SYNTHESIS AND BIOLOGICAL ACTIVITY OF FOUR VASOPRESSIN ANALOGUES SUBSTITUTED IN POSITION 2 BY *p*-FLUORO-PHENYLALANINE

Bernard LAMMEK[a], Izabela DERDOWSKA[a], Gotfryd KUPRYSZEWSKI[a],
Jiřina SLANINOVÁ[b] and Tomislav BARTH[b]

[a] *Institute of Chemistry, University of Gdansk, 80-952 Gdansk, Poland*
[b] *Institute of Organic Chemistry and Biochemistry,*
Czechoslovak Academy of Sciences, 166 10 Prague 6, Czechoslovakia

Received June 27, 1990
Accepted July 30, 1990

Tyrosine in position 2 of AVP, dAVP, [Cpp^1]AVP and [Cpp^1, Val^4]AVP was substituted by *p*-fluorophenylalanine and an effect of this change on agonistic/antagonistic activity in uterotonic, pressor or anti-antidiuretic test was followed. All the prepared analogues were found inhibitory in uterotonic test, however the inhibitory potency was rather low. As far as the pressor activity is concerned, the agonistic potency was slightly decreased (2—4 times), in comparison with the strong decrease of the inhibitory potency.

Structural requirements of neurohypophyseal hormones* for pressor and antidiuretic antagonism are relatively well known, however none of the available antagonists is truly specific for the V_1, V_2 or oxytocin-like receptors[2-4]. Though some of the antagonists of arginine vasopressin (AVP) are highly specific for V_1 and do not block V_2 receptors in vivo, they nonetheless can block oxytocin receptors in uterus[2]. It is known that alkylation of tyrosine residue at position 2 of V_1 antagonists of AVP in many cases greatly enhances the activity and selectivity of the analogues[3]. However the effect of other substituents in this position on V_1 antagonism is not well explored. It was also shown that single substitutions of position 2 of AVP may result in analogues with very interesting pharmacological properties[2-4]. With this in mind we decided to study the effect of the substitution of 4-fluoro-L-phenylalanine (4-F-Phe) for tyrosine in position 2 of AVP and both its agonistic and antagonistic analogues.

Four analogues were synthesized to the above rationale, i.e. [2-(*p*-fluorophenylalanine), 8-arginine]vasopressin ([4-F-Phe^2]AVP, *I*); [1-(2-mercaptopropionic acid),

---

* 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, 3,3-cyclopentamethylene-3-mercaptopropionic acid; dAVP, deamino-8-arginine-vasopressin ([Mpa^1, Arg^8]VP).

2-(*p*-fluorophenylalanine), 8-arginine]vasopressin ([4-F-Phe^2]dAVP, *II*); [1-(1-mercaptocyclohexaneacetic acid), 2-(*p*-fluorophenylalanine), 8-arginine]vasopressin ([Cpp^1, 4-F-Phe^2]AVP, *III*); [1-(1-mercaptocyclohexaneacetic acid), 2-(*p*-fluorophenylalanine), 4-valine, 8-arginine]vasopressin ([Cpp^1, 4-F-Phe^2, Val^4]AVP, *IV*) The synthesized analogues have the following general structure:

$$\overline{\text{X-4-F-Phe-Phe-Y-Asn-Cys}}\text{-Pro-Arg-GlyNH}_2$$

I, X = Cys, Y = Gln *III*, X = Cpp, Y = Gln
II, X = Mpa, Y = Gln *IV*, X = Cpp, Y = Val

Biological potencies of the four new analogues were determined in uterotonic in vitro and pressor tests. The results obtained are presented in Table I. Possible anti-antidiuretic activity of compounds *III* and *IV* was estimated in pilot experiments on conscious rats without a water load.

Table I

Biological activities (rat) of the new vasopressin analogues and some reference compounds.

