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Short communication

Vasodilator effects of C-type natriuretic peptide on cerebral arterioles in rats

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Abstract

The vasodilator effects of C-type natriuretic peptide (CNP) were investigated in isolated rat cerebral arterioles. CNP caused dose-dependent vasodilation, maximally by $10.0 \pm 2.2\%$ at 10^{-6} M. The median effective concentration (EC_{50}) was 5.2×10^{-10} M. In contrast, atrial natriuretic peptide and B-type natriuretic peptide, other members of the natriuretic peptide family, produced little or no vasodilation. Pretreatment with methylene blue (10^{-4} M) abolished CNP-induced vasodilation, whereas pretreatment with N^G-monomethyl-L-arginine or indomethacin did not inhibit vasodilation. Thus, CNP is suggested to cause significant vasodilation in cerebral arterioles via a cyclic guanosine monophosphate-dependent mechanism.

Keywords: CNP (C-type natriuretic peptide); BNP (brain natriuretic peptide); ANP (atrial natriuretic peptide); Cerebral arteriole

1. Introduction

C-type natriuretic peptide (CNP), which was first isolated from porcine brain tissue (Sudoh et al., 1990), is the newest member of the family of natriuretic peptides. This group of peptides includes atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP).

ANP and BNP are found primarily in the atria and ventricles of the heart. In contrast, CNP was initially thought to be present exclusively in the central nervous system. However, recent studies have shown that CNP is present in the circulatory system, such as in vascular endothelial cells (Stingo et al., 1992; Suga et al., 1992), suggesting that it may play a role in the regulation of blood flow.

However, little is known about the effects of CNP on the cerebral circulation, especially the cerebral microcirculation. Therefore, we investigated the effects of CNP on rat cerebral arteriolar vasodilation, comparing its effects with those of ANP and BNP. Furthermore, the mechanism responsible for the vascular effects of CNP on cerebral arterioles was determined by using various inhibitors of vasodilation.

2. Materials and methods

Our protocol followed the guidelines for the care and use of animals in the physiological sciences as established by the Physiological Society of Japan.

2.1. Preparation of arterioles

Cerebral arterioles were isolated and cannulated in an organ bath apparatus. Changes in vessel diameter in response to the extraluminal administration of various agents were measured as previously described (Duling et al., 1981; Dacey and Duling, 1982; Takayasu et al., 1988, 1993). Briefly, penetrating intracerebral arterioles, 53–87 μm (mean 69.4) in diameter and approximately 1000 μm in length, were surgically isolated from the first portion of the middle cerebral artery from the brains of pentobarbital-anesthetized Sprague-Dawley rats ($n = 31$; weight: 300–400 g; Chubu Science Materials, Nagoya, Japan). Vessel segments were transferred to a temperature-controlled chamber on the stage of an Olympus inverted microscope, and one end of the vessel was cannulated, using a glass pipette. After intraluminal blood was washed out, the other end of the vessel was occluded and held with another pipette. The inner vessel diameters were deter-

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mined manually with a micro-scaler system (FOR. A, Model IV-550, Tokyo, Japan) on a video screen.

After cannulation, a constant transmural pressure of 60 mmHg was applied via the cannulating pipette, which was connected to a manometer, and the passive diameter was measured. The external bath solution was then warmed from room temperature to 37–38°C. After approximately 45 min, during which time the solution was changed three or four times, spontaneous tone developed (the control vessel diameter). Vessel responsiveness was then assessed by changing the extraluminal pH from 7.3 to 6.8 or to 7.6. At this stage, the portion of the vessel segment which showed best reactivity was selected, per vessel, for the following measurement.

The physiologic salt solution (PSS) used in these studies was a modified Ringer's solution (millimolar composition: NaCl 144; KCl 3.0; $CaCl_2$ 2.5; $MgSO_4$ 1.4; glucose 5.0; pyruvate 2.0; EDTA 0.02; 3-[*N*-morpholino]propanesulfonic acid (MOPS) 2.0; NaH_2PO_4 1.21). Bovine serum albumin (1.0 g/100 ml) was added to PSS when used as the intraluminal solution, which was maintained at pH 7.3 for all experiments.

