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Adrenomedullin and atrial natriuretic peptide concentrations in normal pregnancy and pre-eclampsia

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Adrenomedullin (AM) is a peptide that elicits a long-lasting vasorelaxant activity, while atrial natriuretic peptide (ANP) has also been shown to be a potent vasodilatory agent. To clarify the possible role of AM and ANP in the physiology of pregnancy and pathophysiology of pre-eclampsia, we measured plasma concentrations of these peptides in non-pregnant women, normal pregnant women and women with pre-eclampsia. A gradual increase in plasma AM was observed as pregnancy progressed. The plasma AM concentrations during the second trimester (12.7 ± 1.4 fmol/ml) were significantly elevated, in comparison with the non-pregnant follicular phase (6.4 ± 0.61 fmol/ml), luteal phase (6.0 ± 0.49 fmol/ml), and the first trimester (6.5 ± 0.8 fmol/ml). The plasma AM concentrations of the third trimester (21.5 ± 1.4 fmol/ml) were significantly elevated when compared with those of the second trimester ($P < 0.05$). Northern blot analysis confirmed the expression of the *AM* mRNA transcript (1.6 kb) in third trimester placentas. In comparison with those observed at term (25.3 ± 4.5 fmol/ml), the plasma concentrations were significantly reduced post-partum (6.4 ± 0.6 fmol/ml). In the third trimester, plasma AM concentrations did not differ significantly between women with pre-eclampsia (17.2 ± 2.3 fmol/ml) and normal pregnant women. In contrast, the plasma ANP concentrations in pre-eclampsia (39.5 ± 7.1 pg/ml) were significantly elevated when compared with those of the normal third trimester (14.4 ± 1.4 pg/ml) ($P < 0.05$). ANP concentrations were reasonably constant throughout the pregnancy.

Key words: adrenomedullin/ANP/pregnancy

Introduction

Adrenomedullin (AM) is a peptide from human pheochromocytoma tissue newly discovered by monitoring its stimulating action on platelet cAMP production (Kitamura *et al*., 1993a). This peptide consists of 52 amino acids with an intramolecular disulfide bond forming a ring structure of six residues, and shares slight homology with calcitonin gene-related peptide (CGRP), a potent hypotensive peptide. Like CGRP, i.v. injection of AM elicits a strong and long-lasting hypotensive effect in anaesthetized rats (Ishiyama *et al*., 1993; Kitamura *et al*., 1993a). The vasodilator action of AM has also been demonstrated in an experiment using perfused mesenteric vessels (Nuki *et al*., 1993).

With regard to the distribution of AM, the adrenal gland prominently expresses the *AM* mRNA, as expected from the origin of the tissue in which this peptide was discovered. However, besides the adrenal gland, considerable mRNA expression has been recognized in much larger organs such as the heart, kidney, and lung (Kitamura *et al*., 1993b). Moreover, a significant concentration of AM has been identified in human plasma by means of specific radioimmunoassay, coupled with liquid chromatography (Kitamura *et al*., 1993a, 1994). These findings suggest that the AM may be a new circulating hormone participating in regulation of the cardiovascular system.

During normal pregnancy, physiological adaptations occur in the mother which assure an adequate blood supply to the fetus. Vascular resistance, mean arterial pressure, and sensitivity to endogenous constrictors are reduced, and cardiac output, heart rate, and blood volume are increased. The mechanisms responsible for maintaining low vascular resistance are still unknown, although several locally produced vasoactive agents have been implicated in regulation of the feto–placental circulation. Atrial natriuretic peptide (ANP) designates a family of peptide hormones secreted by specialized cells in atrial myocytes in a variety of species. These factors have been shown to be potent natriuretic, diuretic and vasodilatory agents. There is no consensus in the literature as to whether ANP is increased in normal pregnancy and in pre-eclampsia. To clarify a possible role of ANP and AM in the physiology and pathophysiology of pregnancy, we examined plasma concentrations of ANP and AM in women during normal pregnancy and pre-eclampsia.

