NECESSITY OF THE DISULFIDE BOND OF VASOPRESSIN FOR ANTIDIURETIC ACTIVITY*

Conrad T. O. Fong, Lawrence Silver** and Daniel D. Louie

Science Research Institute, Oregon State University, Corvallis, Oregon and the Radioisotope Service, Veterans Administration Hospital, Brooklyn, New York

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In our continuing studies on the structure-activity relationship of vasopressin, further experiments were conducted to determine the essentiality of the disulfide bond. Previous investigations on the mammalian kidney (Fong, et al., 1959, 1960) and the toad bladder (Rasmussen, et al., 1960; Schwartz et al., 1960) indicate that a thiol-disulfide exchange or interchange reaction may be necessary for alteration of membrane permeability. This finding suggests that the disulfide bridge of vasopressin in addition to keeping the hormone molecule in the correct stereo-configuration, is involved in the reaction with the receptor thiol resulting in a binding of the hormone to the receptor through a sulfur-sulfur bond.

We have considered it pertinent to desulfurize vasopressin to ascertain directly the necessity of the disulfide bond of vasopressin for antidiuretic activity. Of a number

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of approaches to the problem it was felt that the reduction of the disulfide bond followed by blocking of the sulfhydryl groups with an agent bearing a charged group such as iodoacetate may alter the hormone-receptor interaction through charge and steric effects. Likewise, an uncharged sulfhydryl blocking agent depending on its size, could affect the hormone-receptor interaction by steric effects. Hence, we considered the desulfurization of vasopressin an appropriate approach to the problem.

Arginine vasopressin was prepared by a modification of the method of Ward and Guilleman (1957) from crude pitressin powder*. Arginine vasopressin containing a little lysine vasopressin was reacted with freshly prepared Raney Nickel in absolute ethanol for two hours by a modification of the method of du Vigneaud, et al. (1951). After the desulfurization reaction the nickel was extracted twice with absolute ethanol containing 30% concentrated ammonium hydroxide and the suspension was centrifuged. Both ethanol and ethanolic ammonium hydroxide extracts were combined and evaporated to dryness in vacuo. To remove the Ni^{++} present the residue was taken up in dilute ammonium hydroxide (pH 7.5) and hydrogen sulfide was bubbled in to precipitate nickel sulfide. After filtering off the nickel sulfide the filtrate was concentrated to dryness in vacuo. A fraction of the residue was subjected to acid hydrolysis (6N HCl) and chromatographed on paper (Levy & Chung, 1953). The paper chromatogram revealed alanine resulting from the desulfurization of cystine and the other amino acids found in vasopressin. Only a trace of cystine was evident along

*Kindly supplied by Dr. F. C. Armstrong of Parke Davis & Co.

with a little lysine. To purify the desulfurized arginine vasopressin the residue was taken up in 0.02M ammonium acetate, pH 6.0 and chromatographed on a carboxymethylcellulose column 0.9 x 25 cm using a modification of the method of Ward, et al. (1957). After the initial development of the column with 0.02M ammonium acetate the column was developed with 0.1M ammonium acetate, pH 7.0 followed by 0.2M ammonium acetate, pH 7.0 in order to separate the desulfurized lysine vasopressin from the desulfurized arginine vasopressin. Peptide concentrations were determined spectrophotometrically at 280 mμ. The appropriate fractions were pooled and lyophilized until all the ammonium acetate was removed. The yield of purified desulfurized vasopressin was approximately 20%. Paper chromatography (Levy & Chung, 1953; Hardy et al. 1955) of an acid hydrolyzed sample of desulfurized arginine vasopressin revealed the amino acids found in vasopressin with the exception that alanine was present along with a very small amount of lysine and traces of cystine.

To ascertain the biological activities of the desulfurized vasopressin, antidiuretic and pressor assays were conducted on the dog and on the rat. Pressor assays were also carried out on the chicken. These experiments were conducted with Messrs. L. Sigell and R. Brummett in the laboratory of Professor N. A. David*. The results are shown in the table.

Our preliminary experiments of desulfurized arginine vasopressin on the dog and the rat showed essentially no

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*Department of Pharmacology, University of Oregon Medical School.

TABLE I

Substance	Assay: Dog Pressor	Dog Antidiuretic	Rat Pressor	Rat Antidiuretic
Arginine vasopressin	360	370	340	333
Desulfurized arginine vasopressin	90	ca. 0	33	<0.003

Values are in units/mg

antidiuretic activity. However, pressor assays of desulfurized arginine vasopressin on the dog, rat and chicken showed a significant retention of pressor activity. The pressor activity of the desulfurized arginine vasopressin in the dog was approximately 25% of arginine vasopressin, whereas in the rat it was about 10%. In a preliminary chicken vasodepressor assay* desulfurized arginine vasopressin possessed about 20% of the activity of arginine vasopressin. In these experiments the duration of the pressor effect of desulfurized vasopressin was short and there appeared to be some evidence of tachyphylaxis.

From the results it appears that the disulfide bridge of vasopressin is essential for antidiuretic activity and that it is not essential for pressor activity. This would suggest that the loci of antidiuretic and pressor activities on the vasopressin molecule are not identical.

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