BQ123 (10 μM), failed to evoke any measurable hyperpolarization, although the subsequent addition of acetylcholine did increase the potential (1 μM, increase to -78 ± 1.7 mV; $n = 3$).

4. Discussion

These experiments fail to provide any evidence for endothelin ET_B receptors which are functionally linked to smooth muscle hyperpolarization and relaxation in third and fourth order branches of the rat mesenteric artery. As such, they contrast with the situation in the main branch of the superior mesenteric artery, where Nakashima and Vanhoutte (1993) demonstrated transient endothelium-dependent hyperpolarization in response to either endothelin-1 or -3, and they confirm and extend a previous report that third order mesenteric arteries do not contain endothelin ET_B receptors (Hiley and Poon, 1994).

Nakashima and Vanhoutte (1993) found higher concentrations of endothelin-1 induced depolarization via endothelin ET_A receptors, and that the hyperpolarization could also be stimulated with IRL-1620 (ET_B agonist) and was not altered in the presence of the nitric oxide-synthase inhibitor, N^G-nitro-L-arginine. The latter observation indicated the involvement of an endothelium-derived hyperpolarizing factor (EDHF) distinct from nitric oxide. The experiments were all performed in unstimulated artery segments, but the inference was that smooth muscle hyperpolarization could underlie the relaxation and vasodilatation to endothelin, which has been reported in the mesenteric bed (Douglas and Hiley, 1990; Warner et al., 1989). If this is the case, then our experiments indicate that vasodilatation is unlikely to involve a contribution from hyperpolarization in the two smaller branches of the mesenteric artery.

It seems unlikely that our failure to record any hyperpolarization with endothelin in the third and fourth order branches of the mesenteric bed reflected any damage to the arterial segments, for a number of reasons. First, the size of contractile and relaxant responses to the agonists studied was similar to previous reports, with the relaxation to acetylcholine indicating the presence of a functional endothelium (Garland and McPherson, 1992; Waldron and Garland, 1994; Parsons et al., 1994). Second, these arteries did respond to endothelin, although not through endothelin ET_B receptors. Endothelin ET_A receptor mediated smooth muscle depolarization and contraction was recorded with higher concentrations of endothelin-3 in both the stimulated and unstimulated segments. Finally, our failure to record hyperpolarization did not appear to reflect different experimental conditions to those of Nakashima and Vanhoutte (1993), as mimicking those conditions either by increasing resting tension or recording from smooth muscle cells adjacent to the intima, also failed to reveal any hyperpolarization and relaxation.

In summary, we have found no evidence for endothelin ET_B receptor mediated smooth muscle hyperpolarization and relaxation in small resistance arteries from the rat mesenteric arterial bed, in contrast to larger arteries upstream. These data indicate that the vasodilatation observed in the isolated perfused bed to endothelin may be explained by mechanisms other than smooth muscle hyperpolarization. They also provide further evidence for distinct pharmacological differences between large and small arteries.

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