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*ON THE MECHANISM OF ACTION OF THE ANTIDIURETIC HORMONE (VASOPRESSIN)**

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Evidence available at present indicates that a primary function of the peptide hormones, vasopressin and insulin, is to influence membrane permeability. The chemical basis for this action is ill defined in spite of considerable knowledge concerning the structure and physiological action of these hormones. In our previous investigation findings on the interaction of vasopressin with kidney receptor sites indicated the formation of a covalent bond resulting from a thiol-disulfide exchange reaction.[1] The present paper substantiates this finding and suggests a plausible mechanism of action for the antidiuretic hormone.‡

Materials.—Preparation of tritiated vasopressin (H^3AVP): In our hands, tritiation by the usual Wilzbach method gave low yields and unpredictable specific activities. Hence we resorted to a modified Wilzbach procedure, employing an electric discharge for tritium labeling.[2]

AVP§ obtained from chromatographing beef pitressin powder[3] was introduced into a cup-shaped electrode attached to one-half of a cylindrical pyrex cell. After sealing, the cell was evacuated and tritium gas was allowed to flow in (pressure less than 5 mm Hg). An electric discharge was produced by connecting the electrodes to an AC transformer in series with an autotransformer. The cell was re-evacuated to remove excess tritium and other gases and the tritiated H^3AVP was dissolved in dilute acetic acid and lyophilized. This latter process was repeated three times and the lyophilized protein was then purified twice by chromatography through a CM cellulose column according to the method of Ward and Guilleman.[3] H^3AVP with a specific activity of approximately 400 μc/mg and a biological activity of about 400 pressor units/mg[4] was obtained, indicating a high degree of purification (Fig. 1).

Preparation of acetyl LVP: Acetyl LVP was prepared by reacting LVP (prepared from pitressin powder according to the method of Ward and Guilleman[3]) with acetic anhydride in glacial acetic acid. The acetyl derivative was subsequently purified by chromatographic separation using a CM cellulose column.

Iodination of AVP with tracer I^{131}: Iodination of the hormone was carried out by the usual KI_3 method using 0.2 *M* ammonium hydroxide in place of bicarbonate.[5] The IAVP was purified through a CM cellulose column.

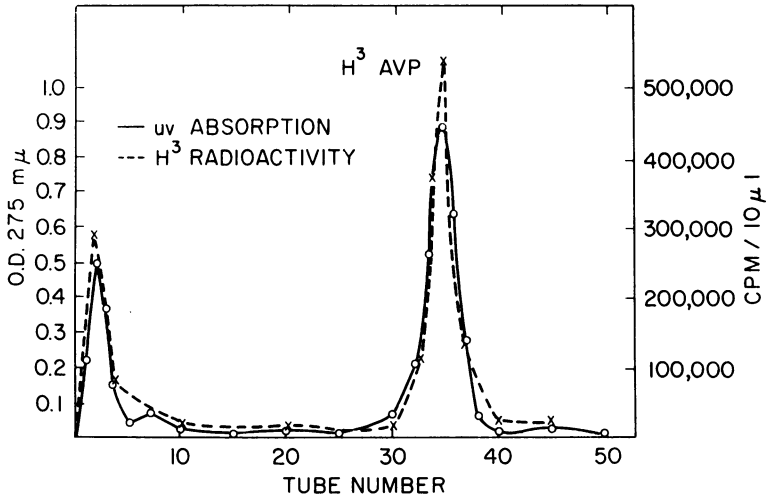

FIG. 1.—Chromatography of H³AVP through CM cellulose column. —— u.v. curve; - - - - radioactivity curve.

Methods.—In vivo binding and separation of hormone-receptor proteins: The following experiment demonstrates in vivo combination of H³AVP and a kidney protein fraction. Partial dissociation of this complex was effected by treatment with cysteine, mercaptoethylamine, and thioglycollate.

