Effect of Intracisternal Injection of Endothelin-1 on Regional Cerebral Blood Flow in Cats

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Abstract

The effect of endothelin-1 on regional cerebral blood flow (rCBF) was investigated in adult mongrel cats. Endothelin-1 was injected into the cisterna magna and rCBF was measured by the hydrogen clearance method before and every 30 minutes for 180 minutes after the injection. Endothelin-1 (10^{-11} mol and 10^{-9} mol) induced a significant decrease in rCBF and an increase in arterial pressure. The effects of endothelin-1 on rCBF and arterial pressure were mediated, at least in part, by Ca^{2+} , because pretreatment with intracisternally injected nicardipine prevented the changes. Cerebral angiograms obtained before and after endothelin-1 injection demonstrated severe constriction of the basilar artery, but constriction of the internal carotid and middle cerebral arteries was mild. Since the rCBF measured was in the territory of the middle cerebral artery, the decrease in blood flow was probably not solely due to the artery constriction. Other mechanisms such as arteriole constriction and/or regulation of rCBF by the central nervous system may also occur.

Key words: endothelin, regional cerebral blood flow, nicardipine

Introduction

In 1982, De Mey and Vanhoutte³⁾ showed that arteriovenous constriction *in vitro* caused by norepinephrine, thrombin, and arachidonic acid is dependent on or enhanced by the endothelium. Anoxic potentiation of norepinephrine-induced vascular contraction is reduced in the absence of endothelium.^{3,4)} Rubanyi and Vanhoutte¹⁶⁾ reported that diffusible factors mediate the constriction mechanism. In 1987, O'Brien *et al.*¹³⁾ discovered the release of a vasoconstrictive peptide from bovine aorta and pulmonary artery endothelial cells *in vitro*, and named it endothelium-derived constricting factor. In 1988, Yanagisawa *et al.*²¹⁾ isolated a novel vasoconstrictor peptide, endothelin, from the supernatant of porcine aorta endothelium culture, with extremely potent vasoconstrictivity. The human endothelin family consists of three distinct isopeptides.⁸⁾ Endothelin-1 is produced by vascular endothelial cells, and endothelin-3 is possibly a neural

form of endothelin.⁸⁾

To clarify the role of endothelin-1 in vasospasm following subarachnoid hemorrhage (SAH), we studied the effect of intracisternal injection of endothelin-1 on regional cerebral blood flow (rCBF) in cats.

Materials and Methods

I. rCBF after intracisternal injection of endothelin-1

Adult mongrel cats of either sex, weighing 2.2–3.8 kg, were anesthetized with halothane and intubated. The right femoral artery was catheterized for continuous recording of systemic arterial pressure and the vein for intravenous injection.

The head was fixed in a stereotactic apparatus and several small burr holes drilled in the left half of the calvarium. The dura was incised and platinum wire electrodes with bared tips 0.5–1.0 mm long were inserted into the cerebral cortex over the para-

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Sylvian area using an operating microscope. The electrodes were fixed and the dura closed by cotton balls and alpha-cyanoacrylate (Aron Alpha A®, Sankyo Co., Ltd., Tokyo). A reference Ag/AgCl electrode was implanted subcutaneously on the right side. After electrode placement, halothane was discontinued. The animals were immobilized by intravenous gallamine administration and connected to a ventilator. Anesthesia was maintained with 25% O₂/75% N₂O, and the respiration adjusted to maintain arterial carbon dioxide partial pressure between 30 and 40 mmHg. Arterial blood gases were intermittently analyzed and the end-tidal CO₂ and O₂ concentrations continuously monitored. About 30 minutes were necessary for the electrode system to become stabilized in the tissue. Hydrogen gas was introduced directly into the endotracheal tube for about 2 minutes (17% O₂, 50% N₂, 33% H₂) and the clearance monitored with an amplifier system and a 6-channel pen recorder. rCBF was calculated from the clearance curves obtained.^{6,15,20)}

After the control rCBF level was measured twice, a 21-gauge butterfly needle was inserted into the cisterna magna with the neck flexed and 1 ml of cerebrospinal fluid (CSF) withdrawn. Endothelin-1 (Peptide Institute, Inc., Minoo, Osaka) dissolved in 1 ml of 0.1 M phosphate-buffered saline (PBS) was then injected *via* the same route and the animal maintained in the head-down position for 10 minutes. The cats were divided into following three groups: Group A, 1 ml of PBS without endothelin-1 was injected (*n* = 5); Group B, the PBS contained 10⁻¹¹ mol of endothelin-1 (*n* = 7); and Group C, 10⁻⁹ mol (*n* = 6). The rCBF was measured every 30 minutes for 180 minutes using 23, 30, and 30 electrodes, respectively. Electrodes causing significant tissue injury or inserted into the white matter as judged by baseline clearance curves were excluded.

