NEW ANALOGS OF ARGININE-VASOPRESSIN CONTAINING β-HOMO-L-AMINO ACID RESIDUES

Krzysztof BANKOWSKI[a], Alexandra MISICKA[a], Tomislav BARTH[b] and Jiřina SLANINOVÁ[b]

[a] *Peptide Laboratory, Department of Chemistry, Warsaw University, 02—093 Warsaw, Poland and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia*

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Four new analogs of arginine vasopressin containing β-homo-L-amino acid residue were synthesized by the solid-phase method. The introduced modifications yielded the following peptides: [β-homoPhe^3]AVP (*I*), [β-homoPro^7]AVP (*II*), [Cpp^1, Tyr(Me)^2, β-homoPhe^3]AVP (*III*), and [Cpp^1, Tyr(Me)^2, β-homoPro^7]AVP (*IV*). Agonistic properties of *I* and *II*, as well as antagonistic properties of *III* a *IV* were decreased, more pronouncedly with analogs substituted in the position 3.

In continuation to our studies on the relationship between chemical structure and biological activity of neurohypophyseal hormones and their analogs, we carried out the synthesis of four new analogs of [8-arginine]vasopressin (AVP). These analogs contain two β-homo-L-amino acid residues*: β-homo-L-phenylalanine (β-homoPhe) and β-homo-L-proline (β-homoPro); β-homoPhe replaces the L-Phe residues in position 3, β-homoPro the L-Pro residue in position 7 of two parent molecules: [8-arginine]vasopressin and [1-β(-mercapto-β,β-cyclopentamethylenepropionic acid), 2-O-methyltyrosine,8-arginine]vasopressin ([Cpp^1, Tyr(Me)^2]AVP). The latter compound is known to be one of the most potent and selective antagonists of vasopressor response to vasopressin[1-3].

The four new analogs are as follows: [3-β-homo-L-phenylalanine, 8-arginine]-vasopressin, [β-homoPhe^3]AVP (*I*), [7-β-homo-L-proline, 8-arginine]vasopressin, [β-homoPro^7]AVP (*II*), [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-O-methyltyrosine, 3-β-homo-L-phenylalanine, 8-arginine]vasopressin, [Cpp^1, Tyr-(Me)^2, β-homoPhe^3]AVP (*III*), and [1-(β-mercapto-β,β-cyclopentamethylenepro-

* All the chiral amino acids are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the recommendations published in Eur. J. Biochem. *138*, 9 (1984). β-homoPhe, 3-amino-4-phenylbutanoic acid; β-homoPro, 2-(2-pyrrolidinyl)acetic acid; Cpp, 3-mercapto-3,3-cyclopentamethylenepropionic acid. All the other symbols are explained in the text.

pionic acid), 2-O-methyltyrosine, 7-β-homo-L-proline, 8-arginine]vasopressin, [Cpp[1], Tyr(Me)[2], β-homoPro[7]]AVP (*IV*).

Replacement of the α-amino acid residue in a peptide chain by the corresponding β-homo-L-amino acid residue enlarges the peptide backbone by one methylene group, maintaining the same configuration at the asymmetric centre and the same structure of all side chains. Thus, analogs with β-homoPhe in position 3 (*I* and *III*) have now 21-member disulfide containing ring and intact tripeptide tail, whereas analogs with β-homoPro in position 7 (*II* and *IV*) are identical to parent compounds in the ring part of the molecule, but have the tripeptide tail shifted over one methylene group toward the carboxy terminus.

Intrigued by the question how these modifications would influence both agonistic and antagonistic properties, we have synthesized compounds *I*–*IV* and determined some of their biological potencies.

