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Analogues of Arginine Vasopressin Modified in Position 2 or 3 with Naphthylalanine: Selective Antagonists of Oxytocin In-vitro

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Abstract

In this study we describe the synthesis and some pharmacological properties of six new analogues of arginine vasopressin (AVP).

Five of the peptides were substituted in position 2 with L-1-naphthylalanine (L-1-Nal) or D-1-naphthylalanine (D-1-Nal); one had D-1-Nal in position 3. All analogues were tested in bioassays for pressor and antidiuretic activity. We also tested the uterotonic activity of the peptides in-vitro. Two of the new peptides were moderately potent V_{1a} and oxytocin antagonists. The modifications proposed resulted in a drop or the removal of antidiuretic activity and in the removal of pressor activity, or conversion into moderate antagonists. Two peptides ([Mpa^1, (L-1-Nal)^2]AVP and [Mpa^1, (D-1-Nal)^2]AVP) which appear not to interact with V_{1a} and V_2 receptors were exceptionally selective oxytocin antagonists in-vitro.

These compounds with selective oxytocin antagonistic activity may be promising candidates for the development of potential tocolytic agents for the prevention of pre-term labour.

The following receptor subtypes for AVP have been characterized to date: V_{1a}, V_{1b} and V_2. V_{1a} receptors modulate the vasopressor actions of AVP. They have been shown to be present in many tissues including brain (Jard et al 1987). V_{1b} receptors stimulate the adrenocorticotropic hormone (ACTH)-releasing effects of AVP from the anterior pituitary (Jard 1988). Both receptor subtypes act via the phosphoinositol pathway. Antidiuretic responses to AVP are modulated by V_2 receptors, present in the renal tubule, particularly the collecting duct. They are linked to adenylate cyclase metabolism (Jard 1988).

Vasopressin agonists and antagonists have been used as pharmacological and therapeutic tools in animal and human physiology and pathophysiology. However, the design and structure–activity studies of analogues that are truly selective for AVP receptors is still a field of great interest. For example, existing V_{1a} and V_2 antagonists also have antioxytocic activity and known blockers of oxytocic receptors possess anti-V_{1a} activity (Manning et al 1987; Chan et al 2000).

Recently, we described the synthesis and some pharmacological properties of analogues having L-1-naphthylalanine (L-1-Nal) or L-2-naphthylalanine (L-2-Nal) in position 3 (Lammek et al 1997). One of the new analogues, [(L-2-Nal)^3(D-Arg)^8]VP, was among the most potent and selective antagonists of the V_{1a} receptors reported to date. Moreover, it was the first V_{1a} antagonist devoid of anti-uterotonic activity, and its high antipressor potency arises without modification of position 1, which was previously thought to be essential for substantial pressor antagonism (Manning et al 1987). Two other peptides [Mpa^1,(L-2-Nal)^3,(D-Arg)^8]VP and [Mpa^1,(L-1-Nal)^3,(D-Arg)^8]VP were highly potent V_2-agonists. The second peptide was exceptionally selective. These results prompted us to investigate further the influence of naphthylalanine on pharmacological properties of AVP analogues. We synthesized and evaluated the biological activity of the following analogues: [(L-1-naphthylalanine)^2]AVP, [(L-1-Nal)^2]AVP (**1**), [(3-mercaptopropionic acid)^1,(L-1-naphthylalanine)^2]AVP, [Mpa^1,(L-1-Nal)^2]AVP, (**2**), [(D-1-

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naphthylalanine)^2]AVP, [(D-1-Nal)^2]AVP, (**3**), [(3-mercaptopropionic acid)^1, (D-1-naphthylalanine)^2]AVP, [Mpa^1,(D-1-Nal)^2]AVP, (**4**), [(1-mercaptocyclohexaneacetic acid)^1,(D-1-naphthylalanine)^2, (valine)^4]AVP, [Cpa^1,(D-1-Nal)^2,Val^4]AVP, (**5**) and [(D-1-naphthylalanine)^3]AVP, [(D-1-Nal)^3]AVP, (**6**).