Materials and methods

Subjects

Ten normal cycling women (five in the follicular phase and five in the luteal phase), 120 women undergoing a normal pregnancy (31

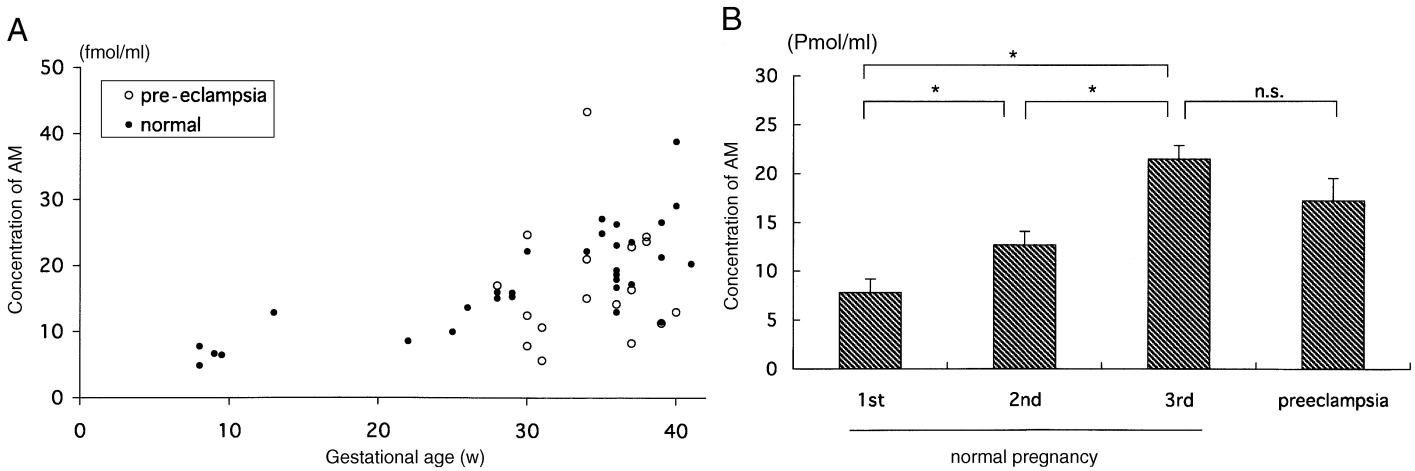

Figure 1. (**A**) Concentrations of adrenomedullin (AM) in plasma of normal pregnant patients and pregnant patients with pre-eclampsia. (**B**) Bars represent mean ± SE. *$P < 0.05$.

for AM) and 16 pregant women with pre-eclampsia were recruited for the study. The normal cycling women had documented cycle lengths of 26–32 days. Pre-eclampsia is diagnosed based on the development of hypertension (blood pressure ⩾140/90 mm Hg) plus proteinuria (300 mg or more of urinary protein per 24 h), pathological oedema that is generalized and overt, or both. None of our patients had a history of any previous hypertensive or medical disorder. Blood was drawn from subjects at random between weeks 4 and 40 of gestation during their routine antenatal clinic visits. The post-partum samples were obtained 4 days post-partum. Blood samples, anticoagulated with EDTA and aprotinin, were kept on ice until centrifuged. After centrifugation, the resulting aliquots of plasma were immediately stored at –80°C until assayed. Informed consent was obtained from all human subjects, and this study was approved by the Gunma University School Institutional Review Board.

Measurement of plasma ANP and AM

The plasma AM concentration was measured by specific radioimmunoassay after extraction and purification as previously described (Kitamura *et al*., 1994). Briefly, 2 ml of plasma were applied to a conditioned Sep-Pak C18 cartridge (Millipore Corporation, Waters Chromatography, Milford, MA, USA), and the column was sequentially washed with 5 ml of isotonic saline, 5 ml of 0.1% (vol/vol) trifluoroacetic acid (TFA), and 5 ml of 20% (vol/vol) acetonitrile in 0.1% TFA. Then, the absorbed material was eluted with 4 ml of 50% (vol/vol) acetonitrile, and the eluate was lyophilized. The residue was dissolved in 0.3 ml of 50 mM phosphate buffer (pH 7.4), and was submitted to radioimmunoassay using the radioiodinated AM and antiserum raised against synthetic AM in rabbits.