Seven rats were hydrated by stomach tube and anesthetized with dilute ethanol by mouth. Approximately 5 μg of H³AVP in 0.5 ml saline were injected into each rat over a 5-min period via a cannulated external jugular vein. Ten minutes after the onset of the injection of H³AVP, at the peak of antidiuretic activity, the kidneys were quickly excised and dropped into liquid nitrogen. These were homogenized with nine volumes of 30 per cent ice-cold sucrose,[6] saturated with NEM to bind sulfhydryl groups and thereby block any interchange reaction, and centrifuged for 20 min at 400 × *g* and 5°C. The residue was suspended in several volumes of 30 per cent sucrose and recentrifuged at 400 × *g*. The precipitate was then rehomogenized in saline containing NEM and recentrifuged at 4000 × *g* for one half-hr. The pellet, containing cell membrane debris and nuclei, was suspended in water, dialyzed against cold tap water and electrodialyzed to remove ionic bonded H³AVP. After electrodialysis acetone was added to the aqueous suspension to a concentration of about 90 per cent and the precipitated proteins were centrifuged and dried in vacuo.

Equal portions of these fractionated kidney proteins were shaken overnight at 37°C with the following reagents made up in 0.1 *M* sodium bicarbonate, adjusted to pH 8.0: (a) bicarbonate control; (b) 0.1 *M* cysteine; (c) 0.1 *M* β-mercaptoethylamine; (d) 0.1 *M* thioglycollate. The treated tissues were centrifuged, in sequence, with water and acetone and dried in vacuo. The dried kidney proteins thus obtained were prepared for tritium analysis by a modified Wilzbach combustion method,[7, 8] using gas proportional counting.

Results.—In a series of fractionations higher specific activity of H³AVP-kidney protein complex was obtained by partial sucrose fractionation than by the original procedure.[1] Table 1 shows a typical experiment in which sucrose fractionation

TABLE 1

H³AVP CONTENT OF SUCROSE FRACTIONATED KIDNEY PROTEINS TREATED WITH VARIOUS THIOL COMPOUNDS IN BICARBONATE AT pH 8

Treatment	Disintegrations per min per mg protein		Average dpm per mg	% H³AVP released
0.1 *M* sodium bicarbonate (control)	42.91	45.09	43.50	...
0.1 *M* cysteine	22.49	22.39	22.45	48.5
0.1 *M* β-mercaptoethylamine	19.81	19.34	19.57	54.5
0.1 *M* thioglycolate	22.61	22.91	22.76	47.7

TABLE 2

H³AVP CONTENT OF FRACTIONATED KIDNEY PROTEINS (1500 × *g*) AFTER TREATMENT WITH THIOL COMPOUNDS IN 8 *M* UREA pH 8

Treatment	Disintegrations per min per mg protein			Average dpm per mg	% H³AVP released
Control	4.8	3.3	4.7	4.3	...
2% Cysteine		2.3		2.3	46
2% β-Mercaptoethylamine		2.3		2.3	46
2% Thioglycolate	2.23		1.47	1.8	58

was employed and Table 2 shows the results from a fractionation procedure essentially as previously described using 8 *M* urea and sodium hydroxide instead of bicarbonate in the thiol-compound treatment. Examination of these tables shows a quantitative difference of bonded H³AVP following thiol-compound treatment of the protein. The release of approximately 46-58% H³AVP indicates a cleavage of a sulfur-sulfur bond by mercaptide ions.

Discussion.—Our experiments suggest a sulfur-sulfur bond in the hormone-receptor interaction. The basis for proposing a specific thiol-disulfide exchange reaction and the evidence in support thereof are:

1. The investigations of Ames and Van Dyke[9] on pitressin and our observations on vasopressin (unpublished results) showed that the loss of antidiuretic activity paralleled the reduction of the hormone disulfide.

2. The studies of Cafruny, Carhart, and Farah[10] on the effects of various hormones on the thiol concentration in kidney cells from hypophysectomized rats showed that treatment with pitressin produced a diminution of sulfhydryl groups in the convoluted portions of the proximal and distal tubules. No changes in thiol concentration in the same kidney tubule sections were observed with growth hormone (a disulfide hormone) and other hormones (thyroxin and thyrotropin). This finding suggests a specific hormone-receptor interaction in the distal convoluted tubule, a segment in which vasopressin-induced water reabsorption is known to occur. The similar finding in the proximal convoluted tubule raises the possibility of a similar hormone-receptor interaction at this site in the nephron, although the functional significance of a proximal permeability change is not apparent.

3. The release of reduced tritiated hormone from hormone-receptor kidney proteins after treatment with thiol compounds (Table 1) indicates a cleavage of a disulfide bond.

4. Toad bladders (*Bufo marinus*) treated with thiol-blocking agents, such as NEM, *p*-chloromercuribenzoate and methylmercuricbromide, showed considerably less disulfide-linked H³AVP than did untreated bladders.[11]

The interaction of the antidiuretic hormone with its receptor may require a specificity dictated by stereochemical and electrostatic effects.

