SYNTHESIS AND SOME PHARMACOLOGICAL PROPERTIES OF SIX NEW ANALOGS OF ARGININE-VASOPRESSIN SUBSTITUTED IN POSITION 2 WITH β-THIENYLALANINE OR PROLONGED ON THE N-TERMINUS WITH 1-ADAMANTANEACETIC ACID

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Six new analogs of arginine-vasopressin, four of them substituted in position 2 with β-thienylalanine, and two prolonged on the N-terminus by acylation with 1-adamantaneacetic acid, were synthesized on chlormethylated resin using Boc strategy and DCC or DCC–HOBt to form peptide bond. The activity of the analogs was fairly low, however, one of the peptides, namely [Cpp[1], Thi[2], Val[4]]AVP, showed selectivity in antiuterotonic, antipressor and anti-antidiuretic effects.

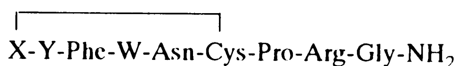
The characterization of oxytocin (OT*) and arginine-vasopressin (AVP) in the early 1950's stimulated not only the fields of synthetic peptide chemistry and peptide endocrinology in general, but also the investigation of structure activity relationships of neurohypophyseal hormones, and in particular the search for structural analogs of these hormones with more specific actions. These studies have lead to synthesis and pharmacological evaluation of many potent and highly selective V_1-antagonists of AVP, but all of these exhibit varying degrees of oxytocin antagonism. In the past decade also many potent V_2-antagonists of AVP have been obtained, but until now, although much progress has been made to improve anti-V_1/V_2 selectivity, the truly selective V_2-antagonists are not available.

* 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, 3-mercaptopropionic acid; Cpp, 1-mercaptocyclohexaneacetic acid; Thi, β-thienylalanine; Aaa, 1-adamantaneacetic acid; dAVP [Mpa[1], Arg[8]]vasopressin.

In the design of AVP analogs the substitutions of Tyr^2 residue alone or in combination with other modifications have been shown in many cases to produce compounds with interesting pharmacological properties[2,3]. However, it is still not clear what quality of the side chain – bulkiness, electronegativity, rigidity etc. – is the most important. In our previous studies we determined how substitutions of L-4-fluoro-phenylalanine (4-F-Phe), L-4-chloro-phenylalanine (4-Cl-Phe) and D-phenylglycine (D-Gly(Ph)) for tyrosine in position 2 affected the activity of some of the agonistic and antagonistic analogs[4,5]. Although these studies did not result in peptides with high activity, we showed that the substitutions may be of value in the future design of selective AVP analogs with agonistic or antagonistic activity. As a continuation of this effort we report on the substitution of β-thienyl-L-alanine for tyrosine at position 2 in the same series of agonistic and antagonistic analogs of AVP as in previous studies. This amino acid has in the side chain a 5-membered aromatic heterocycle with sulfur atom. Thienylalanine is less hydrophilic and polar than tyrosine. Four analogs were synthesized to the above rationale, i.e. [2-(β-thienylalanine),8-arginine]vasopressin ([Thi^2]AVP, *I*), [1-(3-mercaptopropionic acid), 2-(β-thienylalanine), 8-arginine]vasopressin ([Thi^2]-dAVP, *II*), [1-(1-mercaptocyclohexaneacetic acid), 2-(β-thienylalanine), 8-arginine]vasopressin ([Cpp^1,Thi^2]AVP, *III*), [1-(1-mercaptocyclohexaneacetic acid), 2-(β-thienylalanine), 4-valine, 8-arginine]vasopressin ([Cpp^1, Thi^2, Val^4]AVP, *IV*).

Knowing that introduction of 1-adamantaneacetic acid (Aaa) residue into position 1 of linear analogs of AVP results in potent antagonists of this hormone, we found interesting to check how acylation with bulky and lipophilic substituent of N-terminal cysteine residue of AVP or [Tyr(Me)^2]AVP molecules will influence their pharmacological properties. Two analogs designed in such manner were: [*O*-(1-adamantaneacetic acid), 8-arginine]vasopressin (Aaa-AVP, *V*) and [*O*-(1-adamantaneacetic acid), 2(*O*-methyl)tyrosine, 8-arginine]vasopressin (Aaa-[Tyr(Me)^2]AVP, *VI*).

