ANALOGS OF ARGININE-VASOPRESSIN SUBSTITUTED IN POSITION 2 WITH L-4-Cl-PHENYLALANINE OR D-PHENYLGLYCINE

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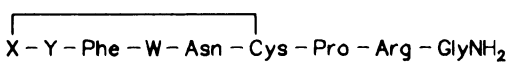
Eight new compounds were designed, synthesized and bioassayed as a part of our studies on the structure–activity relationship of arginine-vasopressin (AVP) analogs. Tyrosine in position 2 of AVP, dAVP, [Cpp[1]]AVP and [Cpp[1],Val[4]]AVP was substituted by L-4-chlorophenylalanine (4-Cl-Phe) or D-phenylglycine [D-Gly(Ph)] and the effect of these changes on agonistic/antagonistic activity in uterotonic and pressor test was followed. All but one of these analogs were found inhibitory in uterotonic test, however in most cases their potency was fairly low. As to the pressor activity the agonistic potency of the 4-Cl-Phe substituted analogs was essentially the same as that having 4-F-Phe in position 2. As far as the potency of antagonists is concerned, 4-Cl-Phe peptides showed significantly higher potency than the 4-F-Phe analogs. All compounds containing D-phenylglycine in position 2 were inactive in the pressor test.

In the design of analogs of [8-L-arginine]vasopressin* (AVP) the modifications and substitutions of Tyr[2] residue alone or in combination with the replacement of the other amino acid residues have been shown to be of particular importance[2,3]. It is known that alkylation of the tyrosine residue at position 2 of the peptide chain of V_1 antagonists of AVP enhances the activity of the analogs in many cases[2,3]. The residue in position 2 is essential for the activity of V_2 antagonists[2,3]. It was also shown that single substitutions of position 2 of AVP may result in compounds with very interesting pharmacological properties[2,3]. In our previous studies we determined how substitutions of L-4-fluorophenylalanine (4-F-Phe) for tyrosine in position 2 affected the activity of some of the agonistic and antagonistic analogs[4]. We replaced Tyr for 4-F-Phe in position 2 of AVP, dAVP, [Cpp[1]]AVP and [Cpp[1],Val[4]]AVP. As far as the pressor activity is concerned the

* 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; dAVP, [Mpa[1],Arg[8]]vasopressin.

substitution studied led, in the case of agonists, to a slight decrease in potency. But in the case of antagonists resulted in dramatic decrease of antipressor potency. We found it interesting that in the case of oxytocic activity, the studied modification converted the character of the compounds from very weak agonism into weak antagonism.

As a continuation of this approach we decided to explore how the substitution of 4-chloro-L-phenylalanine (4-Cl-Phe) or D-phenylglycine (D-Gly(Ph)) for tyrosine at position 2 of agonistic and antagonistic analogs of AVP would affect their pharmacological properties. Eight analogs were synthesized to the above rationale, i.e. [2-(*p*-chlorophenylalanine), 8-arginine]vasopressin ([4-Cl-Phe^2]AVP, *I*), [1-(2-mercaptopropionic acid), 2-(*p*-chlorophenylalanine), 8-arginine]vasopressin ([4-Cl-Phe^2]dAVP, *II*), [1-(1-mercaptocyclohexaneacetic acid), 2-(*p*-chlorophenylalanine), 8-arginine]-vasopressin ([Cpp^1,4-Cl-Phe^2]AVP, *III*) and [1-(1-mercaptocyclohexaneacetic acid), 2-(*p*-chlorophenylalanine), 4-valine, 8-arginine]vasopressin ([Cpp^1,4-Cl-Phe^2,Val^4]AVP, *IV*), [2-D-phenylglycine, 8-arginine]vasopressin ([D-Gly(Ph)^2]AVP, *V*), [1-(2-mercaptopropionic acid), 2-D-phenylglycine, 8-arginine]vasopressin ([D-Gly(Ph)^2]dAVP, *VI*), [1-(1-mercaptocyclohexaneacetic acid), 2-D-phenylglycine, 8-arginine]vasopressin ([Cpp^1, D-Gly(Ph)^2]AVP, *VII*) and [1-(1-mercaptocyclohexaneacetic acid), 2-D-phenylglycine, 4-valine, 8-arginine]vasopressin ([Cpp^1, D-Gly(Ph)^2,Val^4]AVP, *VIII*). The synthesized analogs have the following general structure:



	X	Y	W
I	Cys	4-Cl-Phe	Gln
II	Mpa	4-Cl-Phe	Gln
III	Cpp	4-Cl-Phe	Gln
IV	Cpp	4-Cl-Phe	Val
V	Cys	D-Gly(Ph)	Gln
VI	Mpa	D-Gly(Ph)	Gln
VII	Cpp	D-Gly(Ph)	Gln
VIII	Cpp	D-Gly(Ph)	Val

