p38 MAPK contributes to angiotensin II-induced COX-2 expression in aortic fibroblasts from normotensive and hypertensive rats

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Objective To investigate the effect of angiotensin II on cyclooxygenase-2 (COX-2) expression in aortic adventitial fibroblasts from normotensive [Wistar–Kyoto (WKY)] rats and spontaneously hypertensive rats (SHRs).

Methods Protein expression was determined by western blot, mRNA levels by real-time PCR, transcriptional activity by luciferase assays, superoxide anion (O2·−) production by dihydroethidium fluorescence and prostaglandin E2 by enzyme immunoassay.

Results Angiotensin II (0.1 µmol/l, 0.5–6 h) time dependently induced COX-2 protein expression, this effect being transient in fibroblasts from WKY rats and maintained over time in SHRs. Angiotensin II effect was abolished by valsartan (1 µmol/l), an angiotensin II type 1 receptor antagonist. Angiotensin II-induced prostaglandin E2 production was reduced by valsartan and the COX-2 inhibitor NS398 (1 µmol/l). Angiotensin II increased O2·− production more in SHR than WKY rats. This increase was reduced by apocynin (30 µmol/l) and allopurinol (10 µmol/l), respective nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine oxidase inhibitors. However, angiotensin II-induced COX-2 expression was unaffected by apocynin, allopurinol, tempol (1 mmol/l) or catalase (1000 U/ml). Angiotensin II (2–30 min) induced p38 mitogen-activated protein kinase (MAPK) phosphorylation, transiently in WKY rats but sustained in SHRs. The p38 inhibitor SB203580 (10 µmol/l) reduced angiotensin II-induced COX-2 protein and mRNA levels. The angiotensin II effect was not prevented by inhibition of mRNA synthesis, and angiotensin II was unable to modulate COX-2 transcriptional activity.


Keywords: angiotensin, cyclooxygenase-2, fibroblasts, hypertension, mitogen-activated protein kinase

Abbreviations: COX, Cyclooxygenase; CREB, c-AMP regulatory element-binding protein; DHE, dihydroethidium; ERK1/2, extracellular signal-regulated kinases 1/2; JNK, c-Jun NH2-terminal kinases; MAPKs, mitogen-activated protein kinases; PGE2, prostaglandin E2; ROS, reactive oxygen species; SHRs, spontaneously hypertensive rats; VSMC, vascular smooth muscle cells; WKY, Wistar–Kyoto

Introduction There is increasing evidence indicating that the vascular adventitia, traditionally considered a structural support for the blood vessel, is a critical regulator of vessel wall function in health and disease [1–3]. Adventitia is primarily composed of fibroblasts, collagen and elastin fibers. Vascular fibroblasts produce substantial amounts of reactive oxygen species (ROS) that appear to be involved in the fibroblast proliferation, connective tissue deposition and changes in vascular tone [3] that occur in some cardiovascular diseases.