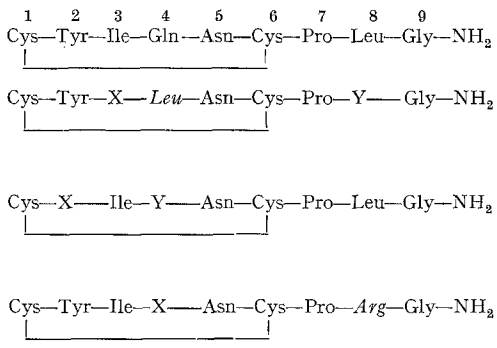


Amino-acids differing from those in oxytocin are in italic type.

afforded the crude peptide IVb which was purified either by partition chromatography[17,18] on Sephadex G-25 using the solvent systems 1-butanol-pyridine-0.2*M* acetic acid (10:7:24) and 1-butanol-ethanol-pyridine-0.2*M* acetic acid (16:1:2:28), or by ion exchange chromatography on Amberlite CG-50 using 0.5 ammonium acetate, pH 6.4, for elution[19].

The [Leu^4]-arginine-vasotocin (IVb) was isolated as the diacetate and characterised by elemental analysis, optical rotation ($[\alpha]_D^{25}$ + 4.0 ± 2°, recalculated for free peptide; c = 1 in 1*M* AcOH), paper electrophoresis at pH 6.0 (m_{Arg} = 0.51) and pH 1.7 (m_{Arg} = 0.52) and by TLC on silica gel and cellulose powder in several solvent systems; in every case the peptide appeared to contain less than 2% of impurities reacting with ninhydrin, the Pauly reagent, or the Sakaguchi reagent. An acid hydrolysate (6*M* HCl, 110°, 72 h) showed the molar ratios: Asp 1.00, Pro 1.06, Gly 0.91, Cys 1.89, Ile 0.99, Leu 1.01, Tyr 0.90, Arg 1.06, NH_3 1.80.

The parmacological properties of this peptide, in particular its natriuretic and diuretic action, are described in the two following communications[20,21].

[17] D. YAMASHIRO, Nature *201*, 76 (1964).
[18] D. YAMASHIRO, D. GILLESSEN and V. DU VIGNEAUD, J. Am. chem. Soc. *88*, 1310 (1966).
[19] J. MEIENHOFER and V. DU VIGNEAUD, J. Am. chem. Soc. *82*, 2279 (1960).
[20] V. PLIŠKA, J. VAŠÁK, M. RUFER and J. RUDINGER, Experientia *29*, 171 (1973).
[21] J. H. CORT, K. M. STRUB, G. HÄUSLER and J. RUDINGER, Experientia *29*, 173 (1973).
[22] F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel (Switzerland).
[23] Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, Hönggerberg, CH-8049 Zürich (Switzerland).

Zusammenfassung. Es wird die Synthese des 4-Leucin-Analogen des Arginin-vasotocins (= [Leu^4, Arg^8]-oxytocin) beschrieben.

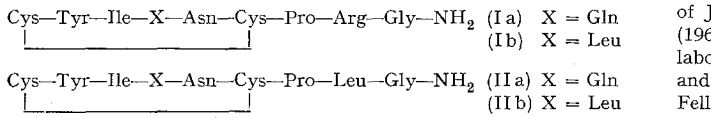
D. GILLESSEN[22], R. O. STUDER[22] and J. RUDINGER[23]

Chemical Research Department, F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basel (Switzerland);
and Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, CH-8049 Zürich (Switzerland), 12 October 1972.

Some Pharmacological Properties of [4-Leucine]-Arginine-Vasotocin

The preceding paper[1] describes the synthesis of [Leu^4]-arginine-vasotocin (Ib), an analogue of the natural hormone arginine-vasotocin (Ia). The pharmacological properties of Ib in some standard assay systems are summarized in this paper and compared with the properties of the parent hormone Ia, of oxytocin (IIa), and of [Leu^4]-oxytocin (IIb). The diuretic and natriuretic properties of Ib are described in the following paper[2].

The action on the uterus was studied with organs from virgin rats in natural oestrus[3] in media[4] without magnesium or with 0.5 m*M* Mg^{2+}; contractions were measured isotonically. Pressor activity was assayed[5] in phenoxybenzamine-treated rats using a pressure transducer, antidiuretic activity in ethanol-anaesthetized, water-loaded rats by a modification[6] of the method of SAWYER[7]. Sodium transport by frog skin was measured as the short-circuit current[8] across isolated ventral skin of *Rana ridibunda* using voltage-clamp equipment[9]. The hydroosmotic effect was determined from the rate of osmotic water flow across frog bladder (*R. esculenta*)[10].



