A Specific Antibody to Vasopressin in a Man with Concomitant Resistance to Treatment with Pitressin

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A 26-year-old man with complete neurogenic diabetes insipidus since age nine was initially treated with vasopressin (Pitressin Tannate in oil). At age 13, its dosages were progressively increased to control the patient’s polyuria; minor allergic symptoms occurred after every such treatment. We incubated serial dilutions of the patient’s plasma with [125I]-labeled arginine-vasopressin and obtained a 50% specific binding for the plasma at a final dilution of 625-fold. Cross-reactivity studies showed that lysine-vasopressin was better recognized by the antibody than arginine-vasopressin. These results were attributed to large concentrations of lysine-vasopressin (pork vasopressin) in the Pitressin.

Additional Keyphrases: radioassay - diabetes insipidus - polypeptide hormones

Occasionally, resistance to treatment with large polypeptide hormones such as insulin is caused by the development of antibodies (1), but small polypeptide hormones such as vasopressin are considered to be much less antigenic. Two previous reports describe circulating antibodies to vasopressin in patients being treated with vasopressin for diabetes insipidus, but resistance to this treatment was not clearly demonstrated (2, 3). In a recent preliminary communication, antibodies to vasopressin were detected in six of 26 patients with central diabetes insipidus (4). Here we describe a patient with complete neurogenic diabetes insipidus, who was being treated with Pitressin Tannate (USP) in oil (PTO) and developed therapeutic resistance and circulating antibodies specifically directed against lysine-vasopressin.1

Clinical Observations and Methods

R.B., a 26-year-old man, was referred to us for resistance to the administration of PTO. He had had neurogenic diabetes insipidus since age nine, manifested by polyuria, polydipsia, and a sodium concentration of 151 mmol/L in the plasma with a concomitant relative density of urine of 1.001. Initially he had been treated with PTO, 5 USP units every second day, but at age 13, the patient noticed a progressive recurrence of his severe polyuria and polydipsia despite regular PTO administration. The PTO dosage was then increased to 10 USP units every day, and then to 20 USP units three times daily, but the polyuria and nocturia persisted. Approximately six months later, every injection of PTO was accompanied by localized erythema and swelling at the injection site as well as face flushing and upper respiratory tract responses such as nose obstruction and sneezing. In October 1983, PTO was discontinued and replaced successfully with 1-deamino-8-arginine-vasopressin (dDAVP). We performed the following investigation three and four months after discontinuation of PTO.

Using freezing-point depression (Osmometer 3DII; Advanced Instruments, Inc., Needham Heights, MA), we determined plasma and urine osmolalities in duplicate on 250-μL samples. The sensitivity of this instrument is ± 1 mOsm/kg and its CV at 290 mOsm/kg is 0.51%. Heparinized plasma for osmometry was collected with as little stasis as possible (5). Plasma sodium was measured with a sodium selective electrode (Nova Biomedical, Newton, MA).

Arginine-Vasopressin Radioimmunoassay

Blood samples collected in chilled tubes containing EDTA were centrifuged (4 °C, 1000 x g, 20 min), and plasma for the determination of arginine-vasopressin (AVP) was extracted by a modification of previously reported methods (6, 7), as follows. Mix 1 mL of thawed sample with 2 mL of cold acetone and centrifuge. Mix the supernate with 5 mL of cold petroleum ether and recentrifuge. Cool to −80 °C and discard the top liquid phase. Thaw the bottom phase and evaporate the solvent at room temperature under a stream of cold air (4 °C). Reconstitute the residue in 750 μL of a 1 g/L bovine albumin solution (Miles, Elkart, IN 46515) containing 1 g of sodium azide per liter as preservative; adjust the pH to 7.2 with Tribase.

The sodium phosphate (0.1 mol/L, pH 7.6) assay buffer contained, per liter, 3 g of NaCl, 1 g of bovine serum albumin (Miles), and 1 g of sodium azide. For standard curves we assayed purified AVP (batch no. BAA 252, 1 mg = 450 int. units; Ferring Pharmaceuticals, S-200 62, Malmo, Sweden), 0.05 to 10 pg per assay tube. We incubated 200 μL of standards in buffer or 200 μL of reconstituted plasma extract, plus 200 μL of antiserum in buffer, in triplicate for the standards and in duplicate for the unknown, for two days at 4 °C; we then added 100 μL of tracer (800 to 1200 counts/min) and incubated for an additional three days. Free and bound fractions were then separated by a dextran–charcoal method (7).

Extracts of plasma from four patients with complete central diabetes insipidus failed to displace tracer and were used regularly as controls.

