Journal of Peptide Science
J. Peptide Sci. **6**: 123–129 (2000)

Non-sequential Vasopressin Peptides. Stereochemistry and Biological Activity

M. ZAORAL[a], I. BLÁHA[b], M. BUDĚŠÍNSKÝ[c], A. MACHOVÁ[c] and J. SLANINOVÁ[c,*]

[a] Na Loukoti 12, 160 00 Prague 6, Czech Republic
[b] PolyPeptide Labs. Ltd, Radiová 1, 100 00 Prague 10, Czech Republic
[c] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo 2, 166 10 Prague 6, Czech Republic

Received 20 August 1999
Accepted 11 September 1999

Abstract: Two cyclic disulfides of structure Cys-Tyr-Arg-Arg-Tyr-Cys-NH_2 (**1**) and Cys-Tyr(Me)-Arg-Arg-Tyr(Me)-Cys-NH_2 (**2**), two nonapeptide derivatives of **1** extended at the *C*-terminal with Pro-Arg-Gly-NH_2 (**3**) or Pro-D-Arg-Gly-NH_2 (**4**) and derivatives of **3** and **4** having Mpr in position 1, i.e. analogs (**5**) and (**6**), respectively, were synthesized, and their stereochemistry and biological activity were studied. All the peptides displayed low dose-dependent uterotonic activity *in vitro* and antidiuretic activity *in vivo*. None of the peptides increased the blood pressure of the experimental animals. Compounds **2**, **4** and **6** showed a low inhibitory effect on AVP pressor activity; compound **6**, in addition, displays a significant and long-lasting vasodepressor effect. NMR measurements indicated the existence of hydrogen bond between the amino acid residues in positions 2,5 and 3,4 of peptides **1** and **2**, and side-chain interactions between amino acid residues in positions 2,3 and 4,5 of peptide **1**. No such side-chain interactions were detected in peptide **2**. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: vasopressin; adiuretin; conformation; analogs; NMR studies; biological activity

INTRODUCTION

DDAVP, [Mpr[1], D-Arg[8]] VP, is a drug [1–4] which under various commercial names (e.g. Minirin, Minrin, Desmopressin, Adiuretin-SD) is widely used for the treatment of diabetes insipidus [2–8], enuresis nocturna [6,9–11], milder forms of haemophilia A and von Willebrand's disease [12–16], in surgery [17–19], clinical diagnostics [6,20–22], etc. The working hypothesis that led to its discovery was based primarily on a comparison of the chemical structure and biological activities of the naturally occurring vasopressins and oxytocins. This approach led to the choice of position 8 for structural changes. The type of structural change was deduced from considerations concerning the mechanism of vasopressin action. It was evident that it is a receptor mechanism, though at the onset of our work, details of it were not known. However, the complementarity of stereospecific hormone–receptor interactions represents an inevitable

Abbreviations: The abbreviations and symbols are according to the IUPAC-IUB-JBNC: see *Eur. J. Biochem.* 1984; **138**: 9–37. Unless stated otherwise the chiral amino acids are of L-configuration. Superscript numbers on residue abbreviations, e.g. Arg[8], refer to positions in vasopressin; designations such as Arg-3 refer to positions in the analogs under study. The abbreviations Mpr for β-mercaptopropionic acid and DDAVP for [1-β-mercaptopropionic acid, 8-D-arginine] vasopressin were used throughout our preceding work and are also used in the present communication. Further abbreviations; Ar, aryl; AVP, arginine vasopressin; 2D-COSY, two-dimensional correlation spectroscopy; DMSO, dimethylsulfoxide; HPLC, high-performance liquid chromatography; LVP, lysine vasopressin; Me, methyl; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; OT, oxytocin; 2D-ROESY, two-dimensional rotating frame NOE spectroscopy; TFA, trifluoroacetic acid; 2D-TOCSY, two-dimensional total correlation spectroscopy.

