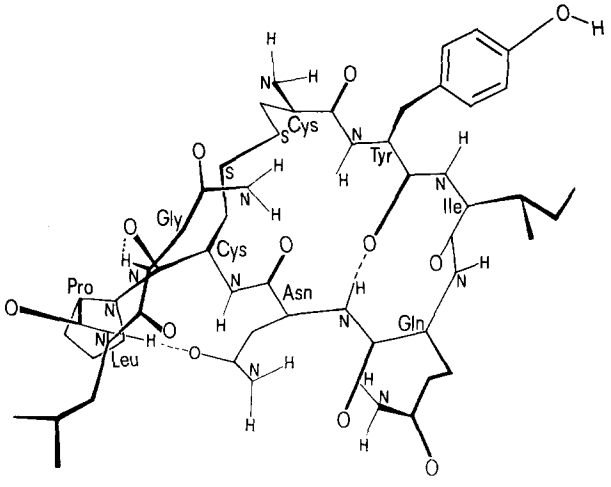
Comparison of some Biological Activities of Arginine Vasotocin and Synthetic Analogs[1,2]

The proposed three-dimensional structure of oxytocin consists of two β-turns, one in the 20-membered covalent ring and one in the linear portion (Figure)[3]. Though detectable conformational differences exist between the naturally occurring neurohypophyseal hormones – and they are fundamental to the differentiation of their biological properties – the backbone conformations of lysine vasopressin[4,5], arginine vasopressin and arginine vasotocin[6] are grossly similar to that of oxytocin. The amino acid residues in positions 3, 4, 7 and 8 of the hormones occupy corners of the β-turns, and the side chains of these residues have only a limited effect on stabilization of the peptide backbone (Figure). As a result the restriction of evolutionary modifications to the side chains in positions 3, 4 and 8 of naturally occurring neurohypophyseal peptides is understandable[7]. It is also the side chains of residues in the corners of the β-turns that are free to engage in intermolecular interactions, including the hormone-receptor interaction.

A corollary of these considerations of the limitations on structural variability of the hormones is that different receptors must exist which recognize particular structural aspects of a given hormone in order to mediate its different activities. Therefore, changes of the side chains of residues in positions 3, 4, 7 and 8 can dramatically enhance one or more hormonal activity, while diminishing or practically eliminating another[4,7,8,9]. In the present study we report on some of the biological activities characteristic of neurohypophyseal hormones for several analogs of arginine vasotocin ([8-arginine]oxytocin) which were synthesized and assayed for their insulin-like activities by SCHILLINGER et al.[10].

The activities of the arginine vasotocin analogs, which are modified in positions 3 or 8, are shown in the Table, and compared to those of arginine vasotocin itself. The substitution of arginine by ε-formyllysine or by citrulline eliminates the possibility for a cationic charge at position 8. There is a significant decrease in both the antidiuretic and pressor activities of [8-formyllysine]oxytocin and [8-citrulline]oxytocin as compared with arginine vasotocin.



Proposed conformation of oxytocin in dimethylsulfoxide

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[2] Neurohypophyseal hormone analogs are denoted in accordance with IUPAC-IUB Tentative Rules on Biochemical Nomenclature, Biochemistry *6*, 362 (1967) and J. biol. Chem. *247*, 977 (1972). All optically active amino acids are of the L-configuration unless otherwise noted. Arginine vasopressin, [8-arginine]vasopressin; lysine vasopressin, [8-lysine]vasopressin; AVT, arginine vasotocin, [8-arginine]oxytocin; Mpr, β-mercaptopropionic acid.

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

[4] R. WALTER, in *Structure-Activity Relationships of Protein and Polypeptide Hormones* (Eds. M. MARGOULIES and F. C. GREENWOOD; Excerpta Medica, Amsterdam 1971), p. 181.

[5] R. WALTER, J. D. GLICKSON, I. L. SCHWARTZ, R. T. HAVRAN, J. MEIENHOFER and D. W. URRY, Proc. natn. Acad. Sci., USA *69*, 1920 (1972).

[6] R. WALTER, A. BALLARDIN, I. L. SCHWARTZ, W. A. GIBBONS and H. R. WYSSBROD, Proc. natn. Acad. Sci., USA, submitted.

[7] R. WALTER, I. L. SCHWARTZ, J. H. DARNELL and D. W. URRY, Proc. natn. Acad. Sci., USA *68*, 1355 (1971).