Plasma ANP was measured using Shiono radioimmunoassay ANP assay kits (Shionogi & Co Ltd, Osaka, Japan) (Yasue *et al*., 1994). This assay system uses two monoclonal antibodies against α-human ANP, one recognizing a carboxy-terminal sequence and the other the ring structure of ANP, and measures α-human ANP by sandwiching it between the two antibodies without extraction of plasma. The minimal detectable quantity of α-human ANP is 5 pg/ml.

Northern blot analysis

Placental tissues were collected from therapeutic Caesarean section materials (38 and 30 weeks of pregnancy). For Northern blot analysis, after the placentas had been removed, and stored in liquid nitrogen, total RNA was extracted by the guanidium thiocyanate method (Chomczynski and Sacchi, 1987). The final RNA pellet was dissolved in diethyl pyrocabonate-treated H_2O and total RNA was quantified by measuring the absorbance of samples at 260 nm. For Northern blot analysis, 15 μg total RNA from each tissue sample was separated by electrophoresis on denaturing agarose gels and subsequently transferred to a nylon membrane (Biodyne; ICN, Glen Cove, NY, USA). Northern blots were hybridized at 68°C with digoxigenin-labelled cRNA probes. Under the standard protocol for the nucleic acid detection kit (Boehringer Mannheim, Indianapolis, IN, USA), membranes were then exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY, USA). Human *AM* cDNAs were subcloned into the Bluescript KS (+) vector and linearized with *Eco*RI. Digoxigenin-labelled *AM* cRNA probes were produced from an 858 bp cRNA by in-vitro transcription with T7 RNA polymerase and the RNA labelling kit (Boehringer Mannheim). A digoxigenin-labelled glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cRNA probe was obtained by the same method.

Statistical analysis

Data are expressed as means ± SE. Comparisons between groups were performed by one-way analysis of variance (ANOVA). The significance of differences between the mean values of the control group and each hypertension group were tested with Duncan's multiple comparison test. $P < 0.05$ was considered to be statistically significant.

Results

The concentrations of plasma AM in normal cycling women were measured, and concentrations in follicular ($n = 5$) and luteal phases ($n = 5$) were not significantly different (6.4 ± 0.61 fmol/ml versus 6.0 ± 0.49 fmol/ml). The concentration of plasma AM during pregnancy is illustrated in Figure 1A. A gradual increase in plasma AM was observed as pregnancy progressed. During weeks 4–16 of pregnancy (first trimester, $n = 5$), the concentration of plasma AM was 6.5 ± 0.8 fmol/ml and not significantly different from that of non-pregnant women, then increased to 12.7 ± 1.4 fmol/ml during weeks 17–28 of pregnancy (second trimester, $n = 5$), to 21.5 ± 1.4 fmol/ml during weeks 29–40 (third trimester, $n = 21$). The second- and third-trimester AM concentrations in pregnant women were higher than those of non-pregnant women. The plasma AM concentrations of the third trimester was significantly elevated in comparison with the first and second trimester ($P < 0.05$). In the third trimester,

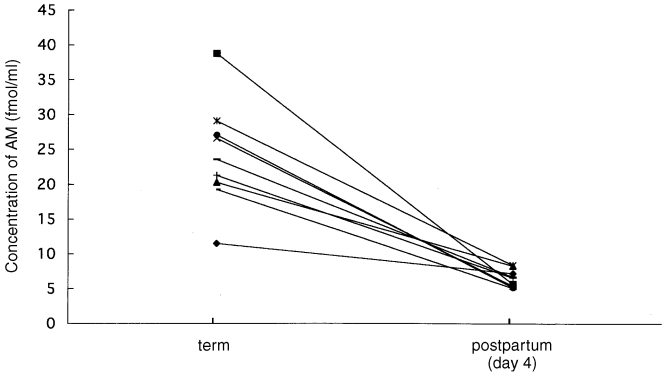

Figure 2. Changes in plasma adrenomedullin (AM) concentrations at term and 4 days post-partum. Symbols represent the nine different patients.

