Dissociation of adrenomedullin concentrations in plasma and cerebrospinal fluid in pregnant and non-pregnant women

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Abstract
Adrenomedullin (AM), a potent vasodilator peptide, has been shown to act within the central nervous system to modulate fluid and electrolyte balance. AM-immunoreactive cells have been found in the anterior pituitary gland and the choroid plexus of humans. In addition, AM activity has been implicated in the regulation of maternal circulation during pregnancy. To determine the relationship between AM concentration in the cerebrospinal fluid (CSF) and plasma, we measured AM levels in CSF and plasma of pregnant (group P, n = 12) and non-pregnant (group NP, n = 10) women scheduled to undergo gynecologic or obstetric surgery. In both groups, the concentration of AM in the plasma exceeded that in the CSF. Plasma AM concentration was significantly higher in pregnant than non-pregnant women (17.3 ± 5.8 vs 5.1 ± 1.4 pmol/l, mean ± S.D.; P < 0.01), whereas CSF AM concentration did not differ between the two groups (1.3 ± 0.9 and 0.9 ± 0.4 pmol/l in groups P and NP respectively). No significant correlation was found between AM concentrations in the CSF and plasma. The present findings suggest that AM is present in the CSF and that its concentration in the CSF is regulated independently from that in the plasma.

Introduction
Adrenomedullin (AM) is a vasodilator peptide originally identified in the tissue extract of a human pheochromocytoma (1). When injected intravenously, it exerts a potent blood pressure-lowering effect in rats. The development of an RIA led to the detection of substantial amounts of AM in multiple tissues, such as adrenal medulla, aorta, lung, heart and central nervous system of humans (2). In addition, expression of AM mRNA has been observed in the human brain (3). An intracerebroventricular injection of AM was reported to inhibit thirst stimulated by water deprivation, and to cause a slow onset but prolonged increase in arterial pressure in anesthetized rats (4, 5). These findings imply that AM may play a role in the central regulation of fluid-electrolyte balance and blood pressure. AM has been shown to be present in the human blood, and plasma AM concentration is elevated in patients with hypertension or congestive heart failure compared with controls. A recent study of normal human pregnancy indicates that AM concentration in the maternal plasma is much higher than in the plasma of non-pregnant women, suggesting that it may be involved in the regulation of the maternal circulation (6). In order to determine the role of AM in the central regulation of cardiovascular homeostasis, it is important to know how AM levels in the cerebrospinal fluid (CSF) are regulated; however, to date, no data are available on AM concentrations in the CSF and whether they are affected by plasma AM. In the present study, we measured AM concentration in both the plasma and CSF in non-pregnant women and pregnant women undergoing cesarean section, to determine the presence of AM in the CSF and the relationship between AM levels in the CSF and plasma.

Subjects and methods
Subjects
This study was approved by our institutional human investigation committee, and written informed consent was obtained from all participants. We examined two groups of patients: group P (n = 12) consisted of pregnant women at 36 to 39 weeks gestation scheduled to undergo primary cesarean section after the diagnosis of cephalopelvic disproportion without uterine contraction, and group NP (n = 10) comprised patients with myoma uteri undergoing hysterectomy. None of the patients had a history of liver, respiratory or kidney disease. All patients received spinal anesthesia in the operating room.
**Experimental protocol**

Systolic and diastolic blood pressure and heart rate in all patients were measured automatically (BSM-8800; Nihon Kohden, Tokyo, Japan) in the operating room 10 min before spinal anesthesia. Serum creatinine and hemoglobin concentrations were also measured before surgery to examine the effect of renal function and hemocoagulation or hemodilution on plasma AM concentration.

Before administration of the spinal anesthetic, blood samples were withdrawn via the antecubital vein, and CSF samples were obtained by lumbar puncture in each patient. A 7 ml sample of blood was collected into a chilled tube containing 1.0 mg/ml disodium EDTA (EDTA-2Na) and 500 kallikrein inhibiting units (KIU)/ml aprotinin, and was immediately centrifuged at 4 °C. Similarly, 1.5 ml CSF was collected into a chilled tube containing EDTA-2Na and aprotinin, and centrifuged. The samples were stored at −80 °C until extraction.

