Advances in CLINICAL CHEMISTRY

Edited by

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New York, New York

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VOLUME 17 • 1975

ACADEMIC PRESS
New York San Francisco London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 58-12341

ISBN 0-12-010317-6

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

Once again, as is always our hope, the Editors have in this volume of the Advances included material which is appropriate in relation to both the clinical aspects of the subject and to technological advances. In fact, the four chapters included in this volume are concerned with such wide fields as diagnosis, therapy, and the etiology of disease, as well as technical advances, which will more commonly be applied in the very near future. The chapters are, we believe, excellent examples of the integral part that the clinical biochemist plays in modern medicine.

The problems associated with inappropriate secretion of antidiuretic hormone have been recognized in the wards for some time. Scheiner, in her review of this topic gives a well-balanced account of the normal physiology and biochemistry of this hormone and the methods for its assay. Having considered the pathophysiology of the syndrome under discussion, she then describes the pathological causes and the clinical aspects, including therapy. The chapter is well rounded-off by a discussion of problems that are still unresolved.

Gamma-glutamyl transpeptidase is becoming of increasing importance in clinical enzymology. The Editors, therefore, feel that they have been particularly fortunate in obtaining a review of this subject by so distinguished an authority as Rosalki. He has dealt very adequately with the action, biological significance, and methods for determination of the transpeptidase, as well as with its distribution in the tissues and body fluids. He has given a clear account of the diagnostic applications of the transpeptidase determination and has concluded his chapter with a description of the current situation in regard to its isoenzymes.

High resolution analysis must inevitably play a greater role in the work carried out in the clinical biochemistry laboratory. Our present preoccupation with so-called biochemical profiles is perhaps somewhat naïve. These investigations have been derived from the commoner tests used in the past, and although much has been written about them, it is perhaps debatable whether they will do much to advance medicine itself. It must be admitted, however, that there is some argument in favor of the statement that such profiles could be time-saving for the laboratory. Recognizing, however, that the fundamental aspects of disease are really involved with metabolic

changes, it is obvious that high resolution analysis is capable of providing much more sophisticated profiles of metabolites themselves and so will offer much in relation to the production of clinical problems. Mass spectrometry is in this respect an important technique, which undoubtedly will be used much more commonly. In his review, Roboz gives a very clear account of the scope of applications and nature of mass spectrometry as well as of the instrumentation and analytical techniques involved in the production of mass spectrographs, including mass fragmentography. Theoretical aspects of the subject are dealt with sufficiently well to enable the average clinical chemist to follow quite easily this clear account of a somewhat complicated field. Reference is made to the combination of mass spectrometry with gas chromatography. The chapter also includes an account of clinical applications in relation to blood gas analysis, detection and quantification of trace elements, and multicomponent analysis of endogenous metabolites in body fluids, which enables the production of metabolic profiles which can be used in the diagnosis of metabolic disorders, as well as in the discovery of new diseases and metabolites which have not been previously recognized. An account is also given of the application of the technique to the analysis of drugs and their metabolites.

Isoelectric focusing techniques in liquids and gels, especially the latter, are having wide application in biochemistry as a whole and are beginning to be used more and more in clinical chemistry itself. In his review, Latner has considered the general theory and various techniques for qualitative and preparative applications; he has also given an account of the present status of electrofocusing in the clinical biochemical situation.

Although the present volume, for reasons outside our control, is somewhat smaller than usual, the Editors hope that the high interest level of its contents compensates for this.

It is, once again, a great pleasure to thank our contributors and publisher for their excellent cooperation, without which this volume would not have been possible.

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A. L. LATNER

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THE RELATIONSHIP OF ANTIDIURETIC HORMONE TO THE CONTROL OF VOLUME AND TONICITY IN THE HUMAN

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1. Introduction

Scope of Chapter

This chapter describes some aspects of normal antidiuretic hormone metabolism. Particular emphasis is placed upon the physiologic control mechanisms for its release. Although the renal and extrarenal effects of antidiuretic hormone (ADH) are described, the cellular and membrane effects of ADH are not included. For a discussion of these topics, the interested reader is referred to the following sources: Leaf, 1967, (L3), Schwartz and Walker, 1967 (S7), Orloff and Handler, 1967 (O2), Stoff *et al.*, 1972 (S24), and Lang and Edelman, 1972 (L1).

