Action of C-type natriuretic peptide in isolated canine arteries and veins

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We characterized the interaction of C-type natriuretic peptide (CNP) with renal and saphenous arteries and renal, saphenous, and femoral veins with and without endothelium in vitro. CNP decreased blood pressure, increased cardiac output, and increased atrial pressure. CNP induced relaxation of isolated canine renal and saphenous arteries and renal, saphenous, and femoral veins with and without endothelium. CNP-induced relaxation was dose-dependent, and it was greater in veins than in arteries with and without endothelium. CNP-induced relaxation was greater in saphenous veins than in renal veins.

METHODS

Organ chamber experiments. Rings of renal, saphenous arteries and veins, and femoral veins obtained from normal mongrel dogs (anesthetized with 30 mg/kg pentobarbital sodium intravenously) were suspended to measure isometric force in organ chambers filled with aerated (95% O2-5% CO2) modified Krebs-Ringer bicarbonate solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25.0 NaHCO3, 0.026 calcium disodium EDTA, and 11.1 dextrose; control solution) at 37°C. In one-half of the rings, the endothelium was removed by gently rubbing the intimal surface with a cotton swab wetted with control solution. Each ring was stretched to the optimal point on its length-tension curve as determined by the maximal tension developed to noradrenaline (3 x 10^-7 M) at each level of stretch. The presence of endothelium was determined at the beginning of the experiment by a relaxation to acetylcholine (10^-6 M) during a contraction to noradrenaline at optimal length. To study responses to the natriuretic peptides, the rings were contracted with phenylephrine (10^-6 M). The peptides were added cumulatively once the contraction had stabilized.

Drugs. The following drugs were used: acetylcholine chloride (Sigma Chemical, St. Louis, MO), human ANP and human CNP (Peninsula Laboratories, Belmont, CA), l-norepinephrine bitartrate (Sigma), and phenylephrine bitartrate (Sigma). All drugs were dissolved in distilled water immediately before the study, and the concentrations are reported as the final molar concentration (M) in the organ chamber.

Statistical analysis. The results are expressed as means ± SE: n equals the number of dogs from which the rings were taken. Rings with and without endothelium were studied in parallel, and Student's t test for paired observations was used to determine statistical significance between responses of rings with and without endothelium and between responses of arteries and veins. Statistical significance was determined at P < 0.05.
RESULTS

CNP produced concentration-dependent relaxations in renal, saphenous, and femoral veins with and without endothelium contracted with phenylephrine (Fig. 1). In femoral and renal veins, relaxations to CNP were significantly greater in rings without compared to with endothelium. In saphenous veins, relaxations of rings without endothelium tended to be greater than relaxations of rings with endothelium; these differences did not reach statistical significance. Maximal relaxations averaged 81 ± 5% of the contraction to phenylephrine in femoral veins without endothelium. ANP did not produce significant changes in tension in renal, saphenous, or femoral veins with or without endothelium. Maximal relaxations to ANP in rings without endothelium averaged −4.5 ± 2.5% in renal veins, −3.2 ± 1.2% in saphenous veins, and −9.1 ± 4.2% in femoral veins; these were not significantly different from responses at 10−10 M ANP.

CNP produced comparable concentration-dependent relaxations in saphenous arteries with and without endothelium; no relaxations were observed in renal arteries (Fig. 2A). The threshold for relaxation was greater (10−7 M) in saphenous arteries than the saphenous veins (10−7.5 M) without endothelium, and maximal relaxation were not different statistically (25 ± 9 and 49 ± 7% in saphenous arteries and veins, n = 6 respectively).

ANP caused concentration-dependent relaxations in renal but not saphenous arteries (Fig. 2B). ANP produced a significant relaxation of 41 ± 6% in rings of renal arteries with endothelium at a concentration of 10−7 M, with no difference in relaxation to ANP in renal arterial rings with and without endothelium.

DISCUSSION

The current study demonstrates for the first time the selective vascular action of the newly identified peptide CNP in isolated canine blood vessels. Specifically, CNP induced greater relaxation in canine renal, saphenous, and femoral veins than arteries. In marked contrast, ANP induced no significant vasoconstrictor effect in renal veins. Interestingly, the vasorelaxation actions of CNP was attenuated in the presence of the endothelium.

These results provide additional evidence that CNP has biological action distinct and separate from ANP. Recent investigations have demonstrated that receptors for CNP are expressed in vascular smooth muscle cells compared with receptors for ANP that are expressed in greater abundance in endothelial cells (15). Furuuya et al. (3) have reported that CNP may be a more potent inhibitor of vascular smooth muscle cell proliferation in vitro compared with ANP. Consistent with these in vitro studies, Stingo et al. (13) have reported that CNP is a potent vasoactive peptide in vivo, which decreases cardiac filling pressures, cardiac output, and arterial pressure in association with increases in systemic guanosine 3′,5′-cyclic monophosphate concentration. The present study extends these previous observations by demonstrating that CNP is a venodilator in vitro, an action that is consistent with the physiological effects observed when the peptides are administered in vivo.

The current study demonstrates that the venodilator action of CNP is endothelium independent and is consistent with the local action of the ANPR-B receptors on the target tissue.
vascular smooth muscle. Indeed, while CNP induced a relaxation in femoral veins, this action was attenuated in the presence of an intact endothelium. The mechanism of this attenuated response is unclear and could be explained by receptor-mediated clearance of CNP by the endothelium by the ANPR-C receptor (6). Alternatively, as the ectoenzyme neutral endopeptidase 24.11 has been localized to endothelial cells and degrades ANP, the endothelium may limit the action of CNP by degrading this peptide also (11). CNP could also enhance the release of endothelium-derived contracting factors, which would antagonize the relaxing action of CNP. Additionally, the endothelium could serve as a diffusion barrier to the smooth muscle cells by binding CNP.

The present study demonstrates that ANP does not initiate marked vasodilator responses in either saphenous arteries or femoral and renal veins with or without endothelium, but ANP does result in a vasodilator response in renal arteries. Previous studies have documented selective regional vasorelaxation effects of ANP in isolated rabbit arteries (renal, thoracic aorta, mesenteric, carotid) and veins (fetal, mesenteric, jugular) in the presence of the endothelium (2). However, no information regarding whether the relaxation was independent of the endothelium was presented in the previous report (2). The current and previous observations are consistent with a heterogeneous vasorelaxing action in vitro for ANP (4) and extend this concept to CNP.

In conclusion, the present study demonstrates that CNP is a potent endothelium-independent dilator of peripheral isolated canine veins. In contrast, ANP has vasodilator actions in peripheral canine arteries. Therefore, the current investigations suggest that CNP with its vasodilator actions has physiological functions distinct from the structurally related peptide ANP.

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