kidney, the predominant mechanisms responsible for maintaining the interstrain activity variation are different in the respective tissues.

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0014-4754/87/11/121209-03$1.50 + 0.20/0

Synthesis of three NH_2-terminally extended arginine-vasopressins with prolonged biological activities

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Summary. The synthesis of three novel AVP-analogues, extended by 1–3 amino acids at their NH_2-termini in accordance with the sequence of the bovine arginine-vasopressin neurophysin II precursor, is reported. The compounds were assayed for their antidiuretic and vasopressor activities with particular attention to the duration of the effects. All compounds showed high potency, based on the intensity, and prolonged effects in both test systems compared with AVP.
Key words. Prolonged vasopressin effect; solid phase peptide synthesis; vasopressin analogue; vasopressin precursor.

Protraction of peptide hormone activity with the aid of synthetic analogues has been attempted and accomplished according to various principles. In the vasopressin field one successful approach involved the preparation of hormonogens[1,2], from which, it was claimed, the hormone was continuously generated by gradual enzymatic degradation[3,4]. Thus, among several NH_2-terminally extended lysine-vasopressins with prolonged effects, Gly-Gly-Gly-lysine-vasopressin has found clinial applications in the treatment of bleeding disorders[5] and is marketed under the name of Glypressin.

With the recent publication of the sequence of the bovine arginine-vasopressin neurophysin II precursor[6], it became feasible to make and examine related vasopressin analogues with primary structures derived from this precursor. This paper describes the synthesis and some biological properties of three such analogues, Ala-AVP (I), Ser-Ala-AVP (II) and Thr-Ser-Ala-AVP (III). In this context it should be pointed out that the human vasopressin-containing gene has now also been sequenced[7]. Human AVP is immediately preceded by Ser-Ser-Ala instead of Thr-Ser-Ala.

Material and methods. The three protected peptides corresponding to the final products I–III were synthesized by the solid phase method[8,9] in the manual mode. When not otherwise stated, the general experimental conditions were as described previously[10]. Starting from 2.42 g of Boc-Gly-resin with a load of 0.62 mmol Gly/g[10], after 8 cycles, including deprotections, neutralizations and couplings with intermittent washings, 4.40 g of Boc-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin was obtained. All coupling steps were monitored by the Kaiser test.[11]. At this stage the resin was divided into 3 equal parts. The first one after another cycle with Z-Ala as carboxyl component, ammonolysis[9], extraction with warm DMF, precipitation with water followed by reprecipitation from DMF-EtOH-ether, gave Z-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (Ia) as a white powder. For further synthetic details and physical data, see table 1. Z-Ser(Bzl)-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-NH_2 (IIa) and Thr(Bzl)-Ser(Bzl)-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (IIIa) were prepared similarly from the second part through 2 cycles with Boc-Ala and Z-Ser(Bzl) and from the third part through 3 cycles with Boc-Ala, Boc-Ser(Bzl) and Boc-Thr(Bzl) as carboxyl components, respectively, followed in the last case by deprotection with HCl/HOAc, with further treatment of the resins as described for compound Ia. Data on IIa and IIIa are also given in table 1.

Aliquots of Ia, IIa and IIIa (200 mg), respectively, were treated in 400 ml of redistilled, sodium-dried ammonia at its boiling point under stirring with sodium, contained in a small-bore glass tube, until the blue color persisted for 30 s. Glacial acetic acid (0.40 ml) was added, the ammonia evaporated in vacuo, the residue dissolved in 0.2% aq. HOAc (800 ml) and titrated with 2 M NH_3 to pH 6.5. Excess $K_3Fe(CN)_6$ (0.01 M, 23 ml) was added gradually under stirring to give a yellow solution which was stirred for 10 min with an anion-exchange resin (Amberlite IR-45, acetate cycle, 20 g damp). After filtering and washing with 0.2% HOAc (100 ml), the combined extract was lyophilized to give a powder which was desalted on a Sephadex G-15 column (115 × 2.9 cm) in 50% HOAc (flow-rate 5.5 ml/h, monitoring at 254 nm). Pertinent fractions were pooled, lyophilized and again chromatographed on Sephadex G-15 (130 × 1.4 cm) in 0.2 M HOAc (flow-rate 3 ml/h) and after pooling and lyophilizing finally chromatographed on Sephadex LH-20 (135 × 1.8 cm) in 0.2 M HOAc to give pure I, II and III, respectively; these are further characterized in table 1.

