

## RADIOIMMUNOASSAY FOR AQUAPORIN-2

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**Objective.** To develop radioimmunoassay for aquaporin-2 (AQP-2).

**Methods.** Anti-AQP-2 antiserum has been raised in New Zealand white rabbits immunized with a conjugate of synthetic AQP-2 peptide (257-271) with bovine serum albumin. Radioiodination of synthetic peptide (tyrosine-AQP2 (257-271) was performed by chloramine T method, followed by purification of radioiodinated material on Sephadex G-25 column.

**Results.** The obtained antibody did not crossreact with vasopressin, pituitary hormones, hypothalamic hormones and neuropeptides. The assay was performed with a double antibody system. The values are expressed as an equivalent of synthetic AQP-2 peptide (257-271). The dilution curve of high AQP-2 urine in radioimmunoassay system was parallel to the standard curve. The recovery percentage of AQP-2 added to urine was about 100 % in this assay system. Intra-assay and inter-assay variation was 4.5 % and 7.2 %, respectively. Mean urinary excretion of AQP-2 was 1.16 ng equivalent of AQP-2 (257-271)/mg creatine and was lower in patients with diabetes insipidus.

**Conclusion.** These data suggest that his assay system is a suitable to measure AQP-2 in urine.

**Key words:** Aquaporin-2 - Immunohistochemistry - Rat

Recently, SASAKI et al. (1994) cloned human aquaporin sDNA (AQP-2), which functions as water channel (FUSHIMI et al. 1993; KING and ARGE 1996; LEE et al. 1997; KNEPPER et al. 1997). Its expression in the kidney is mainly regulated by vasopressin (DEEN et al. 1994; SABOLIC et al. 1995). It has been even reported that an AQP-2 mutation causes nephrogenic diabetes insipidus (DEEN et al. 1995; HOCHBERG et al. 1997; LEE et al. 1997) and urinary excretion of AQP-2 was increased after the administration of vasopressin (SABOLIC et al. 1995).

To study the physiological and pathophysiological significance of AQP-2 in diseases, it is necessary to measure AQP-2 concentrations in urine. A few radioimmunoassay for AQP-2 has been reported (KANNO et al. 1995). The present study was designed to establish a radioimmunoassay system for measuring AQP-2 in urine and elucidate the clinical significance of urinary AQP-2 levels in diseases.

### Materials and Methods

**Subjects:** Thirty two normal subjects and eight patients with diabetes insipidus were subjects to this study. The diagnosis was made according to symptoms, signs and laboratory findings.

**Drugs:** Neuropeptides, hypophysial hormones, pituitary hormone were obtained from the Protein Research Foundation (Japan). Sephadex G-25 was purchased from the Pharmacia (Sweden).

**Preparation of conjugate AQP-2(257-271) to bovine serum albumin:** Peptide corresponding to the following sequences of the AQP-2 (257-271) and tyrosine AQP-2 (257-271) were synthesized by solid phase method employing automatic peptide synthesizer and purified by HPLC: VELHSPQSLPRGTK-AGGQLET or YVELHSPQSLPRGTKA. Synthetic AQP-2 peptide (257-271) was conjugated on an equal weight basis to bovine serum albumin (BSA) by the

**Table 1**  
**A Schematic diagram of assay procedure**

standard or samples	100 µl
antibody (1:500)	100 µl
tyrosine-AQP-2(257-271)-I-125	100 µl
buffer	500 µl
↓	
incubated for 24 hours at 4 °C	
↓	
added second antibody solution µl	
↓	
incubated for 24 hours at 4 °C	
↓	
centrifuged	
↓	
decanted supernatants	
↓	
counted (precipitates)	
↓	
calculated bound/total count (B/T)	

method previously described for anti-GHRH antibody, using glutaraldehyde (MITSUMA et al. 1983). New Zealand white rabbits were immunized with the emulsion of one mg of this conjugate in one ml water and complete Freund's adjuvant (1:2, v/v) which was injected into the foot pad at the intervals of three weeks. Blood was withdrawn one week after each injection and the presence of anti-AQP-2 was checked.