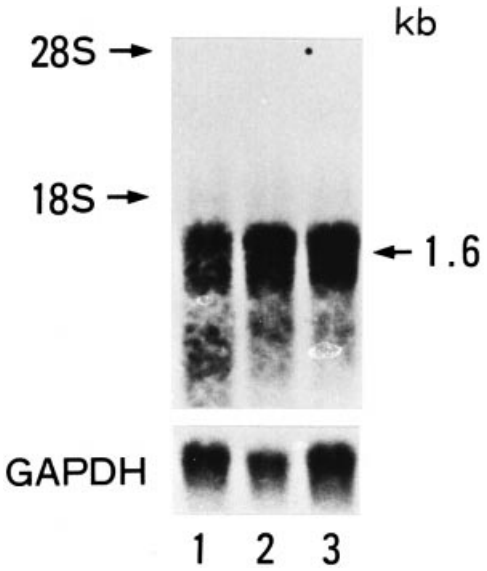

Figure 3. Northern blot analysis for adrenomedullin (*AM*) mRNA in human placenta (lanes 1 and 2, normal 38 week pregnancy; lane 3, 30 week pregnancy with pre-eclampsia). The blots were also probed for using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The Northern blot is representative of three sets of other experiments.

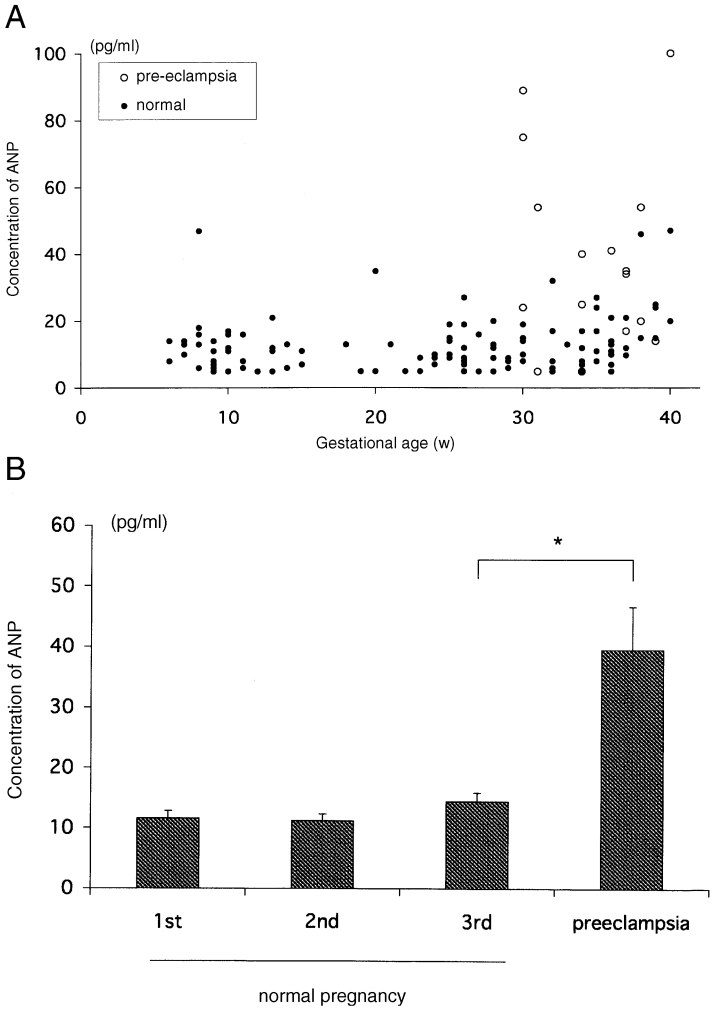

Figure 4. (**A**) Concentrations of atrial natriuretic peptide (ANP) in plasma of normal pregnant patients and pregnant patients with pre-eclampsia. (**B**) Bars represent mean ± SE. *$P < 0.05$.

plasma AM concentrations did not differ significantly between pre−eclampsia (weeks of 28–40 of pregnancy) (17.2 ± 2.3 fmol/ml) and normal pregnancy (Figure 1B).