The tracer was [125I]-labeled vasopressin-8-arginine, monomiodinated (New England Nuclear), specific activity 1820 to 2200 kCi/mol. Nonspecific binding was always <3%.

The antisem (AS-2849), used at a final dilution of 2.5 x 106-fold, was generously provided by J. Durr and M. Lindheimer (Dept. Obstetrics, Gynecology and Medicine, University of Chicago, IL 60637). The cross reactivity of this antisem was less than 8% for lysine-vasopressin and less than 4% for arginine-vasopressin. With this antisem the sensitivity of the assay in our laboratory was always 0.1 pg/assay tube and the 50% displacement was 1.2 pg/tube. The mean analytical recovery of non-radioiodinated vasopressin added to the plasma of patients with central diabetes insipidus was 102 (SD 4). Intra-assay CV for concentrations of AVP in plasma between 2 and 5 ng/L was 5 to 13%. Mean interassay CV for plasma vasopressin values between 0.5 and 18 ng/L was 20%. Characteristics of this antisem and radioimmunoassay have been described (8).

Results

Dehydration test and saline infusion. dDAVP was discontinued for two days and the patient was subjected to a short
dehydration test followed by an infusion of hypertonic saline (30 g/L NaCl): plasma osmolality increased to 310 mOsm/kg H₂O, and the sodium concentration to 152 mmol/L. At the same time urinary osmolality was 80 mOsm/kg H₂O. AVP remained undetectable (<0.5 ng/mL, four determinations) in plasma during the entire procedure. After intranasal administration of 5 µg of DDAVP, the osmolality of the patient’s urine increased rapidly to 540 mOsm/kg H₂O.

Detection of circulating antibodies against vasopressin. Results of the search for detectable concentrations of AVP during the dehydration test in the plasma of this patient were unusual, in that we observed an "inverse" displacement: the amount of 125I-labeled AVP bound to the specific antiserum (AS-2849) used to measure vasopressin was higher in the presence of the extracted plasma from the patient than in the presence of the assay buffer (B/Bo > 100%). This suggested that the plasma of our patient contained a substance binding to vasopressin.

We then incubated for three days serial dilutions of the patient’s plasma with trace amounts of 125I-labeled AVP. In a control experiment, we used plasma from a patient with complete neurogenic diabetes insipidus who had received only DDAVP, never PTO. After three days, free and bound fractions were separated with a dextran-charcoal mixture. The plasma from R.B. showed specific binding of 75% when diluted less than 50-fold. This binding progressively decreased with increasing dilution, reaching 20% at 500-fold dilution. In contrast, we observed no binding in patient’s plasma in the control experiment (Figure 1). As Figure 2 shows, lysine vasopressin was better recognized than AVP. Oxytocin and DDAVP did not displace the tracer from the antibody at the concentrations tested (up to 100 pg/tube).

Discussion

Roth et al. (2) described a patient with central diabetes insipidus treated with PTO who probably developed antibodies to lysine-vasopressin; however, they did not further characterize this antiserum. The progressive replacement of vasopressin preparation originally derived from a mixture of beef and pork glands, with a synthetic preparation of AVP, makes the development of antibodies in patients less likely, AVP being the natural hormone in humans and lysine-vasopressin the antidiuretic hormone in swine. Previously reported antibodies could also have been directed against more complex structures, e.g., neurophysin, or some other nonbiological contaminants. That our patient had clear therapeutic resistance to PTO whereas DDAVP was effective was evidence of an intact end-organ response. Displacement studies showed that this antiserum was directed primarily against lysine-vasopressin.

It is unlikely that the anti-vasopressin antibodies of our patient represented circulating autoantibodies to hypothalamic vasopressin cells, of pathogenic importance and representing an "autoimmune variant" (9). The progressive resistance to vasopressin clearly developed during the patient's treatment with PTO and was not part of his initial clinical presentation. In accordance with our observation, Vokes et al. (4) also found very low incidences of anti-vasopressin antibodies (2.5 ± 0.3% vs 2.3 ± 0.6% in controls) in untreated patients with neurogenic diabetes insipidus.

Currently, probably most, if not all, cases of central diabetes insipidus are treated with DDAVP (10), making a development of antibodies against lysine-vasopressin unlikely; however, our observation also portrays the rapid changes in the understanding and treatment of neurogenic diabetes insipidus. Availability of specific antibodies to AVP raised in laboratory animals, plasma extraction procedures, and reliable tracers with high specific activities affect the diagnosis of patients with nonosmotic polyuria (11).

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References