Inactivation of Deamino-oxytocin, Deamino-carba[1]-oxytocin, [D-Lys[8]]-, and [D-Arg[8]]vasopressins by Kidney Cell Particles

Vasopressin and oxytocin are rapidly degraded by tissue homogenates and certain cell particles mostly on proteolytic or reductive pathways[1–3]. The following mechanisms might be considered (see Figure 1): (1) splitting by non-specific aminopeptidases after reduction of a S-S-bridge[3]; (2) splitting on the N-terminal peptide bond by a specific aminopeptidase (found in human pregnancy serum[4] or renal microsomes[5]); (3) splitting of the C-terminal peptide bond by trypsin- (in case of vasopressin) or chymotrypsin-like enzymes (in case of oxytocin)[6,7]. Potentially, one might therefore expect a prolonged effect in vivo on analogues: (1) which have no disulphide bridge (protection against reduction); (2) which lack an N-terminal amino group (protection against aminopeptidases); (3) which have – in the case of vasopressin – a stabilized peptide bond between arginine (lysine) and glycineamide, e.g. by insertion of a D-amino acid. Such analogues were synthetized during recent years (see Figure 2) and we studied their resistance to degradation by kidney cell particle enzymes and the changes in the half-life of their biological effects in vivo.

Experimental. In this study we used the following peptides: Oxytocin (OT) and Lysine vasopressin (LVP), products of SPOFA-Prague, Deamino-oxytocin (DOT)[8], and Deamino-carba[1]-oxytocin (DCOT)[9] both synthetized by J. RUDINGER and K. JOŠT[9], and [D-Arg[8]]- and [D-Lys[8]]-vasopressin (DAVP and DLVP, respectively), prepared by M. ZAORAL and J. KOLC[10,11].

The incubation mixture contained: the peptide in a concentration of 6.6 μM (oxytocin and its analogues) or 1 μM (vasopressins and their analogues); cell fractions prepared from swine kidney by the method of SCHNEIDER and HOOGEBOOM[12] (dialyzed against a 100-fold volume of 0.01 M Na-phosphate at pH 7.0, 20 h at 4 °C), protein concentration 1.7–7.0 mg/ml; phosphate buffer at pH 7.0, final concentration 0.05 M. The amount of hormone before and after 60 min of incubation was determined by bioassay of antidiuretic activity[13].

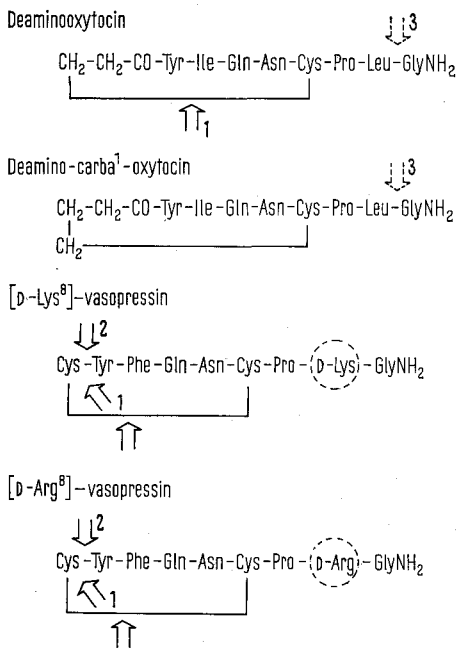

Fig. 1

Table I. Inactivation of oxytocin analogues by kidney cell particles

Substance	Hydrolytic rate constant[a] ($sec^{-1} \times mg\ protein^{-1} \times 10^3$) Homogenate[b] N	D	Microsomes[b] N	D	Cytoplasm[b] N	D
Oxytocin	2.5	9.6	41.6	16.0	18.7	20.8
Deaminoxytocin	1.1	4.1	1.0	16.0	18.7	1.4
Deamino-carba[1]-oxytocin	1.0	1.9	2.7	32.0	0.0	0.0

[a] First order constant of inactivation per mg/ml proteins. [b] N, non-dialysed fraction; D, dialysed fraction.