Compound		Biological activity[a] uterotonic in vitro IU/mg	Biological activity[a] pressor IU/mg
AVP		17	450
[L-Phe^2]AVP		$\sim 0{\cdot}2$	122
[4-F-L-Phe^2]AVP	*I*	$pA_2 = 6{\cdot}7$	193
dAVP		27–63	380
[L-Phe^2]dAVP		$\sim 0{\cdot}3$	29
[4-F-L-Phe^2]dAVP	*II*	$pA_2 = 6{\cdot}6$	90·8
[Cpp^1]AVP		$pA_2 = 8{\cdot}15$	$pA_2 = 8{\cdot}35$
[Cpp^1,4-F-L-Phe^2]AVP	*III*	$pA_2 = 6{\cdot}27$	$pA_2 = 6{\cdot}30$
[Val^4]AVP		—	32
[Val^4]dAVP		—	51
[Cpp^1,Val^4]AVP		$pA_2 = 7{\cdot}34$	$pA_2 = 7{\cdot}97$
[Cpp^1,4-F-L-Phe^2,Val^4]AVP	*IV*	$pA_2 = 7{\cdot}40$	$pA_2 = 6{\cdot}40$

[a] Biological activities of all the previously published analogues were taken from ref.[16].

The introduction of Phe residue at position 2 of AVP alone or in combination with deamination resulted in analogues which possessed about 27·8% and 7·6% of pressor activity of AVP, respectively, but only very low uterotonic activity in vitro[2]. Introduction of 4-F-Phe into this position resulted in lower reduction of pressor activity (42·9% and 23·9% of AVP), however this modification changed the character of the uterotonic potency into low antagonism.

The effect of combining the 4-F-Phe substitution with introduction of 1-mercapto-cyclohexaneacetic acid at position 1, a modification which has proved to be useful and effective in the design of potent V_1 antagonists of AVP (ref.[2]), which are at the same time potent oxytocic inhibitors on uterus, led in the case of both these activities to sharp decrease of inhibitory potency (for 2 orders of magnitude).

Finally, knowing the importance of position 4 for activity and selectivity of the analogues, we found it of interest to evaluate how the modification studied will affect the activity of an analogue where glutamine in position 4 is replaced by valine. As can be seen from Table I the analogue *IV* displayed the same sharp decrease of anti-pressor activity as the analogue *III*, however the antiuterotonic activity was not changed. Pilot experiments that may disclose anti-antidiuretic activity were negative up to the dose of 100 μg per kg of rat.

The four new analogues have in position two *p*-fluorophenylalanine, an amino acid having extremely electron withdrawing substituent in the aromatic ring. This change, i.e. replacement of dissociable OH group by polar function, was introduced into 2 analogues displaying agonistic potency and into 2 antagonists of V_1 receptors. Their oxytocic activity was also determined because the V_1 antagonists are usually very potent oxytocic antagonists in the uterotonic test in vitro. As far as the pressor activity is concerned the substitution studied led in the case of agonists to decrease of the potency, however the decrease was lower than in the case of elimination of the OH group of tyrosine. In the case of antagonists this change led to a dramatic decrease of antipressor potency.

As far as the oxytocic activity is concerned, the studied modification changed the character of the substance from very low agonism into low antagonism, however in the case of antagonists it reduced the inhibitory potency.

EXPERIMENTAL

N,N-Dimethylformamide (DMF) was distilled under reduced pressure, triethylamine (NEt_3) was distilled from ninhydrin. Other solvents and reagents were of analytical grade. Thin layer chromatography was carried out on silica plates (Merck), and the spots were visualized by ninhydrin or iodine. The following solvent systems were used: A, 1-butanol–acetic acid–water (4 : 1 : 5, v/v, upper phase); B, chloroform–methanol (7 : 3, v/v); C, 1-butanol–acetic acid–water–pyridine (5 : 1 : 4 : 5, v/v). For amino acid analysis the peptides (0·5 mg) were hydrolysed with constantly boiling hydrochloric acid (400 μl) containing phenol (20 μl), in evacuated sealed ampoules for 18 h at 110°C. The analyses were performed on a Mikrotechna type AAA 881 analyzer. The optical rotations were measured with a Hilger–Watts polarimeter with an accuracy of 0·01°. The melting point values are uncorrected. The elemental analyses were determined on a Carlo–Erba Model 1106 analyzer.

