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Neuroscience Letters 174 (1994) 47–50

NEUROSCIENCE LETTERS

Increased endothelin-1 in the rabbit model of middle cerebral artery occlusion

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Received 25 January 1994; Revised version received 31 March 1994; Accepted 12 April 1994

Abstract

Endothelin-mediated vasoconstriction may theoretically aggravate ischemic neuronal damage. Although investigators have demonstrated that endothelins are produced by cerebral microvessel endothelial cells, astrocytes and neurons in vitro, whether endothelins are produced during cerebral ischemia is still unclear. The purpose of this study, therefore, was to measure endothelin-1 in brain tissue and plasma following middle cerebral artery occlusion and to examine the relationship between brain tissue and plasma endothelin-1 levels. The middle cerebral artery of rabbits was occluded for 2, 4 or 24 h. The amount of endothelin-1 in both brain tissue and plasma was determined by RIA. The results demonstrate that the concentrations of endothelin-1 in the ischemic brain tissue and plasma are both significantly increased after focal cerebral ischemia ($P < 0.01$). The data confirm that an acute and marked increase of endothelin-1 in brain tissue and plasma is associated with focal ischemic events. The possibility that endothelin-1 has a role in neuronal cell damage following focal ischemia warrants further attention.

Key words: Blood–brain barrier; Endothelin-1; Endothelial cell; Cerebral ischemia; Middle cerebral artery occlusion; Rabbit

Endothelins (ET), a group of vasoconstrictor peptides, were isolated from the supernatant of cultured endothelial cells [22]. Endothelin-1 (ET-1) is found in the CNS [19,20] and is produced by endothelial cells, astrocytes and neurons [6,9,23]. There are specific, high-affinity and high-density binding sites for ET-1 in nearly all regions of the brain [7,19]. ET-1 causes vasospasm in cerebral vessels and reduces cerebral blood flow (CBF) when administered into the cisterna magna, neostriatum and brain parenchyma [12] or when topically applied to exposed brain vessels [13]. ET-1 may not only act as a vasoconstrictor in the brain but may also modulate glial cell growth and mitosis [10], neurosecretion, such as gonadotropin secretion [18], and cerebral glucose metabolism [4]. Recently, several reports have confirmed the increased ET concentrations in plasma and cerebrospinal fluid (CSF) in subarachnoid hemorrhage and acute cerebral infarction but decreased CSF ET concentrations in patients with Alzheimer's disease [8,24,25]. Following transient forebrain ischemia in stroke-prone spontaneously hypertensive rats, Yamashita et al. demonstrated an increased production of ET in the hippocampus [21]. However, it is unknown whether ET-1 is released following focal cerebral ischemia, how early ET-1 is released and from where the ET-1 originates?

In this study, we measured the level of ET-1 in both brain tissue and plasma following middle cerebral artery occlusion (MCAO) in rabbits to determine if the release of ET-1 is related to focal cerebral ischemia, to examine the relationship of ET-1 release between brain tissue and plasma as well as the possible mechanism of ET-1 increase during focal cerebral ischemia.

Twenty-four New Zealand white male rabbits (2.5–3.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). A femoral artery was catheterized to monitor arterial blood pressure and to obtain blood for analysis of blood gases, blood pH and blood glucose. Blood gases were analyzed once during the operation. Animals were

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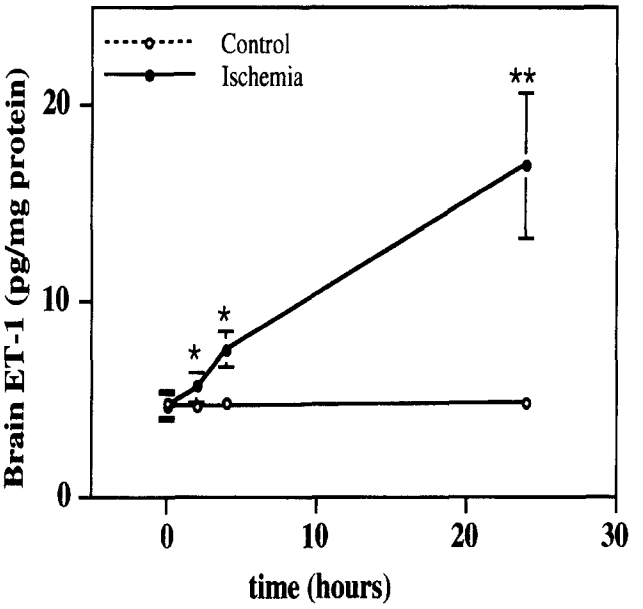
SSDI 0304-3940(94)00322-2

