Impaired Guanosine 3′,5′-Cyclic Phosphate Production in Severe Pregnancy-Induced Hypertension with High Plasma Levels of Atrial and Brain Natriuretic Peptides

Hiroaki ITOH, Norimasa SAGAWA, Hidetaka NANNO, Takahide MORI, Masashi MUKOYAMA*, Hiroshi ITOH*, AND Kazuwa NAKAO*

*Department of Gynecology and Obstetrics, *Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-01, Japan*

Abstract. To investigate the activation of particulate guanylate cyclase in pregnant women with pregnancy-induced hypertension (PIH), plasma levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and guanosine 3′,5′-cyclic phosphate (cGMP) were measured by radioimmunoassays specific to each substance. Ten normal nonpregnant women, and 30 normal pregnant women, 17 pregnant women with mild PIH and 11 pregnant women with severe PIH in the third trimester were included in this retrospective observational study. The diagnosis and classification of hypertension were carried out according to the technical bulletin (No. 91) of the American College of Obstetricians and Gynecologists. In the pregnant women with mild PIH, plasma cGMP levels as well as ANP and BNP levels were significantly ($P<0.05$) higher than those in gestational age-matched normal pregnant or nonpregnant women. But in the pregnant women with severe PIH, plasma cGMP levels were significantly lower than those in pregnant women with mild PIH ($P<0.05$), although plasma ANP and BNP levels were higher than those in pregnant women with mild PIH.

Key words: Atrial natriuretic peptide, Brain natriuretic peptide, Guanosine 3′,5′-cyclic phosphate, Pregnancy-induced hypertension

(*Endocrine Journal* **44**: 389–393, 1997)

THE PLASMA levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), isolated from human cardiac tissue as a natriuretic peptide [1], have been reported to be increased in women with pregnancy-induced hypertension (PIH) [2]. The increases in plasma ANP and BNP levels correlated well with the mean blood pressure [2], suggesting their possible importance in the regulation of circulation in pregnant women.

Natriuretic peptides exert their biological activities through specific natriuretic peptide receptors which have a guanylate cyclase in the intracellular domain and generate guanosine 3′,5′-cyclic phosphate (cGMP) as a second messenger [3, 4]. The plasma cGMP level has been reported to reflect, at least partly, the activation of particulate guanylate cyclase in the vascular wall by ANP and BNP [5]. In the present study, to investigate the activation of the vascular guanylate cyclase system in pregnant women with PIH, we measured plasma cGMP levels as well as ANP and BNP levels in normal pregnant women and in those with PIH complication.

Subjects and Methods

The diagnosis and classification of PIH were made according to the technical bulletin of the American College of Obstetricians and Gynecologists [6]. PIH was classified as mild when the blood pressure was 140/90 mmHg or greater, but less than 160/110 mmHg. Severe PIH was diagnosed when the blood pressure was 160/110 mmHg or greater. All of the pregnant women with severe or mild PIH, investigated in the present study, were complicated with proteinuria. Gestational age-matched normotensive pregnant women (29–37 weeks of gestation) were used as normotensive controls for mild PIH and severe PIH. None of the normal subjects had any history of hypertension, or of renal or cardiovascular diseases. The means and SEM of age and gestational ages of the subjects are listed in Table 1. None of the subjects received any kind of medications other than iron and vitamin B_{12} until the time of collection of blood specimens. In each case, the patient's consent was obtained. Peripheral blood was taken after an overnight fast and 15 min bed rest. Blood samples were immediately transferred to chilled siliconized glass tubes containing aprotinin (1,000 units/m*l*) (Ohkura Pharmaceutical Co., Kyoto, Japan) and ethylenediaminetetraacetic acid disodium salt (EDTA, 1 mg/m*l*), and centrifuged at 1,200 × g for 20 min at 4 °C. The plasma thus obtained was aliquoted and stored at −20 °C until the assay.

Plasma cGMP levels were determined by radioimmunoassay (RIA) as described previously [7] using a commercially available kit (YAMASA cyclic GMP assay kit, Yamasa-Shoyu Co., Chiba, Japan). Inter- and intraassay variations of this system were less than 10%.

Plasma ANP levels were determined by a specific RIA as described previously [1, 2]. Plasma extraction was carried out with a Sep-Pak C18 cartridge (Waters Associates, Milford, MS). The sensitivity of this assay was 0.3 fmol/tube and the 50% inhibitory concentration was 3 fmol/tube. The crossreactivity of this assay with human BNP was less than 0.01%. Inter- and intraassay variations were less than 10%.