Synthesis of the Peptides

The protected peptide precursors required for the synthesis of analogues *I*—*IV* were prepared by the Merrifield method of solid-phase synthesis entirely on the resin[5,6]. First, chloromethylated resin (Bio-Rad, Bio-Beads S × 1, 0·75 mmol Cl/g) was esterified with Boc-Gly to a load of 0·45 mmol/g (ref.[7]). Then Cys(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*V*), Mpa(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VI*), Cpp(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VII*) and Cpp(Bzl)-4-F-Phe-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin (*VIII*) were prepared using solid phase methodology as previously described[5,6,8]. Coupling reactions were mediated either by DCC or DCC–HOBt method[9]. The completeness of each coupling reaction was monitored by the Kaiser test[10]. Boc-Cys(Bzl), 1-benzylthiocyclohexaneacetic acid[11] and 2-benzylthiopropionic acid were each used in the final coupling steps. The protected peptides were cleaved from the resin by ammonolysis[6]. Following evaporation of the solvent, the products were extracted into hot DMF, precipitated with boiling water and left overnight at room temperature. The peptides were collected by filtration, washed with water and dried in vacuo over P_2O_5. The products were purified by dissolving in DMF and reprecipitating with methanol–ethyl ether (1 : 3). The physico-chemical properties of these compounds (*IX*—*XII*) are summarized in Table II.

TABLE II

Physico-chemical data of protected intermediates

Substance	R_F A B	$[\alpha]_D^{21}$ ($c = 1$, DMF) M.p., °C	Formula (M.w.)	Calculated/Found		
				%C	%H	%N
Boc-Cys(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*IX*)	0·57 0·52	−40·0 207—208	$C_{72}H_{99}FN_{15}O_{15}S_3$ (1 529·8)	56·53 56·21	6·52 6·74	13·73 13·51
Mpa(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*X*)	0·53 0·52	−53·4 208—210	$C_{67}H_{89}FN_{14}O_{13}S_3$ (1 413·7)	56·92 56·81	6·35 6·21	13·87 13·69
Cpp(Bzl)-4-F-Phe-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*XI*)	0·54 0·57	−62·6 180—183	$C_{72}H_{97}FN_{14}O_{13}S_3$ (1 481·8)	58·36 58·17	6·60 6·70	13·03 12·98
Cpp(Bzl)-4-F-Phe-Phe-Gln-Val-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (*XII*)	0·55 0·76	−70·0 204—207	$C_{72}H_{98}FN_{13}O_{12}S_3$ (1 452·8)	59·52 59·32	6·80 6·60	12·53 12·41

[1-(1-Mercaptocyclohexaneacetic acid),
2-(*p*-fluoro-L-phenylalanine),8-arginine]vasopressin (*III*)

A solution of the protected acyloctapeptide amide *XI* (252 mg, 0·17 mmol) in sodium dried and redistilled ammonia (400 ml) was treated at boiling point and with stirring with sodium from a stick of the metal contained in a small-bore glass tube until a light-blue color persisted in the solution for 30 s. Dry acetic acid (0·4 ml) was added to discharge the color. The solution was evaporated, the residue dissolved in glacial acetic acid (150 ml) and the solution diluted with methanol (1 000 ml). An excess of solution of I_2 in methanol (0·1 mol/l, 2·5 ml) 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, acetic form, 10 g damp weight). The reaction mixture was filtered through a bed of resin (10 g damp weight). The bed was washed with methanol (100 ml) and the combined filtrate and washings were evaporated under reduced pressure. The resulting material was dissolved in 7 ml aqueous acetic acid (50%) and 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 eluate was fractionated and monitored for absorbance at 254 nm. The fractions comprising the major peak were pooled and lyophilized and the residue (165 mg) 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 vasopressin analogue *III*: yield 59 mg (30·5%, based on the amount of protected peptide used in the reduction-oxidation procedure).

