Study of Immunoreactive 8-Arginine Vasopressin in Human Plasma During Pregnancy and at Delivery : Utilization of a Highly Sensitive Radioimmunoassay with a Reversed Phase C18 Silica Column

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Abstract : *Changes in plasma concentration of immunoreactive(IR) 8-arginine vasopressin (AVP) during pregnancy and at delivery were examined using a highly sensitive radioimmunoassay (RIA). The following results were obtained : (1) The maternal plasma concentrations of IR-AVP gradually decreased during pregnancy and reached a plateau after week 28 up to delivery. From week 28, the plasma concentrations of IR-AVP were significantly lower than those before week 15 (2.4 ± 0.36 pg/ml). Maternal plasma IR-AVP levels at normal vaginal delivery did not differ from those at delivery by elective cesarean section. (2) The umbilical arterial plasma concentrations of IR-AVP at vaginal delivery (390 ± 60.6 pg/ml), which were significantly higher than those of umbilical venous plasma (224 ± 34.1 pg/ml), were markedly higher than at delivery by cesarean section (14.1 ± 9.1 pg/ml). (3) The fact that the concentrations of IR-AVP in the umbilical arterial plasma at delivery, in cases of asphyxia (505 ± 74.1 pg/ml), were significantly higher than those at normal delivery (300 ± 13.3 pg/ml) is noteworthy. These results suggest that : (1) In the mother, AVP acts as an antidiuretic hormone to maintain the homeostasis of physiologically increased total body water and decreased body tonicity, associated with pregnancy. (2) In the fetus, AVP acts as a stress hormone to maintain the homeostasis of cardiovascular and metabolic systems, which are subjected to the influence of labour. (Thai J Obstet Gynaecol 1993;4:89-98.)*

Key words : 8-arginine vasopressin (AVP), pregnancy, normal vaginal delivery, elective cesarean section, asphyxia

8-Arginine vassopressin or antidiuretic hormone is a nonapeptide secreted from the posterior lobe of the pituitary gland, which mainly exerts

an antidiuretic effect through the V2 receptor in low blood concentrations[1], and a vasoconstrictive effect through the V1 receptor at high concentrations in human beings[2].

It is well known that AVP regulates water metabolism and its secretion is also accelerated at the time of stress[3]. In order to evaluate feto-maternal AVP function, we established a highly sensitive radioimmunoassay (RIA) system and measured immunoreactive (IR-) AVP in maternal plasma during pregnancy, when a prominent physiological change occurs in water metabolism, and fetal umbilical plasma at delivery, when a high level of stress would be estimated.

Materials and Methods

Subjects and collection of samples

Blood samples were collected from patients who visited or were admitted to the Osaka University Hospital. They all gave their informed consent to participate in this study. The subjects examined consisted of 4 groups as follows.

Group I : non-pregnant group (12 samples),

Group II : normal pregnancy group (30 samples),

Group III : normal vaginal delivery group (34 samples),

Group III was divided into two further subgroups, which were;

Group III-(a) : non-asphyxia group (27 samples),

Group III-(b) : asphyxia group (7 samples).

Group IV : elective cesarean section group.

In Groups I and II, blood was withdrawn in the sitting position at 9:00-12:00 am. In Groups III and IV, blood samples were obtained 3 minutes after delivery. In the vaginal delivery group III, labour started spontaneously and progressed to vaginal delivery. In the cesarean section group IV, mothers did not eat or drink from 9:00 pm on the day before the operation and received 500 ml of saline intravenously before operation. Spinal anesthesia was used during operation and no severe hypotension was observed. In all cases, blood loss at delivery was less than 500 ml. In the asphyxia group, III-(b), none of the 1 minute Apgar score of newborn infants was more than 7 and cord pH was below 7.20, and in non-asphyxia, III-(a), the 1 minute Apgar score was more than 8 in all cases. In group III-(b), the mean Apgar score was 6.1 ± 0.8, which was significantly lower than that of group, III-(a). There was no significant difference in other parameters, such as, average maternal age, parity, height, weight, blood pressure or neonatal body weight (Table 1).

