SYNTHESIS AND BIOLOGICAL ACTIVITY OF FOUR VASOPRESSIN ANALOGS MODIFIED IN POSITION 3 WITH β-THIENYLALANINE

Jirina SLANINOVA[a], Malgorzata CZAJA[b] and Bernard LAMMEK[b]

[a] *Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 166 10 Prague 6, The Czech Republic*

[b] *Department of Chemistry, University of Gdansk, 80-952 Gdansk, Poland*

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Four new analogs of arginine-vasopressin substituted in position 3 with β-thienylalanine were synthesized on chloromethylated resin using Boc strategy and DCC or DCC-HOBt to form peptide bonds. The activity of the agonists is not much different in comparison to the unsubstituted compounds. However, the potency of one of the antagonists is strongly reduced.

Vasopressin has been a subject of intensive investigation for more than 40 years. During this period a great number of analogs of this hormone modified in various positions was prepared and assayed. However, despite this fact, the role of phenylalanine in arginine vasopressin (AVP)* molecule is still not well explored and the list of AVP analogs modified in position 3 is rather short. Several analogs containing in this position amino acids such as D-Phe, Phe(NH_2), Tyr, Phe(4-Cl), Trp and Phe(4-N_3) were synthesized and assayed (refs[2,3]). From the results obtained so far it is evident that a minimum degree of aromatic character in the side chain in position 3 and an optimal size of this part of the molecule are essential for the interaction with the receptor (ref.[2]). It was also found that the replacement of the Phe^3 residue with Thi^3 in [8-lysine]vasopressin resulted in an analog with the same pressor activity as that of the parent peptide and with the other activities even enhanced (ref.[4]). With all this in mind we have decided to study the effect of the substitution of β-thienylalanine for phenylalanine in position 3 of AVP and its agonistic and antagonistic analogs.

* Unless stated otherwise, all chiral amino acids belong to the L-series. The nomenclature and symbols of the amino acids, their derivatives and peptides obey the published IUPAC recommendations[1]. Mpa, 1-mercaptopropionic acid; Cpp, 1-mercaptocyclohexaneacetic acid; Thi, β-thienylalanine; dAVP [Mpa^1,Arg^8]vasopressin.

Four analogs were synthesized to the above rationale, i.e. [3-(β-thienylalanine),8-arginine]vasopressin ([Thi^3]AVP, *I*), [1-(3-mercaptopropionic acid),3-(β-thienylalanine),8-arginine]vasopressin ([Thi^3]dAVP, *II*), [1-(1-mercaptocyclohexaneacetic acid),3-(β-thienylalanine),8-arginine]vasopressin ([Cpp^1,Thi^3]AVP, *III*), [1-(1-mercaptocyclohexaneacetic acid),2-(*O*-ethyl-D-tyrosine),3-(β-thienylalanine),4-valine,8-arginine]vasopressin ([Cpp^1, D-Tyr (OEt)^2,Thi^3,Val^4]AVP, *IV*).

The synthesized analogs have the following general structure:

X-Y-Thi-W-Asn-Cys-Pro-Arg-GlyNH_2

	X	Y	W
I	Cys	Tyr	Gln
II	Mpa	Tyr	Gln
III	Cpp	Tyr	Gln
IV	Cpp	D-Tyr(OEt)	Val

Pharmacological properties of the new analogs were determined in uterotonic in vitro, pressor and antidiuretic tests.

EXPERIMENTAL

Capillary melting points were determined and are uncorrected. Samples for elemental analysis were dried over phosphorus pentoxide for 12 or more hours at room temperature. The elemental analyses were determined on a Carlo–Erba Model 1106 analyzer.

N,*N*-Dimethylformamide (DMF) was redistilled in vacuum and stored at 4 °C over 4A molecular sieves in darkness. Other solvents and reagents were of analytical grade. Thin layer chromatography was carried out on silica plates (Merck) using the following solvent systems: A, 1-butanol–acetic acid–water (4 : 1 : 5, v/v, upper phase); B, chloroform–methanol (5 : 1, v/v); C, 1-butanol–acetic acid–water–pyridine (15 : 3 : 3 : 10). The detection was performed by ninhydrin or iodine. For quantitative amino acid analysis the peptides (0.5 mg) were hydrolyzed with 6 M HCl containing phenol (20 μl) in evacuated sealed ampoules for 18 h at 100 °C. The analyses were performed on Beckman Model 121 analyzer. The optical rotations were measured at the Na yellow line (586 nm) using a Perkin–Elmer Model 141 polarimeter with an accuracy of 0.01°. High performance liquid chromatography was carried out on a Beckman Model 338 chromatograph with an RF C_{18}, 5 μm column (ODS 4.5 × 150 mm, Ultrasphere plus 4.6 × 4.5 mm precolumn). Solvent systems: S1, 0.1% trifluoroacetic acid; S2, 80% acetonitrile in S1; linear gradient from 30 to 80% of S2 for 15 min, flow rate 1.5 ml/min, detection at 226 nm. An isocratic system was also applied to check the purity. Acetonitrile was of HPLC grade.