With the structure of arginine vasopressin, CyS·Tyr·Phe·Glu(NH_2)·Asp(NH_2)·Cys·Pro·Arg·Gly(NH_2),[12, 13] and all the above considerations in mind, a plausible mechanism for the action of the antidiuretic hormone is:

(*a*) The hormone is attracted to its receptor site by electrostatic interactions of opposite charges, i.e., the positively charged groups of the hormone and the negatively charged groups of the receptor. At short ranges hydrogen bonding, through the tyrosyl hydroxyl group and amide groups of glutamine, asparagine, and glycinamide, emerges as a prominent attractive force. The proper interactions between attractive and repulsive forces bring about an alignment of the hormone with its receptor site.

(*b*) The thiol-disulfide exchange reaction, which is a nucleophilic attack of the receptor mercaptide ion on the hormone disulfide, takes place. This results in a hormone-receptor disulfide. Subsequently, a series of sulfhydryl-disulfide reactions induces alterations of the tertiary structure of membrane proteins. This chain reaction brings about conformational changes in the protein components of the diffusion barrier. The resultant molecular sieve-like structure allows for an increase in the flux of water and certain solutes through the partially "loosened" membrane.

(*c*) The hormone-receptor disulfide bond is ultimately cleaved by a reductase in conjunction with one or more other enzymes.[14, 15] Such a reverse reaction in which the receptor sulfhydryl group is regenerated restores the membrane to its original state and completes the cycle (Fig. 2).

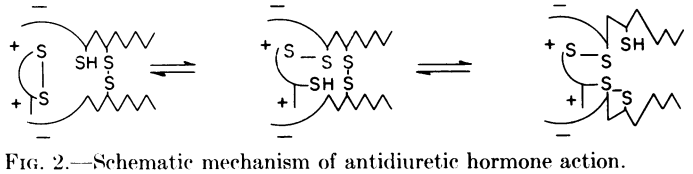

FIG. 2.—Schematic mechanism of antidiuretic hormone action.

A few preliminary experiments were designed to test several points of this hypothesis. These included the elimination of the positively charged groups by acetylation of LVP. This derivative gave an antidiuretic activity (in the rat) of approximately $^1/_{400}$ of the original. Iodination of AVP and LVP resulted in derivatives manifesting about $^1/_{100}$ the antidiuretic activities of the original hormones. This suggests a reduction in hydrogen bonding through the tyrosyl hydroxyl group. These data are consistent with the postulated hypothesis. It is interesting to note the presence of a terminal prolyl peptide chain in a number of hormones (oxytocin, insulin, melanocyte-stimulating hormone): such a chain may contribute to molecular specificity.[16, 17] An examination of structural analogs of vasopressin[18–21] and the correlation of their antidiuretic activities in the dog and rat[22] is consistent with this hypothesis and implies the necessity for a correct molecular fit of the hormone to its receptor. In this mechanism the disulfide of the hormone is considered the essential functional group. A review is available on the role of sulfhydryl-disulfide interchange reactions in biological processes concerned with the phenomena of protein denaturation, blood clotting, and mitosis.[23]

It is interesting that one of the prime functions of insulin is to alter the permeability of insulin-sensitive membranes to sugars.[24] Investigations of Lindley[25] and Fraenkel-Conrat[26] have indicated that the intrachain disulfide bond of insulin is preferentially cleaved on partial reduction and hormonal activity is thus lost. This suggests that a thiol-disulfide exchange reaction similar to that postulated for vasopressin may be involved.

Summary.—Evidence is presented to indicate that a covalent bond is formed in the interaction of vasopressin and its receptor. The disulfide bond thus formed is a result of a thiol-disulfide exchange reaction. A mechanism of action for the antidiuretic hormone is suggested which assumes that this exchange reaction underlies the increased passive transport of water induced by vasopressin.

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‡ AVP and LVP are referred to by synonyms, "vasopressin" and "antidiuretic hormone."

§ Abbreviations used are: AVP, H^3AVP, IAVP, arginine vasopressin, tritiated arginine vasopressin, and iodinated vasopressin, respectively; LVP, lysine vasopressin; CM cellulose, carboxymethyl-cellulose; NEM, N-ethylmaleimide.

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