**Assays**

**AM in plasma and CSF**

AM concentrations in plasma and CSF were measured using an RIA specific for human AM (hAM) (7). Either plasma or CSF was loaded on to a Sep-Pak C_{18} cartridge (Millipore-Waters, Milford, MA, USA) and equilibrated with 5 ml saline. After the cartridge had been washed with 5 ml saline and 10% acetonitrile in 0.1% trifluoroacetic acid (TFA), the absorbed material was eluted with 4 ml 60% acetonitrile in 0.1% TFA and lyophilized to be stored at −80 °C until assayed. The extract was then dissolved in 250 μl RIA buffer (50 mmol/l sodium phosphate buffer (pH 7.4) containing 0.5% BSA, 0.5% Triton X-100, 80 mmol/l NaCl, 25 mmol/l EDTA-2Na, 0.05% NaN_{3} and 500 KIU/ml aprotinin). A 100 μl sample of the dissolved extract was then assayed for hAM. The cross-reactivities of the anti-hAM serum used in this RIA were identical with that of plasma AM (7). The recovery of the position of authentic hAM(1–52), a finding 86.4%.

**Immunoreactive (ir)-AM** To analyze the molecular form of ir-AM, 20 ml CSF was obtained from 10 additional patients who were undergoing hystereotomy or cesarean section. The ir-AM in the CSF extract was characterized by reverse-phase HPLC on a TSK ODS 120A column (Tosoh, Tokyo, Japan). A linear gradient of 10–60% acetonitrile was made in 0.1% TFA for 60 min, and the ir-AM concentration of each fraction was measured by RIA.

**Statistical analysis**

Data are expressed as means ± s.d. The preoperative clinical parameters in the two groups were examined by Student's t-test for unpaired data, and AM levels in plasma and CSF in the two groups were compared using the Mann–Whitney U test. The relationship between two variables was evaluated by Pearson’s correlation. A level of P < 0.05 was considered statistically significant.

**Results**

**Demographics of pregnant and non-pregnant subjects**

Table 1 provides the basal profiles of all participants. All patients recovered uneventfully from surgery. There were no significant differences between the two groups in body weight, height, serum creatinine concentration, hemoglobin concentration and preoperative systolic and diastolic blood pressure.

**Plasma and CSF AM**

As shown in Fig. 1, the plasma AM concentration in group P (17.3 ± 5.8 pmol/l) was significantly (P < 0.01) higher than that in group NP (5.1 ± 1.4 pmol/l), whereas CSF AM concentrations did not differ (1.3 ± 0.9 and 0.9 ± 0.4 pmol/l in groups P and NP respectively). There was no significant correlation between AM concentrations in the plasma and CSF (Fig. 2), and in addition, no significant correlation was found in group P (r = 0.19) and group NP (r = −0.05) when examined separately.

**Ir-AM**

As shown in Fig. 3, a major peak of ir-AM emerged at the position of authentic hAM(1–52), a finding identical with that of plasma AM (7). The recovery of ir-AM during the chromatographic procedure was 86.4%.

**Discussion**

We have shown using RIA and reverse-phase HPLC that AM is present in the CSF of women. Plasma AM concentration was found to be significantly higher in

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<th>Table 1 Patient profiles. Values are means ± s.d.</th>
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<td><strong>Group NP</strong></td>
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*P < 0.05 compared with group NP.
pregnant than non-pregnant women. The concentration of AM in the CSF was significantly lower than that in the plasma in all patients. Plasma AM in pregnant women was three times higher than that in non-pregnant women, suggesting that AM may be associated with the hemodynamic state during pregnancy, which is characterized by an increased fluid volume with low vascular resistance (6, 8). In contrast, CSF AM concentrations did not differ between pregnant and non-pregnant patients. This finding suggests that the concentration of AM in the CSF is regulated independently from AM circulating systemically in the blood.

It has been reported that ir-AM is detected in the human choroid plexus (9); however, the permeability of the blood–brain barrier to AM remains unclear. Although the present results do not necessarily preclude the possibility that plasma AM has access to the neuropil of the subfornical organ or organum vasculosum laminae in the paraventricular lesion where the blood–brain barrier is absent, AM in the CSF may, at least in part, be derived from the choroid plexus. AM immunoreactivity has been detected in the thalamic, hypothalamus and anterior pituitary gland of humans. Specifically, Ueta et al. (10) found AM-ir neurons in the supraoptic nucleus and magnocellular parts of the paraventricular nucleus of the rat, which are known to play important roles in body fluid homeostasis. In addition, Samson & Murphy (11) demonstrated that an intracerebroventricular injection of anti-AM serum augmented saline drinking in rats, a finding that suggests an important role for endogenous AM in CSF or in a paraventricular lesion. The presence of AM in the CSF suggests a possible role for this vasodilator peptide in centrally modulating blood pressure and water-electrolyte balance.
In summary, AM exists in CSF, and its level appears to be regulated in the central nervous system independently from systemic AM.

References


Received 28 July 1998
Accepted 2 September 1998