Symposium on Morbidity of Body Fluid Balance and Its Treatment*

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1. Vasopressin and Related Disorders

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The neuropeptide hormone, arginine vasopressin (AVP), has long been intensively studied from various different viewpoints. The synthesis, secretion, and biologic effects of the hormone had been studied using electrophysiologic and neurophysiologic techniques. With the recent development of techniques involving molecular biology, the details of these mechanisms are now becoming understood. On the other hand, the clinical assessment of posterior pituitary function in patients with polyuria has been also improved using a highly sensitive radioimmunoassay system. The findings of recent fundamental and clinical studies in this area are reviewed.

Functional diagnosis for central diabetes insipidus

We investigated the concentration of unextracted urinary AVP in normal subjects and patients with polyuria for the purpose of clinical application for screening of central diabetes insipidus. A significant correlation was observed between urinary AVP concentration and simultaneously measured plasma AVP in normal subjects. AVP concentration in random urine was also significantly correlated with AVP excretion in 24 hr urine. Therefore, it was suggested that random urinary AVP concentration may reflect the posterior pituitary function. In normal subjects, AVP concentration in random urine was scattered from 9.2 to 470.6 pg/mg Cr (89.5 ± 76.4 pg/mg Cr). In patients with diabetes insipidus, urinary AVP concentration (1.6 to 13.0 pg/mg Cr, 6.9 ± 2.8 pg/mg Cr) was significantly lower than that of normal subjects (Fig. 1). In this study, it is suggested that patients of random urinary AVP concentration below 13.0 pg/mg Cr should be recommended for further examination such as the following hypertonic saline infusion test to diagnose central diabetes insipidus. After the screening by random urinary AVP concentration, hypertonic saline infusion test is necessary for the accurate evaluation of posterior pituitary function (1). Infusion of 5% saline at a rate of 0.05 ml/kg/min for 120 minutes induced an elevation of plasma osmolality (Posm) from 290.3 ± 0.7 to 307.5 ± 2.1 mOsm/kg with a resultant increase in AVP from 2.4 ± 0.4 to 9.9 ± 2.2 pg/ml. During the infusion a highly significant correlation between plasma AVP and Posm was observed. In 22 patients with polyuria, on the other hand, the infusion induced a marked elevation of Posm from 302.6 ± 2.5 to 321.3 ± 2.9 mOsm/kg but caused only a slight or no increase in plasma AVP from the

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basal levels, suggesting that all patients examined had central diabetes insipidus. Based on these results, we recommend the measurement of urinary AVP for a simple screening method and the assessment of plasma AVP during hypertonic saline infusion for final evaluation of posterior pituitary function.

**Molecular aspect in patients with familial central diabetes insipidus**

AVP is generated from preproAVP and this precursor consists of the signal peptide, AVP, neurophysin (NP), and glycoprotein domain. The precursor is encoded by the AVP gene on chromosome 20 which has three exons (2). The first exon encodes the signal peptide, AVP, and the N-terminal region of NP. The second exon encodes the central region of NP, and the third exon encodes the C-terminal region of NP and the glycoprotein domain (Fig. 2). Familial central diabetes insipidus (FDI) is caused by a deficiency of AVP and is transmitted as an autosomal dominant trait. The possibility that a mutation in the AVP gene may be the basis of FDI had been suggested by indirect observations. We reported a single base substitution in exon 2 of the AVP gene in a Japanese FDI pedigree, which results in an amino acid substitution in the NP of the AVP precursor (3). Subsequently, we identified a mutation in exon 1 that changes the residue at the COOH-terminus of the signal peptide (4). Then, we analyzed the AVP gene in four Japanese FDI pedigrees and identified four novel mutations in exon 2 (Fig. 2). One of the mutations predicts a premature termination, and the others result in a single amino acid substitution or deletion (5, 6). More than 20 FDI pedigrees including our cases have been reported to date (7). Interestingly, all mutations with the exception of the signal peptide mutation, are located in exon 2 or 3, suggesting the importance of the NP molecules (Table 1). Moreover, all patients are heterozygous for the mutations, indicating that some mechanisms may be involved in the abolishment of AVP production by the normal allele. The precise mechanisms of how these abnormalities of AVP precursor cause impairment of AVP synthesis have not yet been revealed. To investigate the functional change of the disorder, AtT20 cells were transfected with each mutant AVP cDNA. AVP release in culture media of these transfected cells was significantly decreased compared to control. These findings suggest the following possibilities: 1. The intracellular transport of an abnormal AVP precursor might be impaired because of its conformational changes. Accumulated abnormal precursor could result in the cell degeneration of magnocellular neurons. 2. The abnormal NP generated through posttranslational processing of the abnormal precursor might lack physiologic function. NP is the carrier protein of AVP and is thought to protect AVP from proteolytic degeneration during axonal transport from the hypothalamus to the posterior pituitary.

**References**


![Figure 1. AVP concentration in random urine in normal subjects and in central diabetes insipidus patients.](image1)

![Figure 2. The coding regions of the AVP gene and the structure of the preproAVP. Solid circle (●) indicates the location of mutations identified in familial diabetes insipidus analyzed in our laboratory. SP: signal peptide, GP: glycoprotein.](image2)

| Table 1. Reported Mutations of the AVP Gene and Predicted Changes in the PreproAVP in 22 Pedigrees of FDI |
|-------------------------------------------------|---------------------|---------------------|---------------------|
| 1) AVP gene  | exon 1 | exon 2 | exon 3 |
| number of pedigrees | 4 | 15 | 3 |
| 2) preproAVP | signal peptide | AVP | neurophysin | glycoprotein |
| number of pedigrees | 4 | 0 | 18 | 0 |

AVP gene 5'- EXON 1 - EXON 2 - EXON 3 -3'

preproAVP NH$_2$- SP AVP NP GP COOH
2. Aquaporin-2, a Vasopressin-Sensitive Water Channel, and Nephrogenic Diabetes Insipidus

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Key words: autosomal recessive nephrogenic insipidus, point mutation, compound heterozygote, water permeability

Abstract
Two cases of autosomal recessive nephrogenic diabetes insipidus (NDI) were evaluated. Both cases were found to be compound heterozygote for missense mutations in the aquaporin-2 (AQP2) gene. To determine the structural-functional relationship, the mutated AQP2 proteins, T125M, G175R, A190T, and

Figure 1. Putative membrane topology of AQP2 and site of mutations identified in patients with autosomal recessive NDI. Solid symbols represent the locations of the mutations.

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