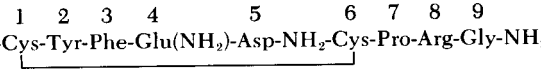
Some of the problems of antidiuretic hormone assay are described. The main focus is upon those clinical conditions characterized by increased antidiuretic hormone. The experimental effects of exogenous ADH are explored. An attempt is made to relate clinical excess to normal control mechanisms.

In the true sense, this is not a review article, since the literature of this complex field simply cannot be covered in one chapter. A complete discussion would entail a description of the relationship of ADH to the renin-angiotension, aldosterone, ACTH-cortisol, cardiovascular and nervous systems. This is clearly not possible. Rather, it is hoped that this article will lead the reader to a clearer physiologic understanding of antidiuretic hormone, its control mechanisms, and its relationship to the clinical states characterized by excess of this peptide.

2. Normal Antidiuretic Hormone (ADH) Metabolism

2.1. ADH Structure and Biologic Occurrence

The naturally occurring antidiuretic hormone of the human is the nonapeptide arginine vasopressin (AVP). The elaboration of the structure of AVP (I) as well as its synthesis have been described by du Vigneaud (D7).



(I)

Oxytocin (oxytocic = rapid birth) is the other naturally occurring human posterior pituitary hormone. It differs in structure from AVP in that isoleucine replaces phenylalanine in position 3 and leucine

replaces arginine in position 8. When the two compounds are compared, AVP has much greater antidiuretic and pressor effects than oxytocin, whereas oxytocin is a more powerful oxytocic and milk-ejecting substance. Both hormones depress blood pressure in birds, the "avian depressor effect," but oxytocin more so than vasopressin. This property is used for oxytocin bioassay (D7, S4).

All mammals have AVP as their sole antidiuretic hormone except for the group *Suina*, in which another substance, lysine vasopressin (LVP), can be found either alone or together with AVP. LVP is identical with AVP except for the substitution of lysine for arginine in position 8. The domestic pig pituitary contains only LVP. The remainder of the vertebrates possess arginine vasotocin. This molecule has the side chain of AVP but the ring structure of oxytocin.

2.2. ADH Production, Storage, Secretion, and Degradation

The supraopticohypophysial tract, which carries AVP, originates in the supraoptic and paraventricular nuclei of the anterior hypothalamus. The supraopticohypophysial fibers terminate in the pars nervosa, or posterior lobe of the pituitary gland. Another vasopressin pathway in the brain has been postulated (P1). It has been shown (S1, S2) that the hypothalamus is the major site for the formation of vasopressin peptide bonds. The hypothalamus is capable of incorporating cysteine-^{35}S into AVP *in vitro*, whereas the pituitary is not. Whether or not a precursor molecule is involved in this synthesis is not clear. The synthesized hormone is carried along the axon with a carrier protein belonging to the class of neurophysins (C1). The hormone–protein aggregates are incorporated into neurosecretory granules, which may be found along the length of the neuron but are most dense at the terminal endings (S2). Stimuli such as dehydration or hyperosmolality, which would ordinarily lead to antidiuretic hormone release, cause a decrease in the number of neurosecretory granules in the posterior pituitary (D8, S2). An increased hypothalamic AVP synthesis rate is found if the stimuli are chronic (S2).

It has been shown in the rat (F8) and in the dog (B13) that the carrier protein of vasopressin can be found in the circulation under resting conditions and that it increased in response to hemorrhage. In the dog, the release was not affected by vagotomy (B13). It was concluded that neurophysin release paralleled that of ADH, and that there might be an equimolecular release of each substance. These data confirmed that the kidneys are the major site for the removal of AVP from the circulation and that neurophysin is also removed

renally. Since the two substances do not occupy the same spatial distribution, that of AVP being twice as large as that of neurophysin, it is suggested that AVP is freer to leave the intravascular space.