EXPERIMENTAL

The protected intermediates *V*–*VIII* of analogs *I*–*IV* were synthesized by the solid-phase method by means of a previously described procedure[4]. Chloromethylated resin (Chemalog, 1% crosslinked S-DVB) was esterified with Boc-glycine to a degree of incorporation of 0·8 mmol/g by the cesium salt method[5]. Boc-β-homo-L-phenylalanine and Boc-β-homo-L-proline were obtained by homologation of the corresponding Boc-amino acids according to the Arndt–Eistert scheme[6]. The peptide chain was elongated using appropriate Boc-amino acids and dicyclohexylcarbodiimide (DCC). Boc-Asn, Boc-Gln and Cpp(Bzl) residues were coupled as *p*-nitrophenyl esters in the presence of N-hydroxybenzotriazole as a catalyst. The N-terminal S-benzylcysteine was introduced as the N-benzyloxycarbonyl derivative. The protected peptides were liberated from the resin as amides by ammonolysis in methanol[7]. All of the protected precursors were purified by the same general method: extraction with hot DMF followed by precipitation with H_2O and precipitation with methanol–ether. The physicochemical properties of the protected peptides are given in Table I. Melting points were determined on a Boetius block and are uncorrected. Optical rotations were measured with Perkin–Elmer 241 polarimeter. Thin-layer chromatography (TLC) was performed on silica gel and following solvent systems were used: A, 1-butanol–acetic acid–water (4 : 1 : 5, upper phase); B, 1-butanol–acetic acid–water (4 : 1 : 1); C, chloroform–methanol (7 : 3); D, 1-butanol–acetic acid–water–pyridine (15 : 3 : 3 : 10). Amino acid analyses were performed on Beckman amino acid analyser. All peptides (protected and free ones) gave the expected amino acid ratios ±3%. All of the new analogs were obtained and purified using the same general procedures: Na in NH_3 was used to deblock each protected precursor as described previously[8], and the resulting disulfhydryl compounds were oxidatively cyclized with $K_3Fe(CN)_6$ (ref.[9]). The free peptides were desalted and purified by gel filtration on Sephadex G-15 (ref.[10]). The physicochemical properties of the new analogs are summarized in Table II.

Bioassay methods: Agonistic and antagonistic potencies of the new analogs were determined in the pressor test on pithed rat, in the uterotonic test in vitro and in the galactogogic test in vivo on ethanol-anaesthetized rats by standard methods described in ref.[11].

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TABLE I

Physicochemical properties of the protected peptides

Peptide	Yield, % M.p., °C	Formula (M.w.)	Calculated/Found % C	% H	% N	$[\alpha]_D^{25}$ $c = 1$, DMF	R_F A	B	C
Z-Cys(Bzl)-Tyr(Bzl)-β-homoPhe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	72·5 216—219	$C_{83}H_{99}N_{15}O_{16}S_3$ .2 H_2O (1 695)	58·81 58·77	5·88 5·62	12·39 11·97	−32·2°	0·64	0·69	0·36
Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-β-homoPro-Arg(Tos)-Gly-NH_2	63·0 202—205	$C_{83}H_{99}N_{15}O_{16}S_3$ .H_2O (1 677)	59·44 59·20	5·95 6·11	12·53 12·88	−27·6°	0·58	0·70	0·45
Cpp(Bzl)-Tyr(Me)-β-homoPhe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2	64·7 194—197	$C_{74}H_{96}N_{14}O_{14}S_3$ .3 H_2O (1 556)	57·12 56·97	6·22 6·68	12·60 12·65	−28·0°	0·65	0·68	0·43
Cpp(Bzl)-Tyr(Me)-Phe-Gln-Asn-Cys(Bzl)-β-homoPro-Arg(Tos)-Gly-NH_2	59·1 173—175	$C_{74}H_{96}N_{14}O_{14}S_3$ .H_2O (1 520)	58·48 58·31	6·37 6·53	12·90 12·45	−51·9°	0·60	0·59	0·38

TABLE II
Physicochemical properties of new analogs

No.	Peptide Yield, %[a]	Formula (M.w.)	Calculated/Found % C	% N	% H	$[\alpha]_D^{25}$ $c = 0{\cdot}3$ 1M AcOH	R_F A	B	C
I	[β-homoPhe^3]AVP	$C_{47}H_{67}N_{15}O_{12}S_2$	49·28	5·99	17·60				
	23·2	.2 $H_2O.C_2H_4O_2$	49·60	6·11	17·60	−50·0°	0·06	0·04	0·23
		(1 194)							
II	[β-homoPro^7]AVP	$C_{47}H_{67}N_{15}O_{12}S_2$	48·99	5·86	18·23				
	46·9	.3 H_2O	48·59	6·02	18·54	+6·3°	0·06	0·04	0·24
		(1 152)							
III	[Cpp^1, Tyr(Me)^2, β-homoPhe^3]AVP	$C_{53}H_{77}N_{15}O_{12}S_2$	52·49	6·49	16·69				
	34·0	.$H_2O.C_2H_4O_2$	52·31	6·47	16·84	−69·3°	0·40	0·45	0·60
		(1 258)							
IV	[Cpp^1, Tyr(Me)^2, β-homoPro^7]AVP	$C_{53}H_{77}N_{15}O_{12}S_2$	51·03	6·31	16·23				
	48·6	.3 $H_2O.C_2H_4O_2$	50·95	6·24	16·59	−23·3°	0·14	0·17	0·38
		(1 294)							

Yields are based on the amounts of protected peptide used in the reduction-reoxidation step in each case.