Synthesis of the Peptides

The protected peptide precursors required for the synthesis of analogs *I – IV* were prepared by the Merrifield method entirely on the resin[5]. First, chloromethylated resin used in the synthesis (Bio-Rad,

Bio-Beads) was esterified with Boc-Gly to the level of 0.45 mmol/g by the method of Gisin[6]. Then, Z-Cys(Bzl)-Tyr(Bzl)-Thi-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*Ia*), Mpa(Bzl)-Tyr(Bzl)-Thi-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IIa*), Cpp(Bzl)-Tyr(Bzl)-Thi-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IIIa*), and Cpp(Bzl)-D-Tyr(Et)-Thi-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IVa*) were prepared using solid phase methodology as previously described[7–9]. The coupling reactions were performed by the use of DCC/HOBt. All reagents were used in 3 molar excess and coupling was monitored by the ninhydrin test[10]. For coupling of Boc-Asn and Boc-Gln, *p*-nitrophenyl esters with addition of *N*-hydroxybenzotriazole were employed. The protected peptides were cleaved from the resin by ammonolysis[11] to give four crude protected amides (*Ib – IVb*). The physico-chemical properties of the compounds (*Ib – IVb*) are summarized in Table I.

The solutions of the peptide intermediates in sodium dried redistilled ammonia was treated at the boiling point with stirring with sodium from a stick of the metal in a small bore glass tube until a light-blue color persisted in the solution for 30 s. Ammonium chloride was added to discharge the color. The solution was evaporated, the residue dissolved in glacial acetic acid (150 ml) and diluted with methanol (1 000 ml). An excess of iodine solution in methanol (0.1 mol/l) was added gradually with stirring. The light yellow solution was stirred for an additional 2 min and then for 10 min with anion exchange resin (Amberlite IR-45, acetate form, 10 g wet weight). The reaction mixture was filtered through a bed of resin (10 g wet weight) and the bed was washed twice with methanol. The combined filtrate and washings were evaporated under reduced pressure. The crude peptides were desalted by gel filtration on Sephadex G-15 (120 × 2.9 cm, 50% AcOH) with a flow rate 6.5 ml/h and further purified on Sephadex LH-20 (120 × 1.4 cm, 30% AcOH) with a flow rate 4.5 ml/h. The peptide was eluted as a single peak. The purity and identity of each peptide was ascertained by thin-

TABLE I
Physico-chemical data of protected intermediates

Compound	Formula M.w.	R_F A B	M.p., °C $[\alpha]_D^{20}$, ° (*c* 0.4, DMF)	Calculated/Found		
				% C	% H	% N
Ib	$C_{77}H_{97}N_{15}O_{16}S_4$	0.50	192 – 195	57.19	6.06	12.99
	1 617.0	0.41	–33.8	57.37	6.04	12.95
IIb	$C_{72}H_{89}N_{14}O_{14}S_4$	0.32	180 – 183	57.56	5.98	13.05
	1 502.4	0.30	–27.6	57.75	5.97	13.10
IIIb	$C_{77}H_{97}N_{14}O_{14}S_4$	0.45	160 – 164	58.89	6.24	12.48
	1 570.5	0.28	–35.1	58.77	6.27	12.53
IVb	$C_{72}H_{95}N_{13}O_{13}S_4$	0.58	174 – 177	58.49	6.49	12.32
	1 478.5	0.53	–12.7	58.72	6.51	12.30

layer chromatography in two different solvent systems and by amino acid analysis. The purity was usually about 97% (HPLC). The physico- chemical properties of the four peptides (*I* – *IV*) prepared in this way are given in Table II.

Pharmacological Methods

Oxytocic activity was estimated in the test in vitro using rat uteri from oestrogen treated rats in media without magnesium[12–14]. The pA_2 values were determined as described[14]. The pressor activity was tested on pithed male rats against standard synthetic AVP (ref.[15]). The pA_2 values were calculated as a negative logarithm of the so called "effective concentration"[14]. The anti-antidiuretic activity was tested on conscious rats as described[16] as an ability of an analog to antagonize endogenous AVP and enhance the urine volume on 6 ml/1.5 h.