Plasma BNP levels were determined by a specific RIA as described previously [1, 2]. Plasma extraction was carried out with a Sep-Pak C18 cartridge. [Tyr^{82}]BNP(83–108) was radioiodinated by the chrolamin T method. Standard BNP or sample was incubated with the monoclonal antibody KY-hBNP-I for 24 h at 4 °C, and [^{125}I][Tyr^{82}]BNP(83–108) was added followed by incubation for an additional 24 h at 4 °C. The free ligand was separated from the antibody-bound ligand by dextran-coated charcoal. The sensitivity of this assay was 0.3 fmol/tube and the 50% inhibitory concentration was 3 fmol/tube. The crossreactivity of this assay with human ANP was less than 0.05%. Inter- and intraassay variations were less than 10%.

Data were expressed as the means ± SEM. Statistical analyses were performed by Student's *t*-test or analysis of variance (ANOVA) followed by Fisher's protected least significant difference test for comparisons of the means of more than two groups. Linear regression analysis was performed with a least squares method.

Table 1. Plasma levels of cGMP, ANP and BNP in normal nonpregnant women, normotensive pregnant women, and pregnant women with mild and severe PIH

group of patients (n)	Age (years)	Gestational age (weeks)	cGMP level (nM)	ANP level (pM)	BNP level (pM)
nonpregnant women (10)	24.5 ± 6.0		1.5 ± 0.4	6.3 ± 1.0	1.8 ± 0.4
normotensive pregnant women (30)	29.5 ± 1.5	33.2 ± 0.5	2.4 ± 0.2	12.1 ± 0.9	3.0 ± 0.5
hypertensive pregnant women					
mild PIH (17)	33.4 ± 2.1	34.9 ± 0.6	3.2 ± 0.3[a]	21.8 ± 3.3[a]	10.3 ± 2.3[a]
severe PIH (11)	32.1 ± 2.7	34.4 ± 1.1	2.0 ± 0.2[b]	33.3 ± 7.7[a,b]	19.9 ± 4.8[a,b]

Values are the means ± SEM. Values with superscript a are significantly different from those of normotensive pregnant women (*P*<0.05). Values with superscript b are significantly different from those of pregnant women with mild PIH (*P*<0.05). ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, guanosine 3′,5′-cyclic phosphate; PIH, pregnancy-induced hypertension.

Results

As shown in Table 1, the plasma cGMP level in normotensive pregnant women at 29–37 weeks gestation was slightly higher than that in normal nonpregnant women, but the difference was not statistically significant. The plasma cGMP level in pregnant women with mild PIH was significantly ($P<0.05$) higher than that in gestational age-matched normotensive pregnant women. In contrast, the plasma cGMP level in pregnant women with severe PIH was significantly ($P<0.05$) lower than that in women with mild PIH, and was not different from that in gestational age-matched normotensive pregnant women. On the other hand, in pregnant women with mild PIH, plasma ANP and BNP levels were significantly higher than those in gestational age-matched normotensive pregnant women ($P<0.05$). The mean plasma ANP and BNP levels in pregnant women with severe PIH were further increased to 33.3 and 19.9 pM, respectively. As shown in Fig. 1A, the plasma cGMP levels in normotensive pregnant women correlated weakly with plasma BNP levels, but not with ANP levels. In women with mild PIH, the plasma cGMP levels correlated well with plasma ANP and BNP levels (Fig. 1B), but in women with severe PIH, there was no significant correlation between cGMP levels and natriuretic peptide levels (Fig. 1C).

Discussion

The present study showed that the plasma cGMP level in the pregnant women with mild PIH was significantly higher than that in gestational age-matched normotensive pregnant or nonpregnant women, but the plasma cGMP level in pregnant women complicated with severe PIH was significantly lower than that in women with mild PIH, and was similar to that in the gestational age-matched normotensive pregnant women. In pregnant women with mild PIH, plasma cGMP levels showed a significant correlation with the plasma levels of ANP and BNP.

