

Vasoconstrictor Action of Endothelin-1
on Feline and Canine Basilar Arteries

エンドセリン-1のネコおよびイヌ
脳底動脈に対する収縮作用

美馬 達夫

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on Feline and Canine Basilar Arteries

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ABSTRACT

We investigated the vasoconstrictor effect of endothelin-1 (ET-1), a recently found vasoconstrictor peptide extracted from the vascular endothelium, on the basilar artery in five cats and five dogs. The caliber of the basilar artery was measured angiographically under anesthesia and compared before and after injection of ET-1 into the vertebral artery or cisterna magna. In cats, 5-500 pmol of ET-1 induced dose-dependent contraction of the basilar artery when injected intracisternally. Within 3 minutes after injection of 500 pmol of ET-1, the caliber of the basilar artery decreased by $73 \pm 4\%$ compared with the pre-injection value. Vasoconstriction was extremely long-lasting: no significant recovery of the caliber of the basilar artery was observed for up to 2 hours after injection. In contrast, infusion of up to 3,000 pmol of ET-1 into the vertebral artery had no significant effect on the caliber of the basilar artery. Similar results were obtained in dogs, although intra-arterial injection of 6,000 pmol of endothelin-1 caused slight dilatation. This might be due to the release of prostacyclin and/or endothelium-derived relaxing factors. Our observations suggest that, in the cerebral artery, ET-1 reaches the

vascular smooth muscle from the adventitial side and causes constriction but is inaccessible to it from the luminal side because of the blood-arterial wall barrier. Long-lasting constriction of the cerebral artery induced by ET-1 raises a possibility that ET-1 is involved in the pathogenesis of cerebral vasospasm.

INTRODUCTION

It is certainly now recognized that the endothelium of cerebral blood vessels serves not only as a barrier that separates the brain from the vascular compartment but also as an organ having a number of important functions.^{1,2} The endothelium of cerebral and non-cerebral blood vessels produces different kinds of diffusible vasoactive substances in response to a variety of stimuli and regulates tone of the vascular smooth muscle.³ Endothelin-1 (ET-1) is a vasoconstrictor peptide recently isolated from the supernatant of cultured porcine aortic endothelial cells.⁴ Consisting of 21 amino acid residues with two intrachain disulfide bonds, ET-1 is one of the most potent and long-lasting vasoconstrictors known so far. Endothelin-2 (ET-2) and endothelin-3 (ET-3) were later found as iso-peptides of ET-1. They are also composed of 21 amino

acid residues.⁵ In in vitro experiments, ET-1-induced constriction of cerebral and non-cerebral arteries observed in various animal species is extremely long-lasting and is hard to eliminate after removal of ET-1 from the perfusion medium.^{6,7}

An interesting question is the possible involvement of ET-1 in the local regulation of cerebral arteries in natural in vivo situations, and more specifically whether or not an excessive production of ET-1 contributes to the development of delayed vasospasm after subarachnoid hemorrhage. We thus investigated the response of feline and canine basilar arteries to intra-arterial and intracisternal administration of ET-1 in intact whole animal preparations.

MATERIALS AND METHODS

Five adult cats weighing 3-4 kg were anesthetized with halothane in N₂O and O₂ and were mechanically ventilated. Blood pressure and blood gases (PaO₂, PaCO₂, and pH) were monitored and maintained within normal ranges throughout the experiment. A catheter was introduced into the vertebral artery at the point where it branched from the subclavian artery. Vertebral angiography was performed

using a bolus injection of 3 ml of Iopamiron (Dai-ichi Pharmaceuticals, Tokyo, Japan). Following control angiography, 3-3,000 pmol of ET-1 dissolved in 3 ml of buffered saline (pH = 7.4), which corresponded to concentrations of 10^{-9} - 10^{-6} M, was cumulatively infused into the vertebral artery taking 2 minutes for each injection. Angiography was performed 3 minutes after each administration of ET-1. At least 30 minutes after the last intra-arterial administration of ET-1, this peptide was injected intracisternally. For an intracisternal administration, 0.5-500 pmol of ET-1 dissolved in 0.5 ml of buffered saline (10^{-9} - 10^{-6} M) was injected through a needle placed in the cisterna magna. To avoid an unphysiological increase in intracranial pressure, the same amount of cerebrospinal fluid was removed before each injection. Angiography was performed 3 minutes after each injection. Following the final intracisternal injection of ET-1, angiograms were taken every 1 hour up to 2 hours.

The caliber of the basilar artery was measured at the following three points on the angiogram; i) at or close to the vertebrobasilar junction (D_1), ii) at the midpoint between the vertebrobasilar junction and the basilar tip (D_2), and iii) at or close to the basilar tip (D_3). Changes

in the caliber of the basilar artery (reduction in the inner diameter of vessels), were expressed as percent of the control diameter, i.e., $[(D'_1 + D'_2 + D'_3) - (D_1 + D_2 + D_3)] \div (D_1 + D_2 + D_3) \times 100\%$, where $(D_1 + D_2 + D_3)$ and $(D'_1 + D'_2 + D'_3)$ denote the sum of calibers at the three points on the control angiogram and on angiograms taken after ET-1 administration, respectively.