All samples were mixed with peptidase inhibitor (EDTA-2Na 1.5 mg/ml of blood) in an ice-chilled plastic test tube, the plasma separated and stored at -20°C until extraction.

Statistical comparisons were made by Mann-Whitney U test and the student t-test, and the significance

Table 1 Comparisons of various parameters of umbilical arterial plasma IR-AVP (mean±S.E.M.)
(*; <0.05 vs (3), **; P<0.01 vs (3))

		maternal age(years)	fetal body weight(kg)	gestational age(weeks)	mean 1min apgar score	UA-AVP (Pg/ml)
(3)	(a) (n=27)	32.3±5.5	3,065±228	39.4±1.3	8.8±0.4	300±43.3
	(b) (n=7)	30.7±4.2	3,066±547	39.2±1.2	* 6.1±0.8	* 505±74.1
(4) (n=10)		36.0±2.2	3,260±276	38.0±0.8	8.9±0.2	** 14.0±9.1

(3); normal vaginal delivey group
(a) non-asphyxia group
(b) asphyxia group
(4) elective cesarean section group

of difference was $p<0.05$.

Measurement of AVP

(a) Antiserum

Antiserum was kindly provided by the Mitsubishi Yuka Science Co, Ltd.[4] It was produced by immunizing New Zealand white rabbits with synthetic 8-AVP and the thyroglobulin complex coupled with carbodiimide. Antiserum was used at the final dilution of 100000.

(b) Iodination of AVP

Standard AVP was purchased from the Peptide Institute, Osaka, Japan and used for iodination. Iodination was carried out by the chloramine T method[5]. Labelled-AVP was purified on a Sephadex G-25 fine column (0.7x20 cm, Pharmacia Fine Chemicals, Upsala, Sweden) and developed with 0.1 mol/l acetic acid.

(c) Extraction of AVP from plasma

Octadecylsilane cartridge (Sep-Pak C18, Waters Associates, Milford, MA, USA) was used for extraction as indicated in Fig. 1.

(d) Radioimmunoassay of AVP

The extracted material (300 μl) in phosphate buffer and 100 μl anti-AVP in 1% (v/v) normal rabbit serum were mixed in a test tube and incubated overnight at 4° C and labelled-AVP (2500 cpm/100 μl) was then added. Free and bound radioactivity was by the addition of 100 μl of a second antibody (anti-rabbit IgG sheep serum; 1:20), 100 μl of NRS

