The Syndrome of Inappropriate Secretion of Antidiuretic Hormone: A Case Report and Characterization of an Antidiuretic Hormone-like Material Isolated from an Oat Cell Carcinoma of the Lung

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SUMMARY

This paper presents a report of a patient with the syndrome of inappropriate secretion of antidiuretic hormone associated with an oat cell carcinoma of the lung. Antidiuretic activity was found on bioassay of plasma and tumor extracts. The active material could be extracted from the tumor by procedures known to isolate the native human peptide, arginine vasopressin. The material passed through a Sephadex G-25 column with a Vc/Vo ratio identical to that of vasopressin; on counter-current distribution, the material possessed a partition coefficient identical to that of purified arginine vasopressin.

INTRODUCTION

It has long been known that neoplasms of endocrine tissues are capable of elaborating excessive quantities of the hormone normally produced by the endocrine organ. The clinical syndromes associated with such tumors are well recognized. Recently, a growing number of papers have described neoplasms of nonendocrine tissues associated with endocrine disorders. The syndromes associated with such neoplasms include Cushings's syndrome, hyperthyroidism, hypercalcemia, hypoglycemia, erythrocytosis, atypical carcinoid syndrome, precocious puberty, and inappropriate secretion of antidiuretic hormone. The subject of endocrine syndromes associated with malignant tumors of nonendocrine origin has been extensively reviewed (7, 21, 32, 33, 36). The hormonal substances have been partially characterized and, in some cases, the evidence indicates that the material produced by the tumor is identical to the naturally occurring hormone; in other cases, the substance, although biologically active, is structurally dissimilar to the natural product.

The present report is that of the twenty-seventh patient reported with the syndrome of inappropriate secretion of ADH (antidiuretic hormone or vasopressin) associated with bronchogenic oat cell carcinoma and demonstrable plasma antidiuretic activity (31). It is the first case in which gel filtration and counter-current distribution studies of the antidiuretic material isolated from the tumor have been carried out.

CLINICAL HISTORY

A 61-year-old white man was admitted to Methodist Hospital with a six-week history of intermittent epigastric pain, anorexia, malaise, a nine pound weight loss, and episodes of irrational behavior. His past and family history and review of systems were noncontributory. He had smoked one pack of cigarettes daily for more than thirty years. Physical examination upon admission was unremarkable. Hematologic studies and urinalysis were within normal limits. Blood urea nitrogen, total serum proteins, serum calcium, and serum phosphorus were all normal. Bromsulphalein retention was 22% after 30 minutes, but other liver function tests were normal. Glucose tolerance, intravenous pyelogram, and radiologic studies of the upper and lower gastrointestinal tract showed no abnormalities. Serum sodium was 112 mEq/liter, chloride 83 mEq/liter, potassium 4.8 mEq/liter, and carbon dioxide combining power 23 mEq/liter. At that time serum osmolality was 234 mOsm/kg, and urine osmolality was 659 mOsm/kg. The possibility of inappropriate secretion of ADH was entertained.

Further studies revealed 24-hour urinary excretion of 17-hydroxy- and 17-ketosteroids at 5.5 mg and 17-ketosteroids at 6.2 mg. A T-3 test was normal. Urine coproporphyrins, protoporphyrins, and uroporphyrins were normal. An EEG revealed only diffuse slowing. Isotope studies demonstrated an increase of total body water with elevation in both extracellular and intracellular fluid compartments with normal total body sodium. Blood was drawn for determination of ADH activity.

A chest X-ray showed changes suggestive of carcinoma of the lung. On bronchoscopy, a lesion of the right upper lobe bronchus was biopsied, confirming the impression of bronchogenic carcinoma. The tumor was judged to be nonresectable, and the hilus was irradiated with 700 roentgens. The tumor decreased in size on X-ray, but the patient's condition deteriorated. When water intake was restricted to less than 100 ml daily, the serum sodium rose to 128 mEq/liter. The patient developed a severe anemia and pancytopenia. Bone marrow

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biopsy showed almost complete replacement by neoplastic cells. Chemotherapy with cyclophosphamide and corticosteroids was initiated with little clinical improvement. The patient expired three months after the initial admission.

At autopsy, which was restricted to the thorax, a tumor mass 1.5 X 2.5 cm was found in the right upper primary bronchus. The tumor was of the undifferentiated small cell, or oat cell, type. There were widespread metastases to both lungs and throughout the mediastinum. Approximately 10 gm of the tumor tissue was taken for biochemical and biologic studies.