In the human, the volume of distribution of AVP has been found to be approximately two-thirds the extracellular fluid volume (F1). The half-life of continuously infused AVP has been found to be 5.6 minutes and the renal clearance to be 1.0 liters per minute (F1). The extent to which AVP is bound to proteins in the circulation is under investigation. AVP has been described as completely bound (L2), not bound at all (L2), or approximately 30 % bound (F1). The degree to which AVP is bound by its own carrier protein in the circulation has yet to be evaluated. The kidneys play a large role, and the liver a lesser one, in the clearance of ADH. The methodologic problems of studying clearance and degradation are beyond the scope of this article, but the reader is referred to Lauson's review (L2) for a thorough discussion.

2.3. Osmolar Stimuli to ADH Release

It has been known for many years that secretion of antidiuretic hormone is related to increased osmolality in the extracellular fluid and that the sensor for detecting this change is located in the distribution of the internal carotid arteries, possibly in the hypothalamus (J1, V2). Verney (V2) showed that hypertonic sodium salts and sucrose administered into the carotid arteries caused antidiuresis and presumably a release of antidiuretic hormone. Similar solutions of urea did not cause ADH release. It was suggested that the reason for this difference might be that urea diffuses rapidly into cells and does not therefore constitute an effective osmotic stimulus for the proposed osmoreceptor.

These experiments infused materials into the vascular side of the blood-brain barrier. Investigating the problem from the other side, by injecting hypertonic solutions into the hypothalamus or into the third ventricle, it was found that antidiuresis occurred (A2). Polydypsia was also elicited by the stimulus. It was concluded that the hypothalamic osmoreceptor system of Verney, which controls the secretion of ADH, could also play an important role in the thirst mechanism by simultaneously sending messages to higher centers. Infusion of saline into third ventricle of the goat caused antidiuresis and a strong drinking response in nonhydrated animals (A2). Sodium bicarbonate infusion caused a lesser drinking response; and long-term sodium acetate, ammonium chloride, urea, or D-glucose infusion into the third ventricle caused no drinking. Although am-

monium chloride injection into the ventricle did not cause thirst, it did lead to ADH release. Urea caused a slight inhibition of water diuresis. In this experimental setting, the stimuli and sites for ADH release and thirst were different.

Intracarotid administration of saline, fructose, and sucrose in the hydrated conscious goat were very effective stimuli for ADH release, but did not cause antidiuresis when applied inside the blood-brain barrier of the third ventricle (E7). However, an equivalent rise in blood tonicity after intracarotid monosaccharide (glucose) administration was not consistently effective as a stimulus for ADH release. Thus the question is raised of whether or not the osmoreceptors in Verney's sense are located outside the blood-brain barrier or in a region of the central nervous system which lacks an effective blood-brain barrier. The conceptual problem encountered in studies of the intraventricular and intravascular administration of substances is that there is no control of intravascular volume during the procedures. The role of volume and its relationship to osmolality have not been carefully enough monitored in these studies.

The studies cited above are characteristic of some of the conflict that exists in the literature concerning the actual site of the osmolality receptor. It has been clearly shown in the normal human (M16) that a definite osmolality-related threshold for vasopressin release exists. After intravenous infusion of 5% saline at 0.05 ml per kilogram per minute, a fall in free water clearance occurs at a plasma osmolality of 288.5 milliosmoles/kg. Intravascular hypertonic saline ought to increase intravascular volume by pulling water from the extravascular extracellular fluid into the intravascular space. This fullness of the intravascular compartment would tend to decrease ADH release, as will be discussed below. Indeed, the osmotic threshold for ADH release has been found to be higher when osmolality is increased by hypertonic saline infusion than when it is increased by water deprivation, which decreases extracellular fluid volume (M15).

2.4. Volume Stimuli to ADH Release

2.4.1. *Atrial Receptors*

In the nonresting, fluid-deprived, normal human subject it has been demonstrated that the blood ADH level changes with position. From a baseline reclining position it increased upon quiet sitting and increased further upon quiet standing (S9). This change was attributed to intravascular volume redistribution, sensed by the atrial

receptor. Share *et al.* (S15), however, found that sodium depletion did not significantly increase plasma ADH, although it did cause a significant rise in plasma renin. Changing from recumbent position to active ambulation had no effect on ADH levels. Overnight food and water deprivation did not cause very high ADH levels although no comparison was made with results obtained when free access to fluids was allowed. These two studies illustrate the difficulties of comparisons in this field, since the kind of activity, the intake of food, salt, and fluid as well as the timing of studies are not comparable.

