Invertebrate neuropeptides resembling vasotocin and some analogues: Synthesis and pharmacological properties

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Received 2 January 1990; accepted 6 February 1990

Summary. The solid phase synthesis of three invertebrate vasopressin-oxytocin homologs: AVP-like factor, F_1[1], ([Leu^2, Thr^4] AVT)[2] isolated from subesophageal and thoracic ganglia of *Locusta migratoria*[3], Arg-conopressin-S[4]. ([Ile^2, Arg^4] AVT), Lys-conopressin-G[4] ([Phe^2, Arg^4] LVT), both isolated from the venom of fish-hunting marine snails of the genus *Conus* and six of their analogues is reported. These analogues are: [Arg^4] AVT, [Ile^2] AVT, [Leu^2] AVT, [Phe^2, Arg^4] AVT, [Arg^4] LVT and [Ile^2, Arg^4] LVT. All peptides were tested for antidiuretic and vasopressor activities.
Key words. Conopressins; AVT-LVT analogues; antidiuretic activity; vasopressor activity; V_2/V_1 selectivity.

The vasopressin-oxytocin peptides were first characterized as neurohypophyseal hormones in mammals. In recent years, knowledge of the physiological roles and phyletic distribution of these peptides has expanded greatly. These peptides are released in tissues other than the pituitary in mammals and in addition, much evidence has accumulated for the presence of these peptides in a number of invertebrates, including insects[1,3,5–10] and molluscs[4,11–13]. To date, the structures of a very few invertebrate vasopressin-oxytocin peptides have been reported[1,4]. In both papers cited[1,4] the sequences of these peptides were given without any information about their pressor or antidiuretic activities.

In this communication we wish to report the synthesis and some biological properties of the AVP-like factor, F_1[1] and two conopressins[4]: Arg-conopressin-S and Lys-conopressin-G. The peptides isolated from *Locusta migratoria, Conus striatus* and *Conus geographus* have a basic residue, Arg or Lys, at position 8, which is correlated with pressor activity in vertebrate hormones and in hundreds of synthetic analogues[14]. All peptides have neutral, hydrophobic residues at position 2, Leu, Ile or Phe. All the vertebrate peptides have an aromatic L-amino acid (Tyr or Phe) at position 2, which is believed to be specifically required for binding to bovine neurophysin[15]. The unique feature shared by both snail peptides is the presence of a basic residue, Arg, at position 4. This gives them a net charge of +3, higher than those of vasotocins (+2), vasopressins (+2) or oxytocin (+1).

The AVP analogues with this substitution have been synthesized: Rekowski et al.[16] reported, that [Arg^4]AVP is very similar to AVP in its antidiuretic and pressor activities, but Arg^4 substitution in a potent vasopressin V_1 antagonist (d(CH$_2$)$_5$AVP)[17] has been reported to turn it into an active V_1 agonist; however, we could not confirm this last observation. We thought it was also worthwhile to explore the effects of Arg^4 substitutions in AVT and LVT alone/or in combination with Leu^2, Ile^2, Phe^2 substitutions. We thus report the synthesis and some pharmacological properties of the AVP-like factor, F_1[1] and the two conopressins[4] (peptides 1–3, table 1) and their six analogues (peptides 4–9, table 2):

1. [2-leucine, 4-threonine]arginine vasotocin ([Leu^2, Thr^4] AVT, AVP-like factor, F_1)
2. [2-isoleucine, 4-arginine]arginine vasotocin ([Ile^2, Arg^4] AVT, Arg-conopressin-S)
3. [2-phenylalanine, 4-arginine]lysine vasotocin ([Phe^2, Arg^4] LVT, Lys-conopressing-G)
4. [4-arginine]arginine vasotocin ([Arg^4] AVT)
5. [2-isoleucine]arginine vasotocin ([Ile^2] AVT)
6. [2-leucine]arginine vasotocin ([Leu^2] AVT)
7. [2-phenylalanine, 4-arginine]arginine vasotocin ([Phe^2, Arg^4] AVT)
8. [4-arginine]lysine vasotocin ([Arg^4] LVT)
9. [2-isoleucine, 4-arginine]lysine vasotocin ([Ile^2, Arg^4] LVT)

