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学 位 論 文

Endothelin secretion in the women with pregnancy
induced hypertension (PIH)

妊娠高血圧症妊婦における血中エンドセリン動態

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Key words : Endothelin, Pregnancy induced hypertension.

Abstract

Endothelin (ET) is a peptide secreted by the vascular endothelial cells and has a strong vasopressor effect. In order to study the pathophysiological significance of ET secretion in hypertension in pregnancy, plasma ET concentrations in the women with pregnancy induced hypertension (PIH) were measured using high sensitive sandwich enzyme immunoassay (Suzuki et al. 1989b). Plasma ET concentrations (pg/ml, $M \pm SD$) were 0.96 ± 0.31 ($n=10$) in normotensive non-pregnant women, and 0.79 ± 0.43 ($n=19$), 0.84 ± 0.33 ($n=25$) and 0.81 ± 0.39 ($n=46$) in the 1st, 2nd and 3rd trimester of normal pregnant women, respectively. In the women with PIH, they were 1.01 ± 0.34 ($n=7$) and 1.67 ± 0.48 ($n=11$) in mild and in severe cases, respectively. The concentrations in the severe cases showed significantly high level in comparison with those in the 3rd trimester of normal pregnant women ($p < 0.001$). Significant positive correlation between plasma ET concentrations and systolic or diastolic blood pressure was observed ($p < 0.001$ and $p < 0.02$, respectively) in cases with normal and PIH pregnancy. In normal pregnant women, plasma ET concentration is somewhat lower than that of normal nonpregnant cases, and it does not show any significant changes according to the course of pregnancy. In PIH women, a mechanism is suspected that promotes secretion of ET resulting in hypertension due to strong contraction of peripheral vessels.

Introduction

Yanagisawa et al demonstrated that vascular endothelial cells secreted a peptide "endothelin", which had a strong vasopressor effect (YANAGISAWA et al. 1989). From the animal experiments, it is reported that endothelin (ET) activates the voltage-dependent Ca ion channels directly or indirectly(GOTO et al. 1989) and causes the stronger and durable vasoconstriction compared with other vasopressor substances ever known, and this effect is very unlikely to be washed out (SAIDA et al. 1989). Also in clinical study, plasma ET concentration is higher in the cases with hemodialysis(TOTSUNE et al. 1989). These results suggest that ET plays an important role to onset or maintenance of hypertension in pregnancy.

Consequently, we measured plasma ET concentrations in the women with pregnancy induced hypertension (PIH) in order to study the significance of ET secretion in the pathologic condition with hypertension in pregnancy.

Subjects and methods

The subjects included 10 normal non-pregnant cases, 90 normal pregnant cases, and 18 pure type PIH cases (7 mild cases and 11 severe cases) at the third trimester of pregnancy. Classifications of PIH(Japan Society of Obstetrics and Gynecology, 1985)is as follows; patients with mild PIH have a diastolic blood pressure between 90-109 mmHg and/or 15 mmHg or more increase of diastolic blood pressure in pregnancy compared with non-pregnant state. These patients have a systolic blood pressure between 140-159 mmHg and/or 30 mmHg or more increase of systolic blood pressure in pregnancy

compared with non-pregnant state. Patients with severe PIH have a diastolic blood pressure greater than 110 mmHg and/or a systolic blood pressure greater than 160 mmHg. After 60 minutes rest, blood pressure was measured, and then blood sample was collected from the antecubital vein in all cases.

After the blood sample was taken into a polypropylene tube contained 2 mg/ml EDTA and 300 KIU/ml aprotinin, plasma was separated immediately by ultracentrifugation, and it was stored at -70°C until ET assay.

Before the estimation of plasma concentration of ET, ET was extracted from this plasma using the Sep-pak C-18 cartridge (Figure 1) and in this extraction method value of recovery rates was 50~60%. The concentrations of extracted ET samples were measured by the sandwich EIA method (SUZUKI et al. 1988, 1989b), and ET values measured by ET-EIA were revised finally according to the recovery rate.

Results

Plasma concentration of ET (pg/ml, $M \pm SD$) was 0.96 ± 0.31 in 10 normal non-pregnant women, 0.79 ± 0.43 in 19 cases at first trimester of normal pregnancy, 0.84 ± 0.33 in 25 cases at second trimester of normal pregnancy and 0.81 ± 0.39 in 46 cases at third trimester of normal pregnancy (Figure 2). On the other hand, plasma concentration of ET in women with PIH pregnancy was 1.01 ± 0.34 in mild cases and 1.67 ± 0.48 in severe cases (Figure 3). The concentration in the severe cases showed significantly high level in comparison with those in normal pregnant women ($p < 0.001$).