The pharmacological properties of the new analogs were determined in uterotonic in vitro and pressor tests. The results obtained together with pharmacological properties of analogs previously published by us (ref.[4]) or others (ref.[5]) are presented in Table I. The introduction of 4-Cl-Phe at position 2 of AVP alone or in combination with deami-

nation, resulted in analogs which possessed about 40% and 20% of the pressor activity of AVP, respectively. These values are similar to the corresponding ones of 4-F-Phe^2 substituted peptides. The antiuterotonic potency of both analogs is also similar as compared with the 4-F-Phe compounds. With respect to peptides *III* and *IV*, the effect of combining the 4-Cl-Phe^2 modification and Cpp^1 substitution or Cpp^1 and Val^4 substitutions led to the significant increase of antipressor activity as compared with the corresponding 4-F-Phe^2 modified analogs. The uterotonic activity of peptides *III* and *IV* is also higher than that of 4-F-Phe^2 analogs. All compounds containing D-phenylglycine at position 2 were inactive in the pressor test in doses up to 0.1 mg/kg, however, analogs *V – VII* showed some antiuterotonic activity. Compounds *III*, *IV*, *VII* and *VIII* were tested for the anti-antidiuretic activity, but only compound *IV* showed a low degree of antagonism. It increased the urine volume to 6 ml/1.5 h only at the dose 0.2 mg/kg. In comparison of the potency of compound *IV* to the potency of its D-diaste-

TABLE I
Biological activities (rat) of vasopressin analogs and some reference compounds *I – VIII*

Compound	No.	Biological activity[a]	
		uterotonic in vitro	pressor
AVP		17	450
[4-F-Phe^2]AVP		$pA_2 = 6.7$	193
[4-Cl-Phe^2]AVP	*I*	$pA_2 = 6.5$	141.7
[D-Gly(Ph)^2]AVP	*V*	$pA_2 = 6.0$	0[b]
dAVP		27 – 63	380
[4-F-Phe^2]dAVP		$pA_2 = 6.6$	90.8
[4-Cl-Phe^2]dAVP	*II*	$pA_2 = 6.7$	96.4
[D-Gly(Ph)^2]dAVP	*VI*	$pA_2 = 6.3$	0[b]
[Cpp^1]AVP		$pA_2 = 8.15$	$pA_2 = 8.35$
[Cpp^1,4-F-Phe^2]AVP		$pA_2 = 6.27$	$pA_2 = 6.30$
[Cpp^1,4-Cl-Phe^2]AVP	*III*	$pA_2 = 6.7$	$pA_2 = 7.69$
[Cpp^1,D-Gly(Ph)^2]AVP	*VII*	$pA_2 = 6.3$	0[b]
[Cpp^1,Val^4]AVP		$pA_2 = 7.34$	$pA_2 = 7.97$
[Cpp^1,4-F-Phe^2,Val^4]AVP		$pA_2 = 7.4$	$pA_2 = 6.40$
[Cpp^1,4-Cl-Phe^2,Val^4]AVP	*IV*	$pA_2 = 6.40$	$pA_2 = 6.84$
[Cpp^1,D-Gly(Ph)^2,Val^4]AVP	*VIII*	0[c]	0[b]

[a] Biological activities (in *IU*/mg) of all the previously published analogs were taken from ref.[4,5]; [b] compound is inactive in the pressor test in doses up to 0.1 mg/kg; [c] compound is inactive in the uterotonic in vitro test in concentration up to $3.3 \cdot 10^{-6}$ mol/l.

reoisomer (ref.[6] and unpublished results) there is a strong decrease of potency in all three assayes in the case of compound *IV*.

The lack of all three activities, the pressor, uterotonic and anti-antidiuretic ones of analog *VIII* which differs from peptide *IV* only by the presence of D-Gly(Ph) instead of 4-Cl-Phe at position 2 and the same low antioxytocic activity of compounds *V* – *VII*, show that D-Gly(Ph) residue at position 2 is not compatible with biological potency. On the other hand these observations may be of value in the design of selective uterotonic antagonists in which D-Gly(Ph)[2] substitution would be combined with other suitable modifications, however, at the present stage it is too early for such a conclusion.

EXPERIMENTAL

N,N-Dimethylformamide (DMF) was distilled under reduced pressure. 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–pyridine (5 : 1 : 4 : 5); C, 1-butanol–acetic acid–water–pyridine (15 : 3 : 3 : 10); D, chloroform–methanol (7 : 3); E, 1-butanol–acetic acid–water (1 : 1 : 1). Capillary melting points were taken 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 18 h at 100 °C. The analyses were performed on a Mikrotechna type AAA 881 analyzer. The optical rotations were measured at the Na yellow line (586 nm) using Hilger–Watts polarimeter with an accuracy of 0.01°. The elemental analyses were determined on a Carlo–Erba Model 1106 analyzer.