Much attention has been devoted to the site of these volume sensitive receptors for ADH release. Partial obstruction of the mitral orifice caused an increase in urine flow in the anesthetized dog (H8). It has been suspected that the diuretic response might be due to a decrease in the rate of antidiuretic hormone release from the neurohypophysis. Goetz *et al.* (G5) showed decreased sodium excretion and urine flow after left atrial tamponade in conscious dogs but were unable to show an increase in plasma ADH, although others have shown ADH increase under the same conditions (M13, S16). Ledsome and Mason (L6), using an intra-atrial balloon in the anesthetized dog again found the expected diuretic response, but could prevent it only by infusing 0.4 mU kg per minute of vasopressin, a higher than physiologic dose. Lower doses did not abolish the diuretic response. They concluded that the diuretic response *could* depend upon a decrease in the concentration of antidiuretic hormone in the circulating blood. Using a similar intraatrial balloon technique, Brennan *et al.* (B18) investigated left and right atrial pressure increases and their relationship to ADH and renin levels. They found that increasing left atrial pressure did decrease ADH levels, but that a rise in right atrial pressure had no effect on ADH; rather it caused a fall in plasma renin.

Paroxysmal tachycardia has been known to be accompanied by diuresis. If the atrium is distended during this period, ADH secretion should be reduced. One recent study (G4) questions this concept since atrial pacing did not change ADH levels, although a diuresis was observed. The carotid sinus and aortic arch were thought to contribute to the reflex which initiates diuresis during tachycardia.

In another study Zehr *et al.* (Z1) anesthetized dogs with surgically created chronic mitral stenosis and subjected them to small nonhypotensive hemorrhage. Normal dogs were used as controls. Control levels af ADH were comparable. Small nonhypotensive hemorrhage

resulted in increased ADH levels in both groups. Although left atrial pressure decreased 2-fold in the bled stenotic group, the ADH level rise was attenuated compared with controls. Despite chronic mitral stenosis, the functional integrity of receptor sites seemed intact. It was concluded that the left atrial pressure–volume relationship had changed with chronic mitral stenosis, so that a larger than normal decrease of left atrial pressure was required to achieve the threshold for ADH release.

Acute atrial distention produced a fall in ADH and relief of acute mitral stenosis caused a 2- to 6-fold rise in ADH, in the dog (M13, S16). Bilateral cervical vagotomy blocked both the inhibition and the rebound of ADH secretion under these acute circumstances.

Since vagotomy does not limit its effect to the heart, the vagi carrying other afferents, Mulcahy *et al.* (M21) studied the effects of cardiac denervation in the dog. Cardiac denervation, as opposed to sham surgery, caused an elevation of plasma and blood volume and a decrease in total body water, the loss being due almost entirely to decreased intracellular water. After denervation the animals' plasma volumes were expanded. A higher control urine flow rate, lower osmolality, and a lower plasma ADH were found when compared with predenervation values. The denervated animals did not significantly lower ADH levels with volume expansion. With fluid restriction, the animals' plasma ADH was lower after denervation than before denervation. Although this study would appear to implicate cardiac (presumably left atrial) afferents in ADH regulation, the contributing roles of the remaining receptors in mediating the ADH fall and volume expansion after cardiac denervation is unclear.

There is substantial evidence that left atrial fullness inhibits ADH secretion, and release of left atrial distention causes a rise in ADH level. There are, however, still some conflicting data. Many methodologic problems are involved, such as the experimental model used, presence or absence and kinds or anesthesia, whether or not positive respiration is used, prior fluid balance of the animal, whether or not the stimulus is acute or chronic, and the ADH assay method used. An interesting and disturbing paper (E8) shows a sharp rise in ADH level following acute hemorrhagic shock. When irreversible shock was continued, ADH levels fell. This fall, found by many others, has been attributed to depletion of neural lobe vasopressin. However these workers demonstrated increased clearance of ADH, as well as increased secretion despite falling levels. It would seem that the problem of ADH levels during left atrial pressure change might be investigated from this point of view.