RESULTS AND DISCUSSION

Four new analogs *I*–*IV* of [8-arginine]vasopressin were synthesized by solid-phase methodology, purified by gel filtration on Sephadex G-15, and assayed for pressor, uterotonic and galactogogic activity (see Experimental). Biological activities are given in Table III.

Incorporation of β-homo-L-amino acid into AVP molecule decreased distinctly the pressor activity. Increase in the ring size to the 21-member ring ([β-homoPhe^3]-AVP) causes much higher decrease of this potency than the prolongation of the tripeptide tail. This is in full agreement with the observation that 20-member ring is critical for obtaining a potent agonist in the pressor assay[3,12]. On the other hand, [β-homoPro^7]AVP is quite a potent pressor peptide (61·1 IU/mg) and it is, in fact, one of the most potent analogs among 7-substituted vasopressins (except [7-(3,4-dehydroproline)]AVP[3,12]). It is interesting to compare relatively high pressor activity of [β-homoPro^7]AVP with the finding that [β-Ala^9,Lys^8]vasopressin appeared to be a weak inhibitor of the pressor response[13]. As it is seen, the same modification (β-alanine may be called β-homoglycine) at the end of the C-terminal tripeptide tail led to drastic changes in pressor properties. It is also known, that deletion of the C-terminal glycinamide from AVP resulted in a complete loss of the pressor activity[14,15]. These findings strongly support the hypothesis that $GlyNH_2$ (ref.[10]) residue is critical for the transduction at the pressor receptor[12].

[8-Arginine]vasopressin exhibits some uterotonic and galactogogic activity. Substitutions of Phe^3 and Pro^7 in AVP by their β-homologs yielded compounds with low potency or without biological potency at all. Again, the change of the ring

TABLE III

Biological activities (I.U./mg) of new arginine-vasopressin analogs

No	Peptide	Pressor test	Uterus in vitro	Galactogogic test	Ref.
	AVP	450	17	77	17
I	[β-homoPhe^3]AVP	5·1	0·0	0·9	*a*
II	[β-homoPro^7]AVP	61·1	0·82	7·0	*a*
	[Cpp^1, Tyr(Me)^2]AVP	$pA_2 = 8{\cdot}62$	$pA_2 = 8{\cdot}13$	—[b]	1
III	[Cpp^1, Tyr(Me)^2, β-homoPhe^3]AVP	$pA_2 = 7{\cdot}00$	$pA_2 = 5{\cdot}95$	—[b]	*a*
IV	[Cpp^1, Tyr(Me)^2, β-homoPro^7]AVP	$pA_2 = 8{\cdot}00$	$pA_2 = 7{\cdot}50$	—[b]	*a*

[a] This paper; [b] not determined.

size exhibits a more pronounced effect on the decrease of activities than the modification of the tail.

[Cpp[1], Tyr(Me)[2]]AVP is one of the most powerful antagonists of the pressor response to vasopressin with its pA_2 value 8·62 (ref.[1]). Moreover, search for antidiuretic antagonists provided even more active antivasopressor analogs[2,3]. It was reported that neurohypophyseal hormone antagonists tolerate removal of the 9-glycine or the 9-glycinamide residue without the loss of antagonistic potency[2,15,16]. Nothing was known about the influence of the change in the ring size in antagonists on their potency. Cpp[1], Tyr(Me)[2], β-homoPro[7]]AVP (pA_2 = 8·00) is found to be a quite potent vasopressor inhibitor. It seems that small modifications of chemical structure in the tripeptide tail do not cause substantial changes in the antagonistic activity. The effect of the increase in the ring size is more pronounced: [Cpp[1], Tyr(Me)[2], β-homo-Phe[3]]AVP retains the antipressor activity with pA_2 drop to 7·00.

All of the antivasopressor analogs exhibit also oxytocic antagonism. Introduction of one methylene group into the peptide ring in [Cpp[1], Tyr(Me)[2]]AVP decreases the antioxytocic activity to a higher degree than the same modification in the tripeptide tail.

It may be concluded that the incorporation of β-homoPhe in the position 3, and β-homoPro in the position 7 in AVP and in the antivasopressor/antioxytocic analog [Cpp[1], Tyr(Me)[2]]AVP yielded peptides with decreased agonistic and antagonistic activities. The modification of the ring part of parent molecules influences biological activities more than the modification of the tripeptide tail.

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