* Correspondence to: Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo sq. 2, 166 10 Prague 6, Czech Republic. E-mail: slan@uochb.cas.cz

component of the receptor mechanism. Consequently, as a tool for changing the ratio of the different hormonal activities of vasopressins, resulting from different hormone–receptor interactions, a change of configuration in position 8 was employed. The third part of the working hypothesis anticipated mutual interactions of the functional groups important for the biological activity [2,23–25] which — eventually — can create a 'functional centrum of higher order' (or active core or center or active conformation in contemporary terms).

1 Cys-Tyr-Arg-Arg-Tyr-Cys-NH_2

2 Cys-Tyr(Me)-Arg-Arg-Tyr(Me)-Cys-NH_2

3 Cys-Tyr-Arg-Arg-Tyr-Cys-Pro-Arg-Gly-NH_2

4 Cys-Tyr-Arg-Arg-Tyr-Cys-Pro-D-Arg-Gly-NH_2

5 Mpr-Tyr-Arg-Arg-Tyr-Cys-Pro-Arg-Gly-NH_2

6 Mpr-Tyr-Arg-Arg-Tyr-Cys-Pro-D-Arg-Gly-NH_2

The first two parts of the working hypothesis proved to be fruitful. Using them as a guide-line, we soon obtained the desired compound (DDAVP), possessing a very high and very specific antidiuretic activity, and later a number of similar substances [2]. The third part of the working hypothesis is treated in this study. We have arranged experiments to prove the consideration that the three parts of the vasopressin molecule, Cys^1, Tyr^2 and Arg^8 participate in the formation of the active structure. On the basis of this idea we have designed and prepared six model peptides **1–6** and studied their biological properties and stereochemistry.

EXPERIMENTAL

Peptides **1–6** were synthesized by the solid-phase method using an Aladin multiple peptide synthesizer (IOCB, Academy of Sciences of the Czech Republic, Prague) and Fmoc/Bu^t-strategy. Each peptide was prepared on 300 mg, (0.14 mmol) of Rink Amide Resin (Novabiochem, Switzerland), the side chain functional groups of Cys, Tyr and Arg being shielded by Tos-, Bu^t- and Pmc-protecting groups, respectively. The peptides were cleaved from the support with a mixture of trifluoroacetic acid (TFA)–water–ethanedithiol–triisopropylsilane (92.5: 2.5: 2.5: 2.5) for 4 h. The disulfide bridge was formed by oxidation with potassium ferricyanide in water (peptide concentration 1 mg/ml, pH 7). The products of the oxidation were desalted on Amberlite CG-50, purified by gradient reverse-phase high-performance liquid chromatography (RP-HPLC) (water–acetonitrile) and characterized by amino-acid analysis and FAB mass spectrometry (Table 1). The purity of peptides prepared was higher than 99%.

Biology

The peptides were tested for antidiuretic potency in conscious male rats in a slightly modified Burn [26,27] antidiuretic assay, for pressor potency in the pressor test according to Dekanski [28] and for uterotonic activity in the *in vitro* uterotonic test according to Holton [29], in Munsick [30] solution, both in the absence and presence of magnesium.

NMR Study

Peptides **1** and **2** were studied by NMR. Proton NMR spectra were recorded on a Varian UNITY-500 spectrometer at 500 MHz frequency in d_6-DMSO solution and referenced to the residual signal of the solvent (δ(DMSO) = 2.50). The spectra were measured at 20, 40 and 60°C. Two-dimensional correlation spectroscopy (2D-COSY) [31] and two-dimensional total correlation spectroscopy (2D-TOCSY) spectra [32,33] (spin lock time 70 ms) were used for the structural assignment of protons of individual residues and two-dimensional rotating