The synthesized analogs *I* – *VI* have the following general structure:



I	X = Cys;	Y = Thi;	W = Gln;	*IV*	X = Cpp;	Y = Thi;	W = Val;
II	X = Mpa;	Y = Thi;	W = Gln;	*V*	X = Aaa-Cys;	Y = Tyr;	W = Gln;
III	X = Cpp;	Y = Thi;	W = Gln;	*VI*	X = Aaa-Cys;	Y = Tyr(Me);	W = Gln;

The pharmacological properties of the new analogs were determined in uterotonic (in vitro), pressor and antidiuretic tests. In the case of compounds *III* and *IV*, for anti-antidiuretic activity as well. The results obtained together with pharmacological properties of some analogs previously published by us[4] or others[5] are presented in Table I. The

introduction of Thi into position 2 of AVP or dAVP, resulted in compounds *I* and *II* which possessed about 9% and 0.2% of the pressor activity of AVP, respectively. This decrease in activity is much higher than in the case of previously by us[4,5] described modifications (4-Cl-Phe and 4-F-Phe in position 2). The decrease in antidiuretic activity is not that profound, in the case of analog *I* negligible, in the case of analogue *II* one order of magnitude. However both new analogs did not show any uterotonic activity which makes them selective agonists towards vasopressin receptors. It is worth to point out, that both corresponding [4-Cl-Phe]^2 and [4-F-Phe]^2 analogs exhibit low antiuterotonic activity[4,5]. As far as peptide *III* is concerned, it is clear that combination of Thi^2 and Cpp^1 substitutions is incompatible with biological activity. However, additional change, i.e. introduction of Val residue into position 4 of analog *III*, results in compound *IV* which did not posses any pressor activity, but exhibits low anti-antidiuretic potency as well as low degree of uterotonic inhibition.

TABLE I
Biological activities (rat, I.U./mg or pA_2) of the new vasopressin analogs and some reference compounds

Compound	Biological activity			
	uterotonic[a]	pressor	antidiuretic	anti-antidiuretic[b]
[Tyr(Me)^2]AVP	pA_2 = 7.44	9.7	386	
Aaa-[Tyr(Me)^2]AVP (*VI*)	0	0[c,d]	<0.1[e]	
AVP	17	450	465 or 100[e]	
[Thi^2]AVP (*I*)	0	39.8	40 – 100[e]	
Aaa-AVP (*V*)	0	0[c]	<0.1[e]	
dAVP	27 – 63	346 – 370	1 300 – 1 745	
[Thi^2]dAVP (*II*)	0	0.95	~10[e]	
[Cpp^1]AVP	pA_2 = 8.15	pA_2 = 8.35	0.033	
[Cpp^1,Thi^2]AVP (*III*)	a i i[f]	0	n.d.[g]	0
[Cpp^1,Val^4]AVP	pA_2 = 7.34	pA_2 = 7.97	0.32	
[Cpp^1,Thi^2,Val^4]AVP (*IV*)	pA_2 = 6.5	0	n.d.[g]	0.3

[a] In vitro. [b] Dose (mg/kg) that enhances the urine volume to 6 ml/1.5 h. [c] In some tests high doses decrease slightly the basal blood pressure, however, they do not decrease the effect of exogenous AVP. [d] Low degree of inhibition 30 min after administration (pA_2 = 6.35). [e] % of AVP. [f] Low intrinsic activity (~1 I.U./mg) causes a permanent contraction; the uterus is later insensitive to oxytocin. [g] Not determined.

As regards pharmacological properties of analogs *V* and *VI*, it is clear that extension of the peptide chain by acylation of aminogroup in position 1 with adamantaneacetic acid residue cancels all the activities.

From the results obtained it is evident that Thi^2 substitution is not advantageous in the design of potent analogs of AVP. On the other hand the gradual decrease in activities in the order uterotonic > pressor > antidiuretic (in the case of peptide *III*) and the quality change from agonism to antagonism (analog *IV* which exhibits low anti-antidiuretic and low antiuterotonic properties and at the same time any pressor activity) may be of a value in the design of selective anti-V_2 antagonists, however at the present stage it is too early for such a conclusion.

EXPERIMENTAL

N,N-Dimethylformamide (DMF) was redistilled in vacuum and stored at 4 °C over 4 Å molecular sieves in darkness. Other solvents and reagents were of analytical grade. Thin layer chromatography was carried out on silica plates (Merck), and the detection was made by ninhydrin or iodine. For TLC the following solvent systems (v/v) were used: A, 1-butanol–acetic acid–water (4 : 1 : 5, upper phase); B, 1-butanol–acetic acid–water–ethyl acetate (1 : 1 : 1 : 1, v/v); C, chloroform–MeOH (7 : 2); D, 1-butanol–acetic acid–water–pyridine (15 : 3 : 12 : 15); E, 1-butanol–acetic acid–water–pyridine (15 : 3 : 3 : 10). Melting points were determined in capillaries and are uncorrected. For quantitative amino acid analysis the peptides (0.5 mg) were hydrolyzed in constantly boiling hydrochloric acid (400 µl) containing phenol (20 µl) in evacuated sealed ampoules for 16 h at 110 °C. The analyzes were performed on a Mikrotechna AAA 881 analyzer. The optical rotations were measured at the Na yellow line (586 nm) with an accuracy of 0.01° using Hilger–Watts polarimeter. The elemental analyses were determined on a Carlo–Erba Model 1106 analyzer.