MATERIALS AND METHODS

Bioassays. For measurement of antidiuretic activity in both plasma and tumor extracts, ethanol-blocked, water-loaded rats were employed (12, 15). Because this is a difficult assay and responses to standards vary among individual animals and in a single animal during the test period, each test dose was bracketed by arginine vasopressin standards. Responses were obtained in some animals to as little as 10 μU of USP Standard arginine vasopressin.

The pithed-rat pressor assay was employed to determine pressor activity of tumor extracts (13). Consistent responses could be obtained with as little as 0.5 mU of USP Standard arginine vasopressin.

Extraction Procedures. The tumor tissue collected at autopsy was minced and placed in chilled acetone. The partially defatted and dried residue was subjected to a modified Kamm procedure (24). The material was first extracted directly into 200 ml of 0.5% acetic acid. The extraction mixture, with brisk stirring, was heated to 90°C for 30 minutes, cooled, and filtered. The procedure was repeated on the residue. The clear filtrates were combined, concentrated, and lyophilized to obtain the acetic acid-soluble portion which was assayed for ADH activity.

The acetic acid-soluble fraction was dissolved in 0.1 M pyridine acetate buffer at pH 4.5. The insoluble portion was removed by centrifugation, and the soluble portion was submitted to molecular sieve filtration on Sephadex G-25 in a column 2 x 100 cm with a flow rate of 37 ml per hour at 24°C (20). The 600-ml effluent was collected in 4.5 ml fractions, and 0.5 ml aliquots were removed from every tenth fraction for assay of antidiuretic activity. The tubes containing ADH-like activity were pooled and lyophilized. Counter-current distribution was done using 20 standard taper stoppered cylinders wired in sequence on a test tube rack; each tube contained 1.5 ml per phase of the system 0.03 M p-toluene sulfonic acid and 2-butanol (47). All transfers were made manually for the upper phase transport to the adjoining tube at each distribution transfer step. After 20 transfers, 0.1 ml of the lower phase was analyzed for protein content by the Folin-Lowry reaction (34). Aliquots were also taken for pressor assay after dilution with distilled water, neutralization of the sulfonic acid, lyophilization, and reconstitution with distilled water.

Material from those tubes subjected to counter-current distribution containing pressor and ADH activity were combined for a thioglycollate inactivation study. The fractions were dissolved in 2.4 ml water; one-half was used as an untreated control with the addition of 1.2 ml of water, and the other half was treated with an equal volume of 0.1 x thioglycollate at pH 7.6, a procedure which has been shown to inactivate arginine vasopressin (3). Pressor activity was assayed at approximately ten-minute intervals after the addition of thioglycollate.

RESULTS

Plasma ADH Activity. Injection of 0.1 and 0.2-ml samples of plasma from the patient on two occasions in a single test animal produced an antidiuretic response equivalent to that produced by an equal volume of a standard solution containing

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800 μU/ml USP Standard arginine vasopressin. Injection of equal volumes of plasma from normally hydrated control subjects failed to produce significant antidiuretic response in the same animal.

**Antidiuretic and Pressor Activity of Tumor Extracts.** Pressor assays of the acetate acid-soluble fraction which weighed 1.05 gm yielded pressor activity equivalent to 0.2 mU of USP Standard arginine vasopressin per milligram of the acetone-dried starting material in four test animals. This is about one-thousandth the concentration present in similar extracts of human posterior pituitary tissue (4).

Aliquots from every tenth tube of the effluent collected from the Sephadex gel filtration were subjected to assays for antidiuretic and pressor activity in four test animals. Significant antidiuretic and pressor responses were present in the region of 250–320 ml of the 600 ml effluent (Chart 1). The V_r/V_o ratio calculated at the midpoint of the band was 2.45, the V_r for the column being 116 and the mean V_o 280. The value for pure arginine vasopressin using this type of column and system has been found to range between 2.15 and 2.7 (40).

The 189-mg fraction with pressor activity was submitted to counter-current distribution to distinguish between arginine and lysine vasopressin. Chart 2 shows the Folin-Lowry curve for peptide concentration after twenty transfers, along with rat pressor activity plotted against tube number. Pressor assays in four different rats yielded identical results. Biologic activity was localized to the area of Tube 7. The calculated partition coefficient (K value) was found to be 0.54, and is identical to the K value for purified arginine vasopressin (41, 47). The K value of lysine vasopressin is 0.32 (41, 47).

