ELSEVIER

European Journal of Pharmacology 330 (1997) 195–198

ejp

Short communication

Effects of adrenomedullin on rat cerebral arterioles

Yoshimasa Mori [a], Masakazu Takayasu [a,*], Yoshio Suzuki [a], Masato Shibuya [a], Jun Yoshida [a], Hiroyoshi Hidaka [b]

[a] *Department of Neurosurgery, Nagoya University School of Medicine, 65 Tsurumai, Showa, Nagoya 466, Japan*
[b] *Department of Pharmacology, Nagoya University School of Medicine, Nagoya, Japan*

Received 22 April 1997; revised 21 May 1997; accepted 23 May 1997

Abstract

The effects of adrenomedullin on isolated rat intracerebral arterioles were investigated and compared with those of calcitonin gene-related peptide (CGRP) and amylin. Adrenomedullin produced dose-dependent vasodilation (maximum dilation $27.1 \pm 2.1\%$ at 3×10^{-7} M, median effective dose (EC_{50}) 1.6×10^{-9} M). CGRP produced similar vasodilation ($19.8 \pm 4.1\%$) at 10^{-7} M with a lower EC_{50} of 2.8×10^{-11} M. Amylin did not cause vasodilation at concentrations up to 10^{-6} M. Adrenomedullin-induced vasodilation was significantly suppressed by CGRP-(8–37). These data suggest that adrenomedullin is a potent vasodilator for arterioles in the cerebral microcirculation that acts through CGRP receptors. © 1997 Elsevier Science B.V.

Keywords: Adrenomedullin; CGRP (calcitonin gene-related peptide); CGRP-(8–37); Amylin; Cerebral arteriole; Cerebral microcirculation

1. Introduction

Adrenomedullin is a novel hypotensive peptide isolated from human pheochromocytoma tissue. Its hypotensive activity is based on its ability to increase the concentration of cyclic AMP in platelets (Kitamura et al., 1993). The amino acid sequence of adrenomedullin shows homology to that of both calcitonin gene-related peptide (CGRP) and amylin. The peptides share a ring structure with a disulfide bond and a C-terminal amide structure. CGRP is a well known vasodilator of cerebral arteries and arterioles (McCulloch et al., 1986; Suzuki et al., 1989) and has been localized to the brain and peripheral nerves, where it functions as a neuropeptide (Rosenfeld et al., 1983). Adrenomedullin was originally found only in small amounts in the brain (Ichiki et al., 1994). However, recent studies have shown that high concentrations of adrenomedullin can be detected in many regions of human brain by using a very sensitive and specific radioimmunoassay and that adrenomedullin mRNA is over-expressed in rat cerebral cortex after focal cerebral ischemia (Satoh et al., 1995; Wang et al., 1995). Moreover, a role for adrenomedullin in the regulation of the circulation has been suggested. Adrenomedullin is present in the blood at relatively high concentrations (Ichiki et al., 1994). The gene expression as well as synthesis of adrenomedullin has been demonstrated in cultured vascular endothelial cells and vascular smooth muscle cells, including those from brain capillaries (Sugo et al., 1994a,b).

We have demonstrated that adrenomedullin is a potent vasodilator peptide in dog cerebral arteries (Baskaya et al., 1995). The smaller arterioles in the cerebral microcirculation play an important role in the control of local cerebral blood flow, and the responses of these cerebral arterioles to vasoactive substances can be different from those of larger cerebral arteries (Dacey and Duling, 1984; Takayasu et al., 1988). Because few data are available concerning the effects of adrenomedullin on the cerebral microcirculation (Lang et al., 1997), we investigated the effect of adrenomedullin on rat intracerebral arteriolar dilation in vitro, and compared the effects with those of the structurally related peptides, CGRP and amylin.

2. Materials and methods

Cerebral arterioles were isolated and cannulated in an organ bath apparatus. Changes in vessel diameter in response to the extraluminal administration of various agents

* Corresponding author. Tel.: (81-52) 744-2353; Fax: (81-52) 744-2360.