II. Pretreatment with nicardipine

To investigate the effect of Ca²⁺ channel blocker on endothelin-1 induced rCBF changes, 0.5 mg of nicardipine (0.5 ml) (Yamanouchi Pharmaceutical Co., Ltd., Tokyo) was administered through a 21-gauge butterfly needle inserted into the cisterna magna after measurement of the control rCBF level. In four cats, the rCBF was measured 30 minutes after nicardipine injection, then PBS without endothelin-1 was administered, and rCBF measurements were repeated every 30 minutes for 180 minutes using 22 electrodes (Group N). In another four cats, 10⁻⁹ mol of endothelin-1 was administered 30 minutes after nicardipine injection, and the rCBF measured using 18 electrodes (Group NE).

III. Cerebral angiography

Adult mongrel cats were anesthetized and intubated, and the right femoral artery and vein were catheterized as described above. The left common carotid artery was catheterized for angiography. The head was fixed in a stereotactic apparatus and the animals were immobilized and ventilated. Angiograms were obtained before and after endothelin-1 injection at predetermined time intervals (before, 30, 60, 120, and 180 minutes after injection). Endothelin-1 doses given were the same as above (0; control, 10⁻¹¹ mol, and 10⁻⁹ mol).

IV. Statistical analysis

Data were presented as mean ± SEM. rCBF changes in the groups were compared using analysis of variance. Differences in rCBF at the same time between Groups A, B, and C were evaluated by analysis of variance and Scheffe's test. Differences in rCBF between Groups N and NE were evaluated by the unpaired Student's *t*-test. rCBF differences before and after endothelin-1 injection were evaluated using the paired Student's *t*-test. Differences at the *p* < 0.05 level were considered statistically significant.

Results

I. rCBF after intracisternal injection of endothelin-1 (Fig. 1)

In Group A, rCBF did not change after PBS injection. However, intracisternal injection of endothelin-1 resulted in a significant decrease in rCBF from 30 to 180 minutes after injection in Groups B and C (*p* < 0.01). In Group B, rCBF 180 minutes after endothelin-1 injection was 82.6 ± 3.6 ml/100 gm/min, about 78% of the baseline value, and significantly lower than that in Group A (*p* < 0.05). In Group C, in which the dose of endothelin-1 was higher, the rCBF at 180 minutes was about 53% of the baseline value. All rCBF values from 30 to 180 minutes in Group C were significantly lower than those in Group A (*p* < 0.001).

II. Pretreatment with nicardipine (Fig. 2)

rCBF increased 30 minutes after intracisternal injection of nicardipine (*p* < 0.01), and thereafter decreased gradually in both groups. Although the secondary flow reduction tended to be larger in Group NE, no significant differences were found.

III. Systemic arterial pressure

In Group A, the systemic arterial pressure remained unchanged after intracisternal PBS injection. The

Fig. 1 rCBF before and after intracisternal injection of endothelin-1. ○: Group A (control), △: Group B (10^{-11} mol), ●: Group C (10^{-9} mol). * $p < 0.05$, ** $p < 0.001$. Reproduced with permission from ref. 11.

Fig. 2 rCBF before and after intracisternal injection of nicardipine (N) and endothelin-1 (E). ○: Group N (nicardipine 0.5 mg), ●: Group NE (nicardipine 0.5 mg + endothelin-1 10^{-9} mol). No statistically significant difference is found between the two groups. Reproduced with permission from ref. 11.

arterial pressure increased after endothelin-1 injection in four animals in Group B and five in Group C. It decreased in only one animal in Group B. Intracisternal endothelin-1 injection produced no changes in arterial pressure in two animals in Group B and one in Group C (Figs. 3 and 4).

Intracisternal nicardipine injection caused a slight decrease in arterial pressure. Pretreatment with nicardipine inhibited the increase in arterial pressure produced by the intracisternal endothelin-1 injection in all animals (Fig. 5).

Fig. 3 Changes in mean arterial blood pressure (MABP) after intracisternal injection of endothelin-1 or the vehicle. * $p < 0.05$ vs. Group A.

Fig. 4 Typical recording of arterial pressure (AP) before and after intracisternal injection of endothelin-1 (10^{-9} mol).

Fig. 5 Typical recording of arterial pressure (AP) before and after intracisternal injection of nicardipine (0.5 mg) (N) and endothelin-1 (10^{-9} mol) (E).

IV. Cerebral angiography

Intracisternal endothelin-1 injection caused severe basilar artery (BA) constriction. In Group B, the diameters of the BA 60 and 120 minutes after the injection were 71% and 71% of the baseline value, and in Group C, 52% and 62%, respectively. However, internal carotid (ICA) and middle cerebral arteries (MCA) constriction was much less severe (89% and 92% of the baseline diameter at 60 and 120 minutes after the injection in Group B, and 84% and 80% in Group C, respectively). The BA constriction lasted for more than 3 hours (Figs. 6 and 7).

Discussion

The present study demonstrated that intracisternal injection of endothelin-1 significantly decreased rCBF and increased arterial pressure in cats. These changes were mediated, at least in part, by Ca^{2+} , because they were prevented by the nicardipine pretreatment.