Although at this point in our studies the active material was far from pure, the quantity remaining was small and further purification was not feasible. The material from Tubes 5 through 9 was pooled for a thioglycollate inactivation study. After incubation with sodium thioglycollate for 90 minutes, virtually all pressor activity had disappeared from the treated fraction while activity in the control fraction remained unchanged.

We have estimated that the 10 grams of tumor tissue removed at autopsy contained antidiuretic hormone activity equivalent to approximately 2 × 10^-4 U of USP Standard arginine vasopressin per milligram of wet tissue. Thus, at the onset of this study, the tissue received for analysis contained a total activity of less than 2 units or 5 μg of vasopressin.

**DISCUSSION**

The structure of vasopressin from human pituitary glands has been established (30). Vasopressin of porcine origin has been found to differ from that of other animals in that it contains a lysine residue in place of arginine in the seven position (16).

The syndrome of inappropriate secretion of ADH has been described in association with a number of clinical conditions, including myxedema (11, 39), acute intermittent porphyria (35, 37), bronchogenic carcinoma (2, 6, 9, 28, 44), intracranial disease (1, 17, 18), and tuberculosis (48), as well as in the absence of any other apparent clinical abnormality (19, 22). Clinically, this syndrome is characterized by persistent hyponatremia in the face of continued renal sodium excretion and urine osmolality greater than that of plasma. There are no signs of dehydration, and renal and adrenal function are normal. The extracellular fluid space is normal or only slightly expanded. The electrolyte abnormalities do not respond to sodium loading but, rather, are corrected by water restriction. The term "inappropriate" secretion of ADH is used because the persistence of ADH activity is not appropriate to the co-existing hypotonic plasma, a condition normally inhibiting ADH release (26).

In the presence of a normal glomerular filtration rate, excretion of a hypertonic urine is presumptive evidence for the presence of ADH. When normal subjects are given sustained elevated doses of vasopressin along with abundant fluids, water intoxication occurs. The signs and symptoms are similar to those seen in patients with the syndrome of inappropriate secretion of ADH with an apparent primary retention of water with dilution of solutes and moderate increase in extracellular fluid volume (23, 25, 29). An extreme increase in fluid volume is prevented by increasing the renal excretion of sodium which results in still further reduction of extracellular fluid osmolality. Thus, constancy of volume is preserved at the expense of toxicity. The urinary sodium loss in these individuals probably involves both an increase of glomerular filtration rate and a decrease of aldosterone secretion in response to the expanded plasma volume (5, 27, 20). On the other hand, when continuous vasopressin is given to normal subjects but fluid intake is restricted, hyponatremia and urinary sodium loss do not occur (23, 26, 27).

Hyponatremia in conjunction with bronchogenic oat cell carcinoma was first reported by Winkler and Crankshaw in 1938 (49). Nearly 20 years elapsed before further note was taken of this association. In 1957, Schwartz and his associates reported two such cases and another in 1960 (42, 43, 44). A number of endocrine and metabolic balance studies were carried out that indicated that the abnormalities present could best be explained on the basis of sustained inappropriate ADH secretion.

Since that time, reports of the association of oat cell carcinoma of the lung and the syndrome of inappropriate secretion of ADH have appeared with increasing frequency. In 1963, Thorn and Transbol described a patient with this syndrome whose urine contained large quantities of an antidiuretic substance (45). This material, excreted in 24 hours, possessed an activity equivalent to approximately 3100 mU of arginine vasopressin. Incubation with thioglycollate destroyed 40% of this activity, and treatment with trypsin destroyed all antidiuretic activity.

Shortly thereafter, a report of a case by Amatruda et al. appeared with careful balance studies demonstrating the characteristic abnormalities of the syndrome (2). Urinary aldosterone levels were normal. Injection of vasopressin produced no increase in urine osmolality. Attempts to inhibit ADH secretion by vagal blockade and by the administration of ethanol and chlorpromazine had no effect on urine osmolality. At autopsy, the primary tumor was extracted and assayed for antidiuretic activity. The dried tumor contained antidiuretic activity equal to approximately 70–350 μU arginine vasopressin per milligram.
The characteristics of the antidiuresis in the bioassay of the tumor extracts were similar to those of arginine vasopressin. Biologic activity was destroyed by incubation with thioglycolate and by boiling with 0.1% sodium hydroxide. The authors suggested that the antidiuretic material might be vasopressin or another polypeptide with antidiuretic activity.