[1] D. GILLESSEN, R. O. STUDER and J. RUDINGER, Experientia *29*, 170 (1973).
[2] J. H. CORT, K. M. STRUB, G. HÄUSLER and J. RUDINGER, Experientia *29*, 173 (1973).
[3] P. HOLTON, Br. J. Pharmac. *3*, 328 (1948).
[4] R. A. MUNSICK, Endocrinology *66*, 451 (1960).
[5] J. DEKANSKI, Br. J. Pharmac. *7*, 567 (1952).
[6] V. PLIŠKA and I. RYCHLÍK, Acta endocrin. *54*, 129 (1967).
[7] W. H. SAWYER, Endocrinology *63*, 694 (1958).
[8] H. H. USSING and K. ZERAHN, Acta physiol. scand. *23*, 110 (1951).
[9] Measurements carried out at the Departement de physiologie, Université de Genève. We wish to thank Dr. R. DE SOUSA for the hospitality of his laboratory and helpful advice.
[10] These and other measurements were performed at the Laboratoire de physiologie cellulaire, Collège de France, Paris, by the technique of J. BOURGUET and S. JARD [Biochim. biophys. Acta *88*, 442 (1964)]. We are grateful to Prof. F. MOREL for the hospitality of his laboratory, to him and Dr. S. JARD for their interest and advice, and to the European Molecular Biology Organisation (EMBO) for a Fellowship to one of us (M.R.).

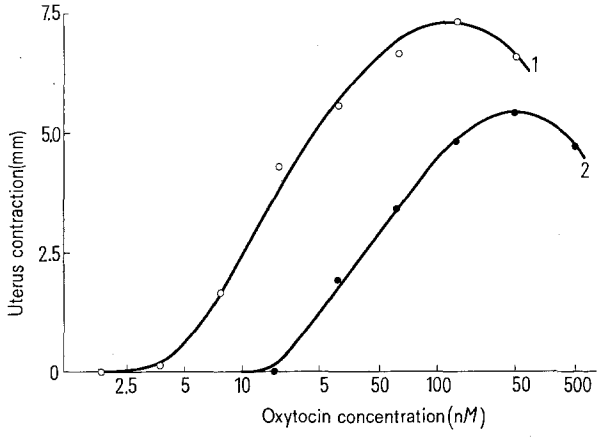
Some biological activities of oxytocin, arginine-vasotocin, and their [4-leucine] analogues

Peptide	Biological activity				
	Uterotonic in vitro (rat)[a] without Mg^{2+}	0.5 m*M* Mg^{2+}	Pressor[a] (rat)	Antidiuretic[a] (rat)	Natriferic[b] (frog skin
Oxytocin[11]	*546 ± 18*	*470*[c]	*3.1 ± 0.1*	*2.7 ± 0.2*	8.15 ± 0.01
[Leu^4]-Oxytocin[12]	*13 ± 1*	—	weakly depressor	diuretic, anti-ADH	—
Arginine-vasotocin[14]	*125 ± 16*	*240*[c]	*245 ± 16*	*250 ± 35*	9.02 ± 0.01
[Leu^4]-arginine-vasotocin	0 (inhibitor) (12)	~1–10[d] (9)	6.1 ± 0.4 (10)	3.2 ± 0.5 (5)	5.85 ± 0.35 (5)

[a] Potencies (± S.E.) are given in IU/mg (literature values; in italics) or in IU/μmol (own results; 1 μmol of the analogue corresponds to 1.035 mg of the dry peptide as free base). In parentheses, number of experiments. [b] Values of pD_2 (negative logarithm of molar concentration eliciting half-maximal response) ± S.E. [c] Calculated from the potencies in the preceding column and the potency ratios given in the literature[4,13]. [d] Concentration range 0.04–0.45 μ*M*; the log-dose–response curves for the analogue and oxytocin are not parallel and the ratio of equipotent doses is therefore concentration-dependent.

The results are summarized in the Table. On uteri taken from rats in natural oestrus and kept in magnesium-free media, [Leu^4]-arginine-vasotocin had no contractile action but inhibited the contractile response to oxytocin. The inhibition persisted for some time after the analogue had been washed out. In the presence of the analogue the log-dose–response curves for oxytocin were displaced to the right: usually the maximal response to oxytocin was also decreased (see figure), suggesting that the inhibition is not purely competitive. As an approximate quantitative index of inhibitor potency, values analogous to pA_2 were calculated[15] from the central portions of the log-dose–response curves and found to be 6.39 ± 0.11 (mean ± S.E.; $n = 6$). In media containing 0.5 m*M* Mg^{2+}, [Leu^4]-arginine-vasotocin generally showed some uterotonic activity but frequently induced tachyphylaxis even when doses were given 8 min apart. The potencies given in the Table are based on comparisons of single doses or a simple bracketing procedure.

