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PHOSPHORAMIDON INHIBITS THE CONVERSION OF INTRACISTERNALLY ADMINISTERED BIG ENDOTHELIN-1 TO ENDOTHELIN-1

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SUMMARY: It is suggested that endothelin-1 (ET-1), a potent vasoconstrictor peptide, is involved in the pathogenesis of cerebral vasospasm following subarachnoid hemorrhage (SAH). We examined the effects of intracisternal administration of big ET-1 on the cerebral arteries in the absence or presence of pretreatment with phosphoramidon, an inhibitor of ET converting enzyme, in anesthetized dogs. After intracisternal administration of big ET-1 (10 μg/dog), the caliber of the basilar artery on the angiogram was decreased to about 59% of the control. This was accompanied by a marked increase in immunoreactive ET in the cerebrospinal fluid. Systemic arterial pressure was markedly elevated following big ET-1 injection. All changes induced by big ET-1 were effectively prevented with phosphoramidon. These data suggest that intracisternally administered big ET-1 is converted to ET-1 and that the generated ET-1 produces cerebral vasospasm and hypertension. A phosphoramidon-sensitive metalloproteinase appears to contribute to this conversion.

Endothelin-1 (ET-1) is a 21-amino acid vasoconstrictor peptide first isolated from the culture medium of porcine aortic endothelial cells (ECs) (1). Yanagisawa et al. (1) proposed that ET-1 is produced by a putative 'ET-converting enzyme' through unusual proteolytic processing between Trp^{21}-Val^{22} of precursor form consisting of 39 amino acid residues, termed big ET-1. Since the vasoconstrictor activity of big ET-1 is much lower than that of ET-1 (2), the conversion of big ET-1 to ET-1 appears to be essential for the pathophysiological significance of ET-1. We have proposed that this conversion of big ET-1 to ET-1 in the vascular ECs is specifically inhibited by phosphoramidon, a metalloproteinase inhibitor (3,4). Furthermore, intravenous administration of this inhibitor to anesthetized rats markedly suppressed the

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big ET-1-induced hypertensive effect without affecting the hypertension induced by ET-1 (5).

Cerebral vasospasm following subarachnoid hemorrhage (SAH) is the major problem dominating the outcome of patients with SAH (6,7). Despite numerous studies on the cause of cerebral vasospasm, its pathogenesis has not been conclusively demonstrated. According to current theories (6,8), vasoconstriction is caused by multiple factors, that is, vasoactive constrictor substances liberated from the subarachnoid clot and/or morphological changes of the arterial wall, such as intimal thickness, smooth muscle necrosis and endothelial cell damage. An intracisternal administration of ET-1 produced a slow onset and prolonged narrowing of the basilar artery (9-13), thereby suggesting an involvement of ET-1 as a causal factor of the vasospasm. If so, then an ET-converting enzyme must exist somewhere in the subarachnoid space and intracisternally administered big ET-1 may act as a constrictor of cerebral arteries. In the present study, we examined the effects of an intracisternal big ET-1 on the cerebral arteries, and the influence of pretreatment with phosphoramidon, an ET converting enzyme inhibitor, on the responses induced by big ET-1.

MATERIALS AND METHODS

In Vivo Study: The experiments were performed on mongrel dogs of either sex weighing 8-12 kg. Under pentobarbital anesthesia (30 mg/kg i.v.) endotracheal intubation was performed and respiration was controled mechanically using a Harvard respirator. Animal heads were fixed with a sterotaxic device and a 21 gauge needle connected to polyethylene tubing was inserted into the cisterna magna. After withdrawal of 3 ml of cerebrospinal fluid (CSF), an equal volume of physiological saline containing 2.3 nmol of big ET-1 (Peptide Institute Inc., Osaka, Japan) was injected intracisternally. Thirty minutes before the cisternal injection of big ET-1, 4 ml of saline or saline containing 2 μmol of phosphoramidon (Peptide Institute Inc., Osaka, Japan) was administered intracisternaly with the same manner. Systemic arterial pressure (SAP) was measured with a Statham pressure transducer connected with a Polygraph System (Nihon Kohden, Osaka, Japan) via the catheter inserted into the right femoral artery. Transvertebral angiography was carried out before the cisternal puncture and 1 hr after the big ET-1 injection. The CSF for a determination of immunoreactive (IR)-ET content was collected at the first cisternal puncture and after the second cerebral artery angiography. The caliber of the basilar artery was measuered at three points on each angiogram with the use of a projector (x10) and expressed as a percent of the control caliber of the artery. The collected CSF was lyophilized and stored at -80 ℃ for assay of IR-ET.

