MIMICKING BIOACTIVE PEPTIDES. [8-N^{β}-GUANIDOACETYL-α,β--DIAMINOPROPIONIC ACID]VASOPRESSIN

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The title compound was prepared by solid phase methodology. It has 34·2 I.U./mg of pressor activity, 5·8 I.U./mg of uterotonic activity and by 3—4 orders of magnitude lower antidiuretic activity than DDAVP.

The molecule of a biologically active compound evidently forms a kind of functional unit[1]. Its structure is differentiated at two or more levels. In principle, it contains elements responsible for its functionality, and a supporting structure. The presence of the functional elements, their nature, spacing and interactions are essential for the biological activity. The supporting structure is important but not essential. Any supporting structures ensuring functionality of the molecule are admissible. Thus compounds with quite dissimilar structures can have the same or similar biological properties*.

Some time ago we demonstrated[3] that peptide segments can mimic terpenoid compounds. The tripeptide ester, H-Ile-Ala-Pab-OEt, was up to twice as active as ±juvabione and had the same activity profile. Later on we showed[4] that lysine in *desamino*--LVP can be replaced by a composite isopeptide** structure, N^{β}-glycyl-α,β-diaminopropionic acid. The analog, [1-β-mercaptopropionic acid, 8-α-amino-β-glycylaminopropionic acid]vasopressin, had a relatively high and specific antidiuretic activity[4]. Since it is impossible to predict the influence of a structural change from a single result only, we have now prepared and investigated an analog of the *amino*-AVP series, [8-N^{β}-guanidoacetyl-α,β-diaminopropionic acid]vasopressin.

* Recommended abbreviations and symbols[2] are used. Further abbreviations: AD, antidiuretic activity; BP, blood pressor activity; UT, uterotonic activity; AVP, [8-arginine]vasopressin; LVP, [8-lysine]vasopressin; DCC, N,N′-dicyclohexylcarbodiimide; HOBt, N-hydroxybenzotriazole; Dap, α,β-diaminopropionic acid; Pab, *p*-aminobenzoic acid; DDAVP, [1-β--mercaptopropionic acid, 8-D-arginine]vasopressin.

** Isopeptide substitution was used in the field of neurohypophysial hormones for the first time by du Vigneaud and co-workers[5—7]. They employed it in the study of the effect of disulfide ring size on biological activities of oxytocin.

The synthesis of the analog was carried out by the solid phase procedure[8]. Using a previously described approach[9] we first prepared Tos-Cys(Bzl)-Tyr-Phe-Gln-Asn--Cys(Bzl)-Pro-Dap(Z)-Gly-(R). The Z group was split off by HBr/AcOH, and the β-amino group was acylated by N^G-Tos-guanidoacetic acid (DCC/HOBt). The protected peptide was split off from the support by ammonolysis. The product obtained was purified by crystallization from aqueous ethanol. The removal of the protecting groups, closure of the disulfide bridge, isolation, purification of the analog, and bioassays were carried out as usual[10]. [8-N^{β}-Guanidoacetyl-α,β-diaminopropionic acid]vasopressin had 34·2 I.U./mg of BP, 5·8 I.U./mg of UT and by 3–4 orders of magnitude lower* AD than DDAVP (ref.[10]) (assayed according to Barth and coworkers[11]). The results obtained clearly show that isosubstitution in position 8 leads also in the amino AVP series to an analog with marked biological activities.

EXPERIMENTAL

Melting points were determined on a Kofler block and are not corrected. Optical activity measurements were carried out in a Perkin–Elmer polarimeter, type 141. The analog was purified by continuous free-flow electrophoresis. The purity of the products was checked by TLC (Silufol, Kavalier Votice, solvent system: tert-butanol–1-butanol–acetic acid–water (2 : 2 : 1 : 1), detection with ninhydrin and by chlorination), by paper electrophoresis (Whatman No 3MM, 700 V (approx. 50 V cm^{-1}), aqueous acetic acid pH 2·5), and by reversed phase HPLC (Separon SI C18, mobile phase 4M-acetic acid–methanol (30 : 70, v/v), UV detection at 280 nm). Samples for amino acid analysis were hydrolysed 20 h/110°C in 6M-HCl and analyzed in a Beckman Spinco model 120B analyzer. Unless stated otherwise the samples for elemental analysis were dried for 24 h at 10 Pa over P_2O_5.

