ENDOTHELIUM-, SOLUBLE GUANYLYL CYCLASEDEPENDENT CONTRACTIONS IN ISOLATED ARTERIES

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OBJECTIVES: This study focuses on the effects of thymoquinone on isolated arteries, by determining the mechanisms underlying the endothelium- and soluble guanylyl cyclase (sGC)-dependent augmentation of contraction that this natural compound causes, comparable to the augmentation described under hypoxic conditions. METHODS: Experiments were designed to study the effect of thymoquinone *ex vivo* in isolated porcine coronary and rat arteries. Rings, with or without endothelium, were suspended in conventional organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide (NO) synthase (L-NG-nitroarginine methyl ester, LNAME), sGC (1H-[1,2, 4]oxadiazolo[4,3-a]quinoxalin-1-one, ODQ), rhoassociated protein kinases (Y-27632) or L-type voltage-gated calcium channels (nifedipine). Some of the control experiments were done under depleted calcium conditions. The rings were contracted with phenylephrine (rat arteries) or prostaglandin F2α (porcine coronaries) and exposed to increasing concentrations of thymoquinone. Selected rings were used to measure cyclic nucleotide levels by HPLC-MS/MS. RESULTS: Thymoquinone caused a sustained further increase in tension in rings with endothelium. This augmentation was prevented by endotheliumremoval, L-NAME and ODQ. Incubation with the NO-donor DETA NONOate in L-NAME-treated rings restored and even increased the contractile response to thymoquinone. Treatment with 8-bromo guanosine 3':5' cyclic monophosphate (cyclic GMP) or pyrophospate did not restore the augmentation by thymoquinone. HPLC-MS/MS measurements revealed that the compound increased the production of inosine 3':5' cyclic monophosphate (cyclic IMP). Y-27632, nifedipine and calcium depletion inhibited the thymoquinone-induced contraction in porcine coronary arteries, but not in rat aortae. CONCLUSIONS: The endothelium-dependent augmentation caused by thymoquinone requires endothelium-derived NO and activation of sGC, as described under hypoxic conditions. In addition, both thymoquinone- and hypoxiainduced augmentations require production of cyclic IMP but not the presence of either pyrophosphate or cyclic GMP. The data suggest that cyclic IMP causes vasoconstriction through calcium sensitization, although the underlying mechanism seems to differ in rat and porcine arteries. Taken into conjunction, thymoquinone can serve as a pharmacological tool to study/ mimic the endothelium-dependent vasoconstrictor effects of intermittent hypoxia, as seen in the clinical setting of sleep apnea patients.