Polyuria associated with an antibody to vasopressin

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Summary

1. A patient with polyuria in whom diabetes insipidus had been diagnosed was treated with Pitressin. Resistance to this therapy developed after 18 months and a circulating antibody to vasopressin was then demonstrated. Withdrawal of therapy led to a fall in titre of the antibody and an increase in maximal urinary concentration.

2. The antibody to vasopressin was associated with the IgA fraction of the serum immunoglobulins and its characteristics are described.

Key words: polyuria, vasopressin antibody.

Introduction

One cause of resistance to treatment with large polypeptide hormones such as insulin is the development of antibodies. Small polypeptide hormones such as vasopressin are not usually considered to be antigenic and there is only one published report of an antibody to vasopressin in man (Roth, Glick, Klein & Petersen, 1966). However, in the development of radioimmunoassays unconjugated vasopressin has been found effective in raising antisera in experimental animals (Edwards, Chard, Kitau, Forsling & Landon, 1972). Moreover, commercially available preparations of vasopressin such as Pitressin (Parke Davis and Co.), prepared from extracts of bovine or porcine posterior pituitary tissue, may contain neurophysin (Martin, 1971) or other extraneous proteins or peptides with which vasopressin could conceivably form antigenic complexes. In this paper we report a case of polyuria in which the patient became refractory to treatment with Pitressin and was found to have an antibody to vasopressin. A preliminary report has been published (Bisset, Black, Hilton, Jones, Kanjanapothi & Montgomery, 1974).

Case report

A 31-year-old man developed polyuria and polydipsia abruptly in July 1967 and was admitted to another hospital. Blood urea, plasma electrolytes, serum calcium, skull and chest X-rays were normal. He produced 1.5–7 l of urine daily with a specific gravity of between 1.000 and 1.010. Urinary specific gravity after fluid deprivation was not above 1.010, but vasopressin reduced the urine volume to less than 1 l/day with an associated specific gravity of 1.019. A diagnosis of diabetes insipidus was made and treatment with Pitressin tannate in oil (5 units daily) controlled his symptoms for approximately 18 months, when polyuria and polydipsia gradually recurred, despite continued administration of Pitressin. About this time he developed intermittent reactions to the injections of Pitressin, consisting of palpitations, facial flushing, vomiting and a tight feeling in the chest. The original diagnosis was questioned and in February 1971 he was referred to St Thomas' Hospital. Investigations performed at that time revealed the following results: haemo-
globin, 15.6 g/100 ml; leucocytes, 8.4 x 10^3/mm^3 with 2.3 x 10^3 eosinophils/mm^3; blood urea, 4.6 mmol/l; plasma creatinine, 88 µmol/l; 24 h creatinine clearance, 105 ml/min; plasma sodium, 148 mmol/l; plasma potassium, 4.1 mmol/l; plasma bicarbonate, 29 mmol/l; plasma osmolality, 305 mosmol/kg; plasma sodium, 148 mmol/l; plasma potassium, 4.1 mmol/l; plasma bicarbonate, 29 mmol/l; plasma osmolality, 305 mosmol/kg; serum calcium, 2.4 mmol/l; serum IgG, 1160 mg/100 ml; serum IgA, 680 mg/100 ml; serum IgM, 70 mg/100 ml.

A water-deprivation test was continued until a loss of 5% body weight had occurred. This reduction in weight was maintained for a further 8 h by allowing him to drink a volume of water equal to that passed as urine. During this period, the highest urinary osmolality achieved was 126 mosmol/kg. An intramuscular injection of 5 units of Pitressin tannate in oil, and a later intravenous injection of 10 munits of arginine vasopressin, failed to produce a rise in the urinary osmolality.

Pitressin injections were stopped in June 1971, and from October 1971 to October 1972 he was treated with a benzothiadiazide diuretic. During the latter part of this period the daily urine volume decreased, a process which continued after the diuretic was withdrawn. A water-deprivation test in November 1972 resulted in a weight loss of only 1.3 kg in 18 h and urinary osmolality reached 700 mosmol/kg.

His subsequent medical history has been dominated by an obsessional neurosis with prominent depressive features which has required psychotherapy. Complaints of thirst and polyuria have almost disappeared and no treatment for diabetes insipidus has been given for 3 years.

The history at the time of referral to St Thomas' Hospital suggested the possibility that the patient had developed an antibody to arginine vasopressin. When the hormone was incubated with the patient's plasma in vitro, it was rapidly inactivated by a factor which was shown to have the characteristics of an antibody and was isolated in the IgA fraction of the immunoglobulins. The effect of this antibody on arginine vasopressin and some related peptides is reported in this paper.

**Methods**

**Blood samples**

Blood (20 ml) was withdrawn from the antecubital vein into plastic syringes and silicon-treated needles. The plasma was separated by centrifugation at 2000 g for 10 min and stored at 4°C.