Table 1. Pharmacological activities of the invertebrate vasopressin-oxytocin peptides and of AVT and LVT

No.	Peptide		Agonistic activities (units/mg) Antidiuretic (V_2)	Vasopressor (V_1)	Selectivity (V_2/V_1)
	AVT[a]		295 ± 25	227 ± 3	1.3
	[Thr^4] AVT[b]		279 ± 25	104 ± 3	2.7
1	[Leu^2, Thr^4] AVT	*Locusta migratoria* (AVP-like factor, F_1)	8.6 ± 0.8	~0.02	430
2	[Ile^2, Arg^4] AVT	*Conus striatus* (Arg-conopressin-S)	16 ± 2	1.14 ± 0.19	14
	LVT[c]		24 ± 3	130 ± 13	0.2
3	[Phe^2, Arg^4] LVT	*Conus geographus* (Lys-conopressing-G)	0.90 ± 0.08	2.91 ± 0.17	0.3

[a] From Manning, M., and Sawyer, W. H., unpublished data; [b] from Manning et al.[27]; [c] from Berde and Boissonnas[26].

The protected and free peptides were synthesized by published procedures[18–23]. The homogeneity of all free peptides was demonstrated by TLC and HPLC. The physicochemical properties of the protected (compounds I–IX) and free peptides (compounds 1–9) are given in tables 3 and 4, respectively. Analogues were assayed for antidiuretic activities by intravenous injection into ethanol-anesthetized and water-loaded rats[24] and for vasopressor activities by intravenous injection into phenoxybenzamine-treated rats under urethane anesthesia[25]. Agonistic activities are expressed in units/mg.

Table 2. Pharmacological activities of AVT and LVT analogues, modified at position 2 and/or 4

No.	Peptide	Agonistic activities (units/mg) Antidiuretic (V_2)	Vasopressor (V_1)	Selectivity (V_2/V_1)
	AVT[a]	295 ± 25	227 ± 3	1.3
4	[Arg⁴] AVT	147 ± 7	62 ± 2	2.4
5	[Ile²] AVT	74 ± 12	14.9 ± 1.2	5.0
6	[Leu²] AVT	16 ± 2	0.91 ± 0.05	17.6
	[Phe²] AVT[b]	109 ± 13	126 ± 15	0.9
7	[Phe², Arg⁴] AVT	53 ± 5	19 ± 1	2.8
	LVT[b]	24 ± 3	130 ± 13	0.2
8	[Arg⁴] LVT	6 ± 1	30 ± 2	0.2
	[Phe²] LVT[b]	1.0 ± 0.1	32 ± 6	0.03
9	[Ile², Arg⁴] LVT	0.45 ± 0.02	0.49 ± 0.04	0.9

[a] From Manning, M., and Sawyer, W. H., unpublished data; [b] from Berde und Boissonnas[26].

Results and discussion

The antidiuretic and vasopressor properties of the three invertebrate neuropeptides are given in table 1. The combination of Leu² and Thr⁴ substitution in the AVT molecule (Peptide 1) gave a peptide with a dramatic reduction of antidiuretic and vasopressor activities. The antidiuretic, pressor selectivity of the AVP-like factor is 330 times higher than that of AVT (V_2/V_1 ratios: ~ 430 and 1.3, respectively). Replacement of Tyr² by Ile² and Gln⁴ by Arg⁴ in Arg-conopressin-S (Peptide 2) also resulted in a dramatic reduction of antidiuretic and vasopressor activities (5.4 % and 0.5 % that of AVT, respectively) and in a 10-fold increase in V_2/V_1 – agonistic specificity. Substitution of Tyr² by Phe² and Gln⁴ by

Table 3. Physicochemical properties of the protected peptides (I–IX)

