High Plasma Levels of Endothelin-1 and Atrial Natriuretic Peptide in Patients With Acute Ischemic Stroke

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The plasma levels of endothelin-1 (ET-1) and atrial natriuretic peptide (ANP) have been measured in 37 patients with acute ischemic stroke, on admission and 3 and 7 days thereafter. The plasma ET-1 levels at the onset of symptoms were about two-fold those observed in age-matched normal volunteers (3.5 ± 2.26 vs 1.54 ± 0.9 pg/mL, respectively; \( P < .001 \)). These levels remained significantly elevated during the 7-day study period. The neurologic deficit was assessed daily by Mathew's modified scale (MS). A significant correlation was found between neurologic status on admission and ET-1 plasma values; patients with worse neurologic status (MS < 45 points) had higher ET-1 plasma values than those with better neurologic status (MS > 45 points) (5.4 ± 2.34 vs 3.05 ± 2.04 pg/mL, respectively, \( P < .05 \)). The plasma ET-1 values did not correlate either with the site of the infarction or with its primary cause (cardioembolic, lacunar, or atherothrombotic). No significant differences were seen in plasma ET-1 concentrations between patients who eventually died and those who survived the acute event. The plasma ANP were about 18-fold higher in ischemic stroke patients on admission than in controls at admission (110.9 ± 29.5 vs 5.84 ± 3.96 pg/mL, respectively, \( P < .01 \)). These values remained significantly elevated on days 3 and 7. There was no correlation between the ANP plasma values and the neurologic status, the site or mechanism of the stroke, or the plasma ET-1 levels. In conclusion, ischemic stroke is associated with marked acute and long-duration increases of ET-1 and ANP. These findings may reflect an enhanced production by damaged endothelial cells within the infarcted tissue and might imply a possible role of ET-1 in the pathogenesis and final outcome of cerebral infarction. The ANP elevation could reflect a vasodilator response to the potent contractile effect of ET-1. Am J Hypertens 1994;7:1085-1089

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Over the last few years the ability of vascular endothelial cells to synthesize and release potent vasodilator and vasoconstrictor substances has been recognized. Yanagisawa and coworkers demonstrated that this vasoconstrictor activity released by the endothelium is related to the formation of a peptide called endothelin.\(^1\) Three forms of endothelin have been characterized: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3).\(^2\) ET-3 is structurally and functionally similar to ET-1 but is mostly detected in, and probably produced by, neural tissue. ET-1 seems to be the most potent vasoconstrictor substance yet identified.\(^3\)

Cerebral microvessels show a marked sensitivity to ET-1\(^4\) and produce this peptide.\(^5\) Endothelin was also found to constrict the intracranial arteries both in vitro and in vivo.\(^6\) Thus endothelial cells may have a

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role in the control of vascular tone in the brain and may participate in the regulation of local blood flow within the brain.

Plasma ET-1 tends to rise after serious clinical insults. This is particularly true in myocardial infarction, after which the concentrations observed are substantially above the normal range.7 The ET-1 concentration is high in the cerebrospinal fluid of patients with subarachnoid hemorrhage, suggesting an involvement of this peptide in the generation of cerebral vasospasm.8 Theoretically, in acute cerebral ischemia there might be an increased secretion of ET-1 and ET-3 from damaged endothelial and neural cells, respectively. The resulting severe and protracted local vasoconstriction may play a role in the pathogenesis and outcome of acute cerebral injury. We therefore measured plasma ET-1 levels in patients during the acute phase of ischemic stroke in order to ascertain whether ET-1 levels might be increased and, if so, whether they correlate with parameters of clinical severity.