We assumed that the hindering effect caused by the naphthylalanine ring would, as in previous cases, have a significant impact on the conformation of the cyclic part of AVP analogues and could thus greatly influence its interaction with AVP receptors. First, we replaced only one position of the AVP molecule to learn how single substitution at position 2 with both L-1-Nal or D-1-Nal would influence the pharmacological properties of the resulting analogues **1** and **3**. In the next two peptides (**2** and **4**) we combined the above modification with deamination which is considered the most effective individual structural change to increase antidiuretic activity (Manning et al 1987). Analogue **5** was designed by substitution of position 2 with D-1-Nal linked with replacement of amino acid residues in positions 1 and 4 with 1-mercaptocyclohexaneacetic acid and valine, respectively. We expected that modification of position 1 should result in antagonistic properties of the analogue, while substitution of position 4 should impart increased selectivity (Manning et al 1987). Finally, we checked how placement of D-1-Nal in position 3 would change the pharmacological properties of the resulting analogue **6**. The structures of the synthesized analogues are shown in Table 1.

Materials and Methods

The optical rotations were measured using a Perkin-Elmer Model 141 polarimeter. For amino acid analysis, the peptides (0·5 mg) were hydrolysed with constantly boiling hydrochloric acid (400 μL), containing phenol (20 μL), in evacuated sealed ampoules left for 18 h at 110°C. The analyses were performed on a Microtechna type AAA881 analyser. The melting point values are uncorrected. TLC was carried out on silica plates (Merck), and the spots were visualized by iodine or ninhydrin. The solvent system used was butan-1-ol–acetic acid–water (4:1:5, v/v), upper phase. The purity of the peptides was also determined by HPLC. Analyses of the analogues were performed on a Gold System Beckman chromatograph with an Ultrasphere ODS column (5 μm; 4·6 × 250 mm) or Vydac C18 column. Solvent system: (i) 0·1% aqueous trifluoroacetic acid (TFA), (ii) acetonitrile–0·1%TFA (80:20, v/v), isocratic system 45% of (ii) for peptide **1**, 42% of (ii) for peptide **2**, 47% of (ii) for peptide **3**, 40% of (ii) for peptide **4**, 46% of (ii) for peptide **5**, 30% of (ii) for peptide **6**, $\lambda = 226$ nm, flow rate 1 mL min^{-1} for analogues **4** and **6** and 2 mL min^{-1} for other peptides.

Table 1. Structures of the synthesized analogues of AVP.

	X-Y-Z-M-Asn-Cys-Pro-Arg-Gly-NH_2			
1	X = Cys,	Y = L-1-Nal,	Z = Phe,	M = Gln
2	X = Mpa,	Y = L-1-Nal,	Z = Phe,	M = Gln
3	X = Cys,	Y = D-1-Nal,	Z = Phe,	M = Gln
4	X = Mpa,	Y = D-1-Nal,	Z = Phe,	M = Gln
5	X = Cpa,	Y = D-1-Nal,	Z = Phe,	M = Val
6l	X = Cys,	Y = Tyr,	Z = D-1-Nal,	M = Gln

The Cpa(Bzl) and Mpa(Bzl) were synthesized in this laboratory by procedures described previously (Schonberg & Iskander 1942; Rekowski & Lammek 1987).

Peptide synthesis

The protected peptide precursors: Z-Cys(Bzl)-(L-1-Nal)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**1a**), Mpa(Bzl)-(L-1-Nal)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**2a**), Z-Cys(Bzl)-(D-1-Nal)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**3a**), Mpa(Bzl)-(D-1-Nal)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**4a**), Cpa(Bzl)-(D-1-Nal)-Phe-Val-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**5a**), Z-Cys(Bzl)-Tyr(Bzl)-(D-1-Nal)-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (**6a**), required for the synthesis of peptides (**1**–**6**) were prepared by the solid-phase method of peptide synthesis entirely on resin (Manning 1968; Lammek et al 1988). Each protected peptide was synthesized by the stepwise coupling of Boc-amino acids to the growing peptide chain on a chloromethylated Merrifield resin. *N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteine, *S*-benzyl-1-mercaptocyclohexaneacetic acid or *S*-benzyl-3-mercaptopropionic acid was used in the final coupling steps. After completion of the synthesis, the protected nonapeptidyl resins or acyloctapeptidyl resins were ammonolysed in methanol (Manning 1968; Lammek et al 1988). After evaporation of the solvent, the products were extracted into hot dimethylformamide (DMF), precipitated with boiling water and left overnight at room temperature (20°C). The peptides were collected by filtration, washed with water and dried in-vacuo over P_2O_5. The products were further purified by dissolving in DMF and reprecipitating with CH_3OH–Et_2O (1:3). The physicochemical properties of

Table 2. Physicochemical data for protected intermediates.