In nine patients whose plasma samples were obtained serially, plasma concentrations of AM at term (25.3 ± 4.5 fmol/ml) declined significantly to 6.4 ± 0.6 fmol/ml post-partum ($P < 0.05$) (4 days after delivery) (Figure 2).

Northern blot analysis showed placentas from normal pregnant women and women with pre-eclampsia expressed *AM* mRNA and the band was ~1.6 kb in size (Figure 3). The concentrations of ANP during pregnancy are shown in Figure 4A. The concentrations remained constant until term; 12.6 ± 1.5 pg/ml in the non-pregnant state, 11.6 ± 1.2 pg/ml ($n = 36$) in the first trimester, 11.2 ± 1.1 pg/ml ($n = 35$) in the second trimester and 14.4 ± 1.4 pg/ml ($n = 49$) in the third trimester. In the third trimester, maternal plasma ANP concentrations from women with pre-eclampsia (39.5 ± 7.1 pg/ml) ($n = 16$) were significantly higher than those of normal women ($P < 0.05$) (Figure 4B).

Discussion

AM is a peptide, first isolated from human pheochromocytoma, which elicits long-lasting vasorelaxation. Endothelial cells also produce AM and patients with hypertension show higher concentrations of plasma AM than normotensive controls, suggesting that AM participates in the physiological regulation of blood pressure and vascular homeostasis (Kitamura *et al*., 1994).

The physiological and pathological role of AM in human pregnancies is not yet fully understood. Reports from other laboratories have suggested that pregnant women have higher concentrations of AM than non-pregnant control subjects (Di Iorio *et al*., 1997; Hata *et al*., 1997). We observed a gradual increase in plasma AM concentrations as pregnancy advanced, and these data confirm and extend previous observations. It has been reported that AM is present in large amounts in amniotic fluid and cord blood (Macri *et al*., 1996), in addition, we have detected the expression of *AM* mRNA in the placentas

from women undergoing a normal pregnancy and those with pre-eclampsia. Although it has been reported that AM remained high in their patients after delivery, there is a significant decrease in plasma AM at 4 days post-partum compared with the concentration at term in our experiment (Hata *et al*., 1997). Taken together, our results suggest that feto–placental tissues contribute to AM production during pregnancy.

Recently, using a subtracted cDNA library derived from undifferentiated and differentiating cytotrophoblast, *AM* mRNA was identified as a novel gene in placenta (Morrish *et al*., 1996). These data showed the *AM* gene to be among those that may have regulatory functions in trophoblast differentiation. Since the placenta produces multiple hormones, one role of AM in the placenta may be to influence the differentiation of trophocytoblasts and thereby affect the secretion of these factors by the placenta. Since the AM concentration in pregnancy appears to rise progressively, augmentation of plasma AM might be relevant to physiological adaptations in the mother which assure an adequate blood supply to the fetus. The mechanisms responsible for maintaining low vascular resistance are still unknown, although AM has been suggested to be one of the vasoactive agents regulating the feto–placental circulation. Further research is needed to elucidate the physiological and pathophysiological roles of this peptide in pregnancy.

In a previous report, while maternal AM concentrations did not differ significantly between women with pre-eclampsia and normal pregnant women, when AM concentrations were increased in amniotic fluid and umbilical vein blood collected from pregnant women with pre-eclampsia (Di Iorio *et al*., 1998). These findings confirm local production of AM and suggest that it may modulate feto–placental haemodynamics through a paracrine mechanism interacting with other vasoactive agents in physiological and pathological states during pregnancy, such as pre-eclampsia.

We have found that maternal plasma ANP concentrations were significantly higher in women with pre-eclampsia than in normal control subjects. These results confirm previous observations (Hatjis *et al*., 1989). ANP is released by the atria and has diuretic, natriuretic, and vasodilator actions. The present study demonstrates that plasma ANP concentrations are elevated in women with pre-eclampsia, but the plasma AM concentrations are not significantly different from the same women with pre-eclampsia in the third trimester. The increase of ANP in hypertension were considered to act as a feedback mechanism increasing sodium excretion and blunting blood pressure elevation. Further investigations are required to elucidate the physiological and pathophysiological role of AM and ANP in pregnancy.

Acknowledgements

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