2.2. Measuring vasodilation of the arterioles

All drugs were dissolved in PSS at a pH of 7.3 and were applied to the extraluminal surface. Luminal changes of the vessels were based on measurements of the inner diameter. The magnitude of vasodilation induced by a peptide is expressed as a percent change in the diameter from the control value.

Dose–response curves for extraluminally applied peptides (CNP-22, CNP-53, ANP-28 and BNP-32) were determined by adding solutions of increasing concentration sequentially to the organ bath. The vessel diameter was allowed to stabilize for 5 min between each change in bath solution.

In order to determine the mechanism of CNP-22-induced vasodilation, changes in luminal diameter were measured in vessels after pretreatment with methylene blue (an inhibitor of guanylate cyclase), N^G-monomethyl-L-arginine (L-NMMA; a nitric oxide synthesis inhibitor), or indomethacin (a cyclooxygenase inhibitor).

2.3. Materials

Synthetic forms of human CNP-22, CNP-53, ANP-28 and BNP-32 were obtained from the Peptide Institute (Osaka, Japan). All other chemicals were of reagent grade or the best grade commercially available. The concentrations of methylene blue (10^{-4} M), indomethacin (10^{-6} M) and L-NMMA (10^{-4} M) used in these studies were determined from preliminary experiments and from values reported in the literature.

2.4. Statistical analysis

Vessel diameters at each agonist dose are expressed as a percentage of the control vessel diameter. Vasoconstriction and vasodilation are expressed as the percent change in diameter from control vessel diameter. Data are reported as the means ± S.E.M. Differences between three or more groups were evaluated by one-way analysis of variance (ANOVA). The Fisher protected least-significant difference (PLSD) multiple range test was used as a post-hoc test. We considered differences significant if $P < 0.05$.

3. Results

The vessels constricted from the passive diameter of 90.9 ± 2.6 μm ($n = 42$) to the control vessel diameter of 68.8 ± 2.1 μm after the development of spontaneous tone. When the extraluminal pH changed, vessels dilated to 118.5 ± 1.2% of the control diameter ($P < 0.001$) at pH 6.8 and constricted to 75.0 ± 1.3% of the control diameter ($P < 0.001$) at pH 7.6. Vessels that responded weakly to the changes in pH (< 10% dilation at pH 6.8 or < 10% contraction at pH 7.6) were discarded. Passive diameters, control diameters, and the arteriolar response to acidosis and alkalosis did not differ between the experimental groups ($P = 0.06$–0.96).

3.1. Vasodilator effects of natriuretic peptides

Extraluminal administration of CNP-22 and CNP-53 (the N-terminal extended form of CNP-22) caused dose-dependent vasodilation to a similar extent in rat cerebral arterioles (Fig. 1). Neither the maximum dilation (10.0 ± 2.2% at 10^{-6} M for CNP-22 and 8.7 ± 1.7% at 10^{-7} M for CNP-53) nor the median effective concentration (EC_{50}) (5.2×10^{-10} M for CNP-22 and 2.5×10^{-10} M for CNP-53) were significantly different between the two peptides

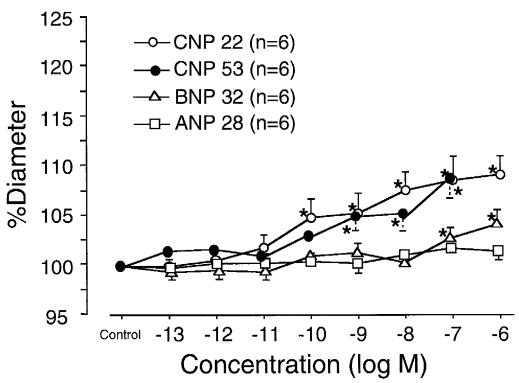

Fig. 1. Dose–response curves for extraluminally applied natriuretic peptides in rat isolated intracerebral arterioles. Each point represents mean vessel diameter expressed as a percentage of control diameter (mean ± S.E.M.). Asterisks indicate significant vasodilation ($P < 0.05$).