Substance	Yield (%)	M.p (°C)	$[\alpha]_D^{20}$ DMF	TLC (R_F)[a]
1a	51.4	202–205	−39·97° c = 1·01	0·73
2a	46·3	183–186	−42·57° c = 0·99	0.44
3a	59·3	195–203	−14·16° c = 0·72	0·73
4a	52·5	201–206	−12.42° c = 0·52	0·73
5a	51·8	198–201	−10·82° c = 1·27	0·75
6a	69·2	238–240	−8·1° c = 1·00	0·58

DMF, *N,N*-dimethylformamide. [a]Butan-1-ol–acetic acid–water (4:1:5, v/v), upper phase.

the compounds **1a–6a** are summarized in Table 2. A solution of the peptide intermediate (**1a–6a**) (0·15 mmol) in sodium-dried and redistilled ammonia was treated at boiling point and with stirring with sodium from a stick of the metal contained in a small-bore glass tube until a light-blue colour persisted in the solution for 30 s, NH_4Cl was added to discharge the colour. The solution was evaporated, the residue dissolved in glacial acetic acid (150 mL) and the solution diluted with methanol (1000 mL). An excess solution of iodine in methanol (0·1 mol L^{-1}) was added gradually with stirring. The light-yellow solution was stirred for an additional 2 min and then for 10 min with anion exchange resin (Dowex 1 × 8, 100–200 mesh, acetate form, 10 g damp weight). The reaction mixture was filtered through a bed of resin (10 g damp weight). The bed was washed with methanol (100 mL) and the combined filtrate and washings were evaporated under reduced pressure. The resulting material was dissolved in 7 mL aqueous acetic acid (30%) and desalted on a Sephadex G–15 column (85 × 3·5 cm) eluted with aqueous acetic acid (30%) with a flow rate of 4·5 mL h^{-1}. The eluate was fractionated and absorbance monitored at 254 nm. The fractions comprising the major peak were pooled and lyophilized and the residue further subjected to gel filtration on a Sephadex LH–20 column (90 × 2·9 cm) eluted with 30% aqueous acetic acid with a flow rate of 2.0 mL h^{-1}. Purification of the fractions comprising the major peak was done by HPLC in the same solvent systems as described for analytical purposes. The peptide was eluted as a single peak.

Lyophilization of the pertinent fractions gave the vasopressin analogue. The purity and identity of each peptide was ascertained by thin-layer chromatography, HPLC, FAB mass spectrometry (molecular ion) and amino acid analysis. The purity of all peptides was between 97 and 99%. The values of molecular ions and results of amino acid analyses were as expected.

Because some of our analogues were found to possess very interesting, but surprising, pharmacological properties we resynthesized [(L-1-Nal)^2]AVP and [Mpa^1,(L-1-Nal)^2]AVP on a methoxybenzhydryl resin using *p*-methoxybenzyl protection for Cys and Mpa. The Mpa derivative was obtained as described previously (Schonberg & Iskander 1942). After synthesis was completed, 1 g of the protected peptidyl or acylpeptidyl resin was treated with 10 mL liquid hydrogen fluoride (HF) containing 1 mL anisole at −70°C and stirred for 50 min at 0°C. After the removal of HF and anisole in-vacuo, the mixture was washed with anhydrous diethyl ether and then with 20% acetic acid. Oxidation and purification of analogues was achieved as described above for peptides **1–6**. The physicochemical and pharmacological properties were found to be identical.

Bioassay methods

The vasopressor and antidiuretic activity of AVP analogues were tested on male Wistar rats, 250–270 g, bred at the Central Experimental Animal Farm of the Silesian Medical University, Poland.