Table 1 Some Characteristics of the Prepared Peptides

Peptide	Yield (mg)	Amino-acid analysis	FAB MS
1	32 (27%)	Cys 1.81, Tyr 2.01, Arg 2.18	861.1 (M+H), 883.2 (M+Na)
2	28 (23%)	Cys 1.81, Tyr 2.01, Arg 2.18	888.1 (M+H), 910.3 (M+Na)
3	52 (32%)	Cys 1.79, Tyr 1.94, Arg 3.13, Pro 1.06, Gly 1.08	1171.5 (M+H), 1193.5 (M+Na)
4	49 (30%)	Cys 1.83, Tyr 1.91, Arg 3.09, Pro 1.09, Gly 1.08	1171.4 (M+H, 1193.4 (M+Na)
5	63 (39%)	Cys 0.85, Tyr 1.84, Arg 3.07, Pro 1.10, Gly 1.14	1156.5 (M+H), 1178.5 (M+Na)
6	42 (26%)	Cys 0.90, Tyr 1.89, Arg 3.11, Pro 1.07, Gly 1.03	1156.4 (M+H), 1178.4 (M+Na)

frame NOE spectroscopy (2D-ROESY) spectra [34,35] (mixing time 300 ms) for sequential analysis and medium range NOE contacts.

RESULTS AND DISCUSSION

The aim of the present work was to verify the third part of the working hypothesis which we had employed for the preparation of vasopressin analogs with very high and highly specific antidiuretic activity, i.e. the idea of the formation of an active conformational center comprising Cys^1, Tyr^2 and Arg^8. To check this idea we have prepared the aforementioned peptides **1–6** and studied their relevant properties, i.e. biological activity and stereochemistry.

Peptides **1** and **3–6** have a common essential primary structure. It contains the *N*-terminal vasopressin dipeptide sequence Cys-Tyr (or Mpr-Tyr) extended by Arg, which is in vasopressin itself at position 8. The 20-membered ring of vasopressin is mimicked by doubling the aforementioned tripeptide Cys-Tyr-Arg (or Mpr-Tyr-Arg) segment (however, the second part of the peptide represents a retrosequence), and closing the disulfide bridge between peptide positions 1 and 6 (analog **1**). Already this analog displayed low but distinct biological activity (Table 2).

The main structural changes employed in our previous study [2] on vasopressin analogs with high and specific antidiuretic activity were D-substitution at position 8 and deamination at position 1. These changes are included in peptides **4–6** in the present study. Should there be a causal relation between the two groups of compounds, then it should be reflected by similarity of the effects elicited. To make the 8-D-substitution possible, the vasopressin tripeptide side chain was attached to the cyclohexapeptide **1**. This modification of **1** strongly reduced its biological activities (see Table 2, peptide **3**), but the change of configuration at position 8 recovered them (peptide **4**). The influence of 1-deamination was similar to that usually observed in the vasopressin and oxytocin series and in our previous studies: potentiation of the biological effects. The uterotonic and antidiuretic activities of peptide **3** were increased, the uterotonic one strongly, the antidiuretic slightly (peptide **5**). The cumulative effect of 8-D-substitution and 1-deamination was also similar in both groups of compounds. In our previous study, it yielded one of the most active compounds antidiuretically, DDAVP; in the present study it led to most active compound as well (peptide **6**).

Compound **6** is the most active peptide of the six model compounds synthesized in the present study, but the most interesting quality is its significant and long lasting vasodepressor effect. Vasopressin and vasopressin analogs showing a depressor effect under special conditions, e.g. when applied directly into different structures of the brain, are well

Table 2 Biological Activities of the Model Peptides

Analog	Activity			
	Antidiuretic % activity of DDAVP[a]	Pressor	Uterus *in vitro* (IU/mg)	
			Without Mg^{2+}	1 mM Mg^{2+}
1	0.0025	0	0.02	0.02
2	n.t.[b]	$pA_2 = 5.6$	0.01	n.t.
3	0.0004	0	0.01	0
4	0.0020	$pA_2 = 6.2$	0.11	0.04
5	0.0008	0	0.11	0.05
6	0.0020	Depressoric[c] $pA_2 = 6.0$	0.30	0.24

