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).

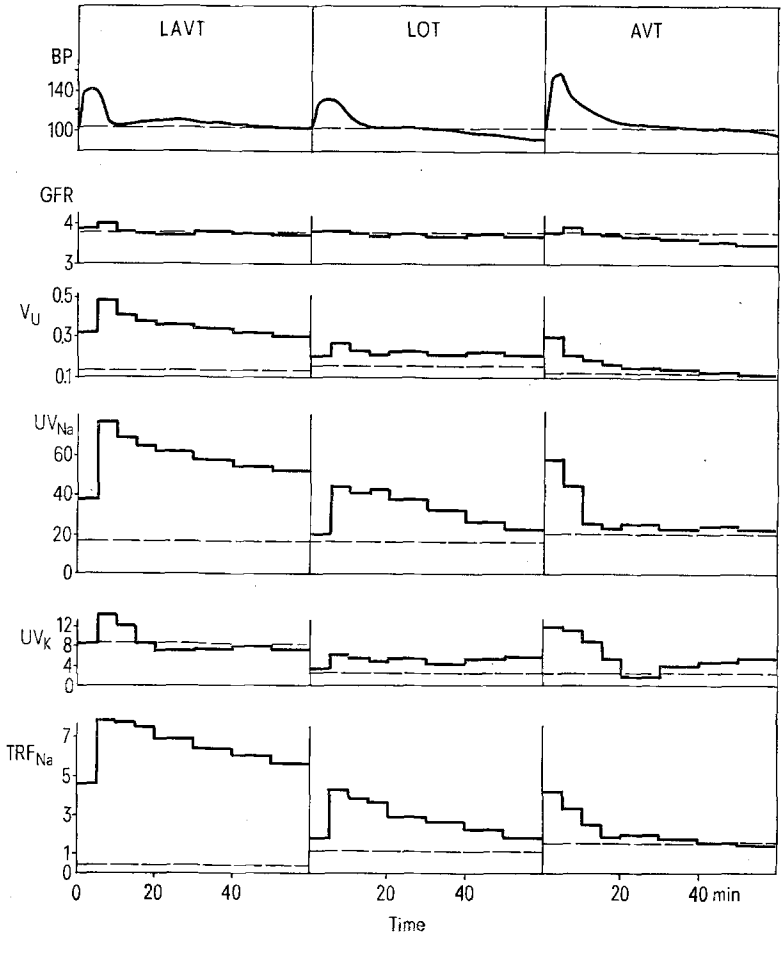


Fig. 1. Renal and pressor responses of chloralosed cats to [Leu$^4$]-arginine-vasotocin (LAVT), [Leu$^4$]-oxytocin (LOT), and arginine-vasotocin (AVT). Each peptide (30 µg/kg, i.v.) given to 1 of 3 different 3-kg cats with similar baseline values of arterial BP and and renal excretion; the pre-injection 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.

saline (for details, see[4]). Each animal was given 1–3 injections; in all, 80 measurements were made in 38 animals. The rats were conscious, 40-day-old females (Füllinsdorf albino outbred) which were starved for 20 h before the experiment but received tap water ad libitum. They were loaded with saline (20 ml/kg body weight) perorally and at the same time injected s.c. with the peptide in saline (2 ml/kg body weight); the control group was injected with saline only. The urine was collected in metabolism cages over 5 h following injection. The dogs were conscious, episiotomized, trained Swiss beagle bitches. They received no food or water during the experiment. The peptide was given i.v. after a 1-h control period. The bladder was emptied at 30-min intervals by means of an indwelling catheter. One dog was used for each of 3 dose levels; the control values were obtained from the same 3 dogs under identical conditions on a different day.