Significant positive correlationship between plasma ET concentrations and systolic or diastolic blood pressure was observed ($p<0.001$, $r=0.49$, and $p<0.02$, $r=0.33$, respectively) in cases with normal and PIH pregnancy(Figure 4 and Figure 5).

Discussion

In normal pregnant women, plasma ET concentration is somewhat lower than that of normal non-pregnant cases, and it dose not show any significant changes according to the course of pregnancy. It can be said to be a phenomenon that the secretion of ET as a strong vasopressor is suppressed in order to maintain the homeostasis in blood pressure in spite of the remarkable hemodynamic changes during normal pregnancy.

On the other hand, in PIH pregnant women, plasma ET concentration was significantly higher than that of normal pregnancy. In PIH women, a mechanism may be suspected that promotes secretion of ET resulting in hypertension due to strong contraction of peripheral vessels. It is well known that there is an ET family consisting of ET-1, ET-2, ET-3 and bigET (bET) (NUCCI et al. 1988; SAITO et al. 1989; SUZUKI et al. 1989a). Thus, it is assumed that the high level of plasma concentration of ET observed in PIH women may be a result of simultaneous measurement of the ET family. However, it has been confirmed that the sandwich EIA assay method used in this study is a system using an antibody recognizing the N terminal and an antibody of the HRP-marked C terminal, which does not measure ET-3 and bET(SUZUKI et al. 1989a). While it has been reported that ET-2 is contained in tissue, it is suspected that ET-

2 does not exist in blood. Accordingly, the high level of plasma ET in PIH women was caused by the increase of ET-1.

Studies on gene expression of ET showed that ET is produced in situ in aortic endothelial cells, placenta and renal cells(KOSAKA et al. 1989), as well as in cultured endothelial cells aorta. It is also reported that ET secretion increases when endothelial cells are impaired, and appearance of mRNA of ET is induced by thrombin, TGF-B, Ca^{++} ionophore and TPA. Therefore, it is indicated that the acceleration of impairment of endothelial cells or abnormalities of blood coagulation system may be present in systemic blood vessels and kidneys of PIH women, resulting in increase of secretion of ET.

In vivo and in vitro experiments, it was reported that ET induced the secretion of thromboxane A_2 and prostacyclin(NUCCI et al. 1988), norepinephrine release and aldosterone synthesis(GOETZ et al. 1988), hANP secretion (FUKUDA et al. 1988), but suppressed renin release(TAKAGI et al. 1988). Therefore, increased ET may lead to further promotion of hypertension through the indirect actions of the pressor and depressor system.

Acknowledgments

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Legends for figures

- Figure1 Method for extraction of endothelin from plasma
- Figure2 Mean levels and standard deviation of plasma immunoreactive endothelin-1 in healthy nonpregnant women (nonP, n=10) and normal pregnant women in each trimester (1st, n=19; 2nd, n=25; 3rd n=46)
n.s. : not significant
- Figure3 Mean levels and standard deviation of plasma immunoreactive endothelin-1 in pregnancy induced hypertension: mild, mild PIH (n=7); severe, severe PIH (n=11) compared with those in nonpregnant women (nonP) and normal pregnant women in the 3rd trimester (3rd)
* : $p < 0.005$, ** : $p < 0.001$, n.s. : not significant
- Figure4 The relationship of plasma endothelin-1 levels to the systolic blood pressure in normal and PIH pregnant women. There is a significant positive correlation (r=0.489, $p < 0.001$) between them.
- Figure5 The relationship of plasma endothelin-1 levels to the diastolic blood pressure in normal and PIH pregnant women. There is a significant positive correlation (r=0.333, $p < 0.02$) between them.