[a] The dose response curves were parallel to that of DDAVP. Doses which produced 200 min long half-life of antidiuresis were compared; in comparison to AVP this corresponds to 0.5–3 IU/mg.
[b] n.t., means not tested.
[c] In the range of doses 1×10^{-2} to 4×10^{-2} per rat, the analog dose dependently decreases blood pressure; the decrease is long lasting. At the same time it inhibits the response to standard doses of AVP.

known [36]. In contrast, a depressor effect observed under the conditions of standard pressor assay, is not a common phenomenon. Among the compounds related to those under the present discussion, [Gly-Cys[1], Tyr(Me)[2]] LVP shows [37] such a short lasting effect. Moreover, it was a potent inhibitor of the pressor effect of vasopressin. The dimer of DDAVP [38], when applied at doses of 0.05–0.1 mg/kg of body weight of the experimental animal (rat), displayed a marked hypotensive effect. Peptide **6**, described in this paper, has most probably the highest depressor activity of the compounds mentioned. Like [Gly-Cys[1], Tyr(Me)[2]] LVP, compound **6** inhibits the pressor effect of vasopressin. Quite recently, Chan *et al.* [39] described another compound displaying this quality, [Pmp[1], D-Tyr(Et)[2], Arg[3], Val[4]]AVP, which has a basic amino acid in position 3 like our compound.

NMR Study of Peptides 1 and 2

The 1D and 2D-NMR measurements on peptides **1** and **2** were carried out at 20, 40 and 60°C. The change of temperature influences the distribution of signals, their prospective overlapping and thus aids collection of NMR data. The common algorithm [40] based on the combination of 1D, 2D-COSY, 2D-TOCSY and 2D-ROESY spectra was employed for the identification of the individual residues and their sequential assignment. The sequential analysis was based on the NOE cross-peaks between the αH and NH proton of neighboring residues (d(αNH_i, H_{i+1})). The chemical shifts (at 20°C) and coupling constants are summarized in Table 3. The NMR spectra of peptides **1** and **2** show similar chemical shifts and coupling constants. Peptide **2** gives in general broader signals at room temperature (20°C). The line-widths of some signals, second order effects, and/or signal overlap prevented determination of some coupling constants.

The basic question is then the existence of a preferred conformation in solution. Small peptides are in general flexible molecules which can adopt many rapidly interconverting conformations in solution. Cyclization dramatically reduces the accessible conformational space. Cyclic peptides containing up to six or seven amino acids prefer only a limited number of backbone conformations (often only one), which are accompanied by more or less preferred side-chain conformations as well. The following NMR criteria can be used to indicate the extent of 'structuring' of the peptide in solution: (a) chemical shifts and coupling constants different from random coil values; (b) different NMR parameters of the same residue in different positions of peptide; (c) different temperature coefficients $\Delta\delta NH/\Delta T$ for individual residues.

The chemical shifts of the α-hydrogens were significantly different from random coil values (see Table 4) except for Tyr-2. In proteins, a sequence of such negative differences indicates a helical segment and positive differences indicate β-structure (chemical shift index [41]). Of the observed backbone coupling constants J(NH,αH) only values for Arg-4 (ca. 5 Hz) are in both peptides markedly lower than random-coil values (7–8 Hz).

The temperature coefficients of chemical shift dependence of amide hydrogens are given in Table 3. They demonstrate the markedly different behavior of the NH protons of Tyr-2 [or Tyr(Me)-2] and Arg-3, whose signals are nearly temperature independent ($\Delta\delta NH/\Delta T = -0.2$ and -0.5 ppb in **1** and -1.5 and -0.5 ppb in **2**), while the NH protons of Arg-4, Tyr-5 and Cys-6 show marked upfield shifts ($\Delta\delta NH/\Delta T = -3$ to 6 ppb in **1** and -2.8 to -7.8 ppb in **2**) with increasing temperature. Such observations indicate protection of Tyr-2 and Arg-3 amide hydrogens from solvent either by steric hindrance or participation in hydrogen bonds [42]. A conformation containing hydrogen bonds indicated is shown schematically in Figure 1.

