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*Short communication*

# Activation of sodium channels is not essential for endothelin induced vasoconstriction

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Abstract. The role of sodium and calcium ions in the vasoconstrictor response of isolated rat aorta and portal vein to synthetically prepared endothelin is investigated. Contractile responses to endothelin, unlike those induced by the sodium channel activator veratridine, are unaffected by tetrodotoxin or by the removal of sodium chloride from the solution bathing the tissue. The responses are the same whether sodium chloride is replaced iso-osmotically with either sucrose or potassium chloride. The endothelin responses in all media are entirely dependent on the presence of extracellular calcium, and can be blocked by 1 μM nitrendipine. These findings offer no support to the idea that voltage activated sodium channels are the primary site of action of endothelin as suggested by sequence homologies to scorpion α-toxins, but are entirely consistent with the possibility that the site of action of endothelin is closely coupled to the calcium channel (Yanagisawa *et al*,1988).

Key words: "Calcium: Endothelin: Aorta: Portal vein: Calcium channels: Sodium channels".

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## INTRODUCTION

The recent discovery (Yanagisawa *et al*, 1988) of a potent endogenous vasoconstrictor peptide, endothelin, from vascular endothelial cells opens up new possibilities in the investigation of the aetiology of hypertension and some forms of coronary and peripheral vasospasm. Endothelin is active in the $10^{-10}$M range, and is thus the most potent vasoconstrictor known. Its 21 amino acid sequence is homologous to scorpion α-toxins. These toxins are thought to prevent inactivation of voltage dependent sodium channels (Catterall 1986). One possiblility therefore is that endothelin also acts at voltage dependent sodium channels either in presynaptic nerve terminals or in the smooth muscle cells themselves (Sturek & Hermsmeyer 1986). This would lead to a maintained cell depolarisation, activation of voltage sensitive calcium channels and hence calcium influx into cells. However, as sodium channels have some resemblance to calcium channels, both belonging to the same superfamily of voltage dependent ion channels (Tanabe *et al*, 1987), another possibility is that endothelin acts directly on the calcium channels (Yanagisawa *et al*, 1988). This communication therefore describes simple experiments which address the question "Does endothelin act via sodium channels?"

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## MATERIALS AND METHODS

Endothelin was prepared and supplied by Cambridge Research Biochemicals Ltd, UK. Aorta and portal vein were obtained from male Sprague Dawley rats (200g - 300g), and the endothelium mechanically destroyed (Furchgott & Zawadzki 1980). Vascular ring segments (2mm) were mounted on stirrups and the tension measured isometrically. The initial tensions were set at 1g and 2g for vein and aorta respectively. Experiments using nitrendipine were performed using sodium lamp illumination. Vascular tissue was bathed in Krebs bicarbonate buffered media equilibrated with 5% $CO_2$/95% $O_2$, or in solutions lacking sodium chloride (iso-osmotic replacement with sucrose or KCl. In solutions lacking calcium, $CaCl_2$ (2.5 mM) was replaced by 2.5 mM $MgCl_2$.