PII S0014-2999(97)01028-5

were measured as previously described (Dacey and Duling, 1982; Takayasu et al., 1988, 1993). Briefly, intracerebral arterioles, 41–101 μm (mean 68.1) 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 secured with another pipette. The inner diameters of the vessels were determined manually with a video microscaler system (FOR. A, Model IV-550, Tokyo, Japan).

After cannulation, a constant transmural pressure of 60 mm Hg 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 (control vessel diameter). Vessel responsiveness was then assessed by changing the extraluminal pH from 7.3 to 6.8 or to 7.6. The portion of the vessel segment which showed greatest reactivity was selected for study.

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; ethylenediaminetetraacetic acid, 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. The intraluminal solution was maintained at pH 7.3 for all experiments. All drugs were dissolved in PSS at a pH of 7.3 and administered to the extraluminal surface. Luminal diameter changes were based on measurements of the inner diameter.

Increasing concentrations (10^{-14} to 10^{-6} M) of adrenomedullin, CGRP, and amylin were sequentially applied to arterioles to generate dose–response curves. Five minutes was usually long enough to obtain the maximum response for each concentration of the peptides before the next concentration was applied. Dose–response curves for CGRP and adrenomedullin were also obtained in vessels pretreated with CGRP-(8–37) (10^{-6} M), a competitive receptor antagonist of CGRP (Dennis et al., 1990), in order to determine the mechanism of adrenomedullin-induced vasodilation.

Human adrenomedullin, human amylin, human α-CGRP, and CGRP-(8–37) were obtained from the Peptide Institute (Osaka, Japan). All other chemicals were of reagent grade.

Vessel diameters at each agonist dose were determined as a percentage of control vessel diameter. Magnitudes of vasoconstriction and vasodilation are expressed as the percent change in diameter from control vessel diameter. These data are reported as the means ± S.E.M. Differences among three or more groups were evaluated by one-way analysis of variance (ANOVA), using the Bonferroni/Dunn's procedure for post-hoc comparisons. Differences in vessel diameter at a given dose of CGRP or adrenomedullin between control and CGRP-(8–37) pretreated arterioles were evaluated by an unpaired *t*-test. We considered differences significant at $P < 0.05$.

3. Results

Vessels developed spontaneous tone and contracted to a diameter of 69.4 ± 1.8 μm from a passive diameter of 90.4 ± 2.2 μm ($n = 31$) after warming to 37–38°C. In response to a pH change, arterioles dilated 18.1 ± 1.0% at pH 6.8 and contracted 14.8 ± 1.3% at pH 7.6. Vessels with poor vasomotor responses to pH changes (< 10% change of diameter) were discarded from further study.

Extraluminal adrenomedullin caused dose-dependent vasodilation, with a maximum increase in luminal diameter of 27.1 ± 2.1% ($n = 9$) at 3×10^{-7} M and an EC_{50} of 1.6×10^{-9} M (Fig. 1). Extraluminal CGRP also produced dose-dependent vasodilation, maximally increasing the luminal diameter by 19.8 ± 4.1% ($n = 6$) at 10^{-7} M with an EC_{50} of 2.8×10^{-11} M. Amylin caused no change in vessel diameter at concentrations between 10^{-9} M and 10^{-6} M. CGRP was a more potent vasodilator in lower doses than adrenomedullin, and there was a significant difference in the EC_{50} for CGRP and adrenomedullin ($P < 0.01$). However, the difference in maximum dilation caused by the two agents was not significant ($P = 0.108$).

