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The new 8-D-homoarginine-vasopressin analogs with strong in vitro and in vivo uterus inhibiting activity

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Introduction

Basicity in the side chain of the amino acid in position 8 is very important for vasopressin-like activities. Surprisingly, this is also advantageous in the design of uterotonic inhibitors. Potent inhibitors derived from oxytocin had bulky substituents on the β-carbon in position 1 and/or the D-configuration of substituted aromatic amino acid in position 2. The inhibitory potency of these analogs is enhanced by a basic amino acid in position 8.

Recently we described the inhibitory activities of vasopressin analogs having D-homoarginine in position 8 and *p*-substituted phenylalanine in position 2 [1]. The considerably high inhibitory activity of these analogs prompted us to combine the above-mentioned modifications with deamination in position 1 [2], and also to investigate the influence of other benzene ring substitutions of phenylalanine. As a consequence we have prepared analogs with *o*-alkyl, multiple and alkoxy-substituted benzene ring.

Results and Discussion

Solid-phase methodology on *p*-methylbenzhydrylamine resin with Boc-strategy was used for the synthesis of nine new analogs of amino (**1–7**) or deamino (**8–9**) vasopressin with non-coded amino acid D-homoarginine in position 8 and mono-, di- or tri-alkyl or *p*-methoxy or *p*-ethoxy substituted D- or L- phenylalanine in position 2. For side-chain protection, nitrogroup (D-Har) and 4-methylbenzyl (Cys, Mpa) were used. Peptides were cleaved from the resin by liquid HF and oxidation of sulfhydryl groups was performed by $K_3[Fe(CN)_6]$. Racemic forms of differently substituted phenylalanines were used during the synthesis and appropriate diastereoisomers were separated by HPLC at the end of the synthesis. The substituted D,L-phenylalanines were prepared via the modified acetamidomalonane method starting from corresponding substituted benzyl chlorides.

Analogs containing L-amino acid in position 2 are significantly more basic (electrophoretic mobility in pyridine-acetate buffer, pH 5.7) than analogs with D-amino acid in the same position. This difference is observable only in analogs with cysteine in position 1. This means that the configuration of amino acid in position 2 controls the pK of the N-terminal amino group. Influence of an amino acid configuration on the pK of its amino group was described [3,4] for oxytocin

Table 1 *Biological activities (rat) of [D-Har[8]] vasopressin analogs (IU/mg of pA_2) with modifications in position 2*

No.	Compounds	Uterotonic		Pressor	Antidiuretic
		In vitro	In vivo		
	AVP	17		412	465
	[D-Har[8]]VP	0.9		0.83	83 (1% dDAVP)
1	[L-Phe(*o*Et)[2], D-Har[8]]VP	$pA_2 = 6.8$	$pA_2 = 6.1$	0	$<10^{-4}$% dDAVP
2	[D-Phe(*o*Et)[2], D-Har[8]]VP	$pA_2 = 8.4$	$pA_2 = 6.9$	$pA_2 = 5.6$	$<10^{-4}$% dDAVP
3	[L-Phe(2,6-diMe)[2], D-Har[8]]VP	$pA_2 = 5.7$	–	$pA_2 = 5.9$	$<10^{-4}$% dDAVP
4	[L-Phe(2,6-diMe)[2], D-Har[8]]VP	$pA_2 = 6.4$	pA2 = 6.5	$pA_2 = 6.1$	–0.1% dDAVP
5	[L-Phe(2,4,6-triMe)[2], D-Har[8]]VP	$pA_2 = 6.1$	0	$pA_2 = 5.8$	$<10^{-4}$% dDAVP
6	[L-Phe(2,4,6-triMe)[2], D-Har[8]]VP	$pA_2 = 8.1$	$pA_2 = 7.5$	$pA_2 = 6.1$	–0.1% dDAVP
7	[D-Tyr(Et)[2], D-Har[8]]VP	$pA_2 = 7.3$	$pA_2 = 6.3$	$pA_2 = 7.2$	$<10^{-4}$% dDAVP
8	[Mpr[1], D-Tyr(Me)[2], D-Har[8]]VP	$pA_2 = 9.0$	$pA_2 = 7.1$	$pA_2 = 6.9$	–
9	[Mpr[1], D-Tyr(Et)[2], D-Har[8]]VP	$pA_2 = 8.8$	$pA_2 = 6.6$	$pA_2 = 7.0$	$<10^{-4}$% dDAVP

analogs with D-cysteine in position 1, but the transfer of this effect over one amino acid residue is a surprising new finding.

Biological activities of the analogs are given in Table 1. Substitution in position 2 led to a substantial decrease in antidiuretic activity. All analogs with substituted phenylalanine in position 2 had either no activity (**1**) or very low inhibitory activity in the pressor test. As in the previous cases [1,2], all analogs are very strong inhibitors of oxytocin action on the uterus in vitro and in vivo. The earlier published activities evidenced that more potent inhibitors result from the combination of D-amino acids in both positions 2 and 8. As analog containing *p*-ethylphenylalanine of D-configuration it was found superior in the *p*-substituted series [1]; by analogy the D-*o*-ethylphenylalanine-containing analog **2** is superior in the *o*-substituted series. In the in vitro test deamino analogs are more potent than amino analogs. Analog **8** is one of the most potent in vitro uterotonic inhibitors described up to now [5]. However, in the uterotonic test in vivo the inhibitory activity is much lower. If we compare the activities in the most commonly used uterotonic in vitro test to the activities in the pressor test, we will find a high selectivity in favour of the antiuterotonic potency. However, if we compare the values from the uterotonic in vivo test and that from the pressor test, which should be considered to be more proper, the values are of the same order of magnitude. It means that the distribution in the organism and/or ion composition plays a big role in the biological potency of these analogs. Analog **6** ($pA_2 = 7.5$) is the most potent in vivo uterotonic inhibitor in the group of amino and deamino analogs of [D-Har[8]]vasopressins and one of the most potent inhibitors described up to now [5].

References

1. Žertová, M., Procházka, Z., Bláha, I., Barth, T., Slaninová, J., Maletinská, L. and Lebl, M., Coll. Czech. Chem. Commun., 55(1990)3000.
2. Žertová, M., Procházka, Z., Barth, T., Slaninová, J., Škopková, J., Bláha, I. and Lebl, M., Coll. Czech. Chem. Commun., 56(1991)1761.
3. Hruby, V.J., Deb, K.K., Spatola, A.F., Upson, D.A. and Yamamoto, D.M., J. Am. Chem. Soc., 101 (1979)202.
4. Blumenstein, M., Hruby, V.J., Yamamoto, D.M. and Yang, Y.C.S., FEBS Lett., 81(1977)347.
5. Lebl, M., In Jošt, K., Lebl, M. and Brtník, F. (Eds.) Handbook of Neurohypophyseal Hormone Analogs, Vol. II, Part 1, CRC Press, Boca Raton, FL, 1987, p. 17.