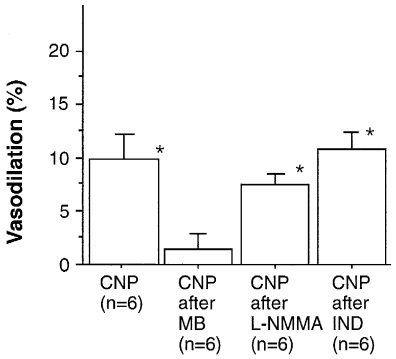

Fig. 2. The effect of pretreatment with methylene blue (MB, 10^{-4} M), N^{G}-monomethyl-L-arginine (L-NMMA, 10^{-4} M), or indomethacin (IND, 10^{-6} M) on vasodilation induced by CNP-22 (10^{-6} M) in rat intracerebral arterioles. Each point represents mean vasodilation expressed as a percentage of control diameter (mean ± S.E.M.). Asterisks indicate significant vasodilation ($P < 0.05$).

($P = 0.67$ and 0.64, respectively). In contrast, ANP-28 had no effect, and BNP-32 produced only slight vasodilation, which was statistically significant at the highest concentrations between 10^{-7} M and 10^{-6} M (maximum dilation of $4.2 \pm 1.3\%$ at 10^{-6} M) (Fig. 1).

3.2. Pretreatment with inhibitors

The underlying mechanism of CNP-induced vasodilation was determined by using arterioles pretreated with various inhibitors (Fig. 2). Pretreatment with methylene blue (10^{-4} M) abolished CNP-induced vasodilation, resulting in no significant change in luminal diameter ($1.5 \pm 1.3\%$ dilation). However, arterioles pretreated with L-NMMA (10^{-4} M) or indomethacin (10^{-6} M) dilated to the same degree as control arterioles in response to CNP-22.

4. Discussion

In the present study, we demonstrated that two forms of CNP, CNP-22 and CNP-53, produced a concentration-dependent vasodilation of rat cerebral arterioles. In contrast, ANP and BNP, other members of the natriuretic peptide family, had little or no effect on vascular tone. Further, the vasodilation induced by CNP was inhibited by pretreatment with methylene blue, a guanylate cyclase inhibitor, but not with an inhibitor of nitric oxide synthesis or a cyclooxygenase inhibitor.

To the best of our knowledge, this is the first report of the effects of CNP on the cerebral vasculature, although heterogeneous effects of CNP on vessels in the systemic circulation have been reported (Bjenning et al., 1992; Wei et al., 1993). Wei et al. (1993) have shown that CNP induces modest relaxation in rings prepared from canine saphenous arteries but not from renal arteries. In contrast, CNP causes significant relaxation in canine veins, including the renal, saphenous, and femoral veins. With regard to the effects of CNP on the cerebral vasculature, we have preliminary data for canine cerebral arteries both in vitro and in vivo. CNP caused concentration-dependent relaxation in an in vitro isometric tension study of canine middle cerebral arterial rings precontracted with prostaglandin $F_{2\alpha}$. Maximum relaxation was obtained with a concentration of CNP of 10^{-7} M ($9.2 \pm 0.9\%$, $n = 7$) in this preliminary study (unpublished data). In an in vivo study, intracisternal injection of 10^{-8} mol of CNP caused angiographically demonstrable dilation (maximum dilation of $16.3 \pm 4.8\%$, $n = 3$) of canine basilar arteries for over 120 min (unpublished data). These preliminary data as well as the present results support our hypothesis that CNP may play a significant role in vasodilation of the cerebral vasculature.

CNP-induced vasodilation was abolished by pretreatment with methylene blue in the present study. Methylene blue is a vital, nontoxic dye that directly inhibits both soluble guanylate cyclase (Gruetter and Kadowitz, 1981) and natriuretic peptide receptor subtype-B (ANP_B) (Shigematsu et al., 1993), suggesting that CNP-induced vasodilation is mediated through either guanylate cyclase or ANP_B. With respect to natriuretic peptide receptors, three subtypes have been identified: subtype-A (ANP_A), subtype-B (ANP_B), and clearance receptor subtype-C (ANP_C). Of the three natriuretic peptides, CNP has the highest affinity for ANP_B (Koller et al., 1991). Drewett et al. (1995) have demonstrated that in rat aortic rings CNP-induced vasorelaxation is blocked by monoclonal antibodies directed against ANP_B. Vigne and Frelin (1992) found that endothelial cells from brain microvessels express large amounts of ANP_B but much less ANP_A, while aortic endothelial cells express relatively more ANP_A. These findings suggest that CNP causes dilation of cerebral arteries and arterioles by binding to ANP_B, which is located more densely in the cerebral vasculature than are other natriuretic peptide receptors. Pretreatment with L-NMMA or indomethacin did not change the degree of CNP-induced vasodilation, suggesting that neither nitric oxide nor prostanoids contribute to CNP-induced vasodilation in cerebral arterioles.

