We have not carried out any experiments which would allow us to determine whether the effect of adrenalectomy on DNA metabolism is due to the absence of corticoids, the stimulation of the pituitary or an effect on the hypothalamus mediated by releasing factors.

It must be emphasized that these experiments have been performed with young rats which are growing rapidly, and do not necessarily apply to adult animals. The hypothalami of young rats contain much more growth hormone releasing activity than the hypothalami of adults.

[18] Acknowledgment. This project was supported in part by a grant from the Israel Cancer Association.

Zusammenfassung. Die Röntgenbestrahlung des Kopfes junger Ratten verursacht eine vermehrte DNS-Synthese im Thymus, was mit der Freisetzung von Wachstumshormon-ähnlichen Substanzen in Zusammenhang gebracht wird.

D. Maor and P. Alexander[18]

Department of Human Microbiology,
Tel-Aviv University,
Tel-Aviv (Israel) and
Chester Beatty Research Institute,
Institute of Cancer Research,
Cliffton Avenue, Belmont,
Sutton (Surrey, England),
13 December 1971.

Competitive Inhibitors of Neurohypophyseal Hormones on Adenylate Cyclase from the Toad Urinary Bladder[1,2]

The three-dimensional structure recently proposed for oxytocin in solution[3] provides a model to correlate the conformation of the molecule with various other aspects of the hormone, e.g., its evolution, biologic activity, antigenicity, immunochemical reactivity, enzymic degradation, etc[4-6]. In the 'cooperative model' of oxytocin[4] (Figure 1) the chemically active groups (3 carboxamide groups and the phenolic hydroxyl group), and the acyclic prolyl-leucylglycinamide moiety, are oriented towards the same side of the 20-membered cyclic component of the hormone. Such an arrangement results in a hydrophilic region, which we believe to be important for the expression of the inherent catalytic activity (i.e. the 'intrinsic activity'[7]) of the hormone[4-6]. Certain structural modifications in this hydrophilic region, particularly if hydrophobic in character, might be anticipated to reduce the catalytic activity of the peptide. An analog in which the modified group(s) were sufficiently large and properly oriented, but did not interfere with the binding of the hormone to the receptor, could be expected to be an inhibitor of the hormone. Structural changes in positions 2, 4 and 9 of oxytocin appear particularly suitable for converting the hormone into an inhibitory analog. The extensive studies in whole animal and various organ preparations with the inhibitor [2-*O*-ethyltyrosine]-oxytocin, and other neurohypophyseal analogs in which the phenolic hydroxyl group is either alkylated or substituted, may be cited as examples (see ref.[8] for a recent summary). The inhibitory property of [2-*O*-ethyltyrosine]-oxytocin is also found in the intact toad urinary bladder[9] and in subcellular preparations of toad bladder epithelium[10], while the neurohypophyseal hormones per se enhance the permeability of the amphibian bladder to water and to certain small molecules[11,12]; strong evidence has accumulated indicating that this hormone-induced process is mediated by cyclic 3′,5′-AMP[10,13,14].

It has also been found that neurohypophyseal hormone analogs which have in common a leucine substitution for the glutamine residue in position 4 of oxytocin or for the serine residue in position 4 of mesotocin, i.e. [4-leucine]-oxytocin[15-17], [2,4-dileucine]-oxytocin[18], and [4-leucine]-

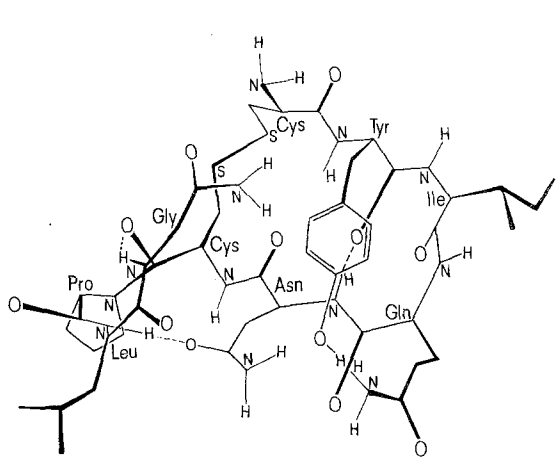

Fig. 1. Schematic representation of the *hypothetical model of the biologically active conformation of oxytocin* ('Cooperative model').

[1] Supported by United States Public Health Service grants No. AM-13567 (RW), by the Life Sciences Foundation, Inc., and by the National Science Foundation No. GB-30716X (VJH).

[2] Neurohypophyseal hormone analogs are denoted in accordance with the IUPAC-IUB [Tentative Rules (Biochemistry *6*, 362, 1967)].