Plasma cGMP is known to be generated from GTP by the particulate guanylate cyclase activated by natriuretic peptides, or by the cytosolic soluble
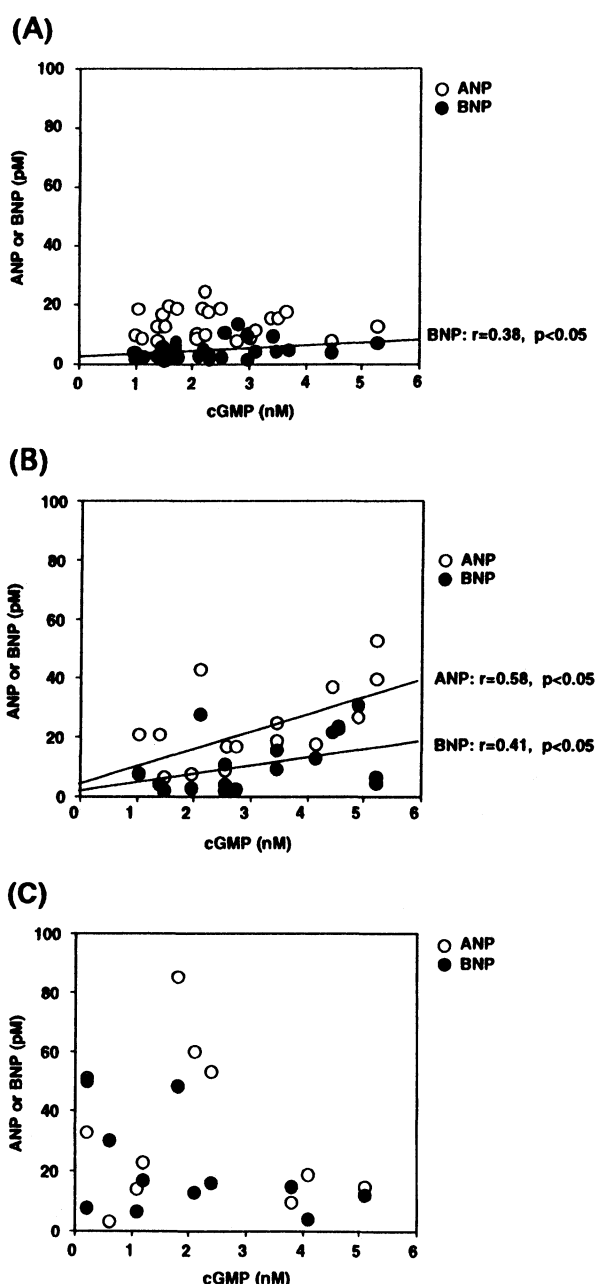

Fig. 1. Correlation between plasma cGMP levels and plasma ANP and BNP levels in normotensive pregnant women (A), in pregnant women with mild PIH (B), and in pregnant women with severe PIH (C). (A): The coefficient (r) of correlation between plasma cGMP levels and plasma BNP levels was 0.38 ($P<0.05$). (B): Coefficients (r) of correlation between plasma cGMP levels and plasma ANP and BNP levels were 0.58 ($P<0.05$) and 0.41 ($P<0.05$), respectively. (C): Correlations between plasma cGMP levels and plasma natriuretic peptide levels were not significant. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, guanosine 3',5'-cyclic phosphate; PIH, pregnancy-induced hypertension.

guanylate cyclase activated by endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO) [4]. When ANP or BNP was administered to patients with cardiovascular disorders, a rise in the plasma cGMP level was noted together with a reduction in blood pressure and an increase in urine output [8, 9]. Moreover, in rats, plasma cGMP levels, increased by intravenous administration of rat ANP (rANP), were attenuated by preadministration of anti-rANP monoclonal antibody [10]. In normotensive pregnant women, the plasma cGMP level correlated only with the plasma BNP level. This may be related to the fact that plasma ANP and BNP levels in these patients were lower than hypertensive women and that in such a condition with low plasma ANP and BNP levels, the basal plasma cGMP level may be influenced by cGMP generated from the nitric oxide - soluble guanylate cyclase pathway. Since plasma ANP and BNP levels were high in women with mild PIH, the high plasma cGMP in the women with mild PIH may be a result, at least in part, of the action of ANP and BNP. Such activation of the cGMP system may result in vasodilation or diuresis and compensate for the hypertension in PIH women.

In pregnant women with severe PIH, however, plasma cGMP levels did not correlate with plasma ANP or BNP levels, and was significantly lower than that in mild PIH ($P<0.05$). Thus in women with severe PIH, plasma cGMP levels were dissociated from plasma ANP and BNP levels, suggesting the impairment of particulate guanylate cyclase activity in the vascular wall in severe PIH. The precise mechanism of the blunted activity of guanylate cyclase could not be determined from the results of the present study. Since down-regulation of natriuretic peptide clearance receptor was reported [11], it is possible that the concentration of particulate guanylate cyclase, the intracellular domain of natriuretic peptide receptor, may be reduced similarly to clearance receptor in these pregnant women with severe PIH. This possibility is currently under investigation in our laboratory.

