Endothelin Acts in Feline and Canine Cerebral Arteries From the Adventitial Side

Tatsuo Mima, MD, Masashi Yanagisawa, MD, PhD,
Taku Shigeno, MD, DrMed, DMSc, Akira Saito, PhD, Katsutoshi Goto, PhD,
Kintomo Takakura, MD, DMSc, and Tomoh Masaki, MD, PhD

We investigated the in vivo vasoconstrictor effect of endothelin, a recently characterized vasoconstrictor peptide from vascular endothelium, in the basilar arteries of five cats and five dogs. Basilar artery caliber was angiographically measured under anesthesia before (control) and after either vertebral artery infusion or cisternal injection of the peptide. In cats, 5–500 pmol endothelin induced a dose-dependent basilar artery contraction in vivo when injected intracisternally; within 3 minutes after injection of 500 pmol endothelin, basilar artery caliber was decreased by 73±4% compared with control diameter before injection. The vasoconstriction was extremely long-lasting; no significant recovery of basilar artery caliber was observed for up to 2 hours after injection. In contrast, infusion of up to 3,000 pmol endothelin into the vertebral artery had no appreciable effect on basilar artery caliber. Similar results were obtained in dogs; vasoconstriction was maintained for as long as 12 hours. Our observations suggest that endothelin acts in cerebral vessels from the adventitial side, not from the luminal side, possibly due to the presence of the blood–arterial wall barrier. The long-lasting nature of endothelin-induced constriction of the cerebral arteries in vivo suggests that the peptide might be involved in the pathogenesis of cerebral vasospasm. (Stroke 1989;20:1553–1556)

The endothelium of cerebral blood vessels is now recognized not only as a physical barrier isolating the brain from the environment but also as an organ having a number of important active functions.1,2 Both cerebral and peripheral endothelium are known to produce different classes of diffusible vasoactive factors in response to a variety of stimuli, thereby regulating vascular smooth muscle tonus.3 Endothelin is a vasoconstrictor peptide that we recently characterized from the supernatant of cultured porcine aortic endothelial cells.4 Consisting of 21 amino acid residues with two intrachain disulfide bonds, endothelin is one of the most potent vasoconstrictors known. Endothelin-induced constriction of cerebral and peripheral arteries from various species in vitro is extremely long-lasting and is characteristically difficult to wash out. In vivo, an intravenous bolus injection of endothelin to anesthetized, conscious rats causes a sustained increase in arterial blood pressure that lasts for >1 hour. Cloning and sequence analysis of endothelin complementary deoxyribonucleic acid from porcine and human endothelial cells has demonstrated that endothelin is produced from an approximately 200-residue prepro-type precursor much like other peptide hormones and that human and mature porcine endothelin are identical.5 The level of preproendothelin messenger ribonucleic acid (mRNA) in endothelial cells is influenced by various chemical and mechanical stimuli, suggesting the importance of endothelin in the local control of vascular tonus.

We are interested in the possible involvement of endothelin in the local regulation of cerebral arteries in vivo. It is of special interest to assume that production of excess endothelin contributes to the development of delayed vasospasm after subarachnoid hemorrhage. We investigated the response of feline and canine basilar artery in vivo to the intraarterial and intracisternal administration of endothelin.

Materials and Methods

Five adult mongrel cats weighing 3–4 kg were anesthetized with halothane in N2O and O2 and were
mechanically ventilated. Blood pressure and blood gases were monitored and maintained within the normal ranges throughout the experiment. A catheter was introduced into the vertebral artery at its branching from the subclavian artery. Vertebral angiography was performed using a bolus injection of 3 ml Iopamiron (Dai-ichi Pharmaceuticals, Tokyo, Japan). Following control angiography of the basilar artery, 3–3,000 pmol endothelin dissolved in 3 ml buffered saline was cumulatively infused into the vertebral artery, with each dose administered over 2 minutes. Angiography was performed 3 minutes after each endothelin administration. At least 30 minutes after the last intra-arterial administration of endothelin, the peptide was injected intracisternally; 0.5–500 pmol endothelin dissolved in 0.5 ml buffered saline was injected via a needle placed in the cisterna magna. To avoid a marked increase in intracranial pressure, the same amount of cerebrospinal fluid was removed before each injection, and angiography was performed 3 minutes after each injection. Following the final intracisternal injection of endothelin, follow-up angiograms were taken for up to 2 hours.

Basilar artery caliber was measured on the angiograms at three points: close to the verebrobasilar junction (D1), at the midpoint between the vertebrobasilar junction and the basilar tip (D2), and close to the basilar tip (D3). Changes in basilar artery caliber, that is, reductions in the inner diameter of the vessel, were expressed as a percentage of control diameter as \[ \frac{(D'_1 + D'_2 + D'_3) - (D_1 + D_2 + D_3)}{(D_1 + D_2 + D_3)} \times 100\% \], where \((D_1 + D_2 + D_3)\) is the sum of the calibers at the three points on the control angiogram and \((D'_1 + D'_2 + D'_3)\) is the sum of the calibers at the three points on angiograms taken after endothelin administration.

Similar experiments were performed in five adult mongrel dogs weighing approximately 10 kg to investigate the effect of endothelin for up to 12 hours. The procedure in dogs was exactly the same as that in cats, except the amounts of endothelin were doubled; for intra-arterial infusion, 6–6,000 pmol in 6 ml buffered saline was given over 2 minutes and for intracisternal injection, 1–1,000 pmol in 1 ml buffered saline was given.