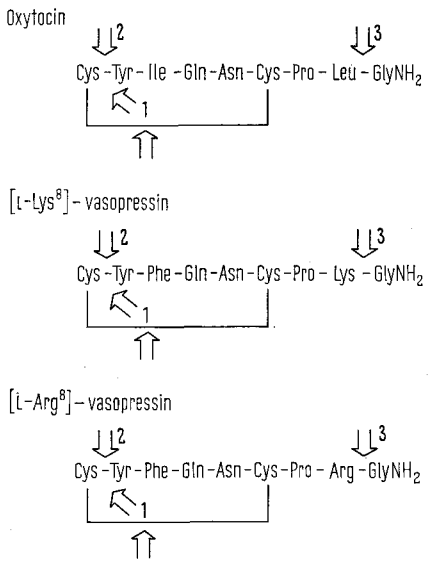

Fig. 2

[1] I. RYCHLÍK, in *Oxytocin, Vasopressin and Their Structural Analogues* (Ed. J. RUDINGER; Pergamon Press, Oxford 1964), p. 153.
[2] S. E. DICKER and A. L. GREENBAUM, J. Physiol. *132*, 199 (1956) and *141*, 107 (1958).
[3] I. BARTOŠEK, V. PLIŠKA, I. RYCHLÍK and F. ŠORM, in *Problemy evolucionnoj i techničeskoj biochimii* (iz. Nauka, Moskva 1964), p. 276.
[4] H. TUPPY and H. NESVADBA, Mh. Chem. *88*, 977 (1957).
[5] H. J. HÜTTER, in preparation.
[6] T. BARTH, V. PLIŠKA and I. RYCHLÍK, Colln. czech. Chem. Commun. *32*, 1058 (1967).
[7] J. B. CAMPBELL, B. THYSEN and FUN SUN CHU, Life Sci. *4*, 2129 (1965).
[8] D. B. HOPE, V. V. S. MURTI and V. DU VIGNEAUD, J. biol. Chem. *237*, 1563 (1962).
[9] K. JOŠT and J. RUDINGER, Colln. Czech. Chem. Commun. *32*, 1229 (1967).
[10] M. ZAORAL, J. KOLC and F. ŠORM, Colln. Czech. Chem. Commun. *32*, 1242 (1967).
[11] M. ZAORAL, J. KOLC and F. ŠORM, Colln. Czech. Chem. Commun. *31*, 382 (1966).
[12] W. C. SCHNEIDER and G. H. HOOGEBOOM, J. biol. Chem. *183*, 123 (1950).
[13] V. PLIŠKA and I. RYCHLÍK, Acta endocr. Copenh. *54*, 129 (1967)

Results and discussion. The results obtained with the group of oxytocin analogues are given in Table I and can be summarized as follows: (1) The kidney homogenate inactivated all three peptides (rate differences were not great between groups) and the inactivation capacity was somewhat increased after dialysis. (2) DCOT was not split by cytoplasm, inactivation of DOT decreased markedly after dialysis of the cytoplasmatic fraction. Since both analogues differ only in the type of bridge which can undergo reduction in DOT but not in DCOT, we may assume the existence of an inactivation via reduction of the S-S-bond. In this cell fraction oxytocin is inactivated apparently by enzymatic systems other than DOT as can be concluded with regard to the effect of dialysis on the inactivation of both peptides. (3) The inactivation of oxytocin by microsomal fraction is decreased after the dialysis in contrast to the inactivation of DOT and DCOT.

The results obtained with the vasopressin group are shown in Table II. Both, the homogenate and microsomes and the cytoplasm split DAVP and DLVP at the same rate as their corresponding L-forms. There were also insignificant differences between dialysed and nondialysed preparations.

As shown by pharmacokinetic evaluation, elimination of analogues in the target organ does not markedly differ from that of the maternal hormone; using the antidiuretic test[13] we found an index of persistence[14,15] for all of these substances equal approximately to unity when doses producing a 10- to 35-min anuria were assayed. None of the above analogues of oxytocin had a protracted uterotonic activity.

These results suggest the following: (1) A two-stage mechanism of inactivation (reduction and aminopeptidase splitting) can be localized in the cytoplasm of kidney cells. (2) In the microsomal fraction there is apparently a combined action of undefined endo- and aminopeptidases (the dialysis removes a low molecular weight cofactor of one system, as well as a dialyzable inhibitor of the other one). (3) A decisive action of trypsin-like enzymes in inactivation of vasopressin can probably be excluded. The physiological significance of these conclusions is uncertain, since it is not known whether neurohypophysial hormones penetrate through the cell membrane into the cytoplasm or into the endoplasmatic reticulum.