Endothelin-1 has a potent vasoconstrictor effect *in vitro* on porcine coronary artery, rat aorta, cat BA, rabbit mesenteric artery, dog mesenteric, femoral and renal arteries, human mesenteric and pulmonary artery branches,²¹⁾ and dog, rabbit and monkey cerebral arteries.¹²⁾ The endothelin-induced contraction in monkey MCA was very long-lasting. About 70% of maximum contractile response to endothelin at 3×10^{-9} M was still present 12 hours after endothelin administration.¹²⁾ *In vivo* experiments have also shown the vasocontractile activity of endothelin.^{1,7)} Ide *et al.*⁷⁾ reported that intracisternal endothelin injection at $0.6\text{--}1.2 \times 10^{-12}$ mol/kg caused a biphasic BA constriction lasting for more than 24 hours in anesthetized dogs. Brain *et al.*²⁾ demonstrated the potent constrictor action of endothelin in the microvasculature by showing decreased regional blood flow after intradermal endothelin injection in rabbits.

In the present study, intracisternal injection of endothelin-1 induced a strong and long-lasting BA

Fig. 6 Angiograms before (left), 30 minutes (center), and 60 minutes (right) after intracisternal injection of endothelin-1 (10^{-9} mol). Note severe constriction of the basilar artery, and mild to moderate constriction of the internal carotid and middle cerebral arteries. Reproduced with permission from ref. 11.

Fig. 7 Changes in the diameter of the basilar artery (left) and of the internal carotid and middle cerebral arteries (right) after intracisternal injection of endothelin-1. ○: Group A, △: Group B, ●: Group C.

constriction. However, arterial contractions of the anterior circulation were slight to moderate compared with the BA. Since the rCBF measured in the present study was in the MCA territory, the flow reduction was probably not due to the ICA and MCA narrowing visualized by angiography alone. Other mechanisms such as arteriole constriction and rCBF regulation by the central nervous system must be considered. Autoradiography has demonstrated specific binding sites for endothelin within the circumventricular structures outside the blood-brain barrier and choroid plexus.¹⁰⁾ ^{125}I -labeled endothelin was mainly absorbed in the hypothalamic and thalamic areas, lateral ventricular region, subfornical organ, globus pallidus, and caudate putamen *in vitro*.¹⁰⁾ Other workers reported that the highest densities of ^{125}I -labeled endothelin binding sites were in

the granular layer of the cerebellum, choroid plexus, hippocampus, and habenular nucleus.⁹⁾ Ferguson and Smith⁵⁾ reported that microinjection of endothelin-1 into the area postrema resulted in dose-dependent biphasic changes in arterial blood pressure. They considered that endothelin-1 had a specific cardiovascular action subsequent to binding within the area postrema not directly associated with the vasoconstrictor action. It is therefore possible that endothelin-1 injected into the cisterna magna was bound to the endothelin receptor in the brainstem and elicited the decrease of rCBF in our study.

Complete inhibition of the endothelin-induced contraction was observed in a Ca^{2+} -free medium *in vitro* and marked attenuation in the presence of the Ca^{2+} -channel blocker, nicardipine.²¹⁾ Nicardipine (10^{-8} M) reverses the contraction of the canine BA induced by 10^{-8} M endothelin, although the return to baseline requires more than 1 hour.⁷⁾ Therefore, the influx of extracellular Ca^{2+} is required for the action of endothelin. Asano *et al.*¹⁾ reported that intracisternal injection of 10^{-11} mol of endothelin markedly reduced BA diameter on the first and third days in dogs, which was reversed by intravenous administration of HA1077, an intracellular Ca^{2+} antagonist. In our study, pretreatment with nicardipine inhibited the rCBF decrease caused by intracisternal injection of endothelin-1. These findings support the hypothesis that the action of endothelin-1 requires the influx of extracellular Ca^{2+} .

Ouchi *et al.*¹⁴⁾ showed intraventricular administration of endothelin induced a dose-dependent increase in mean arterial blood pressure in conscious rats which was significantly suppressed by pretreatment with intravenous phenoxybenzamine. Moreover, intraventricular endothelin considerably increased the plasma epinephrine and norepinephrine concentrations. They concluded that endothelin had a central pressor action possibly mediated, at least partly, by catecholamine release to the peripheral vessels. In the present study, intracisternal injection of endothelin-1 usually induced an increase in arterial blood pressure which was inhibited by nicardipine pretreatment. Therefore, the pressor action of endothelin-1 is also thought to be mediated by Ca^{2+} . The relationship between intrathecal endothelin-1 administration, Ca^{2+} flow and catecholamine release in the systemic circulation remains unclear and requires future study.

The concentration of endothelin-1 in human plasma is 1.59 ± 0.32 pg/ml¹⁹⁾ or 5 pg/ml.¹⁷⁾ The endothelin-1 doses administered in the present study were considerably higher. However, the endothelin

sensitivity of cerebral arteries increased after experimental SAH resulting in blood-arterial wall barrier disruption to horseradish peroxidase.^{12,18)} Therefore, the lower levels of endothelin-1 in the CSF may have a significant effect on rCBF after SAH in humans. Endothelin-1 may then contribute to the genesis of cerebral vasospasm.

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