VASOPRESSIN AND OXYTOCIN ANALOGS WITH INTERCHANGED SEQUENCE OF AMINO ACIDS IN POSITIONS 7 AND 8. SYNTHESIS AND BIOLOGICAL EFFECTS*

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p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-N^G-*p*-toluenesulfanylarginyl-prolyl-glycineamide (*I*) and S-benzylcysteinyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteinyl-leucyl-prolyl-glycine amide (*III*) were prepared by solid phase synthesis. After removal of the protecting groups, closure of the disulfide ring, and purification by continuous free-flow electrophoresis [arginine^7, proline^8]vasopressin (*II*) and [leucine^7, proline^8]oxytocin (*IV*) were obtained. The antidiuretic effect of *II* is markedly higher than its pressor effect; *IV* possesses *c.* 6% of the uterotonic and c. 10% of the galactogogous effect of oxytocin.

The three-dimensional parameters of the side chain of the amino acid occupying the key position 8 are important for the magnitude and relative ratio of the two typical vasopressin effects. A change in shape and length of this chain and its deflection from the original position usually affects more the antidiuretic rather than the pressor effect and the corresponding analogs become antidiuretics more specifically acting than the naturally occurring hormones. Principally a similar effect should exert also the delocalization of the side chain along the backbone of the tripeptide 7–9. We prepared two analogs with the side chain shifted in such a manner, namely [Arg^7,Pro^8]vasopressin (*II*)** and [Leu^7,Pro^8]oxytocin (*IV*). The structure change which product *II* underwent (interchanged sequence of amino acids in positions 7 and 8) markedly differs from the changes to which the side chain had been subjected in our earlier experiments. The preceding alterations merely involved the shape and position of the side chain and were without any deeper effect on the conformation of the tripeptide backbone 7–9. The interchange of amino acids 7 and 8, however, affects both the position of the side chain and the three-dimensional arrangement of the peptide backbone. This interchange alters the position of the carboxyl terminal

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** The abbreviations and symbols common in peptide chemistry were used. All optically active amino acids were of L-configuration.

glycine amide which is very important for the high biological activity of the vasopressins and oxytocins. We expected therefore that products *II* and *IV* would possess relatively low yet for the given purpose sufficing biological effects. The synthesis of the protected linear precursors *I* and *III* was effected in the solid phase by using the usual combination of protecting groups (α-NH_2 Boc, terminal α-NH_2 Tos or Boc, —NH—C(=NH)NH_2 Tos, SH Bzl). The peptides were split off from the resin by ammonolysis and converted into products *II* and *IV* by the usual sequence of operations. The crude products were purified by free-flow electrophoresis. The biological effects were examined in the usual systems and are summarized in Table I. The interchange of the amino acid sequence in position 7 and 8 of arginine vasopressin affected both the vasopressin and oxytocin effects. In the first case the two typical vasopressin effects were decreased by two orders of magnitude, the antidiuretic effect being markedly higher than the pressor effect. The dissociation of both effects can be achieved, at least in principle, also by the shift of the basic amino acid caused by the interchange of amino acids in positions 7 and 8. In the second case, as regards the oxytocin effects, the interchange of amino acids in position 7 and 8 caused a mild yet distinct increase of the uterotonic effect as compared with arginine vasopressin and a decrease of the galactogogous effect by more than one order of magnitude. The shift of the arginine residue toward the cyclic moiety of the molecule practically eliminated the effect of the basic function and the uterotonic effect became a dominant biological property of [Arg^7,Pro^8]vasopressin (*II*). *II* can be regarded as a vasopressin analog with a considerable and considerably specific effect of the oxytocin type and with residual vasopressin effects. The interchange of the amino acids in position 7 and 8 of oxytocin (analog *IV*) caused a decrease of the uterotonic and galactogogous effect by *c.* one order of magnitude; the uterotonic effect was decreased more than the galactogogous effect (the vasopressin effects were not assayed). Unlike in the preceding case the specificity of the uterotonic effect was not increased but decreased.