Atrial natriuretic peptide (ANP) is a potent relaxant of contracted blood vessels; it has been shown to be a unique hormone that regulates blood pressure and fluid homeostasis.9 Recent studies have shown that this hormone regulates sodium transport in vascular endothelial and other cells through activation of guanylate cyclase. This suggests that ANP may regulate water content in tissue, and thus delay brain edema formation by inhibiting sodium transport in capillaries.12 Furthermore, ANP is a systemic vasodilator, blocks angiotensin-induced aldosterone secretion from the adrenal cortex, and inhibits renin secretion,13 all of which are potentially antihypertensive actions. In the postischemic phase of a stroke, a satisfactory level of tissue homeostasis would be recovered by the vasodilator feedback mechanisms. ANP has been proposed, along with both endothelium-derived relaxing factor (EDRF) and calcitonin gene-related peptide (CGRP), as factors to be considered in posts ischemic hyperperfusion.14 The second aim of this study was to investigate plasma levels of ANP in acute ischemic stroke. Since ET-1 has been found to increase ANP secretion,15 we measured plasma ANP concomitantly with ET to further assess the ANP–ET-1 relationship.

PATIENTS AND METHODS

Thirty-seven patients (20 male, 17 female) were studied; the mean age was 70.8 years (range 51 to 92). The identified risk factors were: hypertension, 23 patients (62.1%); dyslipemia, 11 patients (29.7%); tobacco smokers, eight patients (21.6%); and diabetes, seven patients (18.9%). All patients were admitted to the hospital because of sudden-onset focal neurologic deficit. The diagnosis of acute ischemic stroke was confirmed by a complete neurologic workup that included a brain CT scan. All patients with cerebral ischemia due to causes other than atherothrombosis and cardioembolism, such as subarachnoid hemorrhage, intracerebral hemorrhage, hematoma, and complicated migraine, were excluded from the study. Also excluded were patients with overt, severe systemic disease (eg, recent myocardial infarction, cardiogenic shock, severe renal or hepatic failure, severe systemic infection, or nonstabilized myocardial infarction).

The differentiation of atherothrombosis from cardioembolism was based on the finding of either an arterial stenosis/occlusion or a potential source of cardiogenic embolism. A possible cardioembolic mechanism was assumed if a major risk source was present.16 Twelve patients (32%) were included in this category. The diagnosis of lacunar infarcts was established in seven cases (18.9%) on the basis of their clinical features and CT results. In 18 patients (49.1%) the mechanism of the ischemic stroke was related to atherothrombosis of the cranial vessels. The diagnosis of the site of infarction was based on the initial clinical assessment; this was classified as anterior (carotid) in 31 patients (83.7%) and posterior (vertebrobasilar) in six (16.2%). Clinical data of the stroke are presented in Table 1.

The neurologic deficit was assessed by the Mathew scale (MS) as modified by Gelmers et al,17 on admission and on days 3 and 7 thereafter. This is an ordinal scale with upper (100) and lower (0) limits, and comprises 10 items. The mean MS score was 48.76 ± 22.5 on admission, 59.2 ± 24.7 on day 3, and 71.5 ± 17.5 on day 7. Five patients (13.5%) died during the 7-day study period.

Venous blood samples were collected within the first 13.5 ± 9.7 h after the onset of symptoms, and again on days 3 and 7; patients admitted after awakening were considered to have suffered the stroke during the night, and the actual moment was arbitrarily established at 7 AM because of the known circadian variation of cardiovascular events.18 The ET-1 and ANP levels were measured by previously described radioimmunoassay methods.19,20

| TABLE 1. CLINICAL CHARACTERISTICS OF STROKE PATIENTS; SITE AND PATHOGENESIS |
|-----------------------------------|-----------------|
| Number of patients | 37 |
| Age | 70.8 years |
| Sex | 20 male, 17 female |
| Carotid | 31 (83.7%) |
| Vertebrobasilar | 6 (16.7%) |
| Atherothrombosis | 18 (49.1%) |
| Cardioembolism | 12 (32%) |
| Lacunar | 7 (18.9%) |
Twenty-one of the 37 patients were studied on day 3, and 24 of 37 on day 7.