Transitions from agonism to antagonism with changes in the experimental conditions have been observed with other analogues of the neurohypophysial hormones[16] and are interpreted as being due to changes in thestimulus-response coupling[17,18]. Inhibition of the uterotonic response to oxytocin has also been reported for [Leu^2, Leu^4]-oxytocin[19].



Log-dose–response curves for oxytocin alone (1) and in the presence of 0.85 μ*M* [Leu^4]-arginine-vasotocin (2). Uterus from rat in natural oestrus in magnesium-free medium, bath volume 10 ml, isotonic contractions; cumulative dose procedure.

The pressor action of [Leu^4]-arginine-vasotocin was qualitatively similar to that of arginine-vasopressin. In water-loaded rats under the standard assay conditions the analogue had a weak but qualitatively vasopressin-like antidiuretic effect.

[Leu^4]-arginine-vasotocin stimulated sodium transport by the frog skin (*R. ridibunda*) but the concentration required to induce half-maximal response was about 1000 times higher than the corresponding concentration of arginine-vasotocin. The maximal response to the analogue was as high as, or only slightly lower than, the maximal response to the natural hormone. [Leu^4]-oxytocin has been reported[20] to have slight natriferic action on the bladder of the toad (*Bufo marinus*) and to inhibit its response to arginine-vasotocin.

In two experiments, [Leu^4]-arginine-vasotocin was found to increase the osmotic flow of water through the bladder of the frog (*R. esculenta*). The maximal response was about 80%, and the concentration required for half-maximal response about 50-fold as compared with oxytocin. In parallel experiments [Leu^4]-oxytocin elicited a maximal response of 50% or less, and a half-maximal response at a 1000-fold concentration as compared with oxytocin. In the toad bladder (*B. marinus*) [Leu^4]-oxytocin[20] and [Leu^4]-mesotocin[21] had shown no hydroosmotic action but inhibited the response to the natural hormones.

Our finding that [Leu^4]-arginine-vasotocin has appreciable antidiuretic activity in water-loaded rats although

[11] W. Y. CHAN, M. O'CONNELL and S. R. POMEROY, Endocrinology *72*, 279 (1963).
[12] V. J. HRUBY, G. FLOURET and V. DU VIGNEAUD, J. biol. Chem. *244*, *3890* (1969).
[13] H. B. VAN DYKE, W. H. SAWYER and N. I. A. OVERWEG, Endocrinology *73*, 637 (1963).
[14] B. BERDE, R. L. HUGUENIN and E. STÜRMER, Experientia *18*, 444 (1962).
[15] H. O. SCHILD, Br. J. Pharmac. *4*, 277 (1949).
[16] Cf. J. RUDINGER and I. KREJČÍ, in *Handbook of Experimental Pharmacology* (Ed. B. BERDE, Springer-Verlag, Berlin-Heidelberg-New York 1968), Vol. 23, p. 748.
[17] I. KREJČÍ, V. PLIŠKA and J. RUDINGER, Br. J. Pharmac. *39*, 217P (1970).
[18] J. RUDINGER, V. PLIŠKA and I. KREJČÍ, Recent Prog. Horm. Res. *28*, 131 (1972).
[19] V. J. HRUBY and W. Y. CHAN, J. med. Chem. *14*, 1050 (1971).
[20] P. J. S. CHIU and W. H. SAWYER, Am. J. Physiol. *218*, 838 (1970).
[21] P. EGGENA, I. L. SCHWARTZ and R. WALTER, J. gen. Physiol. *56*, 250 (1970).

it is potently natriuretic and diuretic under other conditions[2] bears out the conclusion[22] that the natriuretic and antidiuretic (or anti-ADH) responses to this group of analogues are initiated at different receptors and are, in that sense, unrelated. Moreover, there appears to be no obvious correlation between the gross action of [Leu^4]-analogues and related derivatives on sodium transport by amphibian membranes and their natriuretic potency[23].

Zusammenfassung. Die pharmakologischen Eigenschaften von [Leu^4]-Arginin-vasotocin sind in der Tabelle zusammengefasst. Am isolierten Rattenuterus in magnesiumfreiem Medium wirkt das Peptid als Oxytocinantagonist.

V. Pliška, Jelena Vašák, M. Rufer and J. Rudinger

Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, CH-8049 Zürich (Switzerland), 12 Oktober 1972.

[22] W. Y. Chan and V. du Vigneaud, J. Pharmac. exp. Ther. *174*, 541 (1970). – V. J. Hruby, V. du Vigneaud and W. Y. Chan, J. med. Chem. *13*, 185 (1970). – M. A. Wille, V. du Vigneaud and W. Y. Chan, J. med. Chem. *15*, 11 (1972).

[23] Support from the Swiss National Science Foundation (grant No. 3.424.70) and from the Sandoz Foundation for the Promotion of Medical and Biological Sciences is gratefully acknowledged.