When vessels were pretreated with CGRP-(8–37), the vasodilation elicited by CGRP and adrenomedullin was suppressed (Fig. 2). The dose–response curve for CGRP shifted to the right, with an EC_{50} of 2.5×10^{-8} M. Adrenomedullin, in concentrations between 10^{-14} M and

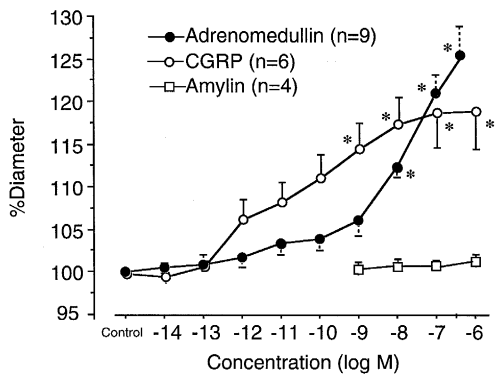

Fig. 1. Concentration–response curves for extraluminally applied adrenomedullin, CGRP and amylin in isolated intracerebral arterioles from rats. Each point represents mean vessel diameter expressed as a percentage of control diameter (mean ± S.E.M.). Asterisks indicate significant vasodilation ($P < 0.05$).

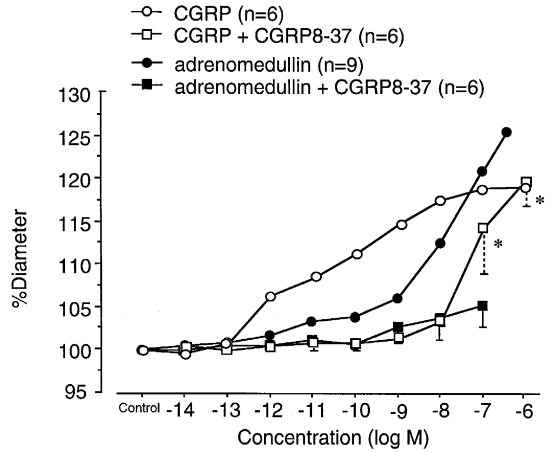

Fig. 2. The effect of pretreatment with CGRP-(8–37) (10^{-6} M) on concentration–response curves for CGRP and adrenomedullin. Each point represents mean vessel diameter expressed as a percentage of control diameter (mean ± S.E.M.). Asterisks indicate significant vasodilation ($P < 0.05$).

10^{-6} M, did not cause vasodilation in arterioles pretreated with 10^{-6} M CGRP-(8–37).

4. Discussion

We demonstrated that adrenomedullin as well as CGRP caused significant dose-dependent vasodilation in rat intracerebral arterioles. In contrast, amylin did not cause vasodilation at concentrations up to 10^{-6} M. Further, adrenomedullin-induced vasodilation was suppressed by pretreatment with a CGRP receptor antagonist, CGRP-(8–37).

The effects of adrenomedullin on the cardiovascular system have previously been examined (Ishiyama et al., 1993; Nuki et al., 1993). The potent vasodilator action of adrenomedullin in the peripheral vascular system accounts for the marked and prolonged systemic vasopressor response to the peptide. We have recently demonstrated that adrenomedullin is a potent vasodilator peptide in dog cerebral arteries (Baskaya et al., 1995). In the present study, we found significant vasodilator effects of adrenomedullin on arterioles, the most distal resistance vessels, in the cerebral microcirculation. These vessels play an important role in the regulation of local cerebral blood flow. The results suggest a role for adrenomedullin in the regulation of local cerebral blood flow. However, the vasodilator potency of adrenomedullin in the cerebral arterioles was approximately one-hundredth that of CGRP. Lang et al. (1997) have recently reported adrenomedullin-induced dilation of rat cerebral pial arterioles in the cranial window, which was suggested to be dependent on the activation of K^+ channels. The effective concentrations of adrenomedullin were similar to ours but they ascribed the low potency of adrenomedullin to extraluminal application of the peptide. Extraluminal application of drugs which directly act on vascular smooth muscle had a greater effect than intraluminal application in our experimental set-up, since drugs gain easy access to the vascular smooth muscle through the very thin adventitia without passing through the endothelial barrier including tight junctions in intracerebral arterioles (Ogura et al., 1991). The potency of adrenomedullin relative to CGRP in cerebral arterioles was less than that reported in other vasculature, in which adrenomedullin has similar or only slightly less potency than CGRP (Nuki et al., 1993; Baskaya et al., 1995).