The physico-chemical properties of this and of the remaining three peptides *I*, *II*, and *IV*, which were prepared in the same way as *III*, 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

TABLE III

Physico-chemical characteristics of AVP analogues

Peptide		R_F A C	$[\alpha]_D^{21}$ (c = 0·5, 1M AcOH)	Amino acid analyses Phe Pro	 Glu Arg	 Asp Gly	 Cys NH_3
[4-F-Phe^2]AVP	(*I*)	0·23 0·58	−70·8	1·02 1·02	1·03 1·04	1·00 1·00	1·93 3·05
[4-F-Phe^2]dAVP	(*II*)	0·28 0·62	−106·8	1·03 1·03	1·02 1·03	1·03 1·00	0·98 3·04
[Cpp^1,4-F-Phe^2]AVP	(*III*)	0·31 0·71	−88·9	1·04 1·02	1·02 1·04	1·02 1·00	0·98 2·05
[Cpp^1,4-F-Phe^2,Val^4]AVP	(*IV*)	0·35 0·73	−89·6	1·02 1·04	1·01 1·03	1·04 1·00	0·98 2·05

pressor activity was tested on pitched male rats against standard synthetic arginine-vasopressin. 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 in ref.[15] as an ability of the analogue to increase the urine volume to 6 ml per 1·5 h.

This work was supported by the Polish Academy of Sciences (Research Grant CPB 06.03.4.1.).

REFERENCES

1. Eur. J. Biochem. *138*, 9 (1984).
2. *CRC Handbook of Neurohypophyseal Hormone Analogs* (K. Jošt, M. Lebl and F. Brtník, Eds), Vol. II, Part 1, p. 17. CRC Press, Boca Raton 1987.
3. Manning M., Grzonka T., Sawyer W. H. in: *The Pituitary* (C. Beardwell and G. Robinson, Eds), p. 265. Butterworth, Kent England 1981.
4. Manning M., Sawyer W. H. in: *Vasopressin* (R. W. Schrier, Ed.), p. 131. Raven Press, New York 1985.
5. Merrifield R. B.: J. Am. Chem. Soc. *85*, 2149 (1963).
6. Manning M.: J. Am. Chem. Soc. *90*, 1348 (1968).
7. Gisin B. F.: Helv. Chim. Acta *56*, 1476 (1973).
8. Manning M., Coy E., Sawyer W. H.: Biochemistry *9*, 3925 (1970).
9. Konig W., Geiger R.: Chem. Ber. *103*, 788 (1970).
10. Kaiser E., Colesott R. L., Bossinger C. D., Cook P. I.: Anal. Biochem. *34*, 595 (1970).
11. Yim N. C. F., Huffman W. F.: Int. J. Pept. Protein Res. *23*, 568 (1981).
12. Holton P.: Brit. J. Pharmacol. *3*, 328 (1948).
13. Munsick R. A.: Endocrinology *66*, 451 (1960).
14. Slaninová J. in: *CRC Handbook of Neurohypophyseal Hormone Analogs* (K. Jošt, M. Lebl and F. Brtník, Eds), Vol. I, Part 2, p. 83. CRC Press, Boca Raton 1987.
15. Lammek B., Wang Y. X., Derdowska I., Franco R., and Gavras H.: Peptides *10*, 1109 (1989).
16. Lebl M., Jošt K., Brtník F. in: *CRC Handbook of Neurohypophyseal Hormone Analogs* (K. Jošt, M. Lebl and F. Brtník, Eds), Vol. II, Part 2, p. 127. CRC Press, Boca Raton 1987.

Revised by the author (J.S.).