Vasopressor activity. Vasopressor assays of AVP analogues were performed in-vivo according to the method of Dekanski (1952) as described by Stürmer (1968). The rats were given 1·75 g kg^{-1} urethane intraperitoneally. Blood pressure stabilization was evoked by phenoxybenzamine in two or three repeated doses (each amounting to 1 mg kg^{-1}, i.v.). Peptides were injected into the cannulated jugular vein in a volume of 0·1 mL (saline) and the cannula was rinsed with 0·2 mL saline. Analogues were injected at 30–40 min intervals. Systolic blood pressure measurements were taken by pressure transducer fixed into the carotid artery.

Antidiuretic activity. Antidiuretic activity of peptides was estimated in-vivo, in rats under ethanol anaesthesia according to Stürmer's modification (Stürmer 1968) of the method of Sawyer (1958). The experiments were carried out under general anaesthesia brought about by the intraperitoneal administration of 125 mg kg^{-1} thiobutabarbital (Inactin, RBI, USA). Peptides were injected into the jugular vein in a total volume of approximately 0·3 mL (including wash) at 40-min intervals. After laparotomy, a polyethylene cannula was introduced into the urinary bladder and ligated. The urine flow was collected directly from the urinary bladder in

subsequent 10-min periods after analogue injection and measured by pipette connected to a vacuum system. Water balance was kept constant for each rat by rehydrating the 0·35% sodium chloride solution through a stomach tube at a volume equal to the excreted urine, adding arbitrary excess (1–2 mL h^{-1}) to compensate for extrarenal water loss. Antidiuretic response was defined as:

$$(V_p/V_o) \times 100$$

where V_o is the urine volume collected in the 10 min period before the injection of AVP or analogue and V_p is the volume of urine collected in the 10 min period starting immediately after injection. Agonistic activity was expressed in IU mg^{-1} through the comparison of regression lines for AVP and related peptide (Feder et al 1991).

Antagonistic activity of AVP analogues was measured by the method of Schild (1947) and expressed as effective dose (ED) and pA_2. ED is the dose of an antagonist which reduces the response to 2 × units of agonist to the response evoked by 1 × unit of agonist. Each peptide was administered in two doses: high, which reduces the response to 2 × units of agonist to less than the response to 1 × unit of agonist, and low, which did not fully reduce the response obtained by 2 × units of agonist. ED50 values were estimated by interpolation on a logarithmic scale between two doses of antagonist and expressed in nmol kg^{-1}. pA_2 values estimated in-vivo represent the negative logarithms (to the base 10) of effective doses divided by an assumed volume of distribution in rats (67 mL kg^{-1}) (Manning & Sawyer 1985). AVP was used as a standard agonist in all assays. Its pressor and antidiuretic activity was accepted as 369 and 323 IU mg^{-1} respectively (Manning & Sawyer 1985).

Bioassay of uterotonic and antiuterotonic activity in-vitro. Antiuterotonic and uterotonic activity were assayed on isolated rat uterus using the modified procedure of Holton (1948). Briefly, virgin female Wistar rats (Mollegard, Denmark), 180–220 g, in proestrus/oestrus stage artificially induced 24 h earlier by injection of oestradiol benzoate (0·8 mg kg^{-1}, i.m.), were used. Oestrus stages were determined by vaginal smears made 1–2 h before each experiment.

The distal parts (about 2 cm) of both uterine horns were removed under Inactin (RBI, USA) anaesthesia (125 mg kg^{-1}, i.p.) and immediately mounted in a 20-mL bathing chamber. The bathing fluid was van Dyke-Hastings solution, in Munsick modification supplemented with Mg^{2+} (Munsick 1960).

The solution had the following composition (mM): NaCl 114.0; KCl 6·2; $NaHCO_3$ 30.0; NaH_2PO_4 1.0; $CaCl_2$ 1.0; $MgCl_2$ 0·5 and glucose 2·8. The reservoir and the bath were gassed with a mixture of 95% O_2 and 5% CO_2. The temperature of the bath solution was 37°C.

Contractions were measured isometrically with a force displacement transducer K-30 (type 351), two-channel bridge amplifier (type 301, Hugo Sachs Electronics; Germany) and recorded by multi-pen recorder R-50 (model 83, Rikadenki Electronics; Japan). The tissue was subjected to a base-line tension of 1 g, and if regular phasic tension development was not maintained during an initial 30 min stabilization period it was discarded.