Figure 1

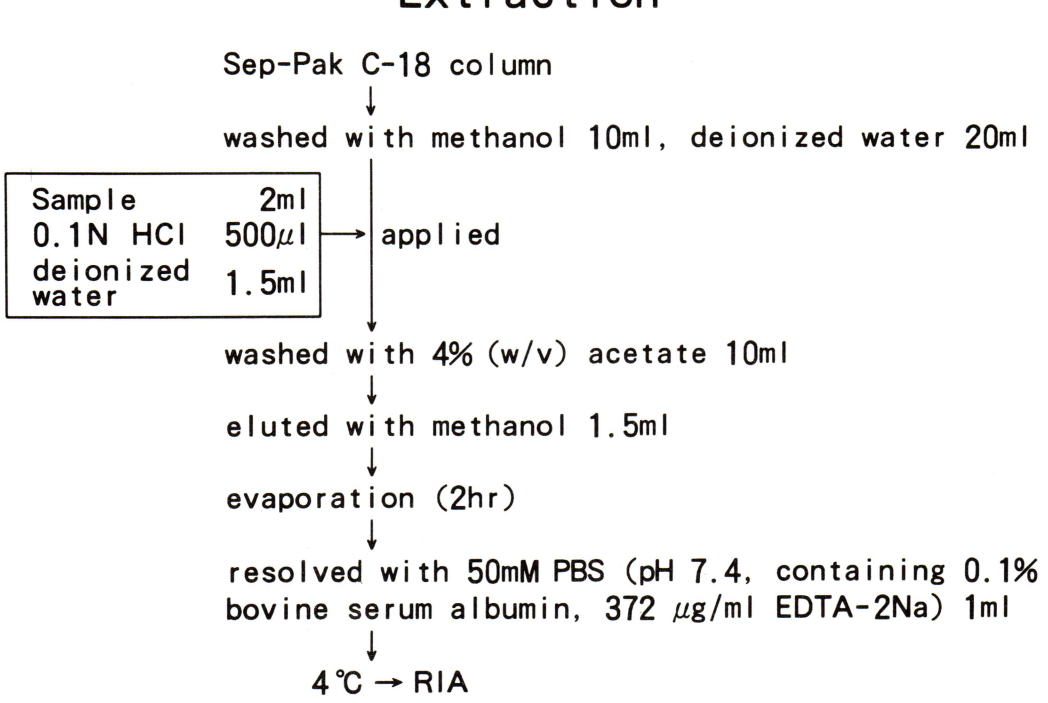
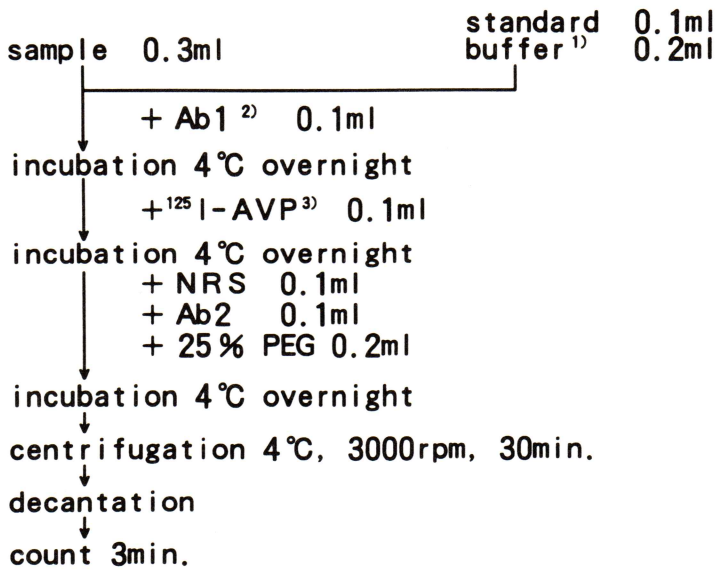


Figure 2



1) assay buffer : 0.1 M PB (0.01% NaN2) containing 0.1% BSA PH 7.4
2) Ab1 : anti-AVP rabbit serum (titer 1 : 100,000 final)
AVP (Ferring A.B.) -porcine thyroglobulin conjugated was injected into female rabbits
3) ^{125}I-AVP : 2500 cpm/ml

and 200 μl of 25% PEG. After overnight incubation at 4° C, the tubes were centrifuged and the radioactivity in the precipitate was measured (Fig. 2).

