HUMAN NEUROPHYSINS DURING PREGNANCY, Puerperium and Menstrual Cycles

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ABSTRACT

To clarify the hypothalamo-posterior pituitary function during pregnancy, puerperium and menstrual cycle, investigations were performed to determine the levels of serum neurophysins by specific radioimmunoassays. Sera from 75 women during normal pregnancy, 17 during postpartum and 49 during the normal menstrual cycle were assayed for Estrogen Stimulated Neurophysin (ESN) and Nicotine Stimulated Neurophysin (NSN). ESN, associated with oxytocin increased during pregnancy and the values decreased to non-pregnant levels after parturition. During menstrual cycle ESN showed three peaks. In contrast, NSN, associated with vasopressin was little changed throughout the menstrual cycle, pregnancy and the puerperium. In this study two neurophysins changed independently, suggesting the hypothesis of “one hormone—one neurophysin.” The data also would indicate the availability of measurement of these two substances on the study of the function of human neurosecretory system.

In 1949, Bargmann,1,2 by using chrom-alum-hematoxylin (CAH) staining technique, observed that there was an intense CAH stain positive material in the paraventricular and supraoptic nuclei of the hypothalamus. This material was identified as the Gomori positive substance of the so-called Van Dyke protein which Van Dyke and his associates3 had isolated from bovine posterior pituitary in 1942. At present this substance is called neurophysin. Neurophysin, the carrier protein of the posterior pituitary hormones, is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and is packed into neurosecretory vesicles binding with oxytocin and vasopressin. A number of
studies in mammals suggest that there are several neurophysins defined as a polypeptide with a molecular weight range of 10,000-25,000.4 Amino acid sequence of bovine neurophysin II composed of 97 amino acids was determined by Walter, et al.5 and that of porcine neurophysin I composed with 92 amino acids was determined by Wuu and his co-workers.6 Human neurophysins were separated into four major neurophysin peptides by preparative disk gel electrophoresis. However by their immunological specificities they were divided into two groups. In 1975 Robinson7 identified two human neurophysins in response to different stimulations. One of them was Estrogen Stimulated Neurophysin (ESN) secreted in response to estrogen administration and another one was Nicotine Stimulated Neurophysin (NSN) secreted in response to cigarette smoking.

There has been a few studies on how these neurophysins are altered during menstrual cycle and gestational period. In order to clarify the hypothalamo-posterior pituitary function in women of reproductive age, the levels of serum neurophysins, ESN and NSN, were measured by specific radioimmunoassay.

MATERIAL AND METHODS

Patient study. The serum samples were obtained from 75 pregnant women (6-40th gestational weeks), 17 at postpartum (1-5th and 28-33rd days after parturition) and 49 during 28 days menstrual cycle in Keio University Hospital and Ohta General Hospital. All are healthy women aged 19 to 33. A 5 ml quantity of blood was collected from the antecubital vein into a dried syringe and transferred to the polyethylene test tube. All blood samples were allowed to clot at 4°C, then the serum was removed and kept frozen at -80°C until it was assayed.

Neurophysins and antibodies. Highly purified human neurophysins, ESN and NSN, and their specific antibodies were kindly granted from National Institute of Arthritis, Metabolism and Digestive Diseases U.S.A.

Radioimmunoassay. Radioiodination of the human neurophysins was accomplished by the chloramin-T method using 1 m Ci of Na125I to iodinate 5 μg of proteins. Purification of the tracer was achieved by successive gel filtration on Sephadex G-25 (0.8×10 cm). After 72 hours of preincubation in the phosphate buffered saline (0.01 M, pH 7.0, containing 1% gelatin), 100 μl of the hot protein (approximately 10000 CPM) was added and the tubes were incubated at 4°C for 5 days. The separation of the bound labeled protein from the free was carried out by double antibody technique adding excess amount of anti rabbit γ-globulin. The serum neurophysins values were expressed in terms of ng/ml serum.
RESULTS

Serum neurophysins levels during normal pregnancy. ESN levels in normal pregnancy from the 6th to 40th week, are shown in Fig. 1. In the early pregnant period, the mean value of ESN was 49.4 ng/ml (6-10 weeks) and it gradually increased to the level of 91.8 ng/ml (36-40 weeks) in the late pregnant period. Fig. 2 indicates NSN in the gestation. The maximal value of NSN was 50.8 ± 0.44 ng/ml (mean ± S.E.) at 21-25 weeks and the minimal value of NSN was 47.3 ± 2.29 ng/ml at 6-10 weeks.