given a 30% O_2/70% NO_2 gas mixture to maintain a P_aO_2 level of ≥90 mmHg. Body temperature was measured with a rectal probe and maintained at 37–37.5°C with a heating pad. The MCAO was performed according to the method described by O'Brien et al. with a minor modification [17]. The middle cerebral artery was exposed at its origin, a small wire hook was inserted under the MCA and then the MCA was coagulated and cut at its origin. Four groups of rabbits (six rabbits in each) were euthanized by decapitation at 0, 2, 4 and 24 h following MCAO. The brains were quickly removed and a 5-mm-thick coronal slice centered around the middle cerebral artery was cut. Tissue samples were obtained from both hemispheres (ipsilateral and contralateral to the occlusion) and stored at −20°C for ET-1 measurements.

Plasma ET-1 levels were measured in all groups of rabbits. Blood was drawn at 0, 2, 4 and 24 h following MCAO and transferred to a chilled polypropylene tube containing 500 U/ml aprotinin (Sigma) and 1 mg/ml EDTA. The samples were centrifuged at 3000 rpm for 15 min at 4°C and then stored at −70°C until assayed. Brain tissue was homogenized in 10 vols. of 0.1 N acetic acid and centrifuged at 3500 rpm for 20 min at 4°C. The supernatants were purified immediately using C^{18} Sep-Pak cartridges (Millipore). Each cartridge was washed with 15 ml 4% acetic acid and the endothelin fractions were then eluted with 2.5 ml 60% acetonitrile in 0.5% ammonium acetate. The samples were dried by evaporation in a speedVac apparatus (Savant, Farmingdale, NY). The dried fractions were reconstituted with 0.25 ml of the RIA buffer. ET-1 was measured using a competitive RIA kit (RIK-6901; Peninsula Lab, Belmont, CA). Duplicate samples of 0.1 ml were assayed according to the manufacturer's instructions. The ET-1 assay has a <10% cross-reactivity with ET-2 and ET-3 and does not cross-react with a variety of other hormones. For each sample assay, all procedures were performed with the tubes and reagents in an ice bath. The level of plasma ET-1 was expressed as ET-1 pg/ml plasma and the level of brain tissue ET-1 was expressed as ET-1 pg/mg protein.

All physiological parameters were in the normal range. There were no significant differences between control and MCAO groups in terms of the mean arterial blood pressure, blood gases (P_aO_2, P_aCO_2 and P_aHCO_3), blood pH and blood glucose.

Fig. 1 shows the changes of ET-1 in the brain samples. In the contralateral hemisphere the ET-1 concentration was similar in all groups. In the ischemic hemisphere, however, the ET-1 concentration was significantly elevated from a value of 4.67 ± 0.28 pg/mg protein in the control group to 5.67 ± 0.59 and 7.57 ± 0.91 pg/mg protein in ischemic hemisphere following 2 and 4 h of MCAO ($P < 0.01$) respectively. By 24 h of MCAO, the ET-1 concentration in the ischemic hemisphere had elevated to 16.91 ± 3.67 pg/mg protein which was four-fold higher than in the control group.



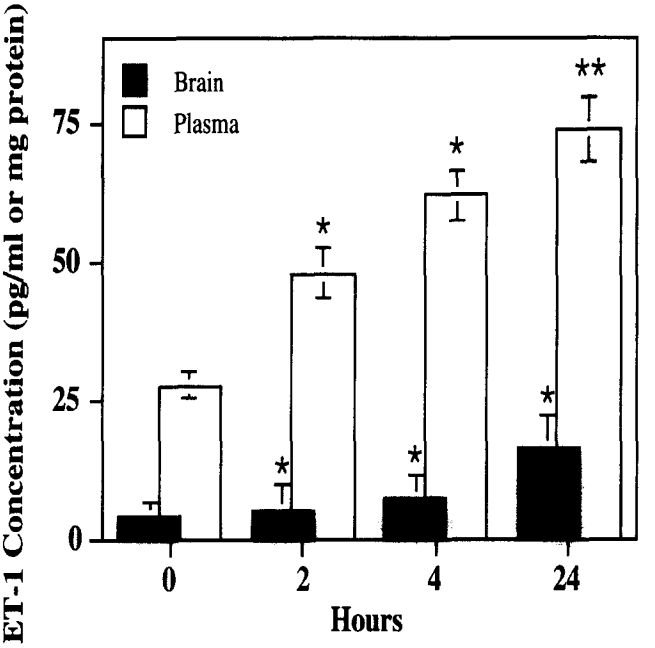


Fig. 1. Changes of ET-1 content in ipsilateral hemisphere (●) and contralateral hemisphere (○) in rabbits. ET-1 content was measured in groups of six rabbits immediately (0 time) and at intervals up to 24 h following MCAO. Values shown are mean ± S.E.M. of differences between values at control and those at subsequent times. $*P < 0.05$, $**P < 0.01$ vs. control group.