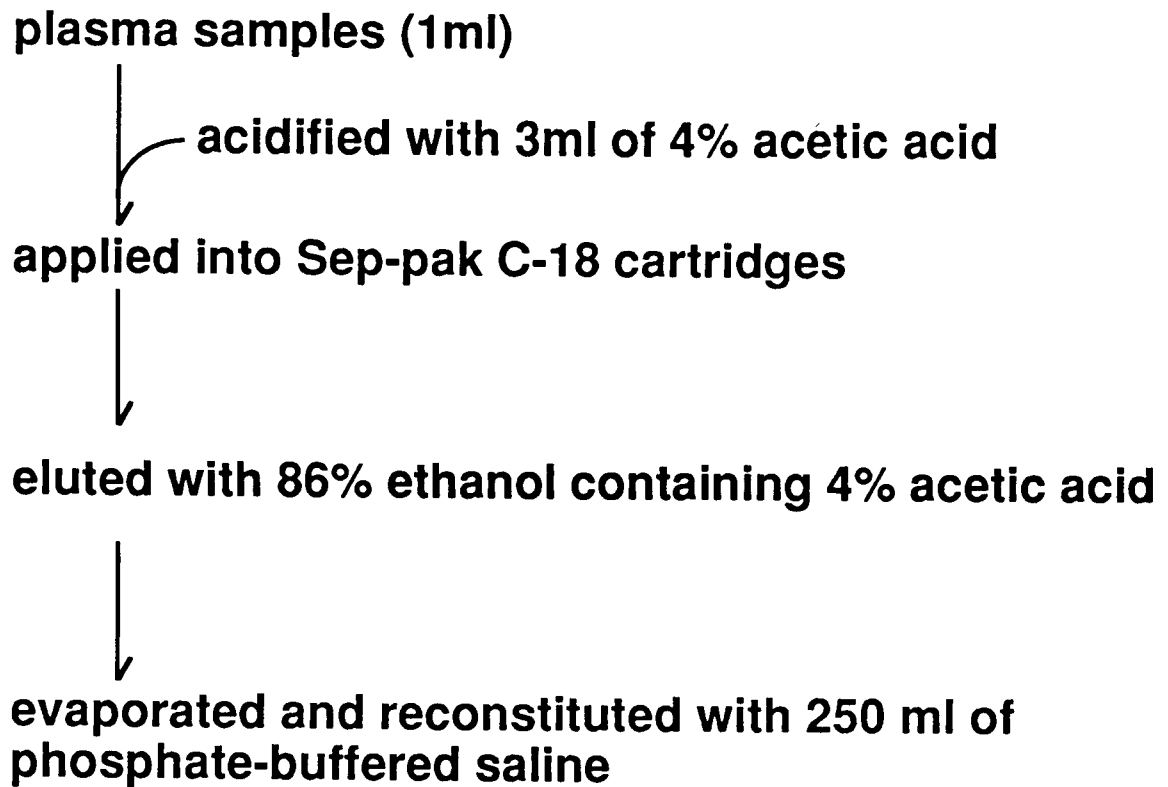


Figure1 Method for extraction of endothelin from plasma

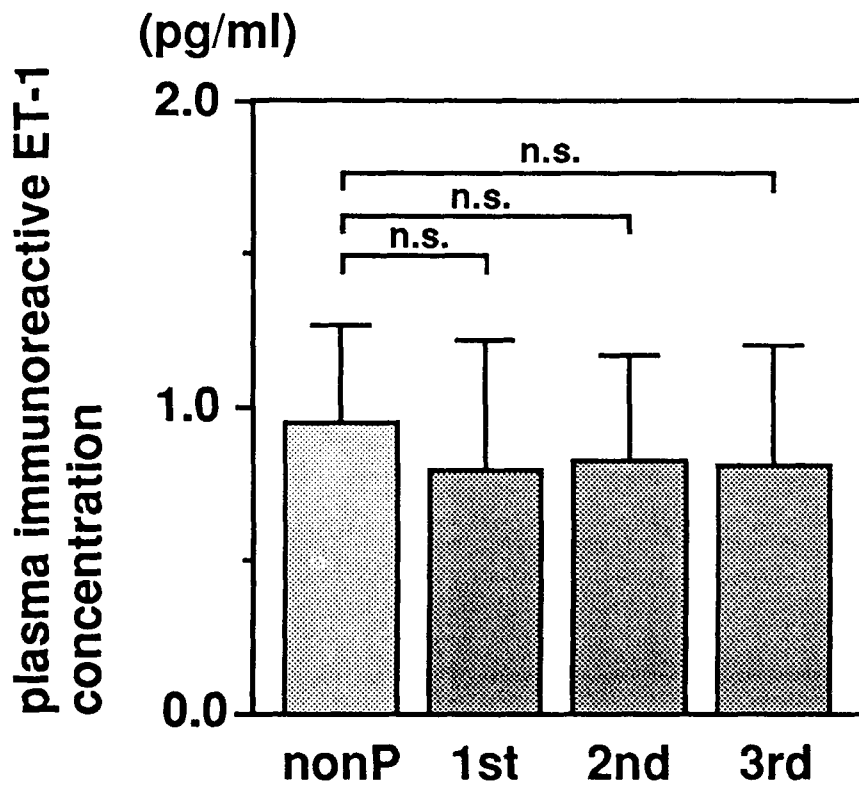


Figure2 Mean levels and standard deviation of plasma immunoreactive endothelin-1 in healthy nonpregnant women (nonP; n=10), normal pregnant women in each trimester (1st, n=19; 2nd, n=25; 3rd, n=46)
n.s., not significant