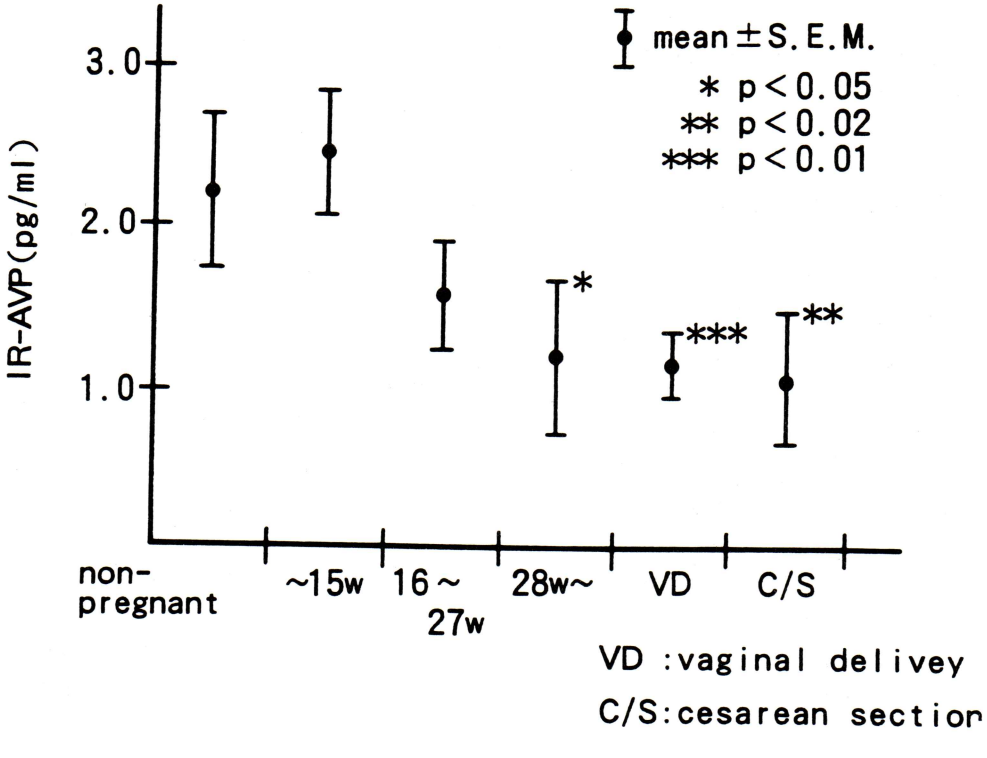
The minimal detectable level of AVP was 0.05 pg/tube and the inter- and intra- assay coefficients of variation were < 7.85% and 8.24%, respectively (n=5). The mean recovery rate, determined by addition of synthetic AVP was 87.1%. The cross-reactivity of antiserum with lysine vasopressin, oxytocin and vasotocin was 100%, < 0.05% and < 0.4%, respectively.

Results

Maternal plasma IR-AVP during pregnancy and at delivery

The maternal plasma concentrations of IR-AVP gradually decreased during pregnancy and reached a plateau after week 28 up to delivery. From week 28, the plasma concentrations of IR-AVP (mean ± SEM) were significantly lower than the one before at delivery by elective cesarean section (Fig. 3).

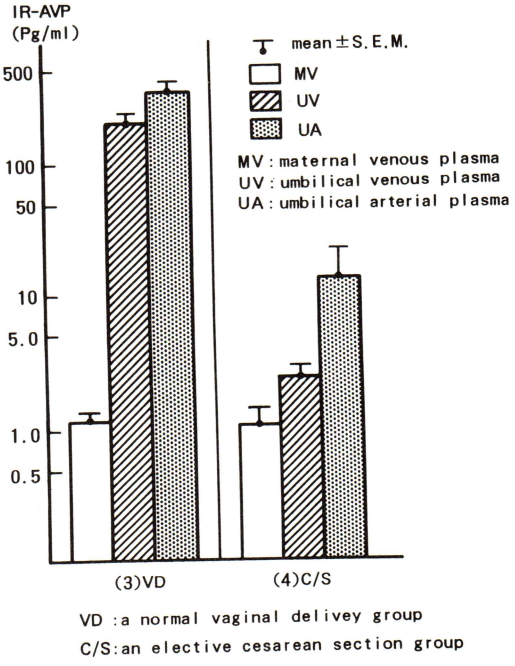
Figure 3 : Maternal plasma IR-AVP levels during pregnancy and at delivey (mean±S.E.M.)
(*: P<0.05, *; P<0.02, *** < 0.01, VS. pregnancy ~ 15w)



Maternal and umbilical cord plasma IR-AVP at delivery

Umbilical cord plasma IR-AVP levels were significantly higher than that of maternal plasma and the difference was pronounced in the vaginal delivery group. The umbilical arterial plasma concentrations of IR-AVP (390 ± 60.6 pg/ml, n=34) were significantly higher than those of umbilical venous plasma (224 ± 34.1 pg/ml, n=29) ($p<0.05$). On the other hand, there was no singificant difference in maternal plasma between vaginal delivery and elective cesarean section groups, but in both umbilical venous and arterial plasma, the IR-AVP concentrations in the vaginal delivery group was markedly higher than those in the elective cesarean group ($p<0.001$) (Fig. 4).

Figure 4. Maternal and umbilical cord plasma IR-AVP levels at delivery (mean±S.E.M.)