In conclusion, we have shown that CNP causes relaxation of rat cerebral arterioles through a cGMP-mediated mechanism. Because CNP is found in the greatest concentrations in the brain, it may function as a regulator of local cerebral blood flow.

References

Bjenning, C., Y. Takei, T.X. Watanabe, K. Nakajima, S. Sakakibara and N. Hazon, 1992, A C-type natriuretic peptide is a vasodilator in vivo and in vitro in the common dogfish, J. Endocrinol. 133, R1.

Dacey Jr., R.G. and B.R. Duling, 1982, A study of rat intracerebral arterioles: methods, morphology, and reactivity, Am. J. Physiol. 12, H598.

Drewett, J.G., B.M. Fendly, D.L. Garbers and D.G. Lowe, 1995, Natriuretic peptide receptor-B (guanylyl cyclase-B) mediates C-type natriuretic peptide relaxation of precontracted rat aorta, J. Biol. Chem. 270, 4668.

Duling, B.R., R.W. Gore, R.G. Dacey Jr. and D.N. Damon, 1981, Methods for isolation, cannulation, and in vitro study of single microvessels, Am. J. Physiol. 241, H108.

Gruetter, C.A. and P.J. Kadowitz, 1981, Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activity by nitroglycerin, sodium nitrate and amyl nitrate, Can. J. Physiol. Pharmacol. 59, 150.

Koller, K.J., D.G. Lowe, G.L. Bennet, N. Minamino, K. Kangawa, H. Matsuo and D.V. Goeddel, 1991, Selective activation of the natriuretic peptide receptor by C-type natriuretic peptide (CNP), Science 252, 120.

Shigematsu, Y., J. Vaughn, C.L. Touchard, E.D. Frohlich, J. Alam and F.E. Cole, 1993, Different effects on natriuretic peptide receptor subtypes in LLC-PK1 and NIH-3T3 cells, Life Sci. 53, 865.

Stingo, A.J., A.L. Clavell, D.M. Heuberlein, C.-M. Wei, M.R. Pittelkow and J.C. Burnett Jr., 1992, Presence of C-type natriuretic peptide in cultured human endothelial cells and plasma, Am. J. Physiol. 263, H1318.

Sudoh, T., N. Minamino, K. Kangawa and H. Matsuo, 1990, C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain, Biochem. Biophys. Res. Commun. 168, 863.

Suga, S., K. Nakao, H. Itoh, Y. Komatsu, Y. Ogawa, N. Hama and H. Imura, 1992, Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-β. Possible existence of 'vascular natriuretic peptide system', J. Clin. Invest. 90, 1145.

Takayasu, M., J.E. Bassett and R.G. Dacey Jr., 1988, Effects of calcium antagonists on intracerebral penetrating arterioles in rats, J. Neurosurg. 69, 104.

Takayasu, M., Y. Kajita, Y. Suzuki, M. Shibuya, T. Ishikawa and H. Hidaka, 1993, Triphasic response of rat intracerebral arterioles to increasing concentration of vasopressin in vitro, J. Cereb. Blood Flow Metab. 13, 304.

Vigne, P. and C. Frelin, 1992, C-type natriuretic peptide is a potent activator of guanylate cyclase in endothelial cells from brain microvessels, Biochem. Biophys. Res. Commun. 183, 640.

Wei, C.-M., L.L. Aarhus, V.M. Miller and J.C. Burnett Jr., 1993, Action of C-type natriuretic peptide in isolated canine arteries and veins, Am. J. Physiol. 264, H71.