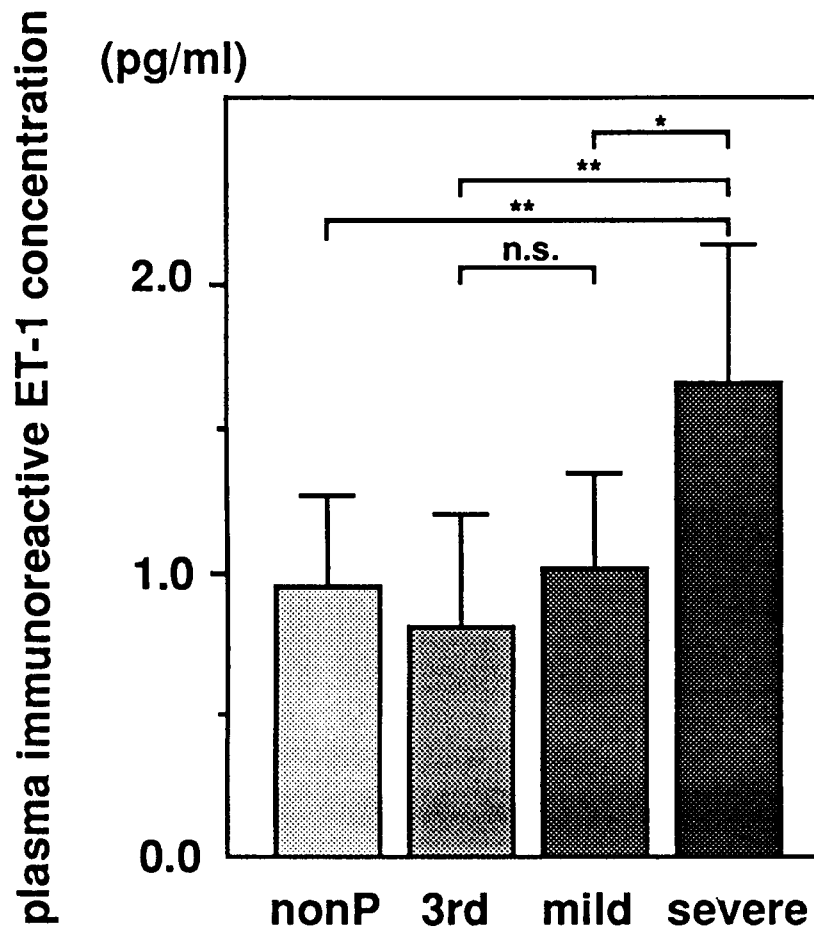


Figure3 Mean levels and standard deviation of plasma immunoreactive endothelin-1 in pregnancy-induced hypertension: mild, mild PIH (n=7); severe, severe PIH (n=11) compared with those in non pregnant women (nonP) and normal pregnant women in the 3rd trimester (3rd)
 *, $p < 0.005$; **, $p < 0.001$; n.s., not significant

