Effect of Endothelin (ET)-1, ET-3 and ET-(16-21) on the Isolated and Perfused Rat Kidney from Normotensive and Spontaneously Hypertensive Rats

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ABSTRACT—We have investigated the effect of endothelin (ET)-1, ET-3 and ET-(16-21) on isolated and perfused rat kidney (IPK). ET-1 and ET-3 produced a similar dose-dependent increase in perfusion pressure of IPK, while ET-(16-21) was completely inactive. The ET-1 effects were greater in spontaneously hypertensive rats (SHR) as compared to normotensive rats (WKYR), whereas the ET-3 effects were greater in the SHR group only at the lowest doses. The pressure response of IPK induced by ET-1 was partially modified by prior application of the nitric oxide synthetase inhibitor L-nitroarginine in both WKYR and SHR.

Keywords: Endothelin, Isolated and perfused kidney, Spontaneously hypertensive rats

Peptides of the endothelin (ET) family exert powerful vascular effects (vasodilation and/or vasoconstriction) that are greatly variable, depending on the type of preparation used (isolated blood vessels, intact animals etc.), species, dose and route of delivery. ET receptors are present in the kidney (1, 2). Several lines of data implicate ETs in renal pathophysiology such as acute renal failure (3, 4). ETs produce powerful vasoconstrictions of isolated renal arteries (5, 6) and an enhanced response to these peptides has been reported in spontaneously hypertensive rats (5). The systemic vasodilator response to ET-1 in rats is blocked by previous administration of nitric oxide synthetase inhibitors (7). ET-1 increases renal vascular resistance in the isolated perfused rabbit kidney (IPK); and at this level, its action is only slightly increased by inhibitors of endothelium-derived relaxing factor, methylene blue and hemoglobin (8).

The aim of this study was to compare the pressor response of the rat IPK to ET-1 and ET-3 in normal and spontaneously hypertensive rats (SHR) and the possible modulatory influence of nitric oxide production by studying the effect of L-nitroarginine on the response to ET-1. In addition, we also investigated the effect of the C-terminal fragment, ET-(16-21), common to both ET-1 and ET-3, which has been reported to possess ET-like agonist activity at some but not all ET receptors (9). Male albino rats, 6-month old normotensive Wistar Kyoto (WKYR) or age-matched spontaneously hypertensive (SHR), Charles-River strain (350–400 g) were anesthetized with ethyl ether, and the kidneys were isolated and perfused as follows: laparatomy was performed via a midline incision; one of the kidneys was exposed; and the renal artery was cannulated with catheter PE G-22, 0.8 mm of diameter. Then the kidney was removed and placed in a wet organ chamber where it was perfused with a solution of normal Tyrode at a constant rate of 3 ml/min by means of a peristaltic pump. The perfusion pressure was measured with a Bentley-Trantec pressure transducer and recorded on a polygraph. After a 30-min stabilization-period, the normal Tyrode solution was replaced with a solution containing increasing concentrations of ET-1 (0.03–3 nM), ET-3 (0.03–3 nM) or ET-(16-21) (up to 1 μM) or with a maximal vasoconstrictor dose of noradrenaline (3 μM). Changes in perfusion pressure (mmHg) were measured as the maximal increase as compared to basal pressure, and solutions containing the contractor agent were replaced when a stable pressure plateau was reached. The mean resting perfusion pressure was 58 ± 2 and 81 ± 5 mmHg (n = 25 for all groups) for WKYR and SHR, respectively. The standard Tyrode solution
had the following composition: 2.7 mM KCl, 1.8 mM CaCl₂, 1.04 mM MgCl₂, 11.9 mM NaHCO₃, 0.42 mM NaH₂PO₄, 136.9 mM NaCl and 11.1 mM glucose. In other experiments L-nitroarginine (300 μM) or thiorphan (10 μM) were added to the perfusion medium. ET-1 and ET-3 were obtained from Peninsula Labs while ET-(16-21) was synthetized using solid phase methods. Statistical analyses were performed by means of Student's t-test for unpaired data. All data in the text are means ± S.E. As shown in the upper panel of Fig. 1, ET-1 induced a dose-dependent increase of perfusion pressure which was significantly larger in SHR than WKYR. Changes in the perfusion pressure induced by ET-1 and ET-3 were not different in WKYR (Fig. 1). ET-3 induced a dose-dependent contraction in the IPK from WKYR which was significantly different as compared to SHR only at the lowest doses (Fig. 1; lower panel). ET-(16-21) was completely inactive up to 1 μM either alone or with the previous addition to the perfusion medium of thiorphan, a known inhibitor of neutral endopeptidase.

The pressor response of the IPK to ET-1 was increased by L-nitroarginine in WKYR only at the lowest doses (Fig. 2, upper panel). Conversely, the prior infusion of L-nitroarginine in SHR produced a decrease in perfusion pressure at the highest doses of ET-1 (Fig. 2, lower panel).

The present findings demonstrate that ET-1 and ET-3 are approximately equipotent for increasing the perfusion pressure of the rat IPK, and that the action of ET-1 is markedly enhanced in SHR. These findings agree with the increased expression of ET-1 and ET-3 binding sites in the kidney of SHR (10) and the larger contractile response to ET-1 of isolated renal arteries of SHR.

![Fig. 1. Effect of ET-1 and ET-3 in normotensive (WKYR, □) and spontaneously hypertensive rats (SHR, ■) on perfusion pressure of the isolated and perfused kidney: data shown are the means ± S.E.; *P < 0.05, as compared to the WKYR-group.](image)

![Fig. 2. Effect of L-nitroarginine on increase in perfusion pressure induced by ET-1 in normotensive (WKYR, upper panel) and spontaneously hypertensive rats (SHR, lower panel): data shown are the means ± S.E.; *P < 0.05 and **P < 0.01, as compared to the controls. □: Control, ■: L-nitroarginine.](image)
animals (5). The failure of the C-terminal hexapeptide, ET-(16-21) to reproduce the pressor effect of the parent peptides points to an important characteristic of the response under study: the existence of ET receptors that can recognize either the N-terminal region of these peptides or require the presence of both N- and C-terminal sequence for their activation. According to what was observed following systemic administration of ET-1 in rats (7), evidence was found for a modulatory role of nitric oxide in the action of ET-1. In fact, L-nitroarginine, a known inhibitor of nitric oxide, enhanced low vasoconstricting doses of ET-1 in WKYR but not in SHR, suggesting that in these latter animals the vasodilating properties produced by nitric oxide are lacking. On the other hand, many factors, such as resting pressure (7) and enzymatic activity, might influence the endothelium-derived substances in the hypertensive state, and further studies need to clarify the interaction between endothelin and nitric oxide. In conclusion, the present findings add further weight to the proposal that ET peptides may be involved in the genesis of hypertension.

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