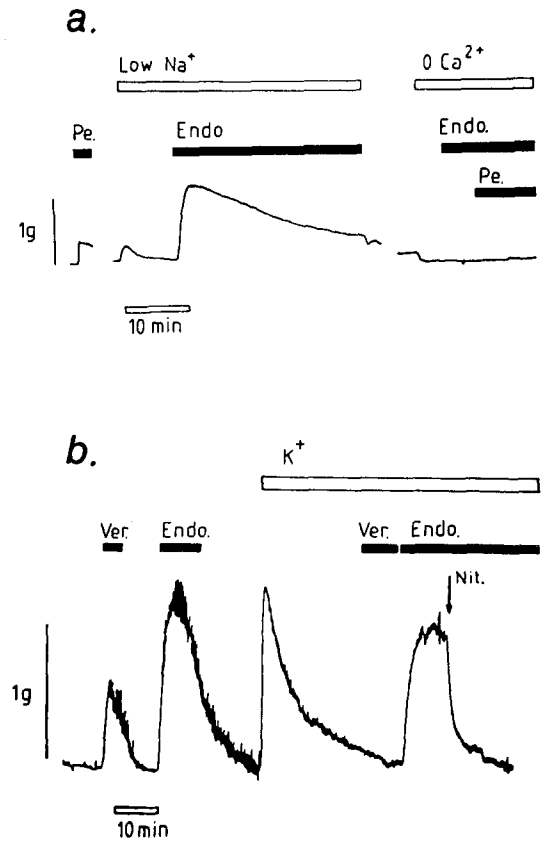


Figure 1.
Isometric tension response of a (a) rat aorta, and (b) rat portal vein ring segments. (a) An aortic ring segment was incubated in Krebs bicarbonate solution containing 2.5 mM $CaCl_2$ and briefly challenged with 1 $\mu$M phenylephrine (Pe). After 10 minutes recovery, and during the time represented by the open bar (Low $Na^+$), the NaCl was iso-osmotically replaced by sucrose and the tissue challenged with 3 nM endothelin (Endo). When the endothelin was removed and after 40 minutes recovery in Krebs solution (as shown by the break in the tension trace), the tissue was bathed in a solution lacking calcium (open bar: 0 $Ca^{2+}$). The tissue was then challenged with both 3 nM endothelin (Endo.) and 1 $\mu$M phenylephrine (Pe).
(b) A portal vein ring segment was incubated in Krebs bicarbonate solution and at the times shown by the closed bars challenged with 10$\mu$M phenylephrine (Pe.), 50$\mu$M veratridine (Ver.), and 10 nM endothelin (Endo). At the time indicated by the open bar ($K^+$.), the ring segment was incubated in a high potassium medium in which the NaCl was replaced iso-osmotically by KCl. Nitrendipine (1$\mu$M) was added at the time indicated by the arrow. Temperature throughout was 37°C.
These data are representative of six experiments using six different animals.

## RESULTS AND DISCUSSION

Both rat aorta and portal vein contract in response to endothelin, the $EC_{50}$ being close to $10^{-9}$M for both. The durations of the contractile responses however are different. The tension developed in the aorta in response to $10^{-8}$M endothelin is relatively sustained, whereas that of portal vein is more transient, the time for the tension to fall to half its peak value being close to 5 minutes. In aorta and portal vein, the effect of endothelin was found to be entirely dependent on extracellular calcium (Fig 1a) and was unaffected by a cocktail of receptor antagonists together with an adrenergic neurone blocker and a cyclo-oxygenase inhibitor i.e. 1 $\mu$M atropine, 10 $\mu$M hexamethonium, 1 $\mu$M mepyramine, 1 $\mu$M naloxone, 1 $\mu$M propanolol, 1 $\mu$M ketanserin, 1 $\mu$M phentolamine, 1 $\mu$M saralasin, 10 $\mu$M guanethidine, and 3 $\mu$M indomethacin (data not shown).

Endothelin elicits contraction in both aorta and portal vein when these tissues are bathed in a medium lacking sodium chloride (iso-osmotic replacement by sucrose) and/or in the presence of 10$\mu$M tetrodotoxin. Figure 1a describes such an effect of sodium removal on the aortic response to endothelin. These data alone suggest that neither sodium channels nor indeed the sodium/calcium exchange mechanism are necessarily involved in the endothelin response. Further evidence against an involvement of voltage dependent sodium channels comes from the finding (Figure 1b) that endothelin can elicit contraction in a fully depolarised tissue, a condition in which the voltage dependent sodium channels are fully inactivated. Figure 1b demonstrates that in Krebs solution portal vein contracts transiently in response to 10 $\mu$M phenylephrine, 50 $\mu$M veratridine, 0.01 $\mu$M endothelin and to a maintained potassium challenge. The response to veratridine, a known sodium channel activator, is abolished by 10 $\mu$M tetrodotoxin. The figure shows that whilst the tissue is depolarised, veratridine cannot elicit contraction whereas endothelin is still effective. This response is blocked by removal of extracellular calcium and by 1$\mu$M nitrendipine. These findings raise the possibility that endothelin is acting in this situation by increasing the open

state probability of a dihydropyridine sensitive calcium channel even during full depolarisation.

Taken together these data argue strongly against the voltage activated sodium channel being the primary site of action of endothelin, but are entirely consistent with the idea that the site of action of endothelin is closely coupled to the calcium channel itself (Yanagisawa *et al.* 1988).

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