[3] D. W. Urry and R. Walter, Proc. natn. Acad. Sci., USA *68*, 956 (1971).

[4] R. Walter, I. L. Schwartz, J. H. Darnell and D. W. Urry, Proc. natn. Acad. Sci., USA *68*, 1355 (1971).

[5] R. Walter, in *Structure-Activity Relationships of Protein and Polypeptide Hormones* (Eds. M. Margoulies and F. C. Greenwood; Excerpta Medica, Amsterdam 1971), part 1, p. 181.

[6] R. Walter and J. M. George, Clin. Res. *20*, 443 (1972).

[7] E. J. Ariëns, Adv. Drug Res. *3*, 235 (1966).

[8] J. Rudinger and I. Krejči, in *Handbook of Experimental Pharmacology* (Ed. B. Berde, Springer-Verlag, Berlin 1968), vol. 23, p. 748.

[9] P. Eggena, I. L. Schwartz and R. Walter, J. gen. Physiol. *56*, 250 (1970).

[10] H.-P. Bär, O. Hechter, I. L. Schwartz and R. Walter, Proc. natn. Acad. Sci., USA *67*, 7 (1970).

[11] V. Koefoed-Johnson and H. H. Ussing, Acta physiol. scand. *28*, 60 (1953).

[12] A. Leaf, Am. J. Med. *42*, 745 (1967).

[13] J. Orloff and J. Handler, J. clin. Invest. *41*, 702 (1962).

[14] S. Hynie and G. W. G. Sharp, Biochim. biophys. Acta *230*, 40 (1971).

[15] W. Y. Chan, V. J. Hruby, G. Flouret and V. du Vigneaud, Science *161*, 280 (1968).

[16] W. Y. Chan and V. du Vigneaud, J. Pharmac. exp. Ther. *174*, 541 (1970).

[17] P. J. S. Chiu and W. H. Sawyer, Am. J. Physiol. *218*, 838 (1970).

mesotocin[9,19] are inhibitors of certain hormone-induced responses in whole animal and organ preparations. In this study we investigated some of the above compounds as well as some new oxytocin analogs. All are characterized by replacement of the glutamine residue in position 4 of the hormone by leucine or isoleucine and by their capacity to inhibit the known action of neurohypophyseal hormones to enhance cyclic 3′,5′-AMP production in adenylate cyclase preparations of toad urinary bladder epithelium. The compounds include [4-leucine]-[20], [2,4-dileucine]-[18], [2,4-diisoleucine]-[21], [2-isoleucine-4-leucine]-[22], and [2-phenylalanine-4-leucine]-oxytocin[23]. (References describe synthesis of analogs).

Methods. The assay procedure used was that of BÄR and HECHTER[24]. In brief, adenylate cyclase preparations consisted of the 600 × *g* fraction of bladder epithelium homogenate of the toad *Bufo marinus*. The reaction mixture contained 45 m*M* *Tris*-HCl buffer (pH 8.0), Mg^{++}, EGTA, 0.1% bovine serum albumin, ATP-α^{32}P, unlabelled cyclic AMP, an ATP regenerating system, AVP or neurohypophyseal hormone analogs as indicated and, where appropriate, varying concentrations of inhibitor. The enzyme reaction was started by the addition of the adenylate cyclase preparation to the reaction mixture. Incubations were carried out at 37°C for 20 min, and were terminated by the addition of carrier solution. Samples were centrifuged, and the supernatant lyophylized and chromatographed on PEI-cellulose thin-layer sheets in 0.3 *M* LiCl.

The spots containing cyclic 3′,5′-AMP, 5′-AMP and ATP were located by UV, cut out, and counted in a toluene scintillation fluid. The percent conversion of ATP to cyclic 3′,5′-AMP and the absolute rates of cyclic 3′,5′-AMP formation were calculated. AVP-stimulated adenylate cyclase responses are corrected for basal activity.

Results and discussion. None of the neurohypophyseal hormone analogs, when tested over a concentration range of 10^{-8} to 10^{-4} *M*, was capable of stimulating the adenylate cyclase activity of the toad bladder. In preliminary experiments using the adenylate cyclase system, we found [4-leucine]-oxytocin (10^{-4} *M*) to be a weak inhibitor of the stimulation of cyclic 3′,5′-AMP production induced by 10^{-6} *M* AVT, which is the natural water balance principle of amphibia; for summary see ref. [25]. Since the affinity of AVT for the hormonal receptor was much greater than that of [4-leucine]-oxytocin, we found that the relative inhibitory properties of this series of [4-leucine]- and [4-isoleucine]-analogs could not be defined in experiments in which AVT was used as agonist. However, when AVP was employed as the hormonal stimulant, also at 10^{-6} *M*, delineation of the relative inhibitory potency of various analogs was possible, as can be seen from the data depicted in Figure 2. The analogs listed in order of decreasing inhibitory activity are as follows: [2,4-diisoleucine]-, [4-leucine]-, [2-isoleucine-4-leucine]-, [2,4-dileucine]- and [2-phenylalanine-4-leucine]-oxytocin.