Antidiuretic assays were performed in anesthetized hydrated

Table 1. Physico-chemical and other data on protected and free peptides

Compound No.	Yield (%)	Melting point (°C)	$[\alpha]_D^{20}$ (°)/c*	TLC on silica (Merck), R_F**	Amino acid analyses***
Ia	91	228–230	−41.5/0.5	0.48^A, 0.69^B, 0.75^C	–
IIa	89	226–229	−31.7/0.7	0.48^A, 0.73^B, 0.74^C	–
IIIa	85	193–196	−34.0/0.5	0.43^A, 0.74^B, 0.83^C	–
I	38	–	−62.7/0.6	0.08^A, 0.22^B, 0.50^D	0.98^A, 2.1^C, 1.01^D, 1.01^E, 0.99^F, 1.01^G, 0.98^P, 0.97^R, 1.01^Y
II	37	–	−77.3/0.4	0.08^A, 0.20^B, 0.48^D	0.99^A, 1.99^C, 1.02^D, 1.00^E, 0.98^F, 1.01^G, 0.98^P, 0.98^R, 1.01^S, 1.01^Y
III	30	–	−89.5/0.5	0.07^A, 0.19^B, 0.46^D	1.01^A, 1.96^C, 1.00^D, 1.01^E, 0.98^F, 1.00^G, 1.01^P, 0.99^R, 0.98^S, 0.99^T, 0.99^Y

*Solvent for Ia, IIa and IIIa was DMF, for I, II and III 1 N HOAc. **Solvent systems were 1-BuOH-HOAc-H_2O, 4:1:5, upper phase (A); 1-BuOH-HOAc-H_2O-pyridine, 15:3:3:10 (B); $CHCl_3$-CH_3OH, 7:3 (C); 1-BuOH-HOAc-H_2O-pyridine, 15:3:12:10 (D).*** The one-letter notation for the amino acids is used.

Table 2. Vasopressor and antidiuretic potencies and indexes of persistence (IP) of N-terminally extended analogues of arginine-vasopressin. The IP values are related to arginine-vasopressin.

X-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

Analogue No. X	Vasopressor activity Potency IU/μmol	IP	Antidiuretic activity Potency IU/μmol	IP
I Ala	147 ± 20.8	2.7 ± 0.6	52.0 ± 4.6	2.9 ± 0.5
II Ser-Ala	109 ± 14.9	2.2 ± 0.3	130 ± 11.1	3.9 ± 0.9
III Thr-Ser-Ala	86.0 ± 14.8	3.3 ± 0.6	48.3 ± 4.0	3.8 ± 0.3

Sprague-Dawley female rats[12]. Potency expressed as intensity was based on the maximal increase of urine conductivity after intravenous injections. Vasopressor assays were performed in anesthetized rats treated with dibenamine using intravenous injections and the maximal pressor response was used as the effect parameter[13]. In both instances AVP was used as the standard and the potency of each analogue was assessed according to a four-point design[14].

Besides the estimation of the antidiuretic and vasopressor potencies based on the intensity, the index of persistence (IP) was also determined[15]. This dimensionless factor is a measure of the duration of the effect of the analogue in relation to a standard. The biological results are summarized in table 2.

Results and discussion. The chemical procedures described above furnished the three peptides, I, II and III, pure by TLC in three systems (table 1). These peptide preparations were used in the pharmacological experiments summarized in table 2.

In comparison with the pressor and antidiuretic (AD) potencies of related NH_2-terminally extended LVP and AVP analogues[1,5,16], the corresponding potencies of our new AVP analogues are high. Thus, the pressor potency of Ala-AVP (peptide I), which is significantly higher than its AD activity, exceeds by far the value previously given for Ala-LVP[16] (147 vs 5.7 IU/μmol). This difference is surprisingly high in view of earlier reported similarities of potencies between analogous LVP and AVP derivatives[17]. Furthermore, some AVP analogues[18], extended at their NH_2-termini with 3 or 4 amino acid residues, give pressor potency values of 10% or less compared with the value of Thr-Ser-Ala-AVP (peptide III). All three peptides (I–III) show protracted rat AD and pressor activities in comparison with AVP as reflected in their IP values (table 2). The new analogues exhibit slightly more persistent effects in the AD assays than in the pressor assays (IP values ranging from 2.9 to 3.9 vs from 2.2 to 3.3). Our IP values are in the same range as those earlier reported for NH_2-terminally extended LVP analogues[16]. For the latter substances there has been shown[19] the existence of an inverse relationship between potency and duration of the effect which would be expected if LVP was generated under the influence of some enzyme(s). In the present study it has not been possible to observe any such relationship since the variation of potency and IP values between our three analogues is small.

However, by extension of the NH_2-terminal part of the AVP molecule we could demonstrate that it is possible to obtain analogues with high potency and at the same time obvious, protracted biological activity. Whether this is achieved via a hormonogenic mechanism and/or caused by the peptides per se will have to be settled by further studies.

This investigation was supported by grants from the Polish Academy of Sciences (CPBP 06.03.4.1. to G.K.) and the Swedish Natural Science Research Council (to U.R.), which are both gratefully acknowledged.

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