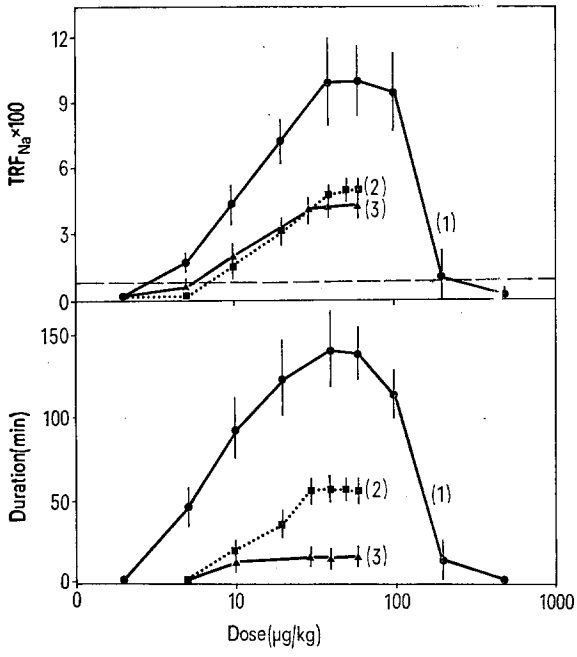


Fig. 2. Dose dependence of the intensity (top) and duration (bottom) of the natriuretic response to [Leu[4]]-arginine-vasotocin (1), [Leu[4]]-oxytocin (2), and arginine-vasotocin (3) in the chloralosed cat preparation. Top: Peak value of $TRF_{Na} \times 100$ (percentage of filtered Na excreted in urine) in response to a given dose vs. log dose. Bottom: Duration of the natriuretic response (from injection to return to baseline) after a given dose vs. log dose. Points are means of 3–5 values, vertical lines S.E. Horizontal dashed line represents the highest acceptable value of baseline TRF under steady-state conditions before samples can be given. The two parent peptides were not administered in doses over 50 µg/kg because of limited supply.

Figure 1 shows the pressor and renal responses in cats to equal doses (30 µg/kg) of [Leu[4]]-arginine-vasotocin and of the two 'parent' peptides, arginine-vasotocin and [Leu[4]]-oxytocin. Of the three peptides vasotocin has the highest and [Leu[4]]-oxytocin the lowest pressor activity (see also[3]); the pressor responses to all 3 peptides were of short duration. The natriuretic responses, expressed either as the total sodium excretion ($UV_{Na}$) or as the tubular rejection fraction ($TRF_{Na}$), in the case of vasotocin showed a similar time course as the pressor response so that little can be said as to a possible intrinsic natriuretic activity of this peptide. However, [Leu[4]]-oxytocin caused a clear increase in natriuresis persisting long after termination of the pressor response. With [Leu[4]]-arginine-vasotocin the increase in natriuresis was far greater in both amplitude and duration.

The dose dependence of the peak natriuretic response and of the duration of the response is shown in Figure 2. Again, [Leu[4]]-arginine-vasotocin is the most potent; the average peak response of 9–11% of the filtered Na load appearing in the urine is a notable degree of activity, particularly when combined with a duration of the response over some 120 min. Both parameters of the response reach a maximum at a dose of about 30 µg/kg; at doses above 100 µg/kg the effect falls off again steeply. This phenomenon may be related to the onset of an antidiuretic activity, as documented in the water-loaded rat[3].

The urinary responses of conscious saline-loaded rats to [Leu[4]]-arginine-vasotocin (Table) show a distinct rise in sodium excretion with a dose of 30 µg/kg (s.c.) and an almost 3-fold increase with 100 µg/kg. The diuretic and kaliuretic effects are somewhat less marked.

In dogs, diuresis and natriuresis set in within 30 min of i.v. application of [Leu[4]]-arginine-vasotocin; preinjection levels were reached again after 3–5 h. Cumulative curves for diuresis, urine osmolality, and sodium and potassium excretion are shown in Figure 3. The increase in the first 3 of these parameters was maximal after a dose of 100 µg/kg, as in the cat preparation.

[4] J. H. CORT, T. DOUŠA, V. PLIŠKA, B. LICHARDUS, J. ŠAFÁŘOVÁ, M. VRANEŠIĆ and J. RUDINGER, Am. J. Physiol. *215*, 921 (1968).

Effect of [Leu[4]]-arginine-vasotocin on renal excretion in conscious, saline-loaded rats

| Dose (µg/kg) | Urine vol. | $Na^+$ concentration | $Na^+$ total | $K^+$ concentration | $K^+$ total |
|---|---|---|---|---|---|
| 0 (control)[a] | 100 | 100 | 100 | 100 | 100 |
| 10 | 104 ± 9.2 | 106 ± 8.0 | 108 ± 8.2 | 40 ± 4.7 | 45 ± 3.5 |
| 30 | 134 ± 7.8 | 118 ± 3.1 | 159 ± 7.7 | 73 ± 7.2 | 102 ± 11.5 |
| 100 | 228 ± 11.4 | 118 ± 7.8 | 275 ± 26.5 | 61 ± 3.3 | 141 ± 7.2 |
| 300 | 223 ± 14.2 | 128 ± 6.7 | 286 ± 17.7 | 76 ± 3.1 | 172 ± 8.3 |
| 1000 | 260 ± 14.0 | 138 ± 7.3 | 357 ± 22.9 | 90 ± 4.5 | 236 ± 10.3 |

Each dose level tested in 20 rats. Results expressed as percentages of control values ± S.E. For experimental details see text.
[a] Control values after injection of saline only were: 5-h urine volume, 13.7 ± 0.93 ml/kg; $Na^+$ concentration, 124.7 ± 7.1 meq/l; 5-h total $Na^+$ excretion, 1.697 ± 0.126 meq/kg; $K^+$ concentration, 70.91 ± 4.69 meq/l; 5-h total $K^+$ excretion, 0.949 ± 0.075 meq/kg.

