Relationship Between Endogenous Antidiuretic Hormone Activity and ACTH Release in Man. (23567)

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On the basis of certain anatomic and physiological observations, the antidiuretic hormone (ADH), vasopressin, has been postulated to be the neurohormone responsible for stimulating ACTH release(1-3). The observation that the intravenous administration of vasopressin in man is associated with a rise in plasma hydrocortisone is in agreement with this hypothesis(4-7). If ACTH release is produced by ADH, one would expect to see increased plasma hydrocortisone levels associated with endogenous ADH release. The present study is concerned with an evaluation of this postulated mechanism of ACTH release. The experiments were designed to answer the following questions: 1. Is the endogenous release of ADH associated with a rise in plasma hydrocortisone concentration? 2. Are increases in plasma hydrocortisone concentration associated with a concomitant release of ADH? Accordingly, a number of stimuli have been employed which are capable of producing an increase either in adrenocortical activity or in ADH activity.

Methods. Thirty-two experiments were performed in 8 male and 7 female normal subjects ranging in age from 18 to 28 years. All tests were begun at approximately 8 A.M. with fasting subjects recumbent in bed. In the studies conducted on males, urine was collected by voluntary micturition. In most of the studies conducted on females, an indwelling urethral catheter was used. Venous blood samples were obtained by means of an indwelling Cournand needle. In all studies carried out during a water diuresis, a total of 1600 ml of water was given by mouth in 4 divided doses over a period of 90 minutes. Urine osmolality was determined by the method of freezing point depression employing an Aminco-Bowman freezing point apparatus. Plasma hydrocortisone was determined by the method of Silber and Porter(8), as modified by Peterson, *et al.*(9). Plasma sugar was determined by the method of Nelson(10).

Results. Fluid Deprivation. Comparative observations on the effect of fluid deprivation and water loading were made in 6 subjects on 2 different days. On both test days fluid deprivation was maintained for 14 hours (6 P.M. to 8 A.M.) preceding the test. On one day fluid deprivation was continued throughout the test, while on the other day endogenous ADH release was suppressed by the administration of 1600 ml of water. During continued water deprivation the mean urine osmolality was 950 mosm./kg H_2O (range: 882-1018) at 8 A.M. and 824 (range: 684-904) at 12 M. That ADH suppression had occurred as a result of water loading was evidenced by the fact that the urine osmolality decreased from a mean of 828 (range: 553-1043) at 8 A.M. to a mean of 125 (range: 84-188) at 12 M. Fig. 1 shows the mean hourly plasma hydrocortisone and urine osmolality values from 8 A.M. to 12 M. on the 2 test days. Despite the persistent ADH activity associated with continuing water deprivation, the plasma hydrocortisone levels decreased in a manner similar to those ob-

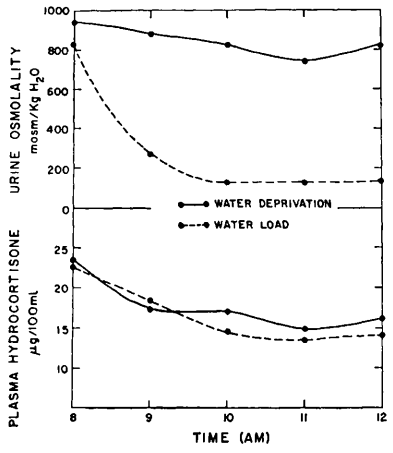

FIG. 1. Effect of fluid deprivation and water loading on plasma hydrocortisone concentration and urine osmolality for six normal subjects. Each plot is the mean for the 6 subjects.

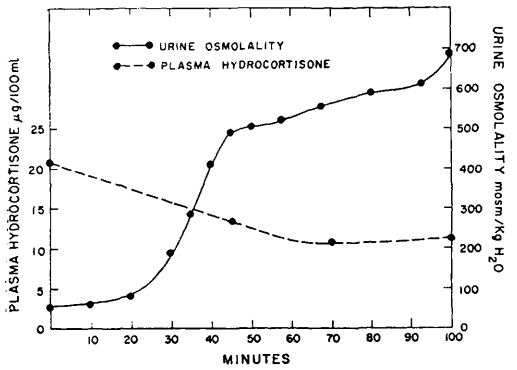

FIG. 2. Effect of 45-min. infusion of 5% saline on plasma hydrocortisone concentration and urine osmolality for three subjects during water diuresis. Each plot is the mean for the 3 subjects.

served during ADH suppression.