The Natriuretic Action of [4-Leucine]-Arginine-Vasotocin

In recent years interest in the natriuretic activity of peptide hormones and their analogues has been stimulated by evidence that a 'natriuretic activity' present in plasma during natriuretic states in animals is due to a peptide and originates in CNS tissue[1] and, on the other hand, by the discovery of a marked natriuretic activity in certain analogues of oxytocin (for references see[2]). In pursuance of these latter findings the 4-leucine analogue of arginine-vasotocin, [Leu^4]-arginine-vasotocin, has been synthesized[2] and its standard pharmacological properties have been examined[3]. This paper reports the diuretic and natriuretic activity of the analogue in cats, rats, and dogs.

Male cats were anaesthetized with chloralose, loaded with 150 m*M* NaCl (10 ml/kg body weight) and continuously infused with 10% mannitol in 10 m*M* NaCl (0.1 ml/min) until urine flow rate and conductivity had reached a steady state; samples were injected i.v. in 0.1–0.5 ml

[1] Cf. J. H. Cort and B. Lichardus, *Regulation of Body Fluid Volumes by the Kidney* (Karger, Basel 1970).

[2] D. Gillessen, R. O. Studer and J. Rudinger, Experientia *29*, 170 (1973).

[3] V. Pliška, J. Vašák, M. Rufer and J. Rudinger, Experientia *29*, 171 (1973).

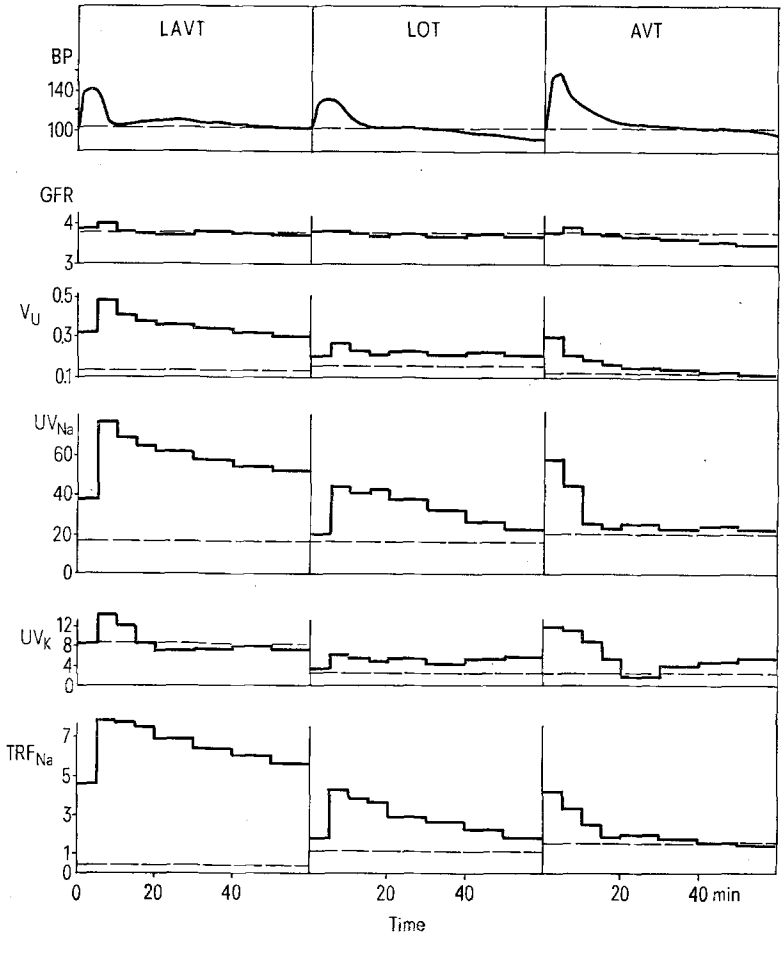


Fig. 1. Renal and pressor responses of chloralosed cats to [Leu^4]-arginine-vasotocin (LAVT), [Leu^4]-oxytocin (LOT), and arginine-vasotocin (AVT). Each peptide (30 µg/kg, i.v.) given to 1 of 3 different 3-kg cats with similar baseline values of arterial BP and and renal excretion; the pre-injection control values for each cat are shown by dashed lines. BP, arterial blood pressure in mm Hg; GFR, glomerular filtration rate as clearance of endogenous creatinine in ml. kg^{-1}. min^{-1}; V_U, urine flow rate in ml. kg^{-1}. min^{-1}; UV_{Na}, total Na excretion in µeq. kg^{-1}. min^{-1}; UV_K, total K excretion in the same units; $TRF_{Na} \times 100$, percentage of filtered Na load appearing in the final urine. Relative activities are related to the areas under the UV_{Na} or TRF_{Na} plots.