2.4.2. *Arterial Receptors*

Major hemorrhage sufficient to cause a fall in mean arterial blood pressure has been shown to cause a rise in ADH level (B15, E8, F8) exceeding that found after relief of atrial distention (M13). Although fall in left atrial pressure may play some role in the response to hemorrhage, undoubtedly other receptors, in the arterial system, are involved. When anesthetized dogs were subjected to cervical vagotomy and the carotid sinuses were isolated and perfused with a pump at constant mean and pulsatile pressures (S12), hemorrhage equal to 40% of the blood volume had only a minimal (75% increase) effect upon plasma vasopressin titer. When the carotid sinuses were perfused by the hypotensive systemic circulation, without pump assistance, there was an 8-fold increase in ADH titer. Thus it would seem that the carotid sinus receptors are involved in the response to hemorrhage and that they receive peripheral input from the left atrial and possibly the aortic arch receptors (B14).

2.4.3. *Tonicity–Volume Interrelationships*

In the preceding sections we have seen that vascular fullness inhibits ADH release and that volume depletion stimulates it. Increased osmolality clearly augments ADH synthesis and release even if there has been concomitant vascular expansion by saline, whereas decreased osmolality inhibits release. The complex relationships between these stimuli have been studied in a variety of ways.

Chronically prepared sheep with indwelling left atrial and jugular venous catheters and permanent carotid loops were studied, unanesthetized, under a variety of circumstances (J2). *Hypotonicity,* with constant volume, mean arterial blood pressure and left atrial pressure, produced a significant fall in plasma ADH with increased urine flow and free water clearance. *Isotonic hypervolemia* caused an increase in left atrial pressure, mean arterial blood pressure, osmolar clearance, free water clearance, and urine flow. Plasma ADH fell. Combined *hypotonicity* and *hypervolemia* caused a marked increase in urine flow and free water clearance with only a slight increase in osmolar clearance. Plasma ADH fell significantly. Minor *hemorrhage* caused a fall in left atrial pressure with no change in mean arterial blood pressure. Pulse pressure was not reported. ADH levels rose with the expected antidiuresis, but this was due to a decrease in osmolar clearance. *Hypotonic hemorrhage* caused a fall in left atrial pressure and a decrease in osmolality. These conflicting stimuli

resulted in no change in plasma ADH. Renal hemodynamics were unchanged during all maneuvers. At the modest levels of stimuli applied during these experiments, neither volume nor tonicity appeared to be dominant. When hemorrhage is massive enough to cause shock, ADH level rises dramatically (M13) and exceeds manyfold the increase caused by prior hypertonic saline infusion (S3).

Dyball and Powell (D8) have shown a significant decrease in rat neurohypophysial vasopressin when 2.5% potassium chloride solution was substituted for drinking water. The amount of depletion was equivalent to that found when 2% saline was drunk. Isosmotic urea caused a slight increase of vasopressin whereas isosmotic dextrose monohydrate caused a significant increase in pituitary vasopressin. Plasma osmolality was increased, but not significantly so, by potassium chloride and urea, but was significantly decreased by glucose ingestion. However, glucose ingestion caused fluid retention while potassium chloride caused relative fluid loss. This latter finding could suggest that the neurohypophysial vasopressin differences might be more related to volume than tonicity.

The site of integration of volume and osmolality information has been investigated by studying the firing rate of cat hypothalamic cells during blood volume change, intracarotid hypertonic saline, and left atrial balloon distention (M7). Only a few cells responded to increase or decrease in blood volume. More cells responded by increasing or decreasing their firing rates when the left atrial balloon was inflated. Two-thirds of the cells studied responded positively or negatively to intracarotid hypertonic saline. Of the cells studied during blood volume and osmotic stimuli, fourteen which responded to hypertonic saline did not respond to blood volume change. When balloon inflation and hypertonic saline were studied together, a large number of cells were found to be responsive to the osmotic stimulus. Some of these also responded to atrial distention. Of the cells responding to atrial pressure, almost all were sensitive to osmolality, but the direction of response for some cells was different; firing rate increased after saline and decreased following atrial balloon inflation. These data suggest a functional hypothalamic integrative system for volume and tonicity.

2.5. Relationship of ADH to Other Stimuli

2.5.1. *Neural Stimuli, Pain, Fear, and Temperature Change*

The complex stress of surgery has been analyzed with respect to circulating ADH levels (M13). Figure 1 maps the typical human