Hypertonic saline. Fig. 2 shows the effect of intravenous infusions of five percent sodium chloride solution on plasma hydrocortisone and urine osmolality values for three subjects during water diuresis. Five percent sodium chloride in volumes from 360 to 440 ml was administered over a 45 minute period. A rise in urine osmolality occurred in each case. The mean urine osmolality prior to the saline infusion was 57 (range: 55-60). During the saline administration the urine osmolality rose to a mean of 690 (range: 675-709). Despite this evidence of endogenous ADH release, the plasma hydrocortisone levels did not rise.

Nicotine. Intravenous injection of 3 mg of nicotine bitartrate (1 mg nicotine base) in 4 subjects during a water diuresis was followed by an increase in urine osmolality in all cases. Mean control urine osmolality was 71 (range: 61-77) rising to a maximum of 399 (range: 276-676) following the injection. Blood for plasma hydrocortisone determinations was obtained at 15, 30 and 60 minutes following the injection of nicotine. In 2 of the 4 subjects, plasma hydrocortisone concentration did not change despite the rise in urine osmolality.

Hand immersion in ice water for periods of 2 to 6 minutes was carried out in 8 subjects during a water diuresis with a mean baseline urine osmolality of 90 (range: 53-134). In 5 subjects urine osmolality increased to a concentration consistent with ADH release (mean: 321, range: 180-447). Two of these 5 subjects evidencing a rise in urine osmolality had no increase in plasma hydrocortisone concentration. One of the 3 subjects who had no increase in urine osmolality manifested a rise of 8 μg/100 ml in plasma hydrocortisone concentration. Thus in 3 of the 8 subjects evidence for simultaneous ADH and ACTH release was lacking.

Insulin-induced hypoglycemia. During water diuresis (mean urine osmolality: 66, range: 62-70) 5 subjects were given intravenous injections of 0.1 U crystalline insulin/kg body weight. This dose of insulin produced an average maximal fall in the plasma sugar of 54 mg/100 ml. A concurrent rise in urine osmolality and plasma hydrocortisone concentration occurred in only one subject. A rise in plasma hydrocortisone concentration occurred in 3 subjects without an associated increase in urine osmolality. The remaining subject manifested a rise in urine concentration of 330 mosm./kg H_2O with no associated rise in plasma hydrocortisone concentration. A composite of all the tests performed under conditions of initial water diuresis is shown in Fig. 5. It is apparent that there is no correlation between change in urine osmolality and change in plasma hydrocortisone concentration.

Discussion. In this study the 2 basic assumptions made are that increases in plasma hydrocortisone concentration and urine osmolality under the conditions of these experiments are valid evidences of ACTH and ADH release, respectively. ACTH release is pre-

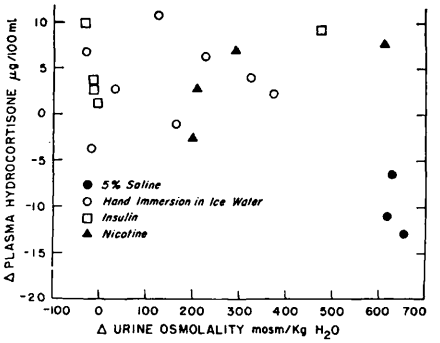

FIG. 3. Comparison of change in plasma hydrocortisone concentration and urine osmolality in four different test situations.

sumed if the plasma hydrocortisone concentration increases 3 μg/100 ml. 30 or more minutes following the test stimulus. This interpretation is based on observations of successive plasma hydrocortisone levels following intravenous injections of placebos in normal subjects under similar experimental conditions(6). A rise of 3 μg 100 ml plasma under the present experimental conditions is statistically significant ($P < 0.05$).

The use of urine osmolality as an index of ADH release under these experimental conditions is based on the following considerations. The average initial urine osmolality of 77 mosm. kg H_2O indicates that ADH secretion was greatly suppressed at the onset of all the tests(11) except fluid deprivation. Persistence of urine dilution of this degree is evidence for continuing suppression of ADH release. On the other hand, increases in urine osmolality, ranging from 115 to 654 have been interpreted as evidence of ADH release. Other factors, such as decreased glomerular filtration rate and increased solute excretion, are known to increase urine concentration in the absence of ADH(12-15). In the present study there was no evidence of an acute increase in solute excretion in any of the experiments with the exception of the intravenous administration of 5% saline. Urine concentrations greater than 600 mosm. kg H_2O observed during water deprivation and associated with the infusion of hypertonic saline during a water diuresis cannot be attributed to acute suppression of glomerular filtration rate(12). Thus the magnitude of the urine osmolality obtained during fluid deprivation and following hypertonic saline administration cannot be accounted for by any mechanism other than ADH release.

