sustained decrease in SNA and BP. In 12 normotensive postmenopausal women, we measured 24-hour ambulatory BP and SNA before and after eight weeks of transdermal estradiol (200 mcg/day), oral conjugated estrogens (0.625 mg/day), or placebo, using a randomized crossover design. The major new finding was that transdermal ERT decreased SNA by 30% (from 40+/−4 to 27+/−4 bursts/min, p = 0.0001) and ambulatory diastolic BP by 5+/−2 mmHg (p = 0.0003), whereas, in the same women, oral ERT was without effect. Because the liver is exposed to supraphysiological concentration of estrogen during oral ERT, we speculate that adverse first-pass hepatic effects of estrogen trigger secondary sympathoexcitation and negate primary inhibitory effect of estrogen. Therefore, we measured serum insulin-like growth factor I (IGF-I), a sympathoinhibitory peptide which is produced in the liver but decreased after menopause. We found that serum IGF-I decreased only with oral ERT (from 148+/−12 to 114+/−9 ng/ml) but was unaffected by transdermal ERT or placebo. In conclusion, transdermal ERT decreases SNA and causes a small but statistically significant decrease in ambulatory blood pressure in normotensive postmenopausal women. Sympathetic inhibition is evident only with transdermal rather than oral ERT whereas the circulating IGF-I is reduced only with oral but not transdermal ERT. These data provide support for the hypothesis that estrogen exerts direct effect on the central nervous system to decrease sympathetic vasoconstrictor activity. However, during oral ERT, the decrease in SNA is opposed by sympathetic overactivity related to IGF-I reduction. Because the effects of transdermal ERT are larger than those of oral ERT, the route of administration may be important consideration in optimizing the beneficial effects of ERT on blood pressure and overall cardiovascular health.

Key Words: estrogen, sympathetic nervous system, blood pressure

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EFFECT OF LONG-TERM TREATMENT ON ENDOTHELIN-1 INDUCED CONTRACTILE RESPONSES AND MAPK ACTIVATION IN RAT CAUDAL ARTERIES
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Endogenous ouabain has been proposed to play a role in the regulation of blood pressure and in the pathogenesis of hypertension, at least in part by modifying vascular reactivity to vasoconstrictor stimuli. To clarify if an altered vascular responsiveness to ET-1 is involved in the pressor effects of ouabain, we infused male Sprague-Dawley rats for 4 weeks with 50 ug/kg/day ouabain and evaluated: a) the blood pressure, and b) the contractile responses and c) MAPK activation of endothelium-deprived rat caudal arteries (RCAs) stimulated with ET-1 in vitro.

Ouabain treatment was made by using Alzet osmotically driven minipumps. Blood pressure was measured by tail cuff plethysmography. In vitro isometric contractile responses to ET-1 were assessed in 2-mm long RCA rings. The effect of Endothelin-1 on MAPK activation was investigated by immunoblot with phospho-specific MAPK antibody and scanning densitometry.

Systolic blood pressure was not affected by ouabain treatment (146 vs 141 mmHg in placebo- and ouabain- treated rats, respectively). Similarly, ouabain treatment did not significantly modify the concentration-response curve to ET-1 (1-100 nM) in RCA rings. Immunoblot analysis revealed the presence of two characteristic bands of 44KD and 42KD, respectively, corresponding to p44ERK1 and p42ERK2 MAPK isoforms in RCAs, with no quantitative difference between control and ouabain-treated rats, at baseline. Exposure of caudal artery to 100 nM Endothelin-1 for 10 min caused a rapid phosphorylation of MAPK in control rats. The increase of phosphorylation of p44ERK1 was 28% in comparison to basal level in ouabain-treated rats and 74% in control rats (p<0.05), that of phosphorylation of p42ERK2 was 2% and 35% respectively in ouabain-treated and in control rats (p<0.01).

These results show that long-term ouabain treatment reduces the stimulatory effect of ET-1 on MAPK in RCAs; this effect is apparently not paralleled by a modification of the responsiveness of these vessels to the peptide, nor by a modification of blood pressure, thereby suggesting new possible roles of endogenous ouabain in the regulation of vascular function.

Key Words: MAPK, ouabain, endothelin-1

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REGULATION OF VASCULAR SMOOTH MUSCLE NITRIC OXIDE SYNTHASE ACTIVITY BY PROTEIN KINASE C
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Under normal resting conditions, little or no nitric oxide synthase (NOS) activity can be measured in vascular smooth muscle cells. However NOS activity may be observed when vascular smooth muscle is exposed to certain stimulants, for example lipopolysaccharide or phenylephrine. The present study was designed to test the hypothesis that under resting conditions vascular smooth muscle NOS activity is under tonic inhibition. The results presented here indicate that under resting conditions, vascular smooth muscle NOS activity may be inhibited by protein kinases.

Phenylephrine (PE) was used to induce concentration dependent contractions of endothelium-denuded aortic rings prepared from Sprague-Dawley rats. The rings were suspended in Sawyer-Bartlestone chambers which were filled with Krebs solution. The chambers were maintained at 37°C, and gassed with 95% oxygen/5% carbon dioxide. A basal tension of 1.0 g was found to produce optimal contractile responses. Absence of any response to acetylcholine was used as the criterion to ensure adequate removal of the endothelium. Pre-treatment of the rings with H-7, a non-specific inhibitor of protein kinases, attenuated the PE-induced response. N-nitro-L-Arginine (L-NNA) and N-methyl-L-Arginine (L-NMA), both NOS inhibitors, as well as aminoguanidine (AG), a selective inhibitor of inducible NOS, all significantly reduced the H-7-induced inhibition of contraction. The effects of L-NNA, L-NMA and AG were all reversed by L-arginine but not D-arginine. H-89, a selective protein kinase A inhibitor, did not significantly inhibit the PE-induced contraction. However, calphostin C, a specific protein kinase C inhibitor, significantly decreased the PE-induced contraction. Direct measurement of NO with NO-sensitive electrodes showed that H-7 and calphostin C both significantly decreased NO production by PE-stimulated aortic rings. These results indicate that under normal conditions vascular smooth muscle NOS activity is inhibited by the action of protein kinase C but not protein kinase A.

Key Words: protein kinase C, nitric oxide, vascular smooth muscle

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VASOACTIVE EFFECTS OF RECOMBINANT HUMAN ERYTHROPOIETIN (RHUEPO) ON THE MESENTERIC ARTERY IN NORMAL, HYPERTENSIVE AND URAEMIC RATS, IN VIVO AND IN VITRO STUDIES
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In about one-third of haemodialysed patients, treatment with rHuEpo induces high blood pressure (BP) or exacerbates the pre-existing hypertension, but it is not known what the mechanism of rHuEpo-caused