RESULTS AND DISCUSSION

Biological activities of the new analogs and of some reference compounds are given in Table III. As can be seen from the Table, in the case of agonists the structural change studied does not influence dramatically the biological activities. In comparison to LVP, in the case of AVP, both oxytocic and pressor activities are slightly decreased. However, different situation can be seen in the case of antagonists. The [Cpp[1]]AVP is de-

TABLE II
Physico-chemical characteristics of protected intermediates

Compound	R_F A C	$[\alpha]_D^{21}$, ° (*c* 0.5, 1 M AcOH)	RT^a min	Amino acid analysis				
				Cys Gly	Tyr Pro	Gln Thi	Val Asn	Arg
I	0.26	+71.3	13.47	1.73	0.97	0.88	–	0.91
	0.42			1.00	1.30	0.83	1.13	
II	0.25	+155.8	11.03	0.87	1.05	1.23	–	0.97
	0.39			1.00	0.88	1.20	1.03	
III	0.23	+196.9	11.18	0.83	0.98	0.82	–	1.15
	0.44			1.00	1.05	0.91	0.97	
IV	0.28	+123.3	13.27	0.85	0.89	–	1.08	1.25
	0.59			1.00	1.09	0.98	0.85	

[a] HPLC retention time. For conditions see Experimental.

scribed in the literature to be very strong antagonist of both oxytocic and pressor activities and the replacement of Phe[3] for Thi[3] resulted in complete loss of this potencies. In the case of another antagonist substituted in several positions[2–4], there is only slight decrease of the antipressor activity and the compound is strong antioxytocic agent. The new peptide remains strong antagonist of the antidiuretic activity of AVP, too. This diverse effect of Thi[3] substitution in the case of the two model antagonists is difficult to explain. The binding site of an antagonist is probably less disturbed in the case of more complicated analog where the antagonism is due to 2 modifications, i.e. Cpp[1] and D-Tyr(Et) changes, than in the case of analogue *III* which has only one modification. Thus, our findings confirm our previous results where we have used thienylalanine to replace tyrosine in position 2 (ref.[17]). Also in this case the replacement canceled the uterotonic and pressor antagonism in the case of CppAVP and strongly decreased the antagonism if a second change – Val[4] was present. One could speculate about the existence of different binding sites for different types of antagonists. Unfortunately too small number of analogs was studied to make definite conclusion.

TABLE III
Biological activities (rat) of the new vasopressin analogs and some reference compounds

Compound	Activity		
	Uterus in vitro[a]	Pressor[b]	Antidiuretic
LVP	4.8 IU/mg	260 IU/mg	260 IU/mg
[Thi[3]]LVP	19.0 IU/mg	243 IU/mg	332 IU/mg
AVP	17.0 IU/mg	412 IU/mg	465 IU/mg
[Thi[3]]AVP	7.6 IU/mg[e]	380 IU/mg[e]	n.d.[d]
dAVP	27 – 63 IU/mg	360 IU/mg	1 300 IU/mg
[Thi[3]]dAVP	16.7 IU/mg[e]	200 IU/mg[e]	n.d.[d]
[Cpp[1]]AVP	pA_2 = 8.15	pA_2 = 8.35	0.033 IU/mg
[Cpp[1],Thi[3]]AVP	0.2 IU/mg[e]	0[c,e]	0[e]
[Cpp[1],D-TyrEt[2],Val[4]]AVP	n.d.[d]	pA_2 = 8.22	pA_2 = 7.81
[Cpp[1],D-TyrEt[2],Thi[3],Val[4]]AVP	pA_2 = 8.3[e]	pA_2 = 7.6[e]	antagonist[f]

[a] Inhibitory activity in the uterus in vitro test in Mg^{2+} free medium expressed as negative decadic logarithm of inhibitor concentration reducing 2 times the half maximal effect of oxytocin. [b] Inhibitory activity in the pressor test on pithed rat, expressed as negative decadic logarithm of the so-called effective concentration reducing the effect of the dose of $1 . 10^{-5}$ mg AVP to the level of $5 . 10^{-6}$ mg AVP, for details see ref.[14]. [c] 0 means inactive up to the dose of $2 . 10^{-2}$ mg per rat. [d] Not determined. [e] This paper. [f] In conscious rat, the dose 5 μg per rat enhances the urine flow from 1.5 ml/1.5 h to 6 ml/1.5 h.

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