The vasodilator effect of adrenomedullin on cerebral arterioles was inhibited by CGRP-(8–37), a CGRP receptor antagonist. Specific receptors for adrenomedullin, which interact with CGRP, have been demonstrated in cultured vascular smooth muscle cells from the rat thoracic aorta (Eguchi et al., 1994). Further, Nuki et al. (1993) reported inhibition of adrenomedullin-induced vasodilation in the mesenteric vascular bed by CGRP-(8–37). A fragment of adrenomedullin lacking the first 12 amino acids, adrenomedullin-(13–53), has similar vasodepressor activity as the intact peptide (Lin et al., 1994). This fragment of adrenomedullin, which shares structural homology with CGRP, is therefore necessary and sufficient to produce a systemic vasodepressor response.

Adrenomedullin along with amylin are members of the structurally related CGRP superfamily. CGRP is widely distributed throughout the nervous system. It is present in sensory nerve fibers that innervate blood vessels and is responsible for adjusting local cerebral blood flow in response to nociceptive signals in the vascular wall (McCulloch et al., 1986). Recent studies have shown that high concentrations of adrenomedullin are detected in many regions of human brain, with the highest concentrations in the thalamus and hypothalamus (Satoh et al., 1995), and that adrenomedullin mRNA is over-expressed in rat cerebral cortex after focal cerebral ischemia (Wang et al., 1995). Active production of adrenomedullin has also been found in cultured endothelial cells from bovine brain capillaries. The production of adrenomedullin in vascular smooth muscle cells is stimulated by cytokines including interleukin-1, tumor necrosis factor, and lipopolysaccharide (Sugo et al., 1995). Adrenomedullin therefore appears to regulate cerebral blood flow by functioning both as a circulating hormone and as a local mediator released from endothelial cells, vascular smooth muscle cells and brain tissue.

References

Baskaya, M.K., Suzuki, Y., Anzai, M., Seki, Y., Saito, K., Takayasu, M., Shibuya, M., Sugita, K., 1995. Effects of adrenomedullin, calcitonin gene-related peptide, and amylin on cerebral circulation in dogs. J. Cereb. Blood Flow Metab. 15, 827–834.

Dacey, R.G. Jr., Duling, B.R., 1982. A study of rat intracerebral arterioles: methods, morphology and reactivity. Am. J. Physiol. 243, H598–H606.

Dacey, R.G. Jr., Duling, B.R., 1984. Effect of norepinephrine on penetrating arterioles of rat cerebral cortex. Am. J. Physiol. 246, H380–H385.

Dennis, T., Fournier, A., Cadieux, A., Pomerleau, F., Jolicoeur, F.B., 1990. hCGRP8-37, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related peptide receptor heterogeneity in brain and periphery. J. Pharmacol. Exp. Ther. 254, 123–128.

Eguchi, S., Hirata, Y., Kano, H., Sato, K., Watanabe, Y., Watanabe, T.X., Nakajima, K., Sakakibara, S., Marumo, F., 1994. Specific receptors for adrenomedullin in cultured rat vascular smooth muscle cells. FEBS Lett. 340, 226–230.

Ichiki, Y., Kitamura, K., Kangawa, K., Kawamoto, M., Matsuo, H., Eto, T., 1994. Adrenomedullin: distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett. 338, 6–10.