**Radioiodination of tyrosine-AQP-2 (257-271) and purification:** Radioiodination of AQP-2 (257-271) was performed with chloramine T according to Greenwood-Hunter method (GREENWOOD et al. 1963). The radioiodinated materials were chromatographed on a Sephadex G-25 (1.0 x 15 cm) and eluted with 0.01 M Phosphate buffer (pH 7.4) collecting 1.0 ml fractions. Two peaks of radioactivity were obtained (Fig. 1). First peak was AQP-2 (15-29)-I-125 and the second peak was free I-125. Specific activity was calculated to be approximately 200 µC/µg.

**Assay buffer:** For the assay 0.01 M phosphate buffer (pH 7.4) with 0.1 % BSA, 0.1 % mercaptoethanol and 0.1 % Triton x-100 was used.

**Assay procedure:** A double antibody radioimmunoassay was performed. A schematic diagram of the methodology is shown in Tab. 1. Values of AQP-2 in urine were expressed as an equivalent of synthetic AQP-2 (257-271).

**Assay method for vasopressin and creatinine:** Plasma vasopressin was measured with a commer-

**Table 2**  
**Relative reactivity\* of hypothalamic hormones, neuropeptides, pituitary, hormones in AQP-2 radioimmunoassay**

AQP-2(257-271)	100
tyrosine-AQP-2(257-271)	100
TRH	<0.001
GHRH	<0.001
Somatostatin	<0.001
LHRH	<0.001
CRH	<0.001
Neurotensin	<0.001
methionine-enkephalin	<0.001
leucine-enkephalin	<0.001
beta-endorphin	<0.001
alpha-neoendorphin	<0.001
beta-neoendorphin	<0.001
Dynorphin	<0.001
Substance P	<0.001
VIP	<0.001
CGRP	<0.001
ANP	<0.001
BNP	<0.001
CNP	<0.001
CCK-8	<0.001
Vasopressin	<0.001
Oxytocin	<0.001
ACTH	<0.001
LH	<0.001
FSH	<0.001
TSH	<0.001
GH	<0.001
Prolactin	<0.001
alpha-MSH	<0.001
beta-MSH	<0.001
gamma-MSH	<0.001
Insulin	<0.001

\*Arbitrary values of AQP-2(257-271) = 100

cially available RIA kit (Mitsubishi Chem. Co., Ltd, Japan). Urinary creatine was measured with autoanalyzer.

**Statistical evaluation:** The differences between control and diabetes insipidus were determined by analysis of variance using ANOVA procedure.

## Results

**Generation of antibodies to AQP-2:** Three out of four rabbits responded to the immunization and developed antibodies at a final dilution of at least

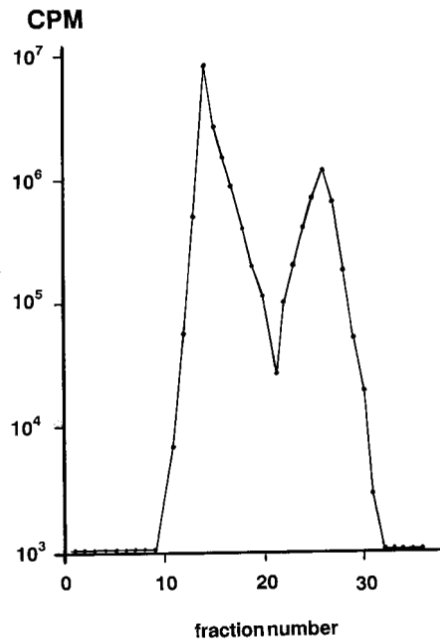


Fig. 1

The elution profile of a radioiodinated tyrosine-AQP-2(257-271) on Sephadex G-25

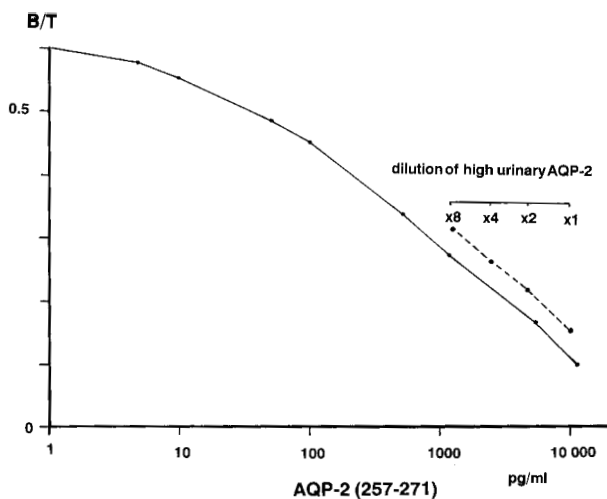


Fig. 2

Standard curve and dilution of high AQP-2 urine

1:1000. The antiserum used in this study was obtained one week after the third injection and showed a specific binding at a final dilution of 1:4000.