The NOE contacts were derived from 2D-ROESY spectra measured at 40 and 60°C for peptide **1** and at 60°C for peptide **2**. Most of the observed cross-peaks represent intraresidual contacts. The remaining contacts are between hydrogens of neighboring (sequential) residues. From the view of possible attractive Tyr-Arg interactions, the most important are contacts between *ortho*-aromatic hydrogens of Tyr and βH and γH of Arg and between *meta*-aromatic hydrogens of Tyr and δH of Arg in both neighboring pairs (Tyr-2, Arg-3 and Tyr-5, Arg-6) of peptide **1**. Analogous contacts between Tyr(Me) and Arg in peptide **2** have not been proven. This may indicate the higher conformational flexibility of peptide **2**. No other contacts between side-chain hydrogens were detected in either peptide.

CONCLUSIONS

Six model peptides were prepared on the basis of the idea that the vasopressin molecule contains an active center comprising Cys[1], Tyr[2] and Arg[8]. Five of the peptides prepared showed distinct and dose-dependent antidiuretic and uterotonic activity but

Table 3 Proton NMR Parameters of Peptides **1** and **2** in d_6-DMSO

Residue	NH	$\Delta\delta$NH/ΔT (ppb)	JNHα	αH	$J\alpha\beta$	βH	$J\beta\beta$	Other H
Peptide **1**								
Cys-1	[a]	–	[a]	4.06	3.3[b] 9.6[b]	3.78 2.87	13.5	–
Tyr-2	8.59	−0.2	~7.5	4.40	5.0 9.4	2.97 2.74	14.0	C_6H_4: 7.03 (*m*-); 6.69 (*o*-); OH: 9.33
Arg-3	8.04	−0.5	~7[c]	~4.11	9.0[c] 5.4[c]	1.69 1.57	[a]	γH: 1.47; δH: 3.08; εNH_2: 7.73
Arg-4	8.35	−5.8	5.2[b]	3.82	~6.8[b] ~6.7[b]	1.56	[a]	γH: 1.42 and 1.32; δH: 3.03; εNH_2: 7.67
Tyr-5	8.32	−6.0	7.4[c]	~4.11	4.5 10.6	3.12 2.90	13.8	C_6H_4: 6.98 (*m*-); 6.67 (*o*-); OH: 9.22
Cys-6	8.26	−3.0	7.4	~4.11	3.8[b] 9.4[b]	3.31 3.25	13.8[b]	$CONH_2$: 7.26 and 6.94
Peptide **2**								
Cys-1	[a]	–	[a]	4.00	3.4[b] 9.5[b]	3.74 2.87	13.7[c]	–
Tyr(Me)-2	8.67	−1.5	[a]	4.42	5.3[c] 9.0[c]	3.08[c] 2.84[c]	14.2[c]	C_6H_4: 7.14 (*m*-); 6.84 (*o*-); OMe: 3.72
Arg-3	8.04	−0.5	6.8[c]	~4.13	9.0[c] 5.4[c]	1.72 1.61	[a]	γH: 1.51; δH: 3.12; εNH_2: 7.83
Arg-4	8.29	−4.2	5.6[c]	3.85	~6.9[c] ~6.5[c]	1.61	[a]	γH: 1.44 and 1.36; δH: 3.04; εNH_2: 7.74
Tyr(Me)-5	8.41	−7.8	7.5[c]	~4.17	4.2[c] 9.1[c]	3.13 2.93	14.0[c]	C_6H_4: 7.10 (*m*-); 6.82 (*o*-); OMe: 3.68
Cys-6	8.28	−2.8	7.6[b]	~4.14	4.2[c] 9.1[c]	3.28 3.20	13.8[c]	$CONH_2$: 7.26 and 6.98

[a] The value of this parameter could not be determined.
[b] J-value obtained at 40°C.
[c] J-value obtained at 60°C.