No.	Protected peptide[a]	Elemental analysis (formula)	Yield[b] (%)	m.p. (°C)	$[\alpha]_D^{25}$, deg. (c = 0.5 DMF)	TLC, R_f A	B	C	D
I	[Leu², Thr⁴] AVT	$C_{75}H_{100}N_{14}O_{15}S_3$	69	213–217	– 24.2	0.68	0.58	0.69	–
II	[Ile², Arg⁴] AVT	$C_{77}H_{105}N_{17}O_{16}S_4 \times 2H_2O$	60	200–204	– 29.0	0.53	0.52	0.73	–
III	[Phe², Arg⁴] LVT	$C_{80}H_{103}N_{15}O_{16}S_4 \times 1H_2O$	10	213–218	– 29.5	0.62	0.69	0.78	–
IV	[Arg⁴] AVT	$C_{87}H_{109}N_{17}O_{17}S_4$	55	197–200	– 31.6	0.55	0.54	0.71	–
V	[Ile²] AVT	$C_{69}H_{95}N_{15}O_{15}S_3$	65	247–249	– 38.8	–	0.47	0.66	0.85
VI	[Leu²] AVT	$C_{69}H_{95}N_{15}O_{15}S_3$	58	239–242	– 36.4	–	0.44	0.68	0.62
VII	[Phe², Arg⁴] AVT	$C_{80}H_{103}N_{17}O_{16}S_4$	43	187–191	– 32.0	0.55	0.52	0.70	–
VIII	[Arg⁴] LVT	$C_{87}H_{109}N_{15}O_{17}S_4 \times 1H_2O$	29	198–203	– 31.0	0.66	0.68	0.77	–
IX	[Ile², Arg⁴] LVT	$C_{77}H_{105}N_{15}O_{16}S_4$	29	206–211	– 30.8	0.67	0.77	0.79	–

[a] The structures of the protected precursors are as follows: Z-Cys(Bzl)-Leu-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (I); Z-Cys(Bzl)-Ile-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (II); Z-Cys(Bzl)-Phe-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2, (III); Z-Cys(Bzl)-Tyr(Bzl)-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (IV); Z-Cys(Bzl)-Ile-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (V); Z-Cys(Bzl)-Leu-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (VI); Z-Cys(Bzl)-Phe-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, (VII); Z-Cys(Bzl)-Tyr(Bzl)-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2, (VIII); Z-Cys(Bzl)-Ile-Ile-Arg(Tos)-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (IX). [b] Yields were calculated on the basis of the glycine content of the starting resin; TLC, A, butan-1-ol – actic acid – water (4:1:1, v/v/v); B, butan-1-ol – acetic acid – water (4:1:5, v/v/v, upper phase); C, butan-1-ol – acetic acid – water – pyridine (15:3:3:10, v/v/v/v); D, butan-1-ol – acetic acid – water (2:1:1, v/v/v).

Table 4. Physiochemical properties of the free peptides (1–9)

No.	Peptide	Yield (%)[a,b]	$[\alpha]_D^{25}$, deg. (c = 0.1, 50 % AcOH)	TLC, R_f A	B	C	D
1	[Leu², Thr⁴] AVT	37	+ 7.0	0.12	0.21	0.32	–
2	[Ile², Arg⁴] AVT	35	– 29.0	0.13 (F)	–	0.11	0.17
3	[Phe², Arg⁴] LVT	29[c]	– 5.0	0.12 (F)	0.08	0.09	0.11
4	[Arg⁴] AVT	51	– 12.0	0.12 (F)	0.04	0.11	0.15
5	[Ile²] AVT	39	+ 9.0	–	0.07	0.17	0.27
6	[Leu²] AVT	41	+ 3.0	–	0.05	0.22	0.42
7	[Phe², Arg⁴] AVT	28	– 12.0	–	–	0.12	0.17
8	[Arg⁴] LVT	36	– 3.0	0.08 (E)	0.05	0.09	0.10
9	[Ile², Arg⁴] LVT	27	– 28.0	–	0.06	0.13	0.10

[a] Yields are based on the amount of protected peptide used in the reduction – reoxidation step in each case; [b] all the free peptides gave the expected amino acid analysis ratios after hydrolysis ± 3 %; [c] peptide 2 was purified by HPLC. HPLC analysis showed 99–96 % purity of all free peptides; TLC, A, B, C and D as in table 3, E, butan-1-ol – acetic acid – water (1:1:1, v/v/v); F, butan-1-ol – acetic acid – water – ethyl acetate (1:1:1:1, v/v/v/v).