Ishiyama, Y., Kitamura, K., Ichiki, Y., Nakamura, S., Kida, O., Kangawa, K., Eto, T., 1993. Hemodynamic effects of a novel hypotensive peptide, human adrenomedullin, in rats. Eur. J. Pharmacol. 241, 271–273.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Lang, M.G., Paterno, R., Faraci, F.M., Heistad, D.D., 1997. Mechanisms of adrenomedullin-induced dilatation of cerebral arterioles. Stroke 28, 181–185.

Lin, B., Gao, Y., Chang, J.-K., Heaton, J., Hyman, A., Lippton, H., 1994. An adrenomedullin fragment retains the systemic vasodepressor activity of rat adrenomedullin. Eur. J. Pharmacol. 260, 1–4.

McCulloch, J.M., Uddman, R., Kingman, T.A., Edvinsson, L., 1986. Calcitonin gene-related peptide: functional role in cerebrovascular regulation. Proc. Natl. Acad. Sci. USA 83, 5731–5735.

Nuki, C., Kawasaki, H., Kitamura, K., Takenaga, M., Kangawa, K., Eto, T., Wada, A., 1993. Vasodilator effect of adrenomedullin and calcitonin gene-related peptide receptors in rat mesenteric vascular beds. Biochem. Biophys. Res. Commun. 196, 245–251.

Ogura, K., Takayasu, M., Dacey, R.G. Jr., 1991. Differential effects of intra- and extraluminal endothelin on cerebral arterioles. Am. J. Physiol. 261, H531–H537.

Rosenfeld, M.G., Mermod, J.-J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., River, J., Vale, W.W., Evans, R.M., 1983. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304, 129–135.

Satoh, F., Takahashi, K., Murakami, O., Totsune, K., Sone, M., Ohneda, M., Abe, K., Miura, Y., Hayashi, Y., Sasano, H., Mouri, T., 1995. Adrenomedullin in human brain, adrenal glands and tumor tissues of pheochromocytoma, ganglioneuroblastoma and neuroblastoma. J. Clin. Endocrinol. Metab. 80, 1750–1752.

Sugo, S., Minamino, N., Kangawa, K., Miyamoto, K., Kitamura, K., Sakata, J., Eto, T., Matsuo, H., 1994a. Endothelial cells actively synthesize and secrete adrenomedullin. Biochem. Biophys. Res. Commun. 201, 1160–1166.

Sugo, S., Minamino, N., Shoji, H., Kangawa, K., Kitamura, K., Sakata, J., Eto, T., Matsuo, H., 1994b. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-α. Biochem. Biophys. Res. Commun. 203, 719–726.

Sugo, S., Minamino, N., Shoji, H., Kangawa, K., Kitamura, K., Eto, T., Matsuo, H., 1995. Interleukin-1, tumor necrosis factor and lipopolysaccharide stimulate production of adrenomedullin in vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 207, 25–32.

Suzuki, Y., Satoh, S., Ikegaki, I., Okada, T., Shibuya, M., Sugita, K., Asano, T., 1989. Effects of neuropeptide Y and calcitonin gene-related peptide on local cerebral blood flow in rat striatum. J. Cereb. Blood Flow Metab. 9, 268–270.

Takayasu, M., Basset, J.E., Dacey, R.G. Jr., 1988. Effects of calcium antagonists on intracerebral penetrating arterioles in rats. J. Neurosurg. 69, 104–109.

Takayasu, M., Kajita, Y., Suzuki, Y., Shibuya, M., Ishikawa, T., Hidaka, H., 1993. Triphasic response of rat intracerebral arterioles to increasing concentration of vasopressin in vitro. J. Cereb. Blood Flow Metab. 13, 304–309.

Wang, X., Yue, T., Barone, F.C., White, R.F., Clark, R.K., Willette, R.N., Sulpizio, A.C., Aiyar, N.V., Ruffolo, R.R. Jr., Feuerstein, G.Z., 1995. Discovery of adrenomedullin in rat ischemic cortex and evidence for its role in exacerbating focal brain ischemic damage. Proc. Natl. Acad. Sci. USA 92, 11480–11484.