The uterotonic activity was measured for 10 min by integration of the area under the response curve after the addition of 5–7 concentrations of oxytocin (0·3–300 nmol L^{-1}) or AVP (10–1000 nmol L^{-1}). The organ bath was washed three times with bathing solution between each application of agonist. The agonistic activity was expressed as pD_2, defined as a negative logarithm of agonist concentration that produces 50% of maximum effect caused by the agonist.

Antagonistic potency of the AVP analogues at a concentration of 10^{-7} mol L^{-1} was estimated by the same procedure, but oxytocin or AVP was given 1 min after peptide administration. Inhibition was expressed as a percentage of the mean response obtained with the agonist given alone at the beginning and given together with antagonists at the end for each estimation. The results are presented as the individual concentration–response curve for each peptide (Microsoft Excel 6.0) (Figures 1 and 2).

To express the antagonistic activity of AVP analogues as a pA_2 value, a similar procedure was used. Three different concentrations of both antagonists of oxytocin or AVP were used which reduced the responses to the agonists by approximately 25, 50 and 75%, respectively. The pA_2 value is defined as the negative logarithm of the molar concentration of an antagonist, which reduces the effect of a concentration of agonist to that of half the concentration. The mean values of concentration ratios for the oxytocin or AVP antagonist pair were calculated by the method of Schild (1947) and plotted in a diagram by regression analysis (Tallarida & Murray 1986). The pA_2 value was obtained from the intercept of the regression line with the abcissa (Tallarida & Murray 1986).

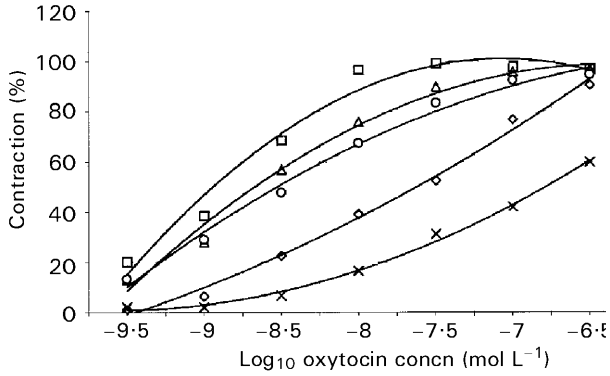

Figure 1. Effect of AVP analogues on uterine contraction caused by oxytocin. Results are expressed as arithmetic means for 4 horns of rat uterus. Oxytocin (□); [L-1-Nal^2]AVP (△); [Mpa1, L-1-Nal^2]AVP (○); [D-1-Nal^2]AVP (◇); [Mpa1, D-1-Nal^2]AVP (×).

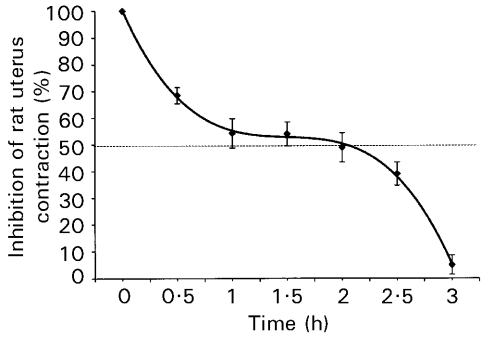

Figure 3. Long-term anti-uterotonic activity of [Cpa^1, D-1-Nal^2, Val^4]AVP in-vitro. Inhibition of uterine contraction was measured at concentrations of 1×10^{-8} mol L^{-1} for oxytocin and analogues (n = 5–10). Results are expressed as arithmetic means for 5–10 horns of rat uterus.

Estimation of pA$_2$ and response time curve for long acting peptide. Anti-uterotonic activity of [Cpa^1,D-1-Nal^2,Val^4] AVP was measured according to the above verified procedure for pA$_2$ calculation with slight modification (Wahrenburg et al 1975; Melin et al 1986).

The long-term action of the peptide allowed only single experiments for each horn of rat uterus and for only two concentrations, for example 1×10^{-8} and 2×10^{-8} mol L^{-1} for measurement of the area under the curve after oxytocin (1×10^{-8} mol L^{-1}) administration. The mean value of pA$_2$ was calculated from horns of 9 rats (Figure 3).