Another source of cGMP is soluble guanylate cyclase, which is activated by the NO from endothelial cells [4, 12]. It is difficult to distinguish cGMP generated by the action of soluble guanylate cyclase from that generated by the particulate guanylate cyclase *in vivo*. It is also possible that the low cGMP level in severe PIH is a consequence of impaired production of NO in the endothelial cells, or that the soluble guanylate cyclase activity in the vascular wall is impaired in severe PIH, but Cameron *et al.* [13] reported that the urinary excretion of NO_x, which is thought to be converted from NO and represents the systemic production of NO, was similar in hypertensive and normotensive pregnant women, suggesting that NO synthesis occurs at similar levels in both groups. Nevertheless, the possibility still remains that the soluble guanylate cyclase activity in the vascular wall is impaired in severe PIH. Further investigation is necessary to elucidate the involvement of the change in soluble guanylate cyclase activity in the pathophysiology of PIH.

The present study demonstrated the activation of guanylate cyclase in pregnant women with mild PIH and impairment of its activity in severe PIH. Such a change of the guanylate cyclase activity during the course of cardiovascular disease has also been suggested in patients with chronic severe heart failure [14, 15] and in chronically hypertensive dogs [16]. At present, the exact sites and mechanisms of the impairment of vascular guanylate cyclase in pregnant women with severe PIH are unknown, but such a failure of cGMP production may become an additional factor in the aggravation of the pathophysiology of PIH.

Acknowledgements

References

1. Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki M, Obata K,

Yasue H, Kambayashi Y, Inouye K, Imura H (1991) Brain natriuretic peptide (BNP) as a novel cardiac

hormone in humans. —Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide—. *J Clin Invest* 87: 1402–1412.

2. Itoh H, Sagawa N, Mori T, Mukoyama M, Nakao K, Imura H (1993) Plasma brain natriuretic peptide level in pregnant women with pregnancy-induced hypertension. *Obstet Gynecol* 82: 71–77.

3. Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K, Imura H (1992) Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinol* 130: 229–239.

4. Wong SKF, Garbers DL (1993) Receptor guanylyl cyclases. *J Clin Invest* 90: 299–305.

5. Gerzer R, Weil J, Strom T, Muller T (1986) Mechanisms of action of atrial natriuretic factor: Clinical consequences. *Klinisch Wochenschrift* 64: 21–26.

6. American College of Obstetricians and Gynecologists (1986) Management of preeclampsia, Technical Bulletin, no. 91. American College of Obstetricians and Gynecologists. Washington, D.C.

7. Steiner AL, Parker CW, Kipnis DW (1972) Preparation of antibodies and iodinated cyclic nucleotides. *J Biol Chem* 247: 1106–1113.

8. Saito Y, Nakao K, Nishimura K, Sugawara A, Okumura K, Obata K, Sonoda R, Ban T, Yasue H, Imura H (1987) Clinical application of atrial natriuretic polypeptide in patients with congestive heart failure: Beneficial effects on left ventricular function. *Circulation* 76: 115–124.

9. Yoshimura M, Yasue H, Morita E, Sakaino N, Jougasaki M, Kurose M, Mukoyama M, Saito Y, Nakao K, Imura H (1991) Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure.

Circulation 84: 1581–1588.

10. Itoh H, Nakao K, Mukoyama M, Yamada T, Hosoda K, Shirakami G, Morii N, Sugawara A, Saito Y, Shiono S, Arai H, Yoshida I, Imura H (1989) Chronic blockade of endogenous atrial natriuretic polypeptide (ANP) by monoclonal antibody against ANP accelerates the development of hypertension in spontaneously hypertensive and DOCA-salt hypertensive rats. *J Clin Invest* 84: 145–154.

11. Kishimoto I, Nakao K, Suga S, Hosoda K, Yoshimasa T, Itoh H, Imura H (1993) Down-regulation of clearance receptor by natriuretic peptides via ANP-B receptor in vascular smooth muscle cells. *Am J Physiol* 265: H1373–H1379.

12. Palmer RMJ, Ferridge AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature (Lond)* 327: 524–526.

13. Cameron IT, van Papendorp CL, Palmer RMJ, Smith SK, Moncada S (1993) Relationship between nitric oxide synthesis and increase in systolic blood pressure in women with hypertension in pregnancy. *Hypertension in Pregnancy* 12: 85–92.

14. Tsutamoto T, Kanamori T, Morigami N, Sugimoto Y, Yamaoka O, Kinoshita M (1993) Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. *Circulation* 87: 70–75.

15. Smits P, Jansen TL, Thien T (1993) Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure [comment]. *Circulation* 88: 811–813.

16. Seymour AA, Assad MM, Sheldon JH, Smith PL, Rogers WL (1995) Atrial natriuretic peptide in chronically hypertensive dogs. *Hypertension* 26: 634–641.