Serum neurophysins levels after parturition. The mean value of serum ESN level was 66.8 ng/ml on 1 to 5 days after parturition and was slightly reduced
Fig. 2  Serum NSN levels in normal pregnancy through 6 to 40 weeks of pregnancy.

Fig. 3  Serum ESN and NSN levels after parturition.
to the value of 58.6 ng/ml on 28 to 33 days after parturition, but these values were not statistically significant (Fig. 3). The mean value of NSN was 46.2 ng/ml on 1 to 5 days after parturition and it was 46.0 ng/ml on 28 to 33 days after parturition (Fig. 3).

Serum neurophysins levels in 28 days-menstrual cycle women. ESN levels in normal menstrual cycle are shown in Fig. 4. Three peaks of ESN were observed, the first was 38.3 ng/ml on the 5-8th day, the second was 33.2 ng/ml on 13 to 16th day and the third was 40.0 ng/ml on 25-28th day. In Fig. 5 the values of serum NSN in the cycle are indicated. NSN values were almost unchanged except for a small rise to 44.6 ng/ml on the 5 to 8th day of menstrual cycle.

Fig. 4 Serum ESN levels in 28 days-menstrual cycle women.
The study of neurophysins in the systemic circulation was first reported in 1967. Fawcett, et al. found heavy peptides, neurophysins, were secreted into the peripheral blood during hemorrhage in dog. In pig, Ginsbury and Jayasna isolated a neurophysin-like material from the blood stream. The radioimmunoassay of neurophysins was first demonstrated by Legros and his co-workers, who reported neurophysins levels in pregnant women. Human neurophysins have been classified by various kinds of research approaches. At present many investigators use the classification according to their response to physiological maneuvers, ESN and NSN. In a few laboratories neurophysins are classified according to electrophoretic mobility, for example, Neurophysin-I. ESN is identi-
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cal to Human Neurophysin-I and NSN is identical to Human Neurophysin-II.

We have shown that ESN levels in pregnancy significantly increased as the pregnancy proceeded and after parturition they decreased to the levels of non-pregnant condition. On the other hand serum NSN mean levels were little changed in the pregnant period and postpartum period. In reasonable agreement with our findings, those of others 10-12 have demonstrated that ESN levels were elevated at the end of pregnancy and fell to the non-pregnant levels after parturition. It could be speculated that the elevation of ESN levels in the terminal period of pregnancy is a positive response to the increased endogenous estrogen, because ESN is secreted by the administration of estrogen. Furthermore the acute reduction of ESN in the first week after delivery might be because of the reduction of the stimulus, presumably estrogen, caused by delivery. It was reported that the serum NSN levels increased acutely at parturition perhaps in response to the stress of delivery,13 however few literatures about the serum NSN levels in the gestational period were reported.

In our experiment, ESN had three peaks, on 5-8th, 13-16th and 25-28th day during menstrual cycle and NSN was little changed except the slight elevation on 5-8th day. Since the second and third peaks of the serum ESN levels are correlative with the peaks of the serum estrogen levels during the cycle, it is presumed that ESN is also elevated in response to the endogenous estrogen during cycle. The first peak of ESN seems not to be linked with estrogen. Originally the term of ESN was first used for purposes of convenience, therefore it would be possible that ESN is secreted in response to other factors, not only estrogen. However so far these are unknown.

The hypothesis "one hormone—one neurophysin" suggests that ESN is associated with oxytocin and NSN is associated with vasopressin. The close association of NSN with vasopressin was demonstrated clinically.14 NSN and vasopressin showed a simultaneous rise in plasma samples after smoking. In contrast, the association of ESN with oxytocin has never been proven in the peripheral blood, since the definite assay of oxytocin has not been established yet. Therefore it is of interest that the levels of ESN during pregnancy seem to increase simultaneously with the levels of oxytocin, measured by recently established method.15 Furthermore it is also of interest that a recent report16 showed there is a peak of oxytocin linking with the first peak of ESN on 5-8th day during menstrual cycle. It could be indicated that ESN and oxytocin have a close correlation in human blood.

Neurophysins were studied as a lipolytic factor, a natriuretic factor and others, however, no physiological and biological function has been established for neurophysins after secretion into the systemic circulation. The most expected
role of the serum neurophysins is the marker of the posterior pituitary hormones. Our findings show that ESN and NSN levels changed independently during pregnancy, puerperium and menstrual cycle and ESN might be associated with oxytocin, suggesting the hypothesis "one hormone—one neurophysin." Furthermore they indicate that two neurophysins could be the marker of the posterior pituitary hormones and would be useful on the study of hypothalamo-posterior pituitary system in reproductive women.

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