Similar experiments were performed in five adult mongrel dogs weighing 8 - 15 kg. Experimental procedures in dogs were essentially the same as in cats, except the amount of administered ET-1 was doubled. For intra-arterial infusion, 6-6,000 pmol in 6 ml of buffered saline (10^{-9} - 10^{-6} M) was given for over 2 minutes. For intracisternal injection, 1-1,000 pmol in 1 ml of buffered saline (10^{-9} - 10^{-6} M) was given.

RESULTS

In cats, intra-arterial infusion of up to 3,000 pmol of ET-1 did not cause any appreciable change in the caliber of the basilar artery (Figure 1). When 300-3,000 pmol (10^{-7} - 10^{-6} M) of ET-1 was administered intra-arterially, arterial blood pressure rose following an initial transient depressor response (Figure 2); the pressor response lasted

for approximately 30 minutes. In contrast, intracisternal injection of ET-1 caused dose-dependent constriction of the basilar artery at concentrations higher than 5 pmol (10^{-8} M). Maximum constriction was obtained at the dose of 500 pmol (10^{-6} M) of ET-1. Constriction of the basilar artery induced by intracisternal injection of 500 pmol (10^{-6} M) of ET-1 was sustained for the 2-hour follow-up period without a significant reduction of the magnitude of constriction. Intracisternal injection of 0.5 ml of buffer solution alone caused no significant change in the caliber of the basilar artery.

Similar constriction of the basilar artery was seen in dogs when 1-1,000 pmol (10^{-9} - 10^{-6} M) of ET-1 was injected intracisternally (Figure 3). In dogs, the vasoconstriction induced by 1,000 pmol (10^{-6} M) of ET-1 was stable over 2 hours and in one dog, for the entire observation period of 12 hours. Intra-arterial infusion of 6,000 pmol of ET-1 (10^{-6} M) did not induce any appreciable constriction of the basilar artery but instead induced slight dilatation. The caliber of the artery returned to its initial level in 3 hours (Figure 3). Interestingly, the temporal pattern of blood pressure response to intra-arterially administered ET-1 was different in cats and dogs. As shown in Figure 2,

the initial transient depressor response was more dominant, but the subsequent pressor response was far weaker in dogs than in cats. At a dose of 500 pmol (10^{-7} M) of ET-1 in dogs, no or very weak pressor response was observed (Figure 2).

DISCUSSION

Our findings disclosed that intracisternally administered ET-1 produced very potent and long-lasting vasoconstriction in feline and canine basilar arteries, although in intact whole-animal preparations. We did not determine its exact threshold concentration of ET-1 for vasoconstriction. Intracisternal injection under the present experimental scheme might have induced cumulative effects and vascular reactions secondary to neurogenic reflexes, and modified the primary effect. However, the actual concentration in the present in vivo experiments is theoretically between the concentrations initially given and that diluted by cerebrospinal fluid. The volume of cerebrospinal fluid in cats is estimated to be 8-13 ml.⁶ Therefore, moderate contraction of feline basilar arteries can be induced by ET-1 at concentrations of 10^{-9} - 10^{-8} M. This value is comparable to the EC_{50} for feline middle

cerebral arteries ($2.6 \times 10^{-10} \text{M}$).⁷ These results suggest that even a very low dose of ET-1 plays an important role in regulating the vascular tone.

In contrast to the findings on the coronary artery, vasoconstriction of the basilar artery occurred when ET-1 was injected intracisternally, but not intra-arterially. Kurihara et al.⁹ reported that 30 pmol/kg of ET-1 administered into canine coronary arteries decreased coronary blood flow by over 90%. Their coronary angiograms demonstrated a strong and sustained vasoconstriction.^{9,10} With respect to the cerebral artery, the effect of ET-1 by intra-arterial infusion seems to be less effective than intracisternal injection because of dilution as a result of high blood flow. However, in our experiments, vertebral arteries did not contract even at the dose of 600 pmol/kg of ET-1. We can say that the response of basilar arteries to ET-1 is different from that of coronary arteries.

The difference in the vascular response between the two vascular beds is likely to be attributed to the blood-arterial wall in the cerebral artery. The endothelial tight junction does not allow ET-1 to freely cross the vascular endothelium.^{11,12} On the other hand, vasoactive substances in the subarachnoid space can easily reach the vascular

smooth muscle via the adventitia of the cerebral vessels, whose barrier function is not well developed.¹¹ If ET-1 is actually produced in the endothelium of cerebral arteries, endogenous ET-1 secreted from the anteluminal surface of the endothelial cells could reach its receptors on smooth muscle cells.