**Separation of plasma fractions**

**Paper electrophoresis.** Portions (80 µl) of the patient's plasma were subjected to paper electrophoresis in barbitone buffer, pH 8.6, for 4 h. The paper was then cut into strips corresponding to the main groups of plasma protein (α1-, α2-, β- and γ-globulins and albumin) and extracted with sodium chloride solution (150 mmol/l). Residual barbitone was removed by dialysis.

**Gel filtration.** This was performed with Sephadex G-200 with a column measuring 70 cm x 2 cm and sodium chloride solution (150 mmol/l) as eluent. Three protein peaks were separated. The first corresponded to the void volume. The second contained predominantly immunoglobulins (IgA and IgG) with a mol. wt. of about 150 000, and the third, albumin with a mol. wt. of about 70 000.

**Ion-exchange chromatography.** A sample of the patient's plasma at 0°C was treated with saturated ammonium sulphate solution to a final concentration of 33 g/l. The globulin precipitate was washed and dialysed against 0.01 mol/l phosphate buffer, pH 6.8. A column of diethylaminoethylcellulose was equilibrated against the same buffer and the sample of globulin applied to the column. Elution was performed with a gradient of phosphate buffer from 0.01 to 0.3 mol/l. Two fractions were collected containing IgG and impure IgA. The concentrations of IgG and IgA in these fractions and in the original sample of plasma were estimated by the method of single radial immunodiffusion (Mancini, Carbonara & Heramens, 1965).

**Incubation of peptides with plasma and plasma fractions**

Neurohypophyseal hormones and the synthetic analogue 1-desamino-8-D-arginine-vasopressin (DDAVP; Zaoral, Kolc & Šorm, 1967) were incubated with neat or diluted plasma or with plasma fractions in a total volume of 2 ml at room temperature. The total amounts incubated were 400-800 μunits of arginine or lysine vasopressin, 800 pg of DDAVP(1) and 400 μunits of oxytocin. All dilutions were made in sodium chloride solution

(1) Abbreviation: DDAVP, 1-desamino-8-arginine-vasopressin.
Antibody to vasopressin (154 mmol/l). Inactivation of the peptides was measured by assaying the antidiuretic or milk-ejecting activity of portions of the incubation mixture taken at various time-intervals after the start of the incubation. Antidiuretic activity was assayed by intravenous injection in water-loaded rats under ethanol anaesthesia in which a constant water-load equivalent to 8% of the body weight was maintained. The method is essentially that described by Dicker (1953) with subsequent modifications (Bisset, 1962; Clark & Rocha e Silva, 1967). Milk-ejecting activity was assayed by retrograde arterial injection in anaesthetized lactating rats (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967).

Materials
The following synthetic peptides were used: arginine vasopressin (Sandoz); lysine vasopressin (Sandoz); oxytocin (Syntocinon, Sandoz); 1-desamino-8-α-arginine-vasopressin (nasal drops; Ferri ng AB, Malmo; 0.1 mg/ml; batch 28071).

Results
The experiments reported in this section were carried out with samples of plasma obtained from the patient at various times between February 1971 and March 1972.

Incubation of peptides with plasma
Arginine vasopressin, lysine vasopressin, DDAVP and oxytocin were all inactivated when incubated with the patient's plasma, the time-course of inactivation depending on the relative proportions of peptide and plasma. An experiment with arginine vasopressin is illustrated in Fig. 1. The profile of the antidiuretic response to 80 μunits was changed after incubation with 0.04 ml of plasma for only 1 min. The onset was slower and the response prolonged, although the maximum intensity was smaller than that observed with 40 μunits of standard vasopressin. After incubation for 35 min the response to 80 μunits of vasopressin was abolished. Incubation for 45 min with five times the amount of plasma from a control subject did not reduce the antidiuretic response to vasopressin. To determine the threshold amount of the patient's plasma required for inactivation, samples of 800 μunits of arginine vasopressin

Fig. 1. Urine flow in a water-loaded rat under ethanol anaesthesia. Flow was measured with a drop recorder which reset to zero every minute. All injections were given intravenously. Each dot indicates the end of the minute during which the injection was made. The values below the tracing show the reduction in flow during the 5 min period from the second to the sixth minute after the injection, expressed as a percentage of the control flow before the injection. V: arginine vasopressin; PP: patient's plasma; CP: control plasma. The times (min) refer to the length of the periods of incubation of vasopressin with plasma.
were incubated with 0.1 ml of serial dilutions of plasma and the mixtures tested for antidiuretic activity after incubation for 60 min. There was no loss of activity with a dilution of 1/80, 40% loss with 1/40, 86% loss with 1/20 and total inactivation with 1/10 dilution. Lysine vasopressin was completely inactivated after incubation of 800 µunits with 0.4 ml of plasma for 80 min. The synthetic analogue, DDAVP, was inactivated more slowly than arginine vasopressin. In an experiment in which samples of DDAVP, was inactivated more slowly than arginine vasopressin. In an experiment in which 280 µg of DDAVP, and the incubation mixtures tested on the same rat, there was about 90% loss of activity with the natural hormone after 60 min but only 50% with the analogue after 90 min. However, almost total inactivation of the analogue was obtained with longer periods of incubation. Incubation of 400 µunits of oxytocin with 0.4 ml of plasma caused a progressive loss of milk-ejecting activity, with no change in the character of the milk-ejection response; inactivation was complete after 90 min. No loss of activity was observed with control plasma.