TABLE I

Biological effects of [arginine^7, proline^8]vasopressin (*II*) and [leucine^7, proline^8]oxytocin (*IV*) [IU/mg]

Compound	Antidiuretic	Pressor	Uterotonic	Galactogogous
II	3·2	<1	20	1·6
IV	—	—	~27	~45

EXPERIMENTAL

All the general experimental details including the instruments used for the measurement and purification have been reported before[1]. Thin-layer chromatography was carried out in the system n-butyl alcohol–tert-butyl alcohol–acetic acid–water (2 : 2 : 1 : 1) on silica gel layer sheets Silufol (Kavalier, Votice, ČSSR). The samples for the analysis were dried 10 h at 100°C and a pressure of 10 Pa.

p-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-N^G-*p*-toluenesulfonylarginyl-prolyl-glycine Amide (*I*)

The synthesis was carried out on the chloromethylated polystyrene resin cross-linked by 2% of divinylbenzene (Calbiochem, Los Angeles, U.S.A.), chlorine content 0·96 mmol/g of resin, 0·35 mmol of Gly/g resin after esterification[2] by Boc-Gly.The synthesis was carried out with 4·28 g of esterified resin (1·5 mmol of Gly) in a manually operated synthetizer following the scheme described earlier[3] (program No 6). The yield was 1·9 g (80·5%), m.p. 162–174°C, after double crystallization from ethanol–water 1·55 g (71%), m.p. 203–205°C, $[\alpha]_D^{20}$ −19·5° (*c* 0·5, dimethylformamide), chromatographically homogeneous. Amino acid composition: Cys(Bzl) 1·93, Tyr 0·97, Phe 1·02, Glu 1·00, Asp 1·04, Arg 1·02, Pro 0·95, Gly 1·05. For $C_{74}H_{91}N_{15}O_{16}S_4 . 1/2\ H_2O$ (1 584) calculated: 56·11% C, 5·85% H, 13·26% N; found: 55·95% C, 5·74% H, 13·04% N.

[Arg^7,Pro^8]vasopressin (*II*)

Protected peptide *I* (400 mg) afforded after reduction by sodium in liquid ammonia, oxidation by potassium ferricyanide, desalting on an ion-exchange column (Amberlite IRC-50), and lyophilization[1] 170 mg of crude product *II*. The yield after purification by continuous free-flow electrophoresis[4] was 52 mg. The lyophilisate contained 79% of product *II* (average value calculated from the nitrogen content and from the polarographic determination of the peptide content[5]), $[\alpha]_D^{20}$ −42° (*c* 0·1, 1M acetic acid), amino acid composition: Tyr 1·03, Phe 0·97, Glu 1·06, Asp 1·08, Arg 0·93, Pro 0·91, Gly 1·02. For $C_{46}H_{66}N_{15}O_{12}S_2 . 1·5\ CH_3COOH.H_2O$ (1 193) calculated: 49·33% C, 6·25% H, 17·62% N; found: 49·21% C, 5·97% H, 17·51% N.

S-Benzylcysteinyl-tyrosyl-isoleucyl-glutaminyl-asparaginyl-S-benzylcysteinyl-leucyl-prolyl-glycine Amide (*III*)

The synthesis was carried out with the resin used for compound *I* which after esterification[6] by Boc-Gly contained 0·7 mmol of Gly/g of resin. The synthesis was carried out with 3 g of the esterified resin (2·1 mmol of Gly), as the synthesis of product *I*. The yield of the crude product split off from the resin by ammonolysis[3] was 1·82 g (73%), m.p. 182–189°C. The yield after double recrystallization from the mixture dimethylformamide–water was 1·47 g (59%), m.p. 204–206°C, $[\alpha]_D^{20}$ −42·4° (*c* 0·13, dimethylformamide), chromatographically homogeneous. amino acid composition: Cys(Bzl) 1·97, Tyr 0·93, Ile 1·03, Glu 0·99, Asp 1·01, Leu 1·03, Pro 0·99, Gly 1·02. For $C_{57}H_{80}N_{12}O_{12}S_2.H_2O$ (1 207) calculated: 56·70% C, 6·84% H, 13·92% N; found: 56·89% C, 6·70% H, 13·62% N.

[Leu^7,Pro^8]oxytocin (*IV*)

Protected peptide *III* afforded after reduction by sodium in liquid ammonia, oxidation by potassium ferricyanide, desalting on an ion-exchange column (Amberlite IRC-50), and lyophilization[1] 177 mg of crude product *IV*. The yield after purification by continuous free-flow electrophoresis[4]

was 61 mg. The lyophilisate contained 84% of compound *IV* (average value calculated from nitrogen content and polarographic determination of peptide content[5]), $[\alpha]_D^{20}$ −50·2° (*c* 0·12, 1M acetic acid), amino acid composition: Cys 1·95, Tyr 0·94, Ile 1·02, Glu 1·01, Asp 1·04, Leu 1·03, Pro 0·98, Gly 1·03. For $C_{43}H_{66}N_{12}O_{12}S_2.CH_3COOH.1{\cdot}5\ H_2O$ (1094) calculated: 49·39% C, 6·72% H, 15·36% N; found: 49·44% C, 6·43% H, 15·08% N.

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