EFFECTS OF RELAXIN ON VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS IN NORMOTENSIVE AND HYPERTENSIVE RATS

Effects of Relaxin on Vascular Smooth Muscle and Endothelial Cells in Normotensive and Hypertensive Rats

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ABSTRACT: We tested the effects of relaxin on $[Ca^{2+}]_i$ response to angiotensin II in smooth muscle (vSMC) and endothelial cells isolated from hypertensive (SHR) and normotensive (WKY) rats. Relaxin markedly reduced the $[Ca^{2+}]_i$ response of vSMCs from WKY, but not from SHR rats. Western blots showed that cGMP-dependent protein kinase G was reduced in vSMCs from SHR as compared with WKY rats. Relaxin also blunted the $[Ca^{2+}]_i$ response in endothelial cells from WKY, but not from SHR rats. However, in endothelial cells from SHR and WKY rats, protein kinase G was nearly unexpressed, thus accounting for an alternative pathway of the intracellular response to nitric oxide and relaxin. Hence, vSMCs and endothelial cells in SHR rats show a deficiency response to nitric oxide that may render them insensitive to relaxin.

KEYWORDS: endothelial cells; smooth muscle cells; SHR; calcium; nitric oxide; protein kinase G

INTRODUCTION

Relaxin has striking vasodilatory properties on reproductive and nonreproductive organs.[1,2] When given systemically to spontaneously hypertensive rats (SHR strain), relaxin reduces systemic blood pressure and blunts the vascular response to vasoconstrictors.[3,4] These properties of relaxin likely rely on multiple mechanisms. *In vitro* studies have shown that relaxin increases the expression of inducible nitric oxide synthase (NOS II) in rat endothelial cells (ECs) and vascular smooth muscle cells (vSMCs). By this pathway, relaxin reduced vasoconstrictor-induced increases in intracellular calcium concentration $[Ca^{2+}]_i$ and promoted cell relaxation.[5,6]

This *in vitro* study was designed to compare the effects of relaxin (porcine) on the $[Ca^{2+}]_i$ response to angiotensin II (AT II) by vSMCs and ECs isolated from spontaneously hypertensive (SHR) rats or normotensive Wistar-Kyoto (WKY) rats.

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MATERIAL AND METHODS

We used eight SHR and nine WKY rats 12–14 wk old. Endothelial cells were isolated from rat hearts by enzymatic digestion and selected from other cardiac cell types by centrifugation and adhesion to plastic surfaces. Aortic vSMCs were isolated by elastase-collagenase digestion of endothelium-free thoracic aortas and used between the second and fourth culture passage. Cells plated on glass coverslips were loaded with fura-2 AM and used for intracellular calcium $[Ca^{2+}]_i$ image analysis assay in control conditions or after a 24-h pretreatment with porcine relaxin (10^{-8} M). Angiotensin II (AT II, 10^{-6} M) was used as the vasoactive stimulus. Untreated and relaxin-treated cell cultures seeded in 25 cm^2 culture flasks were also used for western blot analysis of protein kinase G expression.

Vascular Smooth Muscle Cells

Resting $[Ca^{2+}]_i$ and AT II-induced $[Ca^{2+}]_i$ peak and decay times were similar in SHR and WKY rats. In vSMCs from WKY rats, relaxin (10 nM) decreased the decay time and peak values of $[Ca^{2+}]_i$ transiently. This effect was prevented when the cells were pretreated with the NOS inhibitor L-NAME (10 mM), suggesting the involvement of endogenous nitric oxide generation. Conversely, vSMCs from SHR rats were insensitive to relaxin as well as to L-NAME. By western blotting, vSMCs from WKY rats fairly expressed protein kinase G, whereas those from SHR rats only showed very weak protein kinase G expression.

Endothelial Cells

Resting $[Ca^{2+}]_i$ was higher in SHR than in WKY rats (144 ± 7.4 vs 109 ± 4.0 nM; $P < 0.01$). The AT II-induced increase in $[Ca^{2+}]_i$ was characterized by a fast rise followed by a slower decrease in both WKY and SHR rats. $[Ca^{2+}]_i$ peak and decay times (time needed to return to basal values) were similar in SHR and WKY rats.

In ECs from WKY rats, relaxin (10 nM) significantly decreased the decay time and peak values of $[Ca^{2+}]_i$ transiently. This effect was prevented when the cells were pretreated with L-NAME (10 mM), suggesting involvement of endogenous nitric oxide generation. Conversely, ECs from SHR rats were insensitive to relaxin as well as to L-NAME. By western blotting, ECs from either WKY or SHR rats did not express detectable protein kinase G.

RESULTS AND CONCLUSIONS

This study shows that ECs and vSMCs from SHR rats have a deficiency response to nitric oxide that renders them insensitive to the relaxant action of relaxin. This appears to depend on a defect in the expression of cGMP-dependent protein kinase G by vSMCs, the cells chiefly involved in the regulation of vascular tone, which leads to a breakdown of the nitric oxide-cGMP signaling pathway. These findings support the pivotal role of nitric oxide-cGMP signaling in the vasorelaxant effects of relaxin. Conceivably, the reduction of arterial blood pressure in SHR rats given relaxin systemically reported in previous studies may rely on alternative, nitric oxide-independent mechanisms.

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