Synthesis of Peptides

The chloromethylated resin used in the synthesis (polystyrene crosslinked with 1% of divinylbenzene, Bio-Beads, Bio-Rad) was esterified with Boc-Gly to the level of 0.5 mmol/g by the method of Gisin[7]. Solid

TABLE II
Physico-chemical data of protected intermediates

Compound	R_F		$[\alpha]_D^{20}$, ° (c 1, DMF)	M. p., °C
	A	D (E)		
Ia	0.49	0.39	−37.7	215 – 218
IIa	0.44	0.53	−34.9	224 – 227
IIIa	0.46	0.51	−35.9	192 – 194
IVa	0.55	0.16	−35.4	205 – 211
Va	0.57	(0.71)	−40.2	208 – 210
VIa	0.44	(0.65)	−34.0	197 – 200
VIIa	0.51	(0.73)	−35.8	183 – 185
VIIIa	0.59	(0.75)	−36.0	207 – 209

phase peptide synthesis[8,9] was employed to prepare eight protected analogs of vasopressin: Z-Cys(Bzl)-4-Cl-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*Ia*), Mpa(Bzl)-4-Cl-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IIa*), Cpp(Bzl)-4-Cl-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IIIa*), Cpp(Bzl)-4-Cl-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*IVa*), Z-Cys(Bzl)-D-Gly(Ph)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*Va*), Mpa(Bzl)-D-Gly(Ph)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VIa*), Cpp(Bzl)-D-Gly(Ph)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VIIa*), Cpp(Bzl)-D-Gly(Ph)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VIIIa*). All coupling reactions to form peptide bonds 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[10]. At the end of the synthesis the fully protected peptide was removed from the resin as the amide by ammonolysis[11]. The crude products were dissolved in hot DMF, precipitated with boiling water, collected by filtration and dried in vacuo over P_2O_5.

The physico-chemical properties of these compounds (*Ia* – *VIIIa*) are summarized in Table II.

A solution of the peptide intermediate in sodium dried and redistilled ammonia was treated at the boiling point and stirred with sodium from a stick of the metal in a small-bore glass tube until a light-blue colour persisted in the solution for 30 s. Ammonium chloride was added to discharge the colour. The solution was evaporated, the residue dissolved in glacial acetic acid and the solution diluted with methanol. An excess of solution of iodine in methanol (0.1 mol/l) was added gradually with stirring. The light yellow

TABLE III
Physico-chemical characteristics of AVP analogs

Peptide	No.	R_F A B (C)	$[\alpha]_D^{21}$, ° (c 0.5, 1 M AcOH)	Amino acid analyses				
				Phe Pro	Glu Arg	Asp Gly	Cys NH_3	Val
[4-Cl-Phe^2]AVP	*I*	0.08 0.64	+218.7	1.03 1.02	1.02 1.04	1.02 1.00	1.95 3.03	
[4-Cl-Phe^2]dAVP	*II*	0.17 0.68	+86.8	1.03 1.03	1.03 1.02	1.02 1.00	0.99 3.02	
[Cpp^1,4-Cl-Phe^2]AVP	*III*	0.12 0.72	−54.2	1.04 1.02	1.02 1.04	1.03 1.01	0.98 2.03	
[Cpp^1,4-Cl-Phe^2,Val^4]AVP	*IV*	0.15 0.79	−52.7	1.02 1.04	1.01	1.03 1.00	0.98 1.98	1.00
[D-Gly(Ph)^2]AVP	*V*	0.15 (0.22)	−43.9	1.01 1.07	1.02 0.98	0.99 1.01	2.01 3.03	
[D-Gly(Ph)^2]dAVP	*VI*	0.22 (0.37)	−96.2	1.02 1.03	1.03 1.00	1.01 1.02	1.04 3.04	
[Cpp^1,D-Gly(Ph)^2]AVP	*VII*	0.16 (0.41)	−123.2	1.02 1.02	0.98 1.03	1.04 1.04	1.02 3.02	
[Cpp^1,D-Gly(Ph)^2,Val^4]AVP	*VIII*	0.25 (0.61)	−155.0	1.03 1.04	1.02	1.02 1.03	1.03 2.05	1.01

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 resulting material was desalted on a Sephadex G-15 column (120 × 2.9 cm), eluted with aqueous acetic acid (50%) with flow rate of 6.5 ml/h. The fractions containing the desired compound were combined and lyophilized. The residue was further subjected to gel filtration on Sephadex LH-20 column (120 × 1.4 cm) eluted with aqueous acetic acid (30%) with a flow rate of 4.5 ml/h. The peptide was eluted as a single peak. Lyophilization of the pertinent fractions gave the desired vasopressin analog. The physico-chemical properties of eight peptides which were prepared in this way 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" (ref.[14]). The anti-antidiuretic activity was tested on conscious rats as described in ref.[6] as an ability of the analog to increase the urine volume on 6 ml per 1.5 h.

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