The response contraction of uterus (10 min) to oxytocin (10^{-8} mol L^{-1}) was measured every 30 min from time 0 to 3 h after administration of [Cpa^1, D-1-Nal^2,Val^4]AVP (10^{-8} mol L^{-1}). The response time curve was obtained as a percentage of maximum inhibition response to oxytocin measured in the same time from the control and treated horn of rat uterus. Values were plotted as means ± s.d. for 6–10 horns of uterus (3–5 rats).

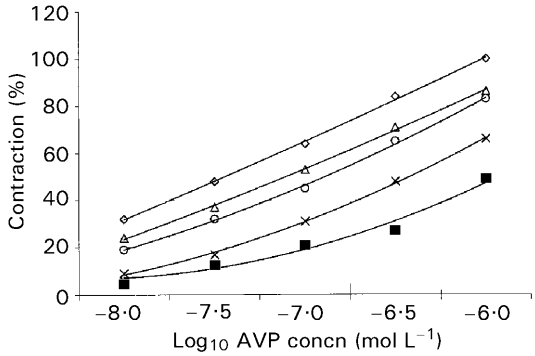

Figure 2. Effect of AVP analogues of uterine contraction caused by AVP. Results are expressed as arithmetic means for 4 horns of rat uterus. AVP (◇); [L-1-Nal^2]AVP (△); [Mpa1, L-1-Nal^2]AVP (○); [D-1-Nal^2]AVP (×); [Mpa1, D-1-Nal^2]AVP (■).

Results

Some pharmacological properties of the new analogues **1**–**6** are presented in Table 3. None of the peptides exhibited pressor or anti-antidiuretic activity.

The vasopressin antagonistic activity of analogues was measured according to method of Schild (1947) as described by Manning & Sawyer (1985). Only two peptides; [(D-1-Nal)^2]AVP (**3**) and [Cpa^1,(D-1-Nal)^2,Val^4]AVP (**5**) with pA$_2$ values of 7·1 and 7·3, exhibited a moderate V$_{1a}$-antagonistic potency; all the others were inactive.

The antidiuretic activity of the analogues was estimated according to Stürmer (1968) and Sawyer (1958). From the results it is clear that the modifications proposed resulted in almost complete removal of interaction of peptides with V$_2$-receptors. Only two analogues, [(D-1-Nal)^2]AVP (**3**) and [(D-1-Nal)^3]AVP (**6**), had negligible antidiuretic activity.

Uterotonic and anti-uterotonic activity was assayed on an isolated rat uterus by the procedure of Holton (1948). In this assay only analogue [(D-1-Nal)^3]AVP showed very low agonistic potency (Table 3). Peptides **1**–**5** with pA$_2$ ranging from 6·9 to 7·93 were oxytocin antagonists. Analogues **1**, **2** and **4** were exceptionally selective. Peptides **1**–**5** also blocked the contraction of rat uterus caused by AVP (pA$_2$ 7·1–8.05). The inhibition of uterine contraction (caused by oxytocin or AVP) in the presence of AVP analogues is presented in Figures 1 and 2. One of the peptides, [Cpa^1,(D-1-Nal)^2,

Table 3. Pharmacological properties of the new analogues of AVP.