Analytical Procedures: Radioimmunoassay (RIA) for ET was performed as described (4). ET-1 antiserum (a generous gift from Dr. M.R. Brown, Department of Medicine, University of California, San Diego) did not crossreact with big ET-1, as described (14). For chromatographic analysis of IR-ET in CSF, a 3 ml aliquot of CSF was extracted with a Sep-Pak C_{18} cartridge (Waters, MA). The

extract was subjected to reverse-phase high performance liquid chromatography (RP-HPLC), using a Capcell-Pak $5C_{18}$-SG300 column (4.6 x 250 mm, Shiseido, Tokyo, Japan) eluted with a linear gradient from 0% to 35% CH_3CN in 0.02% trifluoroacetic acid (TFA) for 15 min, followed by isocratic elution at 35% CH_3CN in 0.02% TFA for 15 min and a linear gradient from 35% to 63% CH_3CN in 0.02% TFA for 15 min. The flow rate was 0.5 ml/min. Each fraction was evaporated and assayed for IR-ET using RIA.

RESULTS

The caliber of the basilar artery on angiograms before the administration of big ET-1 with or without phosphoramidon averaged 1.22±0.12 mm (n=3) and 1.10±0.03mm (n=3), respectively. Representative angiograms in animals treated with or without phosphoramidon are shown in Fig. 1. In animals injected with saline intracisternally, the basilar artery caliber was significantly decreased to 58.8 ±3.9% 1 hour after intracisternal administration of big ET-1 in comparison with the baseline value. In the phosphoramidon treated animals, the reduction of basilar artery caliber induced by big ET-1 was significantly reduced (85.4±1.5%, $P<0.01$ compared to that seen in animals treated only with saline, unpaired t-test).

Following the intracisternal administration of big ET-1, a marked rise in SAP was observed (Fig. 2). In contrast, pretreatment with phosphoramidon effectively suppressed the SAP change induced by big ET-1 (Fig. 2). When the same dose of big ET-1 was administered intravenously, almost no change was observed in SAP (Fig. 2).

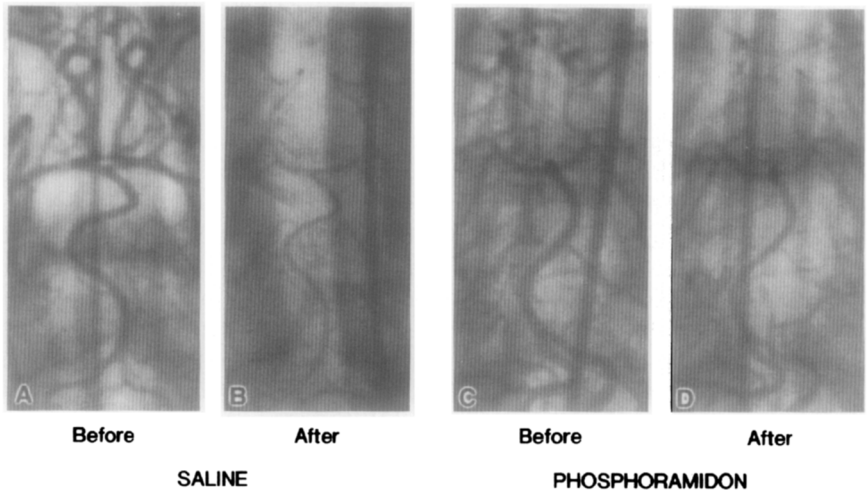


Figure 1. Typical angiograms of vertebral-basilar vessels before (A,C) and 1 hr after (B,D) intracisternal administration of big ET-1 (2.3 nmol) with (right panel) or without (left panel) pretreatment with phosphoramidon (2μmol). Note the remarkable inhibition of big ET-1-induced vasoconstriction in the presence of phosphoramidon.

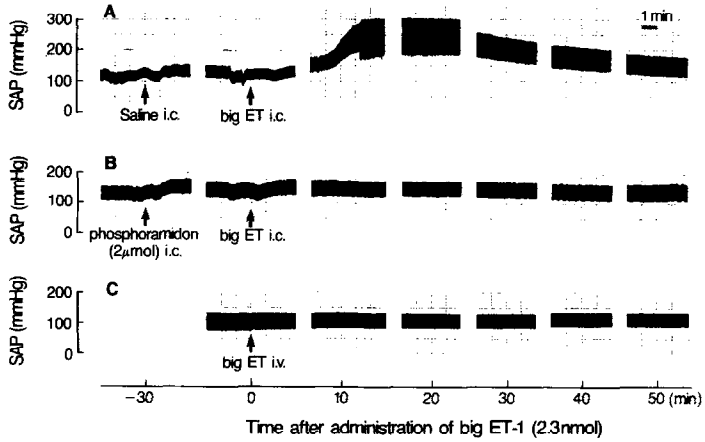
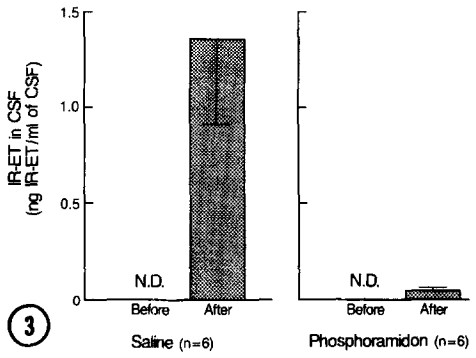


Figure 2. Typical changes in systemic arterial pressure (SAP) after intracisternal (A and B) and intravenous (C) administration of big ET-1 (2.3 nmol). Note that a marked rise in SAP induced by big ET-1 was abolished after phosphoramidon (2 μmol) pretreatment. i.c.: intracisternal, i.v.: intravenous.