**Results**

In cats, intra-arterial infusion of up to 3,000 pmol endothelin did not cause any appreciable change in the basilar artery caliber (Figure 1). When 300–3,000 pmol peptide was administered intrarterially, arterial blood pressure rose following an initial transient depressor response (Figure 2); the pressor response lasted approximately 30 minutes. In contrast, intracisternal injection of endothelin caused a dose-dependent constriction of the basilar

**FIGURE 1.** Graph of mean±SD sequential changes in cat (n=5) basilar artery caliber on angiograms. Intra-arterial infusion of endothelin did not cause any contractile responses. Subsequent injection of endothelin into cisterna magna induced dose-dependent contraction, initiating at 10⁻⁸ M. Maximal contraction elicited by 10⁻⁶ M endothelin lasted during 2 hours of observation.

**FIGURE 2.** Responses of arterial blood pressure to intra-arterial injection of endothelin in cat (upper tracing) and dog (lower tracing). Note slight depressor responses in dog, whereas transient pressor responses were observed in cat.
artery beginning at $10^{-8}$ M, which corresponds to doses as small as 5 pmol in 0.5 ml buffer. Injection of 0.5 ml buffer alone caused no significant change in basilar artery caliber. The maximum constriction was obtained at an endothelin dose of 500 pmol. The basilar artery constriction induced by the intracisternal injection of 500 pmol endothelin was extremely sustained; it lasted with the same magnitude for the entire follow-up period of 2 hours.

A similar in vivo constriction of the basilar artery was seen in dogs when 1–1,000 pmol endothelin was injected intracisternally (Figure 3). In dogs, the vasoconstriction induced by 1,000 pmol endothelin was stable over 12 hours. Intra-arterial infusion of 6,000 pmol endothelin did not induce any appreciable constriction of the basilar artery but instead induced a slight dilatation. Interestingly, the pressor response to intra-arterially administered endothelin, which was dominant in cats, was less evident in dogs. As shown in Figure 2, the response to 500 pmol endothelin was depressor in dogs. Even in doses of up to 5,000 pmol endothelin, the pressor response in dogs was far weaker than that observed in cats.

Discussion

Our findings suggest that, in cerebral arteries in vivo, endothelin acts from the adventitial side but not from the luminal side. This contrasts to previous findings that the perfusion of endothelin into the coronary artery in a Langendorf's preparation of rat heart caused a strong and sustained vasoconstriction. The existence in cerebral arteries of the blood–arterial wall barrier could explain the difference because the endothelial tight junction does not allow substances to penetrate freely across the endothelium. Actually, an autoradiographic study with iodine-125-labeled endothelin has demonstrated that endothelin administered intravenously to rats did not distribute inside the central nervous system except in those areas lacking a blood–brain barrier, such as the median eminence and the subfornical organ. Vasoactive substances present within the subarachnoid space, however, easily accessed the vascular smooth muscle layer via the adventitia of the cerebral vessels, which is not very well developed. If endothelin is actually produced in the endothelium of cerebral arteries, endogenous endothelin secreted from the abluminal surface of the endothelial cells would have access to its receptor on the smooth muscle cells. Our observations, in conjunction with these considerations, suggest that the role of endothelin in the regulation of cerebral arteries is more a local/autacoidal factor than a systemic, circulating factor.

We did not anticipate finding that canine basilar artery slightly but consistently dilated to intraluminal application of endothelin. Therefore, there might be different responses to circulating endothelin in different species, though the peptide itself seems to be widespread in mammals. It has also been reported that regional vasodilatation occurs in response to intravenous endothelin in anesthetized rats. Further, endothelin has been shown to stimulate vasodilatory substances such as prostacyclin and/or endothelium-derived relaxing factor. Although the mechanism of vasodilator responses to intra-arterially administered endothelin in dogs remains to be established, the endothelin-induced release of vasodilators is likely to be involved.

More than a hundred substances have been raised as candidates causing cerebral vasospasm after subarachnoid hemorrhage. Endothelin is a tempting candidate. Cisternally administered in a bolus, endothelin causes a strong and extremely long-lasting cerebral vasoconstriction in vivo. The contraction lasted during the entire follow-up period (12 hours in our dog experiment) without any sign of recovery. This is a salient characteristic of endothelin-induced vasoconstriction, and no other vasoconstrictor substance known to date has a comparable duration of action. Moreover, endothelin provokes contraction of cerebral arteries both in vivo and in vitro at concentrations much lower than other vasoactive agents such as catecholamines. It is tempting to hypothesize that the delayed onset of cerebral vasospasm after subarachnoid hemorrhage is due to the time required for the chain of events that ultimately result in the induction of de novo endothelin synthesis by endothelial cells. Actually, thrombin and transforming growth factor $\beta$, the presence
of both of which might be expected in subarachnoid blood clots and in regions of tissue damage, can induce preproendothelin mRNA in endothelial cells.\textsuperscript{4,13} Furthermore, endothelin could also be involved in the pathogenesis of medial hypertrophy and fibrosis during the chronic stage of cerebral vasospasm since endothelin has recently been shown to possess a potent growth-promoting activity for cultured fibroblasts and vascular smooth muscle cells.\textsuperscript{14,15}

Acknowledgment

We are grateful to Mrs. Reiko Matsu-ura for the animal care.

References


\textbf{Key Words} • blood–brain barrier • cerebral vasospasm • peptides • cats • dogs
Endothelin acts in feline and canine cerebral arteries from the adventitial side.
T Mima, M Yanagisawa, T Shigeno, A Saito, K Goto, K Takakura and T Masaki

Stroke. 1989;20:1553-1556
doi: 10.1161/01.STR.20.11.1553

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/11/1553

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/