Bower and his associates were the first to demonstrate elevated antidiuretic activity in the plasma of a patient with the syndrome of inappropriate ADH secretion and bronchogenic carcinoma (8, 9). Activity in plasma from the patient ranged between 3 and 8 μU/ml, in contrast to normal subjects in whom no activity was detected. No change in urine osmolality was observed after administration of ethanol and of vasopressin to the patient. Extracts of the primary tumor which had received local X-ray therapy contained 0.1-1.0 μU/mg of antidiuretic activity, while extracts of the nonirradiated hepatic metastases contained 6-8 μU/mg of antidiuretic activity. Selective destruction of the posterior pituitary gland by an isolated metastasis was of interest in this patient. Assays were performed in ethanol-blocked rats and in rats with permanent diabetes insipidus produced by stereotaxically placed hypothalamic lesions. Their results were identical in the two groups, indicating the material had intrinsic antidiuretic activity rather than acting primarily as a stimulus for release of ADH.

De Sousa et al. studied activity of an oat cell tumor extract from a patient with the inappropriate ADH syndrome (14). They found that antidiuretic, vasopressor, and milk-ejecting activities of the substance were essentially in the same proportion as arginine vasopressin.

Recently, Utiger has described a patient with oat cell carcinoma of the lung and inappropriate water retention in whom the fluid and electrolyte abnormalities were corrected by excretion of the tumor (46). Radioimmunoassay studies were carried out on tumor extracts using labeled lysine vasopressin and antiserum. The dose response curve of tumor extracts closely paralleled that of a standard curve using arginine vasopressin. The tumor extracts contained activity equivalent to 8-427 μU arginine vasopressin per milligram of wet tissue. These studies would indicate that the tumor tissue contained arginine vasopressin or a closely related peptide.

The present report describes a patient who had the clinical hallmark of the syndrome of inappropriate secretion of ADH. Urine osmolality was consistently greater than that of serum. Hyponatremia in the face of urinary sodium excretion was responsive to water restriction but not salt loading. Determinations of total body water revealed increases in both extracellular and intracellular compartments in conjunction with normal total body sodium. Renal and adrenal function were intact. Tests for porphyria and hypothyroidism were negative. A chest X-ray suggested carcinoma of the lung, and an oat cell carcinoma was found on biopsy and on postmortem examination. Biosays of plasma performed before death revealed excessive antidiuretic activity. Crude extracts of the tumor contained pressor and antidiuretic activity. Activity was destroyed by treatment with thioglycolate, suggesting the presence of a disulfide bond. The material containing this activity could be extracted by the procedure used to isolate arginine vasopressin from the neurohypophysis. The active fraction passed through a Sephadex G-25 column with a Vₒ/Vₑ ratio identical to that of vasopressin, indicating residence of activity in a molecule with physical dimensions and molecular configuration similar to those of vasopressin and oxytocin. When subjected to counter-current distribution in p-toluenesulfonic acid and 2-butanol, the active material possessed a partition coefficient identical to that of purified arginine vasopressin and differing significantly from that of lysine vasopressin.

A number of mechanisms might be postulated to explain how the tumor might be responsible for the induction of a sustained inappropriate secretion of ADH. The theory most widely accepted at the present postulates that the tumor is the site of de novo synthesis of an ADH-like substance. The possibility that the ADH-like material in the tumor was synthesized elsewhere and trapped in the tumor tissue cannot be ruled out. However, if this were the case, one must postulate some protective mechanism for the stabilization of vasopressin in the tumor, for vasopressin in the circulation is normally quickly metabolized and has not been shown to be stored in significant quantities in tissues other than the hypothalamus and posterior pituitary.

We believe the most reasonable interpretation of our results and those of others is that the tumor itself contains the genetic information for the synthesis of arginine vasopressin. This biosynthesis is not controlled by the usual regulating mechanisms, and abnormally high and sustained levels of vasopressin are released into the circulation to produce the clinical manifestations of this syndrome.

Although neoplastic transformation of tissue has been characterized classically by extensive dedifferentiation, in these cases of humoral syndromes associated with tumors of nonendocrine tissues, neoplasia results in the production of rather complex peptide hormones ordinarily considered highly specific to endocrine organs. This apparent discrepancy may be accounted for if the assumption is made that all cells have the same genetic information coded on an identical complement of DNA (32, 33). In the process of their dedifferentiation these neoplastic cells may have lost a genetic repressor mechanism with the consequent unmasking of genetic information normally not expressed. Thus, these rather unusual syndromes provide a compelling link between molecular biology and clinical medicine.

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