no pressor agonistic activity. In contrast, peptides **2**, **4** and **6** inhibited the pressor effect of AVP. Moreover, peptide **6** displayed a significant and long lasting depressor effect. Structural changes which were used by us in the development of DDAVP, i.e. 1-deamination and 8-D-substitution, caused analogous shifts of biological activities when incorporated into the model peptides **4–6**, just as they had produced in the vasopressin series. In analogy with the vasopressin series, methylation of hydroxyl group of

Table 4 Differences Between Observed and 'Random-Coil' Chemical Shifts of α-Hydrogens in Peptides **1** and **2**

Peptide	Cys-1	Tyr-2	Arg-3	Arg-4	Tyr-5	Cys-6
1	−0.58	−0.06	−0.27	−0.56	−0.35	−0.53
2	−0.64	−0.04	−0.25	−0.53	−0.29	−0.50

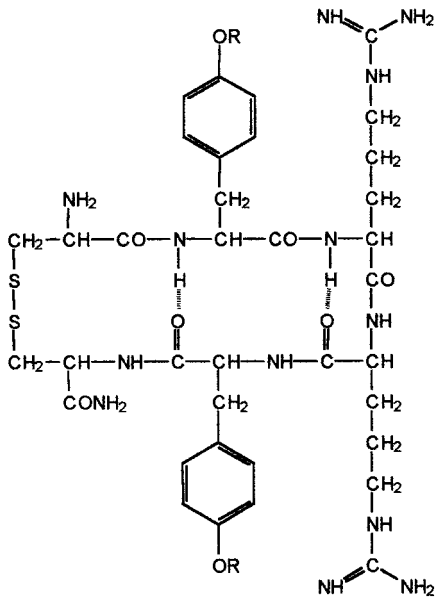

Figure 1 Conformation of peptides **1** and **2** containing hydrogen bonds: **1**, R=H; **2**, R=CH_3.

tyrosine strongly decreased the biological activities of **1**. The peptides under study evidently interacted in a specific way with the corresponding antidiuretic, pressor, and uterotonic receptors.

Our primary concept for the spatial arrangement of DDAVP involved the interaction of the acidic hydroxyl group of tyrosine and the strongly basic guanidine group of arginine. The results obtained in the present study furnished no direct evidence supporting this notion. NMR study provided basic data on the conformation of peptides **1** and **2**. It demonstrated the presence of hydrogen bonds between amino acid residues 3,4 and 2,5. Peptide **1** showed NOE contacts between side chains of the neighboring amino acid pairs Tyr-Arg and Arg-Tyr. No such contacts were detected in peptide **2**, which contains an *O*-methylated tyrosine residue. The observed NOE contacts are not directly related to our problem as they concern interactions of neighboring amino acid residues. However, such contacts may be expected to occur also in cases of conformational closeness of the mentioned residues in sequentially distant positions.

The results obtained show that the relationship between the model peptides and natural vasopressins is causal, and not only casual. They support the idea of the presence of an active center in the vasopressin molecule, comprising Cys^1, Tyr^2 and Arg^8, and show that this idea was more than a mere working hypothesis.

Acknowledgements

The authors are indebted to Dr A. Holý, director of the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, and to Dr. M. Flegel from Ferring – Léčiva a.s., for continuous support of this project. This project was partly supported by Grant Agency of the Czech Republic, grant No. 204/99/1453.