Umbilical arterial plasma IR-AVP in case of neonatal asphyxia

It was noteworthy that the umbilical arterial plasma at delivery in cases of asphyxia (505 ± 74.1 pg/ml, n=7) was significantly higher than that at normal delivery (300 ± 13.3 pg/ml, n=27) (Table 1).

Discussion

There have been several reports concerning the relationship between plasma AVP level and maternal plasma osmotic pressure[6], or fetal hypoxia[7], from either the maternal or fetal point of view. However, there are few reports concerning AVP from these points of view in both. Besides, the sensitivity of the measurement of plasma AVP has not always been satisfactory because the AVP concentration in human plasma is quite low.

We have established a highly sensitive RIA system to evaluate feto-maternal AVP function with different modes of delivery and fetal asphyxia from both maternal and fetal points of view.

A striking increase in water metabolism and a mild decrease of plasma osmotic pressure occurs physiologically in pregnancy. Renal plasma flow increases about 45% and cardiac output also increases about 40% in the first trimester and reaches

a plateau which is maintained up to week 30[8]. Plasma osmotic pressure after 10 weeks pregnancy decreases to 10 mOsm/kg less than in the non-pregnant status (280-282 mOsm/kg, monpregnant; 273 ± 0.5 mOsm/kg, pregnant; 277.0 ± 0.5 mOsm/kg, delivery), and maintains this plateau level up to delivery[9]. Coinciding with this, the plasma osmotic pressure threshold of AVP secretion from the posterior lobe pituitary decreases[6].

Our finding that the plasma concentration of IR-AVP is significantly lower than that before week 15 suggests that AVP plays an impotant role in maintaining increased volume of maternal blood circulation and decreased osmotic pressure during pregnancy.

In a recent study, AVP RNA expression in the hypothalamo-neurophyseal system during pregnancy did not differ from that in the non-pregnant[10], but the plasma vasopressinase (cystine amino peptidase) level and inactivation of AVP in the placenta increased after the third trimester of pregnancy[11]. These results support the conjecture that an increase of AVP metabolic rate causes a decrease of plasma AVP concentration and contributes to the promotion of water metabolism during pregnancy. Our observation that maternal plasma IR-AVP levels at normal vaginal delivery did not differ from those at delivery by cesarean section suggest that stress such as labour pain, does not necessarily reach the threshold of accelerating AVP secretion in pregnant women without serious complications, such as PIH or massive hemorrhage at delivery.