When the IR-ET in the CSF was measured by RIA, there was a marked increase in IR-ET following the administration of big ET-1, in each animal (Fig. 3). However this increase in IR-ET was almost completely prevented by treatment with phosphoramidon (Fig. 3).

To further characterize the IR-ET in CSF after big ET-1 administration, we examined the elution profile of pooled CSF on RP-HPLC coupled with RIA. As shown in Fig. 4, the elution profile revealed one major IR-ET component



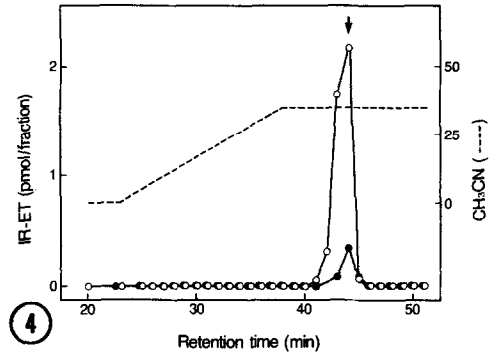


Figure 3. Changes in IR-ET in CSF before and 1 hr after intracisternal administration of big ET-1 (2.3 nmol), with (right panel) or without (left panel) pretreatment with phosphoramidon (2 μmol). Values are means$\pm$SE. N.D.: not detectable.

Figure 4. RP-HPLC profile of dog CSF extract. Arrow points to the retention time of synthetic ET-1. ○: CSF from animals received saline, ●: CSF from animals received phosphoramidon.

corresponding to the elution position of synthetic porcine ET-1. In the presence of phosphoramidon, a marked decrease in IR-ET was observed.

DISCUSSION

Our results indicate that intracisternally administrered big ET-1 is converted to ET-1 and produces a vasoconstrictor response in cerebral arteries, accompanied by a marked rise in ET-1 content in the CSF. The ET-1 generated in the subarachnoid space appears to lead to a marked and temporal elevation in SAP. In addition, these responses to big ET-1 were effectively prevented by intracisternal treatment with phosphoramidon. These findings suggest that an ET-1 converting enzyme is present somewhere in the vascular wall or subarachnoid space to produce ET-1 and that this conversion of big ET-1 to ET-1 is effectively inhibited by phosphoramidon, a metalloproteinase inhibitor. With respect to the site of conversion, we can rule out conversion of big ET-1 to ET-1 in the CSF. When big ET-1 was incubated in collected freshly CSF, we detected no ET-immunoreactivity, thereby indicating that there is no converting activity in the CSF (data not shown).

Recent studies (3-5, 9, 15) revealed that phophoramidon specifically inhibits the conversion of big ET-1 to ET-1, under physiological conditions. Intravenously administered big ET-1-induced hypertension in anesthetized (5) or conscious (15) rats was almost completely blocked by phosphoramidon. In cultured ECs, the secretion of ET-1 is markedly suppressed by this agent at a concentration of 10^{-4} M (4). In the present study, 2μmol of phosphoramidon and 2.3 nmol of big ET-1 were injected into the cisterna magna, in which the drug concentration was roughly calculated at 10^{-4} M and 10^{-7} M, respectively (the volume of CSF in the dog is about 20 ml). It is unclear whether the residual vasoconstriction seen in the animals treated with phosphoramidon is due to incomplete inhibition of the conversion and/or a vasoconstrictive activity of big ET-1 itself.

In addition to the angiographic cerebral vasoconstictor action, big ET-1 produced a marked rise in SAP. These findings are in agreement with those reported in experiments in which the effects of ET-1 on cerebral arteries were examined (10-13). It seems likely that ET-1 generated in the subarachnoid causes a paralysis of the vasomotor center or has a direct action on the central nervous system like a neurotransmitter and/or a neuromodulator, since intravenous administration of the same dose of big ET-1 exhibited almost no effect on SAP.

There are some reports showing that the changes in IR-ET content in the CSF or plasma from patients with SAH were related to the occurence of angiographic vasospasm and neurological dysfunction (16, 17). Enhanced sensitivity to ET-1

was observed in isolated arterial strips from rats with SAH (18). Furthermore, ET-1 was reported to cause a cerebrovasoconstrictor result similar to that induced by bloody CSF after SAH in human cerebral arteries *in vitro* (19). Although there are few reports demonstrating the origin(s) of liberated ET-1 and the site(s) generating ET-1 in the subarachnoid space after SAH, our results suggest that the big ET converting pathway is present somewhere in the subarachnoid space, except the CSF, and that the inhibition of this pathway by phosphoramidon effectively prevents the severe vasoconstriction due to ET-1. More recently, we found that an intracisternal administration of phosphoramidon effectively suppresses the developement of cerebral vasospasm in the canine "two-hemorrhage" model (Matsumura et al. submitted). Whether enhanced conversion of big ET to ET participates in the pathogenesis of cerebral vasospasm and whether the supression of this conversion can lead to prevention of cerebral vasospasm remains to be determined.

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