[8] R. WALTER, T. YAMANAKA and S. SAKAKIBARA, Proc. natn. Acad. Sci., USA, in press.

[9] As a contrast, it is apparent that modifications which significantly change the average peptide backbone conformation will render the hormone unrecognizable by *any* receptor (or might enhance the fit to *all* receptors) and thus will, in first approximation, tend to affect all activities in a similar direction.

[10] E. SCHILLINGER, O. LOGE, E. SCHRÖDER, E. KLIEGER and K. LÜBKE, Eur. J. Biochem. *27*, 473 (1972).

Comparison of biological activities of arginine vasotocin with those of analogs possessing amino acid substitutions in positions 3 and 8[a]

	Uterotonic (rat)[b]	Vasodepressor (fowl)[c]	Pressor (rat)[d]	Antidiuretic (rat)[e]
[Arg^8]oxytocin[f] (arginine vasotocin)	155	285	245	250
[D-Arg^8]oxytocin	50 ± 2	7.7 ± 0.5	0.28 ± 0.02	360 ± 10
[Lys (form)^8]oxytocin	170 ± 20	320 ± 30	35 ± 1	27 ± 1
[Cit^8]oxytocin	330 ± 30	427 ± 27	33 ± 3	9 ± 2
[Mpr^1, *Nle*^{3}, Arg^8]oxytocin	14 ± 1	42 ± 1	40 ± 2	162 ± 7
[Mpr^1, *Val*^{3}, Arg^8]oxytocin	22 ± 1	56 ± 3	13 ± 2	13 ± 2
[Mpr^1, *Pro*^{3}, Arg^8]oxytocin	0.02 ± 0.01	0.25 ± 0.01	0.12 ± 0.03	0.44 ± 0.10

[a] Biological activities are expressed as USP units/mg ± SEM on the basis of the molecular weight of the anhydrous peptide. [b] Uterotonic activities were assayed on the isolated uteri of rats in natural estrus, determined on the morning of the assay by vaginal smear, by the method of HOLTON[18] as modified by MUNSICK[19], utilizing Mg^{++}-free van Dyke-Hastings solution. [c] Avian vasodepressor assays were performed on conscious chickens[20] by the method of COON[21]. [d] Pressor activity was determined in atropinized, urethane-anesthetized male Sprague-Dawley rats as described in the U.S. Pharmacopeia[22]. [e] Antidiuretic activity was determined in inactin- and ethanol-anesthetized, hydrated male Sprague-Dawley rats according to a modification of the method of JEFFERS et al.[23]. [f] Values taken from ref. [24].

In contrast, the oxytocic activities of [8-formyllysine]oxytocin are retained at a level equal to those of AVT, and the same activities of [8-citrulline]oxytocin are actually enhanced, giving rise to a striking differentiation of oxytocic and pressor-antidiuretic properties for both analogs.

A change in the stereochemistry of the arginine residue in position 8 of arginine vasotocin from an L to a D configuration results in a substantial increase in the antidiuretic activity while essentially eliminating the pressor activity. This differentiation of activities with [8-D-arginine]vasotocin is considerably more dramatic than previously observed with [8-D-arginine]vasopressin[11], [8-D-lysine]vasopressin[11] and other vasopressin analogs with an amino acid residue of D configuration in position 8[12–14]. These results have all been interpreted to indicate a more stringent requirement for a complementary charge interaction at the pressor receptor, compared to the antidiuretic receptor[7].

In the series of analogs with 3-position modifications, the N-terminal amino group is deleted. In general, deamino oxytocin compounds show increased uterotonic activity (e.g.[15]) and deamino vasopressins exhibit a high ratio of antidiuretic-to-pressor activities (e.g.[16]). The substitution of norleucine for isoleucine in [1-deamino, 3-norleucine]arginine vasotocin results in relatively high antidiuretic activity with retention of significant oxytocic activities. The replacement of isoleucine by valine is accompanied by a large reduction of all tested activities, and proline is an even less compatible replacement for the isoleucine residue. Provided that only amino acid residues with aliphatic side chains are considered as substitutions in position 3 it is likely that the decreases in potency reflect differences in binding of the analog to receptor[17], although dose response studies in appropriate in vitro assay systems are required to ascertain this point.

In conclusion, modifications of the side chains of 'corner' residues of the proposed β-turns of oxytocin – in this case residues in positions 3 and 8 – can selectively affect biological activities of the neurohypophyseal hormone analogs, although by the influence these modified side chains may have on the 'catalytic center' of the proposed 'biologically active' conformation of oxytocin[4,7] they may exert synergistic rather than strictly additive effects.