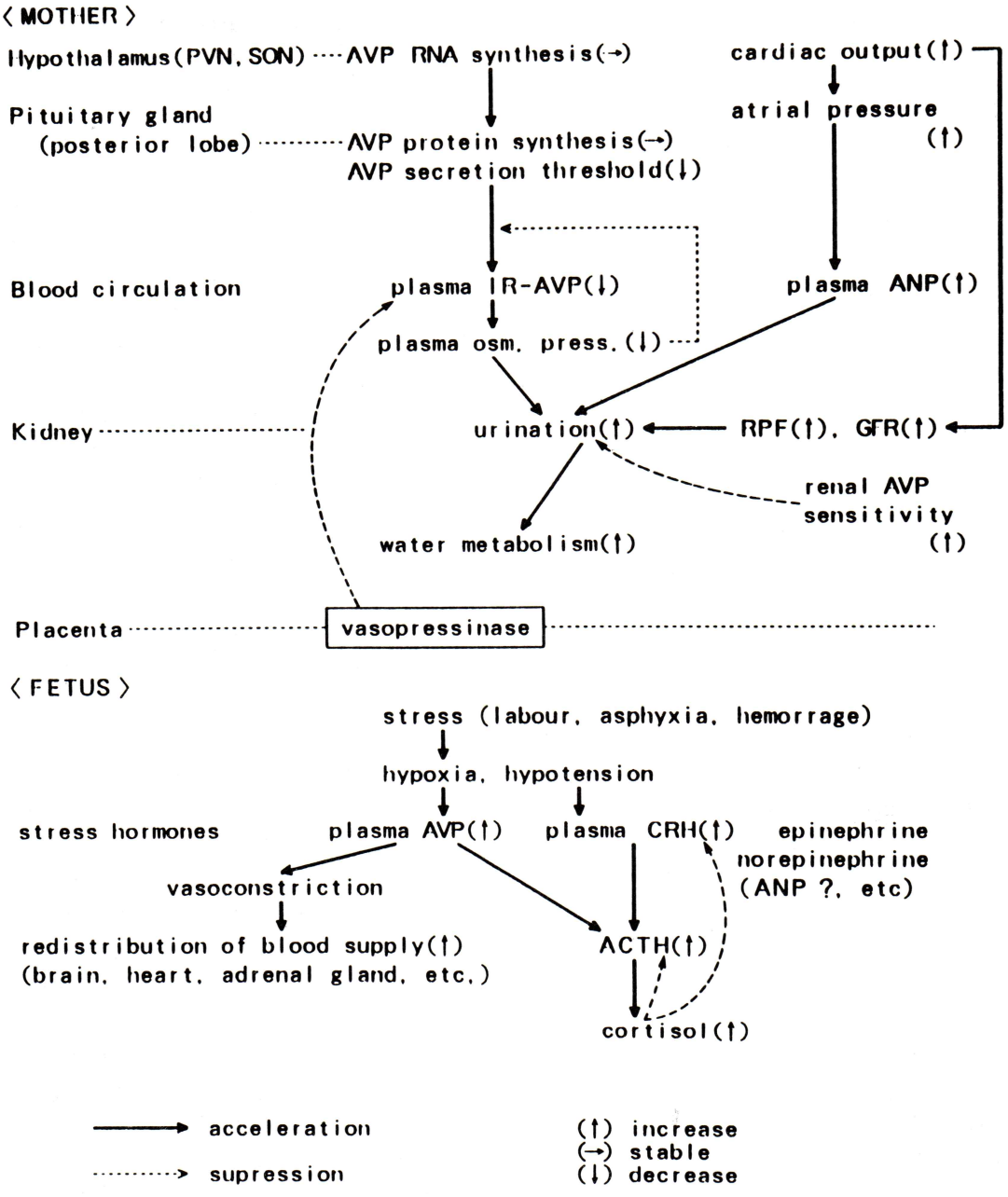
Human natriuretic peptide (hANP), which has a reverse effect against AVP of natriuresis and decreases blood pressure by relaxing vascular smooth muscle, increases during pregnancy[12], and inhibits vasopressin secretion[13]. These facts suggest that this hormone will have a relationship to AVP, on water metabolism, during pregnancy.

AVP plays a role in the fetal cardiovascular response to stress, such as hypoxia or hypotension, as a consequence of hemorrhage. It is postulated that there is no shift of AVP between fetus and the mother as the placenta has a large amount of vasopressinase and inactivates AVP[11]. Accordingly, the high concentration of IR-AVP in the umbilical cord plasma, is of fetal production, so that, the fetus itself secretes AVP in response to the stress of labour, in order to maintain cardiovascular homeostasis. In asphyxia, further secretion of fetal AVP would be accelerated, and contribute to the redistribution of blood flow and the selective supply of oxygen and nutrition to the important organs, such as, brain, heart or adrenal glands.

In addition to epinephrine, norepinephrine and AVP, corticotropin releasing hormone (CRH) is being investigated recently as a stress hormone. CRH as well as AVP directly effects the anterior lobe of the pituitary gland and regulates ACTH secre-

Figure 5.

HYPOTHESIS OF FETO-MATERNAL AVP FUNCTION DURING PREGNANCY AND DELIVERY



tion. We have previously reported that umbilical cord plasma IR-CRH also increases in cases of asphyxia[14]. Taking these facts into consideration, AVP may play an important role in the fetus as a stress hormone.

In other reports, AVP was shown to accelerate hANP secretion[15], and the umbilical arterial plasma concentration of IR-hANP at vaginal delivery was significantly higher than the venous plasma[16].

In summary, there must be a complex interaction among several stress hormones during pregnancy and AVP may play a key role in maintaining the homeostasis of cardiovascular and metabolic systems (Fig. 5).

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