Fig. 2. Changes of ET-1 content in brain tissue (■) and plasma (□) in rabbits. ET-1 content was measured in groups of six rats immediately (0 time) and at intervals up to 24 h following MCAO. Values shown are mean ± S.E.M. of differences between values at 0 time and those at subsequent times. $*P < 0.01$, $**P < 0.001$ vs. control group.

The changes of plasma ET-1 concentration is shown in Fig. 2. In the control group the plasma ET-1 content

was 27.57 ± 2.28 pg/ml. Following 2 h of MCAO, it increased to 48.25 ± 4.49 pg/ml (175% of control). The plasma ET-1 level increased in a time-dependent fashion reaching to 62.49 ± 4.43 pg/ml at 4 h and 73.75 ± 5.83 pg/ml at 24 h following MCAO.

In the present study we have demonstrated an increase of ET-1 level both in the ischemic area and plasma following MCAO in rabbits. In the first 4 h of MCAO, ET-1 levels were increased in the ipsilateral hemisphere but the amount was not high. By 24 h of MCAO, however, the level of ET-1 increased dramatically in the ipsilateral hemisphere and was four-fold higher than in the control hemisphere. Our studies have also demonstrated that the plasma ET-1 level is increased gradually after MCAO in rabbits.

The source of the ET-1 in the ischemic hemisphere is uncertain. It is not present in the blood stream as similar concentrations are not found in the non-ischemic hemisphere. The increased tissue ET-1 is also not likely to have come from the blood stream. The concentrations reached after 24 h of MCAO, 17 pg/mg protein, corresponds to a concentration of ~2 ng/ml brain water (assuming 1 mg brain protein to 7.5 mg brain water), 30-fold higher than the plasma concentration found at a similar time point. So, even though the blood–brain barrier may have been disrupted after 24 h of MCAO [15] and present ET that can penetrate through the CNS [11], an influx of plasma ET-1 is unlikely to be the cause of the higher tissue ET-1 in the ischemic hemisphere.

The two other potential sites of ET-1 production in the ischemic hemisphere are the brain vascular endothelium and the brain parenchyma. Endothelin was first discovered being produced by cultured endothelial cells [22] but Takahashi et al. have demonstrated that the presence of immunoreactive ET (irET), ET mRNA and ET receptors in the brain tissue, although the distributions of irET, ET mRNA and ET receptors were different in the brain regions [19]. Following cerebral ischemia, brain vascular endothelial cells may be injured, thus synthesis of ET-1 could increase in these regions [5].

The cause of the ischemia induced increase in plasma ET-1 found here and also seen by other authors is still unclear [25]. However, it may be a secondary response to the increase in brain ET-1 [16]. Suprapharmacological doses of ET-1 have been ascribed neuromodulator and paracrine functions [3,12]. It causes a systemic sympathoadrenal activation, including a rise in plasma catecholamine and vasopressin level [14], that might stimulate peripheral organs to release ET into the circulation.

A number of studies have examined whether ET-1 might play a role in ischemic brain injury. Injection of high doses of ET-1 (43–430 pmol/rat) into the caudate putamen has produced local brain ischemia in rats [1,2] which may be due to a reduction in local CBF [13]. The concentrations used in those experiments (~20–200 pg/g wet weight if the endothelin distributes within the whole brain) are two to three orders of magnitude higher than the tissue concentrations found in the ischemic tissue in this study. However, if the ET-1 is generated by the vascular endothelium, it is possible that local concentrations of ET-1 may be much higher than found in the whole ischemic tissue and they might be high enough to decrease CSF and perhaps potentiate the ischemic brain injury. ET could produce a locally deregulated metabolic state by reducing CBF while stimulating tissue metabolism simultaneously in the same region. This condition may aggravate the ischemic injury [4]. Also as noted above, smaller amounts of ET injected intraventricularly may have an effect on systemic parameters including the release of vasoconstrictors and activators of the sympathetic nervous system. In the ipsilateral hemisphere, a local increase of ET-1 may be an important factor in the pathogenesis and outcome of cerebral ischemia which may be due to the release of excitotoxic amino acids, and increased glial and neuronal intracellular calcium.

We thank R. Keep, Crosby Neurosurgical Research Laboratories, University of Michigan Medical Center, Ann Arbor, MI, for many helpful comments and suggestions, and K. Donahoe for editorial assistance in the preparation of this manuscript.

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