Characteristics of the inactivating factor in plasma

The factor in the patient’s plasma which inactivated arginine vasopressin retained its potency after storage of the plasma at 4°C for 2 years. It was not removed by dialysis in Visking tubing for 24 h. Acidifying and boiling diluted plasma destroyed its capacity to inactivate vasopressin. If vasopressin had first been inactivated by plasma, acidifying and boiling the incubation mixture resulted in almost complete restoration of the antidiuretic activity. This was shown by an experiment in which incubation of 400 µunits of arginine vasopressin for 3 h with 0.1 ml of the patient's plasma had caused at least 88% inactivation. After further incubation for 2 h, the mixture was acidified to pH 3 with 20% acetic acid, placed in a boiling-water bath for 5 min, cooled, neutralized and assayed for antidiuretic activity. This amounted to 86% of the original content of vasopressin. Enzymatic inactivation of vasopressin would be expected to be irreversible. The regeneration of activity in this experiment is consistent with the dissociation of a complex formed between the hormone and an antibody. Such a complex should be precipitated by ethanol (Edwards et al., 1972). To test this possibility, 20 µunits of arginine vasopressin and 20 µunits of oxytocin were added to 5 ml of the patient's blood and extracts prepared by the method of Bisset, Hilton & Poisner (1967). In this method, plasma is separated by centrifugation of the blood for 5 min, the plasma proteins are precipitated by adding twice the volume of ethanol and the ethanolic supernatant is concentrated by vacuum distillation. The recovery of the added vasopressin was 0.9% and that of the added oxytocin, 8.6%. Recoveries from a control sample of blood extracted simultaneously were 56% and 88% respectively. However, when 800 µunits of arginine vasopressin were incubated with 4 ml of the patient's plasma until no detectable antidiuretic activity remained, and the plasma was acidified to pH 3 with 20% acetic acid before extraction with ethanol, 65% of the vasopressin added was recovered.

Incubation of peptides with plasma fractions

The fractions obtained by paper electrophoresis were tested against lysine vasopressin. Inactivation was produced by the fraction containing γ-globulins but not by those containing α1-, α2- or β-globulin or albumin. The three fractions obtained by gel filtration were tested against arginine vasopressin. The fraction containing predominantly IgA and IgG caused inactivation, but not the void volume from the column eluate, nor the fraction representing the albumin peak.

An experiment to differentiate between IgA and IgG, which had been partially separated by ion-exchange chromatography, is illustrated in Fig. 2. Incubation of 80 µunits of arginine vasopressin with 0.02 ml of plasma for 30 min produced partial inactivation, the profile of the antidiuretic response resembling that illustrated in Fig. 1. Incubation of 80 µunits for 30 min with a volume of the IgA fraction containing the same amount of IgA (10 µg) as 0.02 ml of plasma produced an almost identical antidiuretic response. After incubation with the IgA fraction for 120 min the vasopressin was completely inactivated. In contrast, the response to 80 µunits of vasopressin incubated with a volume of the IgG fraction corresponding to 0.02 ml of plasma was almost indistinguishable from the response to 80 µunits of standard vasopressin. This experiment indicated that the factor in plasma inactivating arginine vasopressin could be quantitatively recovered from a fraction of the plasma proteins consisting predominantly of IgA.

A progressive decline in the inactivating potency
Antibody to vasopressin

FIG. 2. Rat urine flow. For the key to abbreviations etc., see Fig. 1. IgA and IgG: fractions separated from the patient's plasma by ion-exchange chromatography, the volumes used containing the same amounts of IgA and IgG respectively as 0.02 ml of unfractionated plasma (PP).

of the patient's plasma was evident from a comparison of experiments in which samples obtained at different times were incubated with a standard amount of 400 μunits of arginine vasopressin. The experiment illustrated in Fig. 1 was carried out with a sample of plasma obtained in February 1971; 0.2 ml caused more than 50% loss of antidiuretic activity after 1 min and total loss after 35 min. In September 1972, 0.4 ml of plasma caused only 50% loss after 70 min. In September 1973, 1 ml of plasma caused no loss after 2 min, and only 75% loss after 90 min; incubation for 180 min was required for total inactivation. Endogenous antidiuretic activity equivalent to 1.8 μunits of vasopressin/ml was detected in an extract of the plasma obtained in September 1973. The extract was prepared by the method of Ratcliffe & Edwards (1971) (see Bisset & Jones, 1975), in which vasopressin is adsorbed to glass beads and eluted with aqueous acetone. To provide the optimum pH for adsorption, the plasma is first acidified to about pH 3; according to the results reported in the previous section this would be expected to dissociate any vasopressin bound to antibody in the patient's plasma.