REFERENCES

1. Zaoral M, Kolc J, Šorm F. Synthesis of 1-deamino-8-D-γ-aminobutyrine-vasopressin, 1-deamino-8-D-lysine-vasopressin and 1-deamino-8-D-arginine-vasopressin. *Collect. Czech. Chem. Commun.* 1967; **32**: 1250–1257.
2. Zaoral M. Vasopressin analogs with high and specific antidiuretic activity. *Int. J. Peptide Protein Res.* 1985; **25**: 561–574.
3. Andersson KE, Bengtsson B, Paulsen O. Desamino-8-D-arginine vasopressin (DDAVP): Pharmacology and clinical use. *Drugs Today* 1988; **24**: 509–528.
4. Zaoral M. Research, development and practice utilization of DDAVP. *Cs. Pediat.* 1998; **53**: 506–511 (in Czech).
5. Vávra I, Machová A, Holeček V, Cort JH, Zaoral M, Šorm F. Effect of a synthetic analogue of vasopressin in patients with diabetes insipidus. *Lancet I* 1968; 948–952.
6. Lichardus B, Némethová V, Lehotská V. Ispolzovanie DDAVP pri lečenii gipofysarnogo diabeta, nočnogo enureza i pri bystrom opredelenii koncentracionnoj funkcii poček. *Sov. Med.* 1967; **10**: 106–109.
7. Marek J, Loutocký A, Pacovský V, Zaoral M. Ten year experience with DDAVP in treatment of diabetes insipidus. *Endocrinologie* 1978; **72**: 188–194.
8. Seckl JR, Dunger DB. Diabetes insipidus. Current treatment recommendations. *Drug* 1992; **44**: 216–224.
9. Birkášová M, Birkáš O, Flynn MJ, Cort JH. Desmopressin in the management of nocturnal enurensis in children: a double-blind study. *Pediatrics* 1978; **62**: 970–974.
10. Kovács L, Lehotská V, Kernová T, Šašinka M. Primárna nočná enuréza – staronový problém. *Detský lekár* 1994; **4**: 3–7.
11. Monothematic issue No. 3 devoted to Enuresis nocturna. *Česko-Slovenská Pediatrie* 1995; **50**: 131–171.
12. Cash JD, Gader AMA, Da Costa J. The release of plasminogen activator and factor VIII by LVP, AVP, DDAVP, AT II and OT in man. *Br. J. Haematol.* 1974; **27**: 363–364.
13. Manucci PM, Paretti FI, Holmberg L *et al.* Studies on the prolonged bleeding time in von Willebrand's disease. *Lab. Clin. Med.* 1976; **88**: 662–671.

