beneficial synergistic effects upon the blood pressure as well as the vasculature.

Key Words: Angiotensin II, Reactive Oxygen Species, Nitric Oxide

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HMG-COA REDUCTASE INHIBITOR IMPROVES ENDOTHELIAL DYSFUNCTION IN MINERALOCORTICOID HYPERTENSION BY INHIBITION OF RHOA
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Statins reduce cardiovascular morbidity and mortality. These beneficial effects are not fully explained by their lipid-lowering action. As such, we investigated the impact of a new statin, rosuvastatin, on endothelial function, the key event in early atherogenesis, in an experimental model of normocholesterolemic hypertension. Hypertension was induced in Wistar-Kyoto rats by inhibition of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) with Glycyrrhizic acid (GA). 11β-HSD2 provides mineralocorticoid receptor specificity for aldosterone by metabolising glucocorticoids to their receptor inactive 11-dehydro derivatives. GA was added to the drinking water (3 g/L) for 4 weeks. Endothelial-dependent relaxation in isolated aortic rings to acetylcholine (ACh, 10-10 -10 -5 mol/L) and sodium nitroprusside (SNP, 10-10 -10 -5 mol/L) was measured. In addition, vascular reactivity to ET-1 was increased by GA (p<0.05 vs control), but not affected by rosuvastatin. The within-assay coefficients of variation were 12, 5, 3 and 0.5% for ET-1 concentrations of 0.84, 1.5, 2.3, 5.2 and 9.9 fmol/ml respectively. Between-assay coefficients of variation for two human control plasma containing 1.0 fmol/ml (n=8) and 1.2 fmol/ml ET-1 (n=7) were 8% and 10% respectively. Assay accuracy was demonstrated by the consistency of recoveries of added ET-1 and by the linearity of ir-ET-1 concentrations measured in serially diluted plasma extracts (r=0.99). No ir-ET-1 was detected when albumin buffer was extracted instead of plasma (buffer blank). Using this method, we found increased ir-ET-1 levels in plasma of three experimental rat models of hypertension. (i) Plasma ir-ET-1 concentrations were significantly higher in stroke-prone spontaneously hypertensive rats (SP-SHR) than in normotensive Wistar rats. (ii) DOCA-salt hypertensive rats exhibited 4 times higher ir-ET-1 levels than sham operated control rats. (iii) One kidney-one clip (1K-1C) hypertensive rats showed moderately increased ir-ET-1 levels compared to sham operated controls. In contrast, the ir-ET-1 levels in plasma of SHR were half that of normotensive Wistar rats. In two kidney-one clip (2K-1C) Goldblatt hypertensive rats, the plasma ir-ET-1 concentrations were not different from sham-operated control rats. The plasma ir-ET-1 concentrations of 37 healthy human subjects were 0.85 ± 0.26 fmol/ml (mean ± SD). We conclude that the present assay reliably measures plasma immunoreactive ET-1 levels in rats and in human subjects. Normal plasma ET-1 concentrations in humans and conscious rats are in the low picomolar range.

Key Words: Endothelium, Vasoconstrictor, Hormone

OR-54
PLASMA IMMUNOREACTIVE ENDOTHELIN-1 LEVELS IN HYPERTENSIVE RATS AND HUMAN SUBJECTS
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Endothelin-1 (ET-1) is an endothelium-derived potent vasoconstrictor peptide of 21 amino acids. To establish reference values in different forms and models of hypertension and in human subjects an assay for plasma ET-1 was optimized. Immunoreactive (ir-) ET-1 is extracted by acetone from 1 ml plasma and subjected to a sensitive sandwich type enzyme linked immunosorbent assay. The detection limit for plasma ET-1 (3 SD above zero readings) is 0.05 fmol/ml. Mean recoveries of the 1, 2, 5, 10 fmol of ET-1 added to 1 ml plasma (n=5, each) were 66, 75, 85 and 92% respectively. The within-assay coefficients of variation were 0.18, 0.19* 7.6, 10**, 261, 11**, 295*, 1142, 62, 385, 1122 and 99 fmol/ml respectively. Using this method, we found increased ir-ET-1 levels in plasma of three experimental rat models of hypertension. (i) Plasma ir-ET-1 concentrations were significantly higher in stroke-prone spontaneously hypertensive rats (SP-SHR) than in normotensive Wistar rats. (ii) DOCA-salt hypertensive rats exhibited 4 times higher ir-ET-1 levels than sham operated control rats. (iii) One kidney-one clip (1K-1C) hypertensive rats showed moderately increased ir-ET-1 levels compared to sham operated controls. In contrast, the ir-ET-1 levels in plasma of SHR were half that of normotensive Wistar rats. In two kidney-one clip (2K-1C) Goldblatt hypertensive rats, the plasma ir-ET-1 concentrations were not different from sham-operated control rats. The plasma ir-ET-1 concentrations of 37 healthy human subjects were 0.85 ± 0.26 fmol/ml (mean ± SD). We conclude that the present assay reliably measures plasma immunoreactive ET-1 levels in rats and in human subjects.

Key Words: Angiotensin II, Reactive Oxygen Species, Nitric Oxide

OR-55
IDENTIFICATION OF DOMINANT NEGATIVE RAT RAMPs ABLE TO INHIBIT ENDOGENOUS ADRENOMEDULLIN RECEPTOR FUNCTION
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Adrenomedullin (AM) exerts a wide variety of biological effects, including potent vasorelaxation. Its receptors were recently shown to be heterodimers comprised of a novel accessory protein, the receptor activity-modifying protein (RAMP), and the calcitonin receptor-like receptor (CRLR). When CRLR is co-transfected with RAMP2 or -3, the two proteins are transported together to the plasma membrane where they function as an AM-specific receptor. Recently, we have shown that seven amino acids of human (h)RAMP2 (86-92) and hRAMP3 (59-65) are essential for high-affinity agonist binding to hAM receptors. Interestingly, both seven-residue segments are located between three conserved residues (Trp, Cys and Tyr) and the three ones are common to humans, rats and mice. In this study, we examined whether seven-residue segments situated between these residues conserved in both rat (r)RAMP2 and rRAMP3 (amino acids 93-99 and 58-64, respectively) are key determinants of agonist binding to AM receptors, and then tested whether their deletion mutants can act as dominant negative RAMPs.