Discussion

The investigations described in the patient demonstrate that a state of vasopressin-resistant polyuria was associated with a circulating factor which inactivated vasopressin. Our results lead to the conclusion that this factor was an antibody contained in the IgA fraction of the immunoglobulins. It is of interest to consider possible mechanisms by which the antibody arose. One possibility is that the patient had diabetes insipidus and that the antibody was formed in response to administered Pitressin. In favour of this suggestion is the effectiveness of Pitressin, admittedly in large doses, in controlling the polyuria for some 18 months after diagnosis. This was followed by the development of reactions to injections of this preparation and increasing resistance to its antidiuretic action. Against this interpretation is the evidence of good neurohypophyseal function now. Although idiopathic diabetes insipidus may fluctuate in its severity, such a prolonged period of essentially normal water balance without treatment as the patient has experienced over the last 3 years would be unusual. Moreover, there is no evidence of any disease which might have led to a self-limited period of diabetes insipidus. Was diabetes insipidus caused by the development of an antibody to endogenous vasopressin? Against this possibility are the steady decline in antibody titre after stopping Pitressin injections, together with the good neurohypophyseal function over the last 3 years.

Many aspects of the problem would best be explained by postulating that the patient initially developed compulsive polydipsia, the antibody arising in response to administered Pitressin. The initial good response to Pitressin therapy is unusual in patients with compulsive polydipsia (Barlow & de Wardener, 1959) but this explanation seems the most plausible, although a firm conclusion is not warranted. The high antibody titre was maintained as
long as Pitressin was continued, but withdrawal of treatment led eventually to a marked reduction in titre of the antibody, associated with an ability to secrete hypertonic urine in response to water deprivation.

Partial purification of the factor responsible for the patient's vasopressin resistance in 1971 suggested that it belonged to the IgA fraction of the immunoglobulins. Injected antigens usually induce IgG or IgM antibodies and IgA is believed to be associated principally with immune mechanisms associated with body surfaces. We cannot explain the distribution of the patient's antibody, particularly as he did not receive vasopressin by nasal spray or insufflation.

An attempt was made to investigate the specificity of the antibody for arginine vasopressin. The synthetic analogue 1-desamino-8-D-arginine-vasopressin (DDAVP) differs from the natural hormone, arginine vasopressin, in having no terminal NH₂ group in the hemicystine residue in position 1 and D-arginine in place of L-arginine in position 8. The NH₂ group is necessary for the binding of the natural hormone to its carrier protein neurophysin (Hope & Pickup, 1974). It was thought that, if this group were also involved in binding to an antibody in the patient's plasma, DDAVP might not be bound and, since it has high antidiuretic with negligible pressor potency (Vávra, Machová, Holeček, Cort, Zaoral & Šorm, 1968), it might have been of therapeutic value to the patient. DDAVP was inactivated by plasma although at a slower rate than arginine vasopressin. This suggests that neither the terminal NH₂ group in position 1 nor L-arginine in position 8 is critical for binding. The inactivation of lysine vasopressin, which contains lysine in place of arginine in position 8, confirms that this part of the molecule is not essential for binding.

Oxytocin, which differs from vasopressin in containing isoleucine in place of phenylalanine in position 3 and leucine in place of arginine in position 8, was also inactivated by plasma. If a single antibody is involved in these reactions, the inactivation of oxytocin again excluded position 8 as a binding site and suggests that position 3 is not critical. It is possible, however, that a second antibody might have formed to a trace of oxytocin present as an admixture with vasopressin in Pitressin.

The detection of an antibody to vasopressin in the patient's plasma has been of pharmacological value. The plasma has been used to identify the antidiuretic activity detected in extracts of blood collected during haemorrhage as due to vasopressin (Bisset, 1976) and to identify vasopressin in extracts of a bronchial carcinoma from a patient with the suspected syndrome of inappropriate secretion of antidiuretic hormone (Bisset & Jones, 1975). The differential diagnosis of polyuria often depends on the correct interpretation of a water-deprivation test. Failure to concentrate the urine in response either to water deprivation or to the subsequent injection of vasopressin would usually be attributed to a renal tubular defect. Investigation of the case reported in this paper has revealed that a similar pattern of response may be produced by a circulating antibody to vasopressin.

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References