14. Gader AMA, Da Costa J, Cash JD. A new vasopressin analogue and fibrinolysis. *Lancet II* 1973; 1417–1418.
15. Contributions in *Desmopressin in Bleeding Disorders*, Proc. NATO Adv. Res. Workshop, 1992. Mariani G, Manucci PM, Cattaneo M (eds). NATO ASI Series, Series A: Life Sciences, Vol. 240. Plenum Press: New York and London, 1993.
16. Cattaneo M. Review of clinical experience of desmopressin in patients with congenital and acquired bleeding disorders. *Eur. J. Anaesthesiol.* 1997; Suppl. 14: 10–14.
17. Lethagen S. *DDAVP (Desmopressin) and Hemostasis*. Doctoral Thesis, Department of Coagulation Disorders, Malmö, 1992.
18. Lethagen S. Desmopressin — a haemostatic drug: state-of-the-art review. *Eur. J. Anaesthesiol.* 1997; Suppl. 14: 1–9.
19. Hunt BJ. Desmopressin and bleeding during invasive surgery. *Eur. J. Anaesthesiol.* 1997; Suppl. 14: 42–47.
20. Némethová V, Lichardus B, Lehotská V. Erfahrungen mit einem DDAVP-Schnelltest zur Besimmung der Konzentrationsfähigkeit der Niere. *Monatschr. Kinderheil.* 1977; **125**: 165–167.
21. Janda J, Marek V, Reitspiesová K, Šikut M, Korous J. Standardizovaný koncentrační test s Adiuretinem Spofa u školních dětí. *Česko-Slovenská Pediatrie* 1982; **37**: 285–288.
22. Ohta M, Kimura T. Pitressin test and DDAVP test. *Nippon Rinsho* 1997; **55**: 303–306.
23. Zaoral M. *Studie v oblasti vasopresinových analogů*. Thesis, Institute of Organic Chemistry and Biochemistry, Prague.
24. Zaoral M, Kolc J, Šorm F. [8-α,β-diaminopropionic acid]-, [8-D-α,β-diaminopropionic acid]- and [8-D-ornithine]vasopressin. *Collect. Czech. Chem. Commun.* 1970; **35**: 1716–1726.
25. Zaoral M. Vztahy mezi strukturou látek a jejich biologickou účinností. In *Edice Macro N-13, cyclus Organic Chemistry*, Vol. 12, Bláha K, Tichý M (eds). Institute of Macromolecular Chemistry, Prague, 1985, 143–171.
26. Burn HJ, Finney DJ, Goodwin LG. *Biological Standardization*, 2nd edition. Oxford University Press: Oxford, 1950.
27. Vávra I, Machová A, I.Krejčí. Antidiuretic action of 1-deamino-8-D-arginine vasopressin in unanesthetized rats. *J. Pharmacol. Exp. Ther.* 1974; **188**: 241–247.
28. Dekanski J. The quantitative assay of vasopressin. *Br. J. Pharmacol.* 1952; **7**: 567–572.
29. Holton P. A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Br. J. Pharmacol.* 1948; **3**: 328–334.
30. Munsick RA. Effect of magnesium ions on the response of the uterus to neurohypophyseal hormones and analogues. *Endocrinology* 1960; **66**: 451–457.
31. Aue WP, Bartholdi W, Ernst RR.Two-dimensional spectroscopy. Application to nuclear magnetic resonance. *J. Chem. Phys.* 1976; **64**: 2229–2246.
32. Braunschweiler L, Ernst RR. Coherence transfer by isotropic mixing: application to proton correlation spectroscopy. *J. Magn. Reson.* 1983; **53**: 521–528.
33. Bax A, Davis DG. MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy. *J. Magn. Reson.* 1985; **65**: 355–360.
34. Bothner-By AA, Stephens RL, Lee J-M, Warren CD, Jeanloz RW. Structure determination of a tetrasaccharide: transient nuclear Overhauser effects in the rotating frame. *J. Am. Chem. Soc.* 1984; **106**: 811–813.
35. Bax A, Davis DG. Practical aspects of two-dimensional transverse NOE spectroscopy. *J. Magn. Reson.* 1985; **63**: 207–213.
36. Papsuevich OS, Chippens GI, Michailova SV. Biological activity and function of neurohypophyseal hormones. In *The Neurohypophyseal Hormones* (in Russian). Latv. Acad. Sci.: Zinatne, Riga, 1986, chapt. 2, pp. 42–43.
37. Zaoral M, Šorm F. Synthesis of Gly-Cys^1-lysine-vasopressin and Gly-Cys^1-Tyr(Me)^2-lysine vasopressin. *Collect. Czech. Chem. Commun.* 1965; **30**: 2812–2816.
38. Flegel M, Barth T, Zaoral M. Isolation and characterization of side products in [1-deamino-8-D-arginine]vasopressin synthesis. In *Peptides 1976*, Loffet A (ed.), Univ. Brussels: Brussels, 1977, 511–515.
39. Chan WY, Wo NC, Stoev S, Cheng LL, Manning M. Discovery of novel selective hypotensive vasopressin peptides. A new vasodilating vasopressin receptor? *Adv. Exp. Med. Biol.* 1998; **449**: 451–453.
40. Wüthrich K. Sequence-specific resonance assignment in proteins, in *NMR of Proteins and Nucleic Acids*. John Wiley & Sons: New York, 1986; chapt. 8, pp. 130–161.
41. Wishart DS, Sykes BD, Richards FM. The chemical shift index: A fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. *Biochemistry* 1992; **31**: 1647–1651.
42. Ohnishi M, Urry DW. Temperature dependence of amide proton chemical shifts: the secondary structure of gramicidin S and valinomycin. *Biochem. Biophys. Res. Commun.* 1969; **36**: 194–202.