Interestingly, intraluminal application of 6,000 pmol of ET-1 (10^{-6} M) slightly but consistently dilatated basilar arteries in dogs but not in cats. Recently it has been reported that ET-1 stimulates and releases vasodilator substances, such as endothelium-derived relaxing factors (EDRFs) and/or prostacyclin.^{13,14,15} The initial depressor response has been thought to be due mainly to EDRFs because indomethacin did not inhibit depressor response.¹² The release of prostacyclin is also a possibility. Intravenous administration of ET-1 in dogs resulted in a dose-dependent transient hypotension accompanied by an elevation of plasma 6-keto prostaglandin $F_{1\alpha}$ levels.¹⁵

Our results suggest that ET-1-induced release of vasodilators differs among animal species. Vasodilation is marked in dogs but not in cats. In dogs, intracisternal administration of 10-100 pmol of ET-1 (10^{-8} - 10^{-7} M) might induce endothelial cells to release EDRFs and/or

prostacyclin, and partially offset the vasoconstriction. At high doses of ET-1 (10^{-6} M), its vasoconstrictor action might surpass the effect of vasodilator substances and result in constriction of basilar arteries.

Ide et al.¹⁶ have reported that low doses of ET-1 resulted in a biphasic change in the caliber of canine basilar arteries. In our experiments, cumulative administration of ET-1 may interfere with vasoconstriction at the time of delayed dilatation of the previously injected ET-1. The difference in the response of ET-1-induced release of vasodilators might explain the fact that the initial hypotension is dominant but the subsequent hypertension is less prominent in dogs.

Undoubtedly, ET-1 is a potent vasoconstrictor. But in some animal species it may cause a release of vasodilators, such as EDRFs and/or prostacyclin, which may also participate in the local regulation of the vascular tone. In pathological conditions such as subarachnoid hemorrhage, the imbalance between the primary contractile and secondary dilatory responses, both induced by ET-1, may underlie pathogenesis of the cerebral vasospasm.

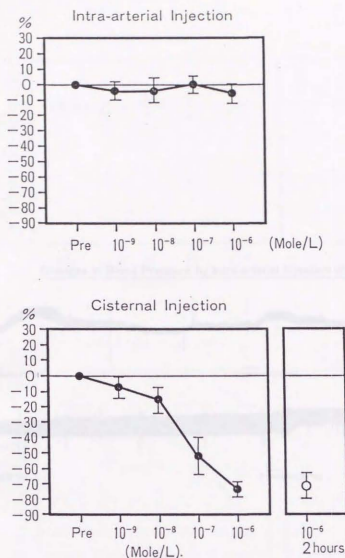


FIGURE 1. Changes in the caliber of the basilar artery in five cats (mean \pm SD). Intra-arterial infusion of ET-1 did not cause contractions. Subsequent injection of ET-1 into the cisterna magna induced dose-dependent contraction from at 10^{-8} M. Maximal contraction elicited by 10^{-6} M of ET-1 lasted by 2 hours of observation.

Changes in Blood Pressure by Intra-arterial Injection of ENDOTHELIN

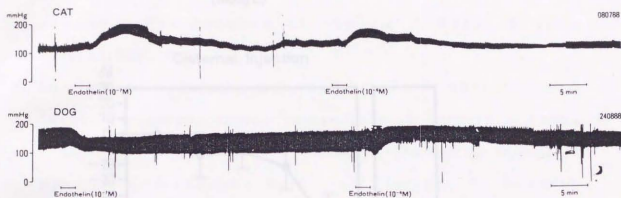


FIGURE 2. Responses of arterial blood pressure to intra-arterial injection of endothelin in cats (upper tracing) and dogs (lower tracing). Initial depressor responses were evident in dogs, whereas following pressor responses were dominant in cats.

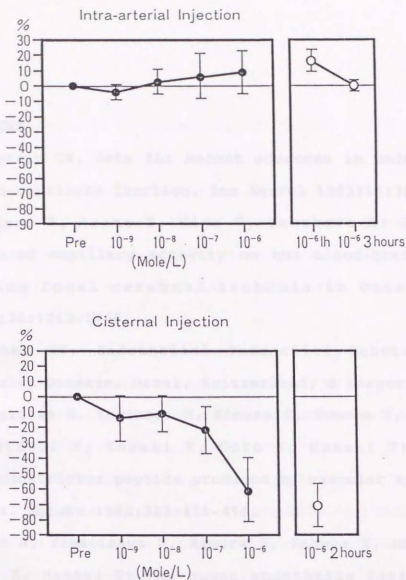


FIGURE 3. Changes in the caliber of the basilar artery in five dogs (mean \pm SD). Note the vasodilatation 1 hour after intra-arterial infusion of 10^{-6} M of ET-1. The caliber of the basilar artery subsequently returned to its initial level in 3 hours. Intracisternal injection of ET-1 caused dose-dependent contraction, which lasted throughout the observation period, for up to 2 hours.

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