The increase in urine osmolality that results from hand immersion in ice water averaged 231 mosm./kg H_2O. The duration of the period of increased urine osmolality was at least 60 minutes in 3 subjects, 50 minutes in one subject, and 30 minutes in one subject. In view of the short duration of the hemodynamic response (<5 minutes) and the prolonged duration of the antidiuresis, such changes are interpreted as evidencing ADH release rather than a persistent reduction of glomerular filtration rate sufficient to cause changes of this magnitude.

In the insulin studies the persistence of a very low urine osmolality in 3 of the subjects indicates a continued suppression of ADH despite the rise in plasma hydrocortisone concentration.

The magnitude of the increase in urine osmolality following the injection of nicotine could conceivably result from hemodynamic changes in some of the studies. However, the duration of the response (at least 30 minutes) makes an ADH-mediated response the more likely interpretation.

Summary. 1) The hypothesis that ADH is the neurohormone responsible for ACTH release has been investigated in normal human subjects by simultaneous determination of urine osmolality and plasma hydrocortisone levels in various test situations. 2) The data indicate that in normal human subjects endogenous ADH release may occur without an increase in ACTH release. Furthermore, it has been shown that an increase in ACTH release may occur without evidence of ADH release. These observations fail to support the concept that endogenous ADH release stimulates the release of ACTH.

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Distribution of CO and Radiochromium in Blood and Tissues of Rabbit and Dog. I. Carbon Monoxide.* (23568)

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Comparative studies on methods for measuring blood volume have shown that the volume of distribution of carbon monoxide (CO) is 12 to 16% greater than that of radioactively tagged red cells in splenectomized dogs(1) and human subjects(2). The discrepancy has been attributed to accumulation of CO in extravascular pigments, chiefly myoglobin, as proposed by Haldane and Smith(3), but the sites of accumulation of CO have never been demonstrated directly. We have analyzed homogenates of various tissues from rabbits and dogs after simultaneous administration of CO and autogenous red cells tagged with radiochromium (Cr^{51}), using the radioactivity of each homogenate as an index of its blood content, and found the principal sites of CO accumulation to be red skeletal muscle and myocardium.

Methods. Fifteen ml of blood from each animal was tagged with Cr^{51}, and the cells were stored overnight in saline or their own plasma at 5°C(2). Tracheotomy was done under narcosis with sodium pentobarbital (Nembutal), and the airway was connected to a small rebreathing system for administration of measured amounts (15 to 100 ml) of CO gas(4). Ten ml of the animal's own Cr^{51}-tagged cells was injected into a vein. Twenty minutes later, blood was drawn from the heart or a large vessel into tubes containing Wintrobe's oxalate mixture. The animals were killed by injection of Nembutal or by exsanguination. Pieces of heart, spleen, lung, liver, kidney, skeletal muscle, brain, bone and bone marrow were excised, wiped dry and homogenized. The blood was analyzed for CO by the palladium reduction method of Wennesland(5), and 3 g portions of homogenates were analyzed by a modification of a procedure used for blood clots(6). Five ml of a 1% solution of pepsin powder‡ in 1 N H_2SO_4 was added to each portion of homogenate to accelerate liberation of CO. Six to 8 hours were needed for complete liberation of the gas and reduction of an equivalent amount of $PdCl_2$. After the iodometric titration was completed, each sample was brought to a total volume of 12 ml by addition of distilled water. Its radioactivity in counts per second was measured in a flange-type scintillation counter. A total of 4,096 counts was made. The counting error was ± 2%. The error of the analysis of CO in homogenates was assumed to be the same as that in the analysis of blood clots(6). To obtain the desired accuracy in the tissue analyses the animals were given relatively larger doses of CO and of tagged cells than are used in measurements of blood volume. The CO content in volumes % was compared to the radioactivity in counts per second to obtain CO:Cr^{51} ratios for each tissue homogenate (R_t) and for the blood of each animal (R_b). For each tissue homogenate the ratio, R_t:R_b, was calculated. With no

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