Vasopressin biosynthesis

The concept of neurosecretion[1] implicates the supraoptic and paraventricular nuclei as the sites for the synthesis of vasopressin. This hormone is presumed to be bound to a carrier "neurosecretory material" which is supposed to move in a protoplasmic flow via the supraoptico-hypophysial tract to the posterior pituitary lobe where storage and release occurs[2]. In the experiments reported below, we attempted first to demonstrate the isolation (in isotopically homogeneous form) of small samples of vasopressin labelled *in vivo*, and second to obtain some biochemical information concerning the anatomical site(s) of synthesis of this hormone.

Unanesthetized dogs (5–6 kg) were given highly labelled [^{35}S]cystine by continuous infusion via the jugular vein over 15, 24 and 36 h. The infusate contained $0.5–1.0 \cdot 10^{10}$ counts/min and consisted of an acid hydrolysate of protein prepared from *Escherichia coli* grown in the presence of carrier-free $^{35}SO_4^{=}$ and unlabelled methionine[3]. Under the selected conditions of growth and hydrolysis, about 60% of the counts were shown by chromatography on paper[3] and on Dowex 50[4] to be associated with cystine. When the infusion was complete, the animals were sacrificed and the hypothalamus and neurohypophysis excised and prepared separately for the isolation of labelled vasopressin. Since these tissues contained a total of only 5–50 μg pressor material, approximately 1 mg of highly purified beef (arginine) vasopressin was added as carrier to each tissue extract. It had been established in separate experiments that dog vasopressin was separable from synthetic lysine vasopressin* and indistinguishable from arginine vasopressin by chromatography on carboxymethylcellulose. The purification procedure consisted of extraction of the homogenized tissues in 0.1 M acetic acid (100°, 5 min), removal of soluble protein by precipitation with trichloroacetic acid, which was removed by ether extraction. Vasopressin was isolated from this clear extract by ion-exchange chromatography, first on carboxymethylcellulose[5] and then on an XE-64 resin[6] using continuous elution gradients. In separate experiments performed in the absence of carrier it was shown that vasopressin, isolated in this manner from hypothalamic and neurohypophysial tissue obtained from dogs, had a biological potency of approximately 300–400 USP Pressor Units/mg**. Isotopic purity was established by rechromatography, paper ionophoresis, and performic acid oxidation and isolation of oxidized vasopressin. The mean values for the specific activity of hypothalamic and posterior pituitary vasopressin obtained in the three experiments are presented in Table I. Although these data do not preclude the hypothalamus as the site of synthesis of vasopressin the ratios of specific activity of hypothalamic to pituitary vasopressin obtained after these relatively short periods of continuous labelling would appear to be inconsistent with a theory of neurosecretion consisting of a simple homogeneous two-compartment system[7,8]. Assuming that the previously estimated rate of synthesis of vasopressin in the dog[9] of approximately 1–5 mU/h is correct then the theoretically calculated ratios[7] would be several hundred times the experimentally observed values.

This work is currently being extended to a more detailed kinetic study of the

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* This material was part of a gift by Dr. VINCENT DU VIGNEAUD to Dr. GEORGE SAYERS.

** Pressor assays were performed in the rat using USP posterior pituitary Reference Standard with a stated potency of 0.47 U/mg; mg refers to mg of protein measured by the FOLIN procedure[10] against bovine serum albumin as standard.

TABLE I

INCORPORATION OF [^{35}S]CYSTINE INTO VASOPRESSIN

Expt.	Time of infusion h	Posterior pituitary		Hypothalamus		Ratio of specific activities H.P
		Total units	Counts/min/unit	Total units	Counts/min/unit	
1*	16	16.0	8.0	3.2	23.0	2.9
2*	24	19.0	14.2	1.6	28.4	2.0
3*	36	13.0	9.7	2.7	31.1	3.2

* Specific activities have been corrected for dilution by carrier and radioactive decay; they represent the mean values for the purification steps outlined (see text) *e.g.* Expt. 2, post. pit. spec. act. after XE-64, 15.4; paper ionophoresis, 15.3; rechromatography on carboxymethylcellulose and dividing the biologically active peak into 3 fractions, 13.5, 12.6, 15.0; Expt. 3, post. pit. gave spec. activities of 11.0, 9.8, 11.4, 7.5, 9.0 respectively; Expt. 1 given as counts/min/2.5 μg (approx. 1 unit vasopressin) of oxidized vasopressin eluted from paper after ionophoresis and measured colorimetrically by the FOLIN procedure[10].

biosynthesis of vasopressin both *in vivo* and *in vitro* as well as to an investigation of the intracellular sites of synthesis and storage of this hormone.

This work was supported by a grant from the National Institutes of Health, U.S. Public Health Service, A 2650.

I wish to express my gratitude to Drs. BERNE, ROYCE, and SAYERS of the Department of Physiology for their generous advice and assistance with some of the bioassay and operative procedures.

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Received May 11th, 1959

Chemical nature of the DFP-binding site of pseudocholinesterase

DFP is known to phosphorylate a number of esterases and proteases at their enzymically active sites. Information on the chemical nature of the active sites of these enzymes has been derived from degradation of the DFP-inhibited enzymes and analysis of the P-containing peptides produced.

In the present note we report the analysis of a P-peptide obtained from DFP-inhibited pseudocholinesterase. An enzyme preparation, obtained from about 800 l