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Hypertension Increases Contractile Responses to Hydrogen Peroxide in Resistance Arteries through Increased Thromboxane A_2, Ca^{2+}, and Superoxide Anion Levels

Ana Belén García-Redondo, Ana María Briones, Amada Elia Beltrán, María Jesús Alonso, Ulf Simonsen, and Mercedes Salaices

Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain (A.B.G.-R., A.M.B., A.E.B., M.S.); Departamento de Ciencias de la Salud III, Universidad Rey Juan Carlos, Alcorcón, Madrid, Spain (M.J.A.); and Department of Pharmacology University of Aarhus, Aarhus C, Denmark (U.S.)

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ABSTRACT

This study investigated the mechanisms underlying the response to hydrogen peroxide (H_2O_2) in mesenteric resistance arteries from spontaneously hypertensive rats (SHRs) and normotensive Wistar Kyoto (WKY) rats. Arteries were mounted in microvascular myographs for isometric tension recording and for simultaneous measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), superoxide anion (O_2^-) production was evaluated by dihydroethidium fluorescence and confocal microscopy, and thromboxane A_2 (TXA_2) production was evaluated by enzyme immunoassay. H_2O_2 (1–100 μM) induced biphasic responses characterized by a transient endothelium-dependent contraction followed by relaxation. Simultaneous measurements of tension and Ca^{2+} showed a greater effect of H_2O_2 in arteries from hypertensive than normotensive rats. The cyclooxygenase (cox) inhibitor, indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-H-indole-3-acetic acid] (1 μM); the COX-1 inhibitor, SC-58560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole] (1 μM); the thromboxane (TXA_2) synthase inhibitor, furegrelate [5-(3-pyridinylmethyl)-2-benzofurancarboxylic acid, sodium salt] (10 μM); and the TXA_2/prostaglandin H_2 receptor antagonist, SQ 29,548 ([1*S*-[1.α.,2.α.(*Z*),3.α.,4.α.]]-7-[3-[[2-[(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid)) (1 μM) abolished H_2O_2 contraction in arteries from WKY rats but only reduced it in SHRs. The O_2^- scavenger, tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt) (1 mM), and the NADPH oxidase inhibitor, apocynin (4′-hydroxy-3′-methoxyacetophenone) (0.3 mM), decreased H_2O_2 contraction in arteries from SHRs but not in WKY rats. H_2O_2 induced TXA_2 and O_2^- production that was greater in SHRs than in WKY rats. The TXA_2 analog, U46619 [9,11-di-deoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$ (0.1 nM–1 μM)], also increased O_2^- production in SHR vessels. H_2O_2-induced TXA_2 production was decreased by SC-58560. H_2O_2-induced O_2^- production was decreased by tiron, apocynin, and SQ 29,548. In conclusion, the enhanced H_2O_2 contraction in resistance arteries from SHRs seems to be mediated by increased TXA_2 release from COX-1 followed by elevations in vascular smooth muscle $[Ca^{2+}]_i$ levels and O_2^- production. This reveals a new mechanism of oxidative stress-induced vascular damage in hypertension.

Reactive oxygen species (ROS) like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) have been suggested as mediators of vascular structural and functional alterations observed in hypertension (Lacy et al., 2000; Paravicini and Touyz, 2006; Alvarez et al., 2007). Several sources of O_2^- have been described. Among them, xanthine oxidase, uncoupled nitric-oxide synthase, and cyclooxygenase (COX) can produce O_2^- in different conditions (Touyz, 2003). However, it is well established that at the vascular level, NADPH oxidase is the main source of O_2^- (Touyz, 2003; Lyle and Griendling, 2006). Dismutation of O_2^- by superoxide dismutase produces H_2O_2, a

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ABBREVIATIONS: ROS, reactive oxygen species; O_2^-, superoxide anion; H_2O_2, hydrogen peroxide; COX, cyclooxygenase; $[Ca^{2+}]_i$, intracellular calcium concentration; TXA_2, thromboxane A_2; MRA, mesenteric resistance artery; SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto; KHS, Krebs-Henseleit solution; indomethacin, 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-H-indole-3-acetic acid; SC-58560, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole; SQ 29,548, [1*S*-[1.α.,2.α.(*Z*),3.α.,4.α.]]-7-[3-[[2-[(phenylamino) carbonyl] hydrazino] methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid); furegrelate, 5-(3-pyridinylmethyl)-2-benzofurancarboxylic acid, sodium salt; tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt; apocynin, 4′-hydroxy-3′-methoxyacetophenone; NS-398 *N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide; AM, acetoxymethyl ester; U46619, 9,11-di-deoxy-11α,9α-epoxymethano prostaglandin $F_{2\alpha}$ (0.1 nM–1 μM); ANOVA, analysis of variance; PSS, physiological saline solution.