N^G-*p*-Toluenesulfonylguanidoacetic Acid Dicyclohexylammonium Salt

p-Toluenesulfonyl chloride (3·7 g, 20% excess) in 30 ml of acetone was added portionswise to a stirred and cooled (0°C) solution of 1·9 g (16·22 mmol) of guanidoacetic acid in 8·1 ml of 2M--NaOH. The pH of the reaction mixture was maintained at 12 by addition of 2M-NaOH. After completion of the reaction, the reaction mixture was extracted with ether (3×), concentrated in vacuo, acidified (HCl) to pH 2 and the product was extracted with EtOAc (3×). The combined extracts were washed with H_2O (2×), dried over Na_2SO_4, the solvent was distilled off and the residue dried in vacuo. The residue was dissolved in a small volume of EtOAc and an excess of dicyclohexylamine was added. The salt which had separated was filtered off and recrystallized twice from EtOAc–MeOH. Yield 5·1 g (69·5%), m.p. 218–220°C. For $C_{22}H_{36}N_4O_4S$ (452·6) calculated: 58·38% C, 8·02% H, 12·38% N; found: 58·14% C, 8·00% H, 12·35% N.

N-*p*-Toluenesulfonyl-S-benzylcysteinyl-tyrosyl-phenylalanyl-glutaminyl-asparaginyl--S-benzylcysteinyl-prolyl-N^G-*p*-toluenesulfonylguanidoacetyl-α,β-diaminopropionyl--glycine Amide

Chloromethylated styrene-divinylbenzene peptide resin (2% divinylbenzene, 5·9% Cl/g, Calbiochem, Los Angeles, U.S.A.) was esterified by Boc-Gly (KF procedure[12]) (0·7 mmol Gly/g). The

* AD of the analog was in the range 35–200 I.U./mg.

synthesis was carried out in a manually operated synthesizer according to a previously described scheme. From 1·43 g of the peptide resin 0·96 g (60%) of crude, protected, linear nonapeptide amide was obtained after ammonolysis of the peptide resin, m.p. 155—156°C. The product was reprecipitated twice from aqueous EtOH, m.p. 167—169°C. Amino-acid analysis: Tyr 0·93, Phe 0·99, Glu 1·07, Asp 1·04, Pro 1·03, Gly 1·01. (Under the used conditions Cys(Bzl) and Dap were incompletely separated). For $C_{74}H_{90}N_{15}O_{17}S_4.2\ H_2O$ (1 626) calculated: 54·66% C, 5·83% H, 12·92% N; found: 54·82% C, 5·66% H, 12·81% N.

[8-N^{β}-Guanidoacetyl-α,β-diaminopropionic acid]vasopressin

The linear, protected nonapeptide amide, obtained in the preceding experiment was reduced, oxidized, desalted and purified according to a previously described procedure[9]. From 500 mg of the linear, protected nonapeptide amide 233 mg of the crude and 92 mg of the purified product were obtained. The peptide content in the lyophilisate was 74·4%, $[\alpha]_D^{20}$ −47·7° (*c* 0·2, 1M-AcOH). The product behaved as a homogeneous compound in paper electrophoresis and in HPLC. Amino-acid analysis: Tyr 0·91, Phe 0·91, Glu 0·98, Asp 0·96, Pro 1·04, Dap 1·02, Gly 1·09. For $C_{46}H_{66}N_{15}O_{13}S_2.3\ CH_3COOH.2\ H_2O$ (1 317) calculated: 47·40% C, 6·27% H, 15·94% N; found: 47·65% C, 5·98% H, 16·13% N.

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