Thirty-six age-matched (55 to 78 years) normal subjects served as controls. The control (normal) ET-1 levels in our laboratory were 1.54 ± 0.9 pg/mL; ANP normal values were 5.84 ± 3.96 pg/mL.

The Student's *t* test for unpaired data was applied to assess the statistical significance of differences between patients and normal control groups. Correlation coefficients were calculated by linear regression analysis to evaluate the degree of linear association between measured variables, using the R-Sigma program in a personal computer. *P* < .05 was considered statistically significant. All measured values are expressed as means ± SEM.

**RESULTS**

The ET-1 levels were significantly increased in patients with ischemic stroke as compared to the normal control subjects. On admission, the ET-1 levels in patients were about twofold those observed in normal volunteers (3.50 ± 2.26 pg/mL vs 1.54 ± 0.9 pg/mL, *P* < .001). These levels remained significantly elevated on days 3 (4.28 ± 3.14 pg/mL) and 7 (2.91 ± 2.18 pg/mL) (*P* < .001 and *P* < .01, respectively) (Figure 1). The differences in the ET-1 levels from day 1 to day 7 in the patient group were nonsignificant.

The admission ET-1 levels were higher in patients with more severe neurologic deficits (MS score < 45) than in those with better neurologic status (MS score > 45): ET-1 was in the first case 5.40 ± 2.34 pg/mL vs 3.05 ± 2.04 pg/mL in the second (*P* < .001). This difference remained statistically significant on day 3 (MS < 45, 7.33 ± 2.36 pg/mL vs MS > 45, 3.82 ± 3.10 pg/mL, *P* < .05).

There were no significant differences in the ET-1 levels between strokes of carotid or vertebrobasilar location, nor between embolic (3.22 ± 1.93 pg/mL), lacunar (2.99 ± 1.05 pg/mL), or atherothrombotic (3.65 ± 2.48 pg/mL) infarctions. In addition, no significant differences in the ET-1 levels were observed between patients who eventually died and those who survived the acute ischemic event. Furthermore, there was no significant correlation between the ET-1 levels and either age, sex, or arterial blood pressure.

The ANP levels were found to be significantly higher in stroke patients than in normal volunteers. On admission, the ANP levels in the patients were 18-fold those of the control subjects (110.9 ± 29.5 pg/mL vs 5.8 ± 3.4 pg/mL, *P* < .01). These values remained significantly elevated on days 3 and 7 (86.7 ± 32.5 pg/mL and 147.5 ± 41.6 pg/mL, *P* < .01 in both cases).

No significant correlation was found between the ANP levels and the neurologic deficit as assessed by the MS score, nor between the ANP and ET-1 levels. There were no differences in ANP levels between the patient subgroups with atherothrombotic, cardioembolic, or lacunar infarctions. Neither did age, sex, arterial blood pressure, or eventual death correlate with the ANP plasma levels.

**DISCUSSION**

The major finding in our study is that ET-1 and ANP levels were both significantly elevated in acute ischemic stroke. The ET-1 increases were more pronounced during the first 24 h after the onset of symptoms, and they tended to correlate significantly with the severity of neurologic deficit. Furthermore, ET-1 remained elevated over the 7-day study period, thus confirming the initial findings.

The mechanism for this increase in circulating ET-1 remains unknown. The ET-1 gene contains a consensus sequence for acute-phase reactant elements; thus, the increased ET-1 production might be attributable to the patient's hypoxic condition or stress-induced release of an acute-phase reactant during acute cerebral infarction. Alternatively, the excess ET-1 may result from the excessive local ET-1 production induced by the ischemic insult and arising from injured endothelial cells of the involved cerebral microvessels. On the other hand, the possible role of elevated thrombin concentrations within the ischemic region is yet to be determined. Our results, however, cannot ascertain whether or not the increased ET-1 levels reflect local overproduction or are derived from the systemic circulation.