Arg^4 in Lys-conopressin-G (peptide 3) decreased activities: the antidiuretic and vasopressor potencies were 3.7 % and 2.2 % that of LVT. Practically speaking, there was no difference in the V_2/V_1 selectivity of this peptide as compared with LVT.

The pharmacological properties of the new AVT and LVT analogues are given in table 2. Replacement of Gln^4 by Arg^4 in AVT and LVT has reduced both pressor and antidiuretic activities, i.e. about 50 % – 25 % that of AVT and LVT (Peptides 4 and 8). Arg^4 modification in the AVT molecule increased the antidiuretic/pressor selectivity almost twofold (V_2/V_1 ratios: 2.4 and 1.3, respectively), (Peptide 4), but did not change the V_2/V_1 selectivity of peptide 8 as compared with LVT. Substitution of Phe^2 for Tyr^2 in AVT and LVT produced a significant reduction of the pressor and antidiuretic potencies in both compounds and was reflected in lower V_2/V_1 selectivities (data from ref. 26). It is worth noting that replacement of Tyr^2 by Ile^2 or Leu^2 in the AVT molecule resulted in substantial gain in V_2/V_1 ratios: from 1.3 (AVT) to 5 ([Ile^2] AVT) and 17.6 ([Leu^2] AVT). The combination of Ile^2 and Arg^4 modifications in AVT and LVT (peptides 2 and 9) clearly showed the importance of Ile^2 substitution for the increase of V_2/V_1 selectivities in analogues obtained, i.e. $V_2/V_1 = 14$ ([Ile^2, Arg^4] AVT) and $V_2/V_1 = 2.4$ ([Arg^4] AVT), also 0.9 ([Ile^2 Arg^4] LVT) and 0.2 ([Art^4] LVT). Again, the combination of Phe^2 and Arg^4 substitutions in AVT and LVT (peptides 7 and 3) has proved that Phe^2 replacement in these analogues has very little influence on antidiuretic/pressor selectivities, i.e. $V_2/V_1 = 2.8$ ([Phe^2, Arg^4] AVT and $V_2/V_1 = 2.4$ ([Arg^4] AVT), also 0.3 ([Phe^2, Arg^4] LVT) and 0.2 ([Arg^4] LVT).

Comparing the selectivities of [Ile^2] AVT ($V_2/V_1 = 5$), [Leu^2] AVT ($V_2/V_1 = 17.6$) with that of AVT ($V_2/V_1 = 1.3$), also [Leu^2, Thr^4] AVT ($V_2/V_1 = 430$) with that of [Thr^4] AVT ($V_2/V_1 = 2.7$) suggests that the combination of Leu^2 and Arg^4 substitutions in the AVT and LVT molecules (possibly also Leu^2 and Thr^4 in LVT) might give analogues with dramatically increased antidiuretic/pressor selectivities.

Acknowledgement. This work was supported by research grants from the National Institute of General Medical Sciences (No. GM-25280) and the National Institute of Diabetes and Digestive and Kidney Diseases (No. DK-01940).

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2 Abbreviations. The symbols for the amino acids and peptides are in accordance with the Recommendations 1983 of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Eur. J. Biochem. *138* (1984) 9). Other abbreviations used: AVT, 8-arginine vasotocin; LVT, 8-lysine vasotocin; Z, benzyloxycarbonyl; Bzl, benzyl; Tos, tosyl; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography.
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0014-4754/90/070771-03$1.50 + 0.20/0