Zusammenfassung. Nachweis, dass die Substitution in Positionen 3 und 8 im Arginin Vasotocin ([8-Arginin]-Oxytocin) selektive Veränderungen verschiedener biologischer Aktivitäten hervorruft, was am Modell der Oxytocinkonformation diskutiert wird.

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[11] M. ZAORAL, J. KOLC and F. ŠORM, Collect. Czech. chem. Commun. *32*, 1242 (1967).
[12] M. ZAORAL, J. KOLC and F. ŠORM, Collect. Czech. chem. Commun. *35*, 1716 (1970).
[13] M. ZAORAL and M. FLEGEL, Collect. Czech. chem. Commun. *37*, 3350 (1972).
[14] M. ZAORAL and F. ŠORM, Collect. Czech. chem. Commun. *31*, 310 (1966).
[15] D. B. HOPE, V. V. S. MURTI and V. DU VIGNEAUD, J. biol. Chem. *237*, 1563 (1962).
[16] R. D. KIMBROUGH, JR., W. D. CASH, L. A. BRANDA, W. Y. CHAN and V. DU VIGNEAUD, J. biol. Chem. *238*, 1411 (1963).
[17] J. RUDINGER, V. PLIŠKA and I. KREJČÍ, Rec. Progr. Horm. Res. *28*, 131 (1972).
[18] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[19] R. A. MUNSICK, Endocrinology *66*, 457 (1960).
[20] R. A. MUNSICK, W. H. SAWYER and H. B. VAN DYKE, Endocrinology *66*, 860 (1960).
[21] J. M. COON, Archs int. Pharmacodyn. *62*, 79 (1939).
[22] *The Pharmacopeia of the United States*, 17th Rev. (Mack Printing Co., Easton, Pa. 1965), p. 749.
[23] W. A. JEFFERS, J. J. LIVEZEY and J. H. AUSTIN, Proc. Soc. exp. Biol. Med. *50*, 184 (1942).
[24] R. L. HUGUENIN and R. A. BOISSONNAS, Helv. chim. Acta *43*, 182 (1962).
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Hyperglucagonemia, Hypocalcemia and Diminished Gastric Blood Flow-Evidence for an Etiological Role in Stress Ulcer of Rat

In the past many pathophysiological aspects of stress induced ulcers in man and experimental animals have been studied intensively. At present, despite increased knowledge of the role which various factors possibly involved might play, such as histamine and the magnitude of gastric mucosal perfusion, this disorder is far from being fully understood.

Pancreatic glucagon (pGl) is known to suppress both gastric acid secretion[1,2] and blood perfusion[3]. In addition to hypoglycemia and amino acids infusion[4], this hormone is released from the pancreas by stress[5]. Hypothesizing that pGl might be a important substance during the events finally leading to mucosal lesions and ulcers, we studied several groups of rats using local oxygen pressure (pO_2) in gastric mucosa as a reliable index for blood perfusion (described in detail elsewhere[6]) and its dependency upon pGl concentration. In order to clarify whether pGl also may be functionally related to actual serum calcium and gastrin, these parameters were measured in intact and adrenalectomized rats.

Materials. 3 groups of male SPF Wistar rats, approx. 200 g body wt. (Mus Rattus GmbH., Brunnthal/Germany) were randomly divided into intact, sham-operated (laparotomy only) and adrenalectomized animals, 1 moiety serving as control. The other half was stressed by the restraint technique[7] at room temperature for 24 h. Food

[1] T. M. LIN and G. F. SPRAY, Archs int. Pharmacodyn. *191*, 88 (1971).
[2] E. KOKAS, S. H. KAUFMAN and J. C. LONG, Z. vergl. Physiol. *74*, 315 (1971).
[3] T. M. LIN and M. W. WARRICK, Gastroenterology *61*, 328 (1971).
[4] A. OHNEDA, M. SATO and K. MATSUDA, Tohoku J. exp. Med. *107*, 241 (1972).
[5] S. R. BLOOM, P. M. DANIEL and D. J. JOHNSTON, Q. Jl. exp. Physiol. *58*, 99 (1973).
[6] W. SCHELLERER, P. O. SCHWILLE and P. HERMANEK, Langenbecks Arch. Chir., Suppl. Chir. Forum, in press (1974).
[7] D. A. BRODIE and H. M. HANSON, Gastroenterology *38*, 353 (1960).