This situation is similar to that observed in myocardial infarction, in which elevated plasma ET-1 levels have been reported. In fact, it is also possible and conceivable that the increased peripheral plasma ET-1 levels reflect much higher ET-1 concentrations within the area of cerebral infarction. In addition, our
findings of increased plasma levels of ET-1 despite the very rapid clearance of this substance from the blood (60% within the first minute, mainly through the lung) further suggest that substantial increases in ET-1 production may be involved.

On the other hand, taking into account that ET-1 is a potent and long-acting vasoconstrictor peptide, and that it will, at low and threshold concentrations (which do not elicit any significant vasomotor response), potentiate the response to other vasoconstrictor hormones such as exogenous norepinephrine, norepinephrine released from adrenergic nerve endings, or serotonin, the peptide may contribute to enhanced contractility of the atherosclerotic cerebral blood vessel wall. ET-1 may thus cause constriction of collateral vessels and contribute to a vicious circle with further reduction in regional blood flow, enhancement of the severity and size of the infarction, and worsening of the neurologic outcome.

The present study shows that ET-1 and ANP levels in patients with acute stroke were elevated and remained so for at least 1 week after the onset of symptoms. Furthermore, these data suggest that ET-1 could be considered a marker of disease severity in acute ischemic stroke. Ziv et al recently reported similar results, however, their study was designed with both a shorter period of observation and smaller number of patients.

The increase of ET-1, either locally produced or derived from the systemic circulation, may therefore be deleterious to the already-injured neural tissue. Two recent publications have shown that experimentally induced brain ischemia in the rat elicited a significant increase in ET immunostaining in a degree-dependent manner. The possibility that ET has a role in the development of neuronal cell death following transient forebrain ischemia warrants further attention.

There are, however, several intrinsic mechanisms which limit the effects of ET-1. The first level of control is at the stage of ET-1 synthesis; the pre-proET-1 mRNA is particularly short-lived and this may be a safeguard against overproduction. The vasoconstrictor action of ET-1 can be opposed by the concomitant release of prostacyclin, ANP, and nitric oxide (NO), all of which are potent vasodilators. In vitro pharmacologic studies suggest that ET-1 releases NO directly and at concentrations lower than those required for vasoconstriction. In vitro studies in perfused rat atrioocytes have demonstrated that ET-1 stimulates human ANP release, and this may counteract the potent vasoconstrictor effect of ET-1. It is possible that the very high ANP levels observed in the patient group in our study might reflect a vasodilator response to the potent vasoconstrictor effect of ET-1. Furthermore, ANP acts directly on the central nervous system to inhibit brain water and sodium accumulation in ischemic brain edema; this action is probably related to its inhibitor effect on sodium transport in brain capillaries. In addition, ANP has been shown to modulate intracellular electrolyte content through the activation of guanylate cyclase. In cultured astroglia, ANP increases intracellular guanosine 3’-5’cyclic monophosphate (cGMP), thus regulating sodium content. ANP could act as a protective factor in the setting of ischemic stroke via both this antiedema and vasodilator effect.

We have observed that the ET-1 levels increase with age (0.54 ± 0.39 pg/mL at age 20 to 50 years, n = 100, v 1.54 ± 0.9 pg/mL at age 55 to 78 years, n = 39; P < .01). In the present study, the acute ischemic stroke patients evidenced significantly elevated plasma ET-1 levels as compared to both young normal subjects and the age-matched control group. Although the actual importance and significance of ET-1 and ANP in the regulation of ischemic stroke is not completely understood, the present results suggest that the elevation of ET-1 levels may be an important factor in the ensuing further reduction in regional blood flow, enhancement of the severity of the size of the infarcted tissue, and worsening of the neurologic outcome. The role of ANP in counteracting the vasoconstrictor action of ET-1 also remains to be elucidated. It is clear that further work is needed to clarify these important issues.

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REFERENCES