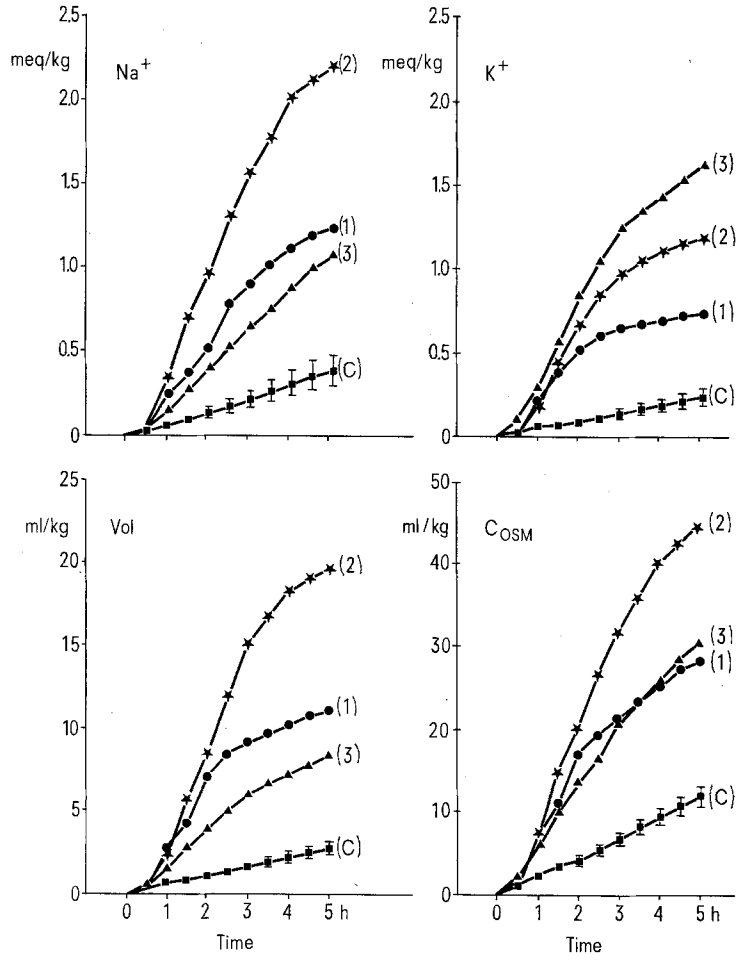


Fig. 3. Renal responses of conscious dogs to 3 dose levels of [Leu[4]]-arginine-vasotocin: cumulative values up to 5 h for total amounts of sodium, potassium, urine, and solutes. Doses (i.v.) 30 µg/kg (1), 100 µg/kg (2), and 300 µg/kg (3), each dose given to 1 dog. Control values (C) obtained from the same 3 dogs on another day (vertical bars give S.E.).

Our results with [Leu[4]]-arginine-vasotocin cannot be directly compared with those recorded for [Leu[4]]-oxytocin and some related oxytocin derivatives[5–8] because of the different experimental conditions used in the two studies (in particular, saline vs. water loading and the longer period of measurement in our experiment). However, direct comparison of [Leu[4]]-oxytocin with [Leu[4]]-arginine-vasotocin in cats (Figures 1 and 2) indicates that in this species at any rate the 4-leucine analogue of arginine vasotocin is the most potent natriuretic peptide of this series to date.

*Zusammenfassung.* [Leu[4]]-Arginin-vasotocin wirkt sowohl an Katzen (Chloralose-Narkose, Mannit-Diurese) als auch an wachen Ratten (mit 0.9%-iger NaCl-Lösung belastet) und wachen nicht vorbehandelten Hunden natriuretisch und diuretisch.

J.H. Cort[9], K.M. Strub[10], G. Häusler[10] and J. Rudinger[11]

*Laboratory of Peptide Biology,*
*Institute of Organic Chemistry and Biochemistry,*
*Czechoslovak Academy of Science,*
*Praha 4–Krč (Czechoslovakia); and*
*Department of Experimental Medicine,*
*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.*

[5] W. Y. Chan, V. J. Hruby, G. Flouret and V. du Vigneaud, Science *161*, 280 (1968).
[6] V. J. Hruby, V. du Vigneaud and W. Y. Chan, J. med. Chem. *13*, 185 (1970).
[7] W. Y. Chan and V. du Vigneaud, J. Pharmac. exp. Ther. *174*, 541 (1970).
[8] M. A. Wille, V. du Vigneaud and W. Y. Chan, J. med. Chem. *15*, 11 (1972).
[9] Laboratory of Peptide Biology, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Praha 4-Krč (Czechoslovakia).
[10] F. Hoffmann-La Roche & Co. Ltd., Department of Experimental Medicine, CH–4002 Basel (Switzerland).
[11] Institute of Molecular Biology and Biophysics, Swiss Federal Institute of Technology, Hönggerberg, CH–8049 Zürich (Switzerland).