**Specificity of antiserum:** The specificity of anti-AQP-2 is shown in Tab. 2. No crossreactivity was observed with neuropeptides, hypothalamic hormones, pituitary hormones.

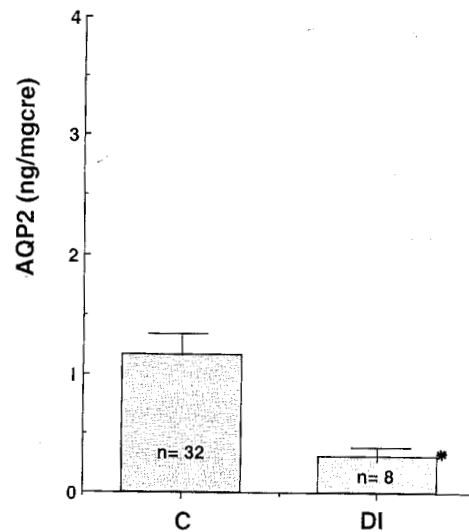


Fig. 3

Urinary AQP-2 in the control (C) and diabetes insipidus (DI)

**Standard curve dilution curves of high AQP-2 urine:** A typical standard curve, which used AQP-2 (257-271) for standard, is shown in Fig. 2. The detection limit in this system was 10 pg/ml and parallel curves were obtained for the dilution of high urinary sample with high level of AQP-2 (Fig. 2).

**Recovery of AQP-2 (257-271) added to the urine:** Known amounts of AQP-2 (257-271) added to the urine were measured with the present RIA system. Recovery percentage was approximately 100 %. The intra-assay and interassay variation was 4.5 and 7.2 %, respectively.

**Clinical results:** Mean urinary excretion of AQP-2 was 1.16 ng/mg creatinine. In central diabetes mellitus, urinary excretion of AQP-2 was significantly lower as compared to normal subjects (Fig. 3). Urinary AQP-2 concentration correlated with plasma vasopressin concentrations and it was inversely correlated with plasma osmolality (data not shown).

## Discussion

A radioimmunoassay for AQP-2 has been developed. Anti-AQP-2 was raised in New Zealand white rabbits immunized with a conjugate of synthetic peptide (AQP-2, 15-29) to BSA. The antibody which was used in this study did not cross-react with neuropep-

tides, hypophysial hormone, pituitary hormone. These data indicate that this antibody is specific to AQP-2 and is suitable for the measurement of AQP-2 in radioimmunoassay system. Dilution of high AQP-2 urine is parallel to the standard curve which was constructed with synthetic peptide (AQP-2, 257-271). This indicates that synthetic peptide (AQP-2, 257-271) can be used for standard and the values found may be expressed as equivalent to this peptide. Radioiodination was performed with chloramine T method and elution profile showed two peaks; first peak is AQP-2-I-125, second peak is free I-125. First peak was used for radioactive-ligand. The results of recovery experiment, intraassay and interassay variation suggest that this assay system is suitable to measure AQP-2 in urine.

The present study demonstrated that mean urinary AQP-2 concentrations are 1.16 ng/mg creatine in normal subjects and are lower in central diabetes mellitus. These data confirmed previous reports in which AQP-2 was present in urine and its concentrations in urine were lower in central diabetes mellitus (KANNO et al. 1995; SAITO et al. 1997). We also found that urinary AQP-2 concentration was correlated with plasma ADH and urinary AQP-2 concentration was inversely correlated with plasma osmolarity. It has been reported that urinary excretion of AQP-2 is regulated with vasopressin (DEEN et al. 1994; SABOLIC et al. 1995). The present study confirmed this report. As mentioned above, nephrogenic diabetes insipidus caused by an aquaporin-2 mutation (DEEN et al. 1995; HOCHBERG et al. 1997). The present data and previous reports taken together suggest that the measurement of urinary AQP-2 concentrations are useful index to study the action of vasopressin and water balance disorders.

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