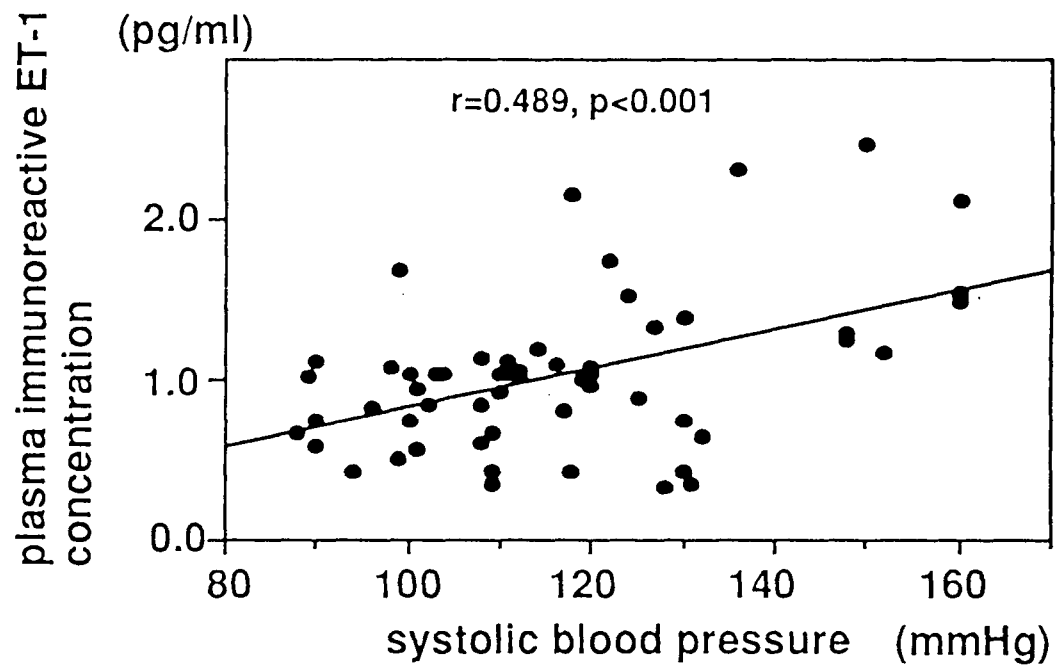


Figure 4 The relationship of plasma endothelin-1 levels to the systolic blood pressure in normal and PIH pregnant women. There is a significant positive correlations ($r=0.489, p<0.001$) between them.

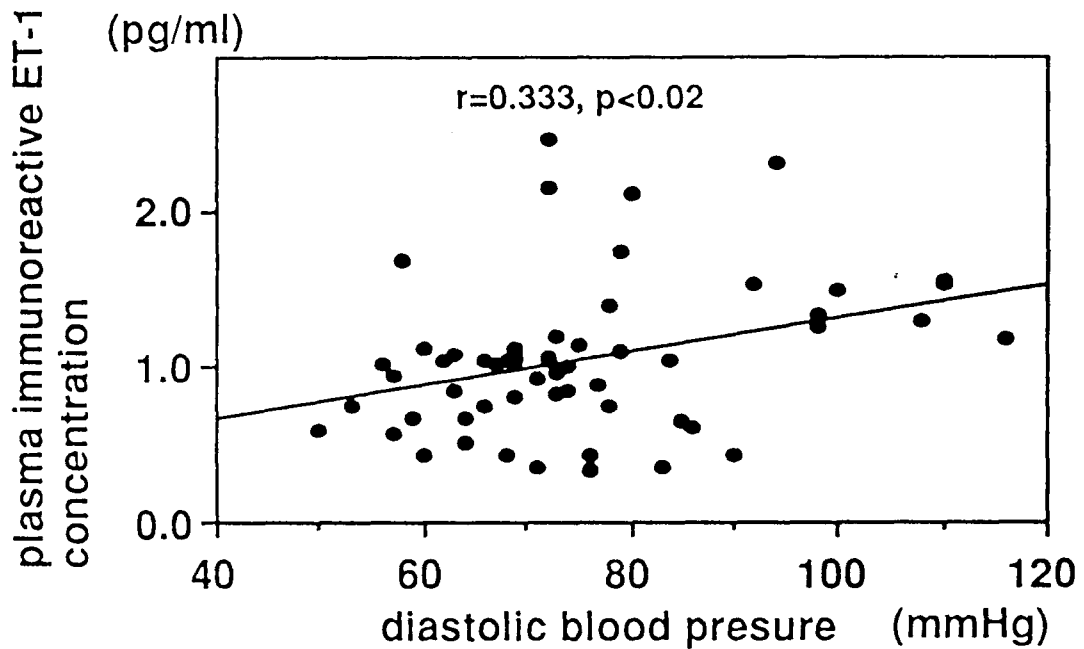


Figure 5 The relationship of plasma endothelin-1 levels to the diastolic blood pressure in normal and PIH pregnant women. There is a significant positive correlation ($r=0.333, p<0.02$) between them.