Synthesis of the Peptides

The chloromethylated resin used in the synthesis (polystyrene crosslinked with 1% of divinylbenzene, Bio-Rad, Bio-Beads) was esterified with Boc-Gly to the load of 0.5 mmol/g by the method of Gisin[6]. Solid phase peptide synthesis[7,8] was employed to prepare six protected analogs of vasopressin: Z-Cys(Bzl)-Thi-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*Ia*), Mpa(Bzl)-Thi-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*IIa*), Cpp(Bzl)-Thi-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*IIIa*), Cpp(Bzl)-Thi-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*IVa*), Aaa-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*Va*), Aaa-Cys(Bzl)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*VIa*). All coupling reactions were mediated either by DCC or DCC–HOBt, except those involving the carboxyl groups of asparagine and glutamine, which were allowed to react in DMF as their nitrophenyl esters. The completeness of each coupling reaction was monitored by the Kaiser test[9]. At the end of the synthesis the fully protected peptide was removed from the resin as the amide by ammonolysis[10]. Following the solvent evaporation, the products were extracted into hot DMF, precipitated by boiling water and left overnight at room temperature. The peptides were purified by dissolving in DMF and reprecipitating with methanol–diethyl ether (1 : 3). The physicochemical properties of the compounds *Ia* – *VIa* are summarized in Table II. Each protected peptide was deblocked by means of sodium in liquid ammonia, as previously described[11]. The resulting disulfhydryl compounds were oxidatively cyclized with iodine in methanol (0.1 mol/l) to give analogs *I* – *VI*. The crude peptides were desalted by gel filtration on Sephadex G-15 (50% AcOH) and purified on

TABLE II
Physico-chemical data of protected intermediates

Compound	R_F A	R_F B	$[\alpha]^{20}$, ° (*c* 0.5, DMF)	M.p., °C
Ia	0.48	0.66	–34.4	198 – 200
IIa	0.44	0.64	–29.8	199 – 202
IIIa	0.45	0.65	–30.8	182 – 185
IVa	0.34	0.63	–34.3	198 – 200
Va	0.49	0.40 (C)	–28.1	227 – 230
VIa	0.48	0.33 (C)	–32.7	229 – 232

TABLE III
Physico-chemical characteristics of AVP analogs

Compound	R_F A (E)	$[\alpha]^{21}$, ° (*c* 0.5, 1 M AcOH)	Amino acid analysis					
			Phe Pro	Glu Arg	Asp Gly	Cys NH_3	Val Thi	Tyr
[Thi^2]AVP (*I*)	0.12 (0.23)	–64.5	1.05 1.08	1.04 1.05	1.07 1.00	2.06 3.08	– 1.05	–
[Thi^2]dAVP (*II*)	0.14 (0.50)	–151.3	1.02 1.06	1.07 1.05	1.06 1.00	1.04 3.06	– 1.06	–
[Cpp^1,Thi^2]AVP (*III*)	0.25 (0.43)	–108.8	1.06 1.04	1.08 1.05	1.05 1.00	1.08 3.05	– 1.06	–
[Cpp^1,Thi^2,Val^4]AVP (*IV*)	0.33 (0.63)	–68.6	1.08 1.06	– 1.06	1.08 1.00	1.04 2.07	1.07 1.08	–
Aaa-AVP (*V*)	0.31 (0.75[a])	–5.4	1.04 1.02	0.97 1.03	1.03 1.00	2.06 3.06	– –	0.97
Aaa-[Tyr(Me)^2]AVP (*VI*)	0.23 (0.72[a])	–88.2	1.02 1.03	1.03 1.02	1.01 1.00	2.04 3.03	– –	1.04

[a] In solvent D.

Sephadex LH-20 (30% AcOH). The purity and identity of each peptide was ascertained by thin-layer chromatography in two different solvent systems and by amino acid analysis. The physico-chemical properties are given in Table III.

Pharmacological Methods

Anti-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. The pA_2 values were calculated as a negative logarithm of the so called "effective concentration"[14]. The antidiuretic activity was determined in conscious rats according to modified Burn method[15,16]. The anti-antidiuretic activity was tested on conscious rats as described in ref.[17] as an ability of the analog to increase the urine volume on 6 ml per 1.5 h.

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