Table II. Inactivation of vasopressin analogues by kidney cell particles

Substance	Hydrolytic rate constant[a] ($sec^{-1} \times mg\ protein^{-1} \times 10^{-3}$)					
	Homogenate		Microsomes		Cytoplasm	
	N	D	N	D	N	D
[L-Lys^8]-Vasopressin	3.4	11.0	25.0	25.4	9.7	16.5
[D-Lys^8]-Vasopressin	10.4	9.4	19.9	9.9	19.6	21.7
[L-Arg^8]-Vasopressin	10.4	11.1	28.2	25.4	19.6	12.5
[D-Arg^8]-Vasopressin	7.7	11.5	45.1	40.7	20.3	15.0

[a] Explanation see Table I.

Zusammenfassung. Die Inaktivierung von Carba-, Deamino- und 8-D-substituierten Analogen der neurohypophysäuren Hormone durch Schweinenierenhomogenate verläuft mit der gleichen Geschwindigkeit wie die des Oxytocins und Vasopressins.

T. BARTH, H. J. HÜTTER[16], V. PLIŠKA and F. ŠORM

Institute of Organic Chemistry and Biochemistry, Czech. Academy of Sciences, Praha 6 (Czechoslovakia), 20 December 1969

[14] V. PLIŠKA, Arzneimittel-Forsch. *16*, 886 (1966).
[15] V. PLIŠKA, Europ. J. Pharmac. *5*, 253 (1969).
[16] Medizinische Universitäts-Klinik, Rostock (DDR).

Behaviour of Glycogen and Related Enzymes in the Sertoli Cell Syndrome

The presence and the behaviour of glycogen and of 1–4 amylophosphorylase (1–4 AP) in human testis under normal and pathological conditions has already been described in an earlier report[1,2]. There is evidence that intratubular glycogen represents an important source of energy for spermatogenesis[3–9] whereas the presence of this substance in the cytoplasm of some peritubular cells (muscle cells, Ross[10]) seems to demonstrate that glycogen is also involved in tubular motility[11].

Among the pathological conditions of the human testis, the 'germinal aplasia' or 'Sertoli cell Syndrome'[12] seems to be characterized from a histochemical point of view, by the scarcity or absence of intratubular glycogen and 1–4 AP, and by the large amounts of both substances in the peritubular zone[1]. The present paper reports the results of further investigations on histochemical behaviour of glycogen and the 1–4 AP in this particular condition.

Material and methods. Bioptic specimens were obtained from testes of 14 subjects who were found to be affected by complete absence of seminiferous epithelium (Sertoli cell syndrome). Eight of them gave no history of exogenous

[1] A. FABBRINI, M. RE and G. SPERA, Experientia *24*, 789 (1968).
[2] A. FABBRINI, M. RE and C. CONTI, J. Endocr., in press (1969).
[3] E. GIERKE, Beitr. path. Anat. *98*, 351 (1937).
[4] J. P. ARZAC, J. clin. Endocr. *10*, 1465 (1950).
[5] H. ELFTMANN, in *Studies on Testis, and Ovary, Eggs and Sperm* (Ed. E. T. ENGLE and CH. C. THOMAS; Springfield 1952), p. 26.
[6] R. E. MANCINI, J. NOLAZCO and F. A. DE LA BALZE, Anat. Rec. *114*, 127 (1952).
[7] C. P. LEBLOND and Y. CLERMONT, Am. J. Anat. *90*, 167 (1952).
[8] C. P. LEBLOND and Y. CLERMONT, Ann. N.Y. Acad. Sci. *55*, 548 (1952).
[9] W. MONTAGNA, Ann. N.Y. Acad. Sci. *55*, 629 (1952).
[10] M. H. Ross and I. R. LONG, Science *153*, 1271 (1966).
[11] E. C. ROOSEN-RUNGE and F. D. BARLOW, Am. J. Anat. *93*, 143 (1953).
[12] E. B. DEL CASTILLO, A. TRABUCCO and F. A. DE LA BALZE, J. clin. Endocr. *7*, 493 (1947).