The finding that [4-leucine]-oxytocin is an inhibitor of the response induced by neurohypophyseal hormones, not only in the intact toad bladder[17] but also in the toad bladder adenylate cyclase system, lends further support to studies correlating early events in hormone action (e.g., adenylate cyclase stimulation) with the hormone-induced organ response (i.e., hydroosmotic effect). Hence it can be expected that the above peptides, with the exception of [2-phenylalanine-4-leucine]-oxytocin, are weak antagonists of neurohypophyseal hormones in the intact toad urinary bladder, and that, continuing this series, the most potent inhibitor could well be the known [4-isoleucine]-oxytocin.

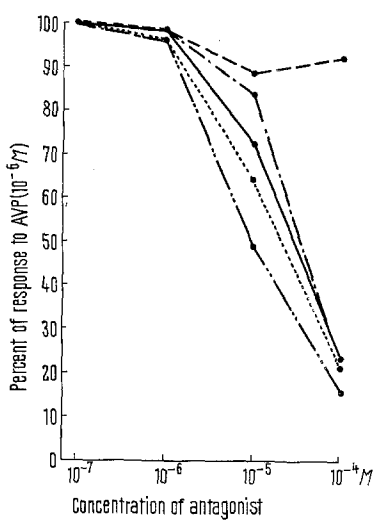

Fig. 2. Typical inhibitory effects of neurohypophyseal hormone analogs on arginine-vasopressin-induced adenylate cyclase activation in broken cell preparations of toad urinary bladder epithelium. The percentage of response to AVP (10^{-6} *M*) is plotted along the ordinate, and increasing concentrations of the analog along the abscissa. Listed in order of decreasing inhibitory effect, the compounds tested were: [2,4-diisoleucine]-oxytocin(----); [4-leucine]-oxytocin (---); [2-isoleucine]-oxytocin (—); [2,4-dileucine]-oxytocin (---); and [2-phenylalanine-4-leucine]-oxytocin (—). Each curve represents a composite of results from 3 independent adenylate cyclase preparations. See text for assay conditions.

Zusammenfassung. Adenylat-Cyclase in plasmamembranreichen Präparationen des Epitheliums von der Harnblase der Kröte wird durch Arginine-Vasopressin aktiviert. Diese Aktivierung wird durch mehre Oxytocinanaloge, die alle eine Substitution des Glutaminrestes in Position 4 des Hormones durch Leucin oder Isoleucin gemeinsam haben, kompetitiv gehemmt. Der stärkste Antagonist der Serie ist [2,4-diisoleucin]-Oxytocin und die antagonistische Aktivität nimmt in der Reihenfolge [4-Leucin]-, [2-Isoleucin-4-leucin]-, [2,4-Dileucin]- und [2-Phenylalanin-4-leucin]-Oxytocin ab. Die Resultate werden an Hand der Oxytocinkonformation diskutiert.

R. WALTER, MADELEINE A. KIRCHBERGER and V. J. HRUBY

Department of Physiology, the Mount Sinai Medical and Graduate Schools of the City University of New York, 5th Avenue and 100th Street, New York (N.Y. 10029), and Department of Chemistry, University of Arizona, Tucson (Arizona 85721, USA), 28 February 1972.

[18] V. J. HRUBY and W. Y. CHAN, J. med. Chem. *14*, 1050 (1971).
[19] J. RUDINGER, O. V. KESAREV, K. PODUSKA, B. T. PICKERING, R. E. DYBALL, D. R. FERGUSON and W. R. WARD, Experientia *25*, 680 (1969).
[20] V. J. HRUBY, G. FLOURET and V. DU VIGNEAUD, J. biol. Chem. *244*, 3890 (1969).
[21] V. J. HRUBY, V. DU VIGNEAUD and W. Y. CHAN, J. med. Chem. *13*, *185* (1970).
[22] F. A. MUSCIO, M. S. Thesis, University of Arizona, 1972.
[23] V. J. HRUBY, C. M. GROGINSKY, P. M. GITU, D. SABA and W. Y. CHAN; manuscript in preparation.
[24] H.-P. BÄR and O. HECHTER, Analyt. Biochem. *29*, 476 (1969).
[25] H. HELLER; in *Comparative Endocrinology* (Eds. U. S. VON EULER and H. HELLER, Academic Press, New York 1963), vol. 1, p. 26.