Analogue	Pressor ($IU\,mg^{-1}$)	Antivasopressor pA_2	Antidiuretic ($IU\,mg^{-1}$)	Uterotonic pD_2	Anti-uterotonic pA_2	
					Oxytocin	AVP
AVP[a]	369 ± 6	–	323 ± 16	$7{\cdot}38 \pm 0{\cdot}05$ (n = 4)	–	–
[Cpa[1],Val[4]]AVP[b]	NA	$7{\cdot}97 \pm 0{\cdot}06$ (n = 8)	$0{\cdot}32 \pm 0{\cdot}02$ (n = 4)	–	$7{\cdot}34 \pm 0{\cdot}07$	–
[Mpa[1],]AVP[c]	346	–	1745	63	–	–
[(L-1-Nal)[2]]AVP (**1**)	NA (n = 4)	NA (n = 4)	NA (n = 4)	NA	$6{\cdot}90 \pm 0{\cdot}12$ (n = 6)	$7{\cdot}10 \pm 0{\cdot}06$ (n = 4)
[Mpa[1],(L-1-Nal)[2]]AVP (**2**)	NA (n = 4)	NA (n = 4)	NA (n = 4)	NA	$7{\cdot}03 \pm 0{\cdot}12$ (n = 11)	$7{\cdot}20 \pm 0{\cdot}07$ (n = 4)
[(D-1-Nal)[2]]AVP (**3**)	NA (n = 4)	$7{\cdot}1 \pm 0{\cdot}53$ (n = 8)	$(13{\cdot}3 \pm 3{\cdot}07) \times 10^{-3}$ (n = 8)	NA	$7.47 \pm 0{\cdot}15$ (n = 8)	$7{\cdot}78 \pm 0{\cdot}09$ (n = 4)
[Mpa[1],(D-1-Nal)[2]]AVP (**4**)	NA (n = 4)	NA (n = 4)	NA (n = 4)	NA	$7{\cdot}69 \pm 0{\cdot}07$ (n = 6)	$7{\cdot}94 \pm 0{\cdot}23$ (4)
[Cpa[1],(D-1-Nal)[2],Val[4]]AVP (**5**)	NA (n = 4)	$7{\cdot}3 \pm 0{\cdot}41$ (n = 8)	NA (n = 4)	NA	$7{\cdot}93 \pm 0{\cdot}21$ (n = 9)	$8{\cdot}05 \pm 0{\cdot}12$ (n = 4)
[(D-1-Nal)[3]]AVP (**6**)	NA (n = 6)	NA (n = 6)	$(6{\cdot}5 \pm 2{\cdot}4) \times 10^{-3}$ (n = 12)	$5{\cdot}89 \pm 0{\cdot}09$ (n = 6)	–	–

Val[4]]AVP (**5**), showed protracted antioxytocic effect, having a half-time of approximately 2 h (Figure 3).

Discussion

Previously we reported that the hindering effect caused by the bulky naphthyl moiety in position 3 of AVP analogues had a significant impact on the bioactive conformations of molecules and thus influenced their interaction with V_{1a}, V_2 and oxytocic receptors. Some of the new analogues designed in this manner exhibit interesting and in some cases surprising pharmacological properties (Lammek et al 1997). In this study we wanted to determine how the substitution at position 2 or 3 of AVP (compounds **1**, **3** and **6**) or some of its agonistic (peptides **2** and **4**) or antagonistic analogues (peptide **5**) with L-1-naphthylalanine or D-1-naphthylalanine would change the pharmacological properties of the resulting compounds.

Substitution of Tyr^2 in the AVP molecule with L-1-Nal gave peptide **1** which did not interact with V_{1a} and V_2 receptors. However, it showed weak anti-uterotonic activity in-vitro. Inversion of the configuration of the Nal residue introduced into position 2, converted analogue **1** into a moderately potent V_{1a}-blocker (peptide **3**). This compound exhibited negligible antidiuretic activity and, as in the previous case, low anti-uterotonic potency. It is worth stating that peptide **3** was designed without modification of position 1, which was until recently thought to be essential for substantial pressor antagonism (Manning et al 1987). Combination of $(D\text{-}1\text{-}Nal)^2$ substitution with introduction of Cpa and Val in positions 1 and 4, respectively, resulted in peptide **5**, with antipressor potency only slightly higher than that of peptide **3**. However, the anti-uterotonic activity of this analogue was increased significantly. In this case, Cpa modification, which was intended to increase anti-V_{1a} activity, was not supportive; however, it did enhance antiuterotonic potency. In addition, $[Cpa^1,(D\text{-}1\text{-}Nal)^2Val^4]$AVP exhibited a more protracted antioxytocic effect in-vitro than previously observed (Figure 3). This compound may be considered to be a derivative of $[Cpa^1,Val^4]$AVP and, comparing their activity, it is clear that the two peptides did not differ dramatically.

Another single substitution which we proposed was replacement of Phe^3 with D-1-Nal, analogue **6**. It resulted in almost complete removal of all tested activities. This peptide exhibited only negligible antidiuretic activity, as well as weak uterotonic antagonism (Table 3). Both $(D\text{-}1\text{-}Nal)^2$ and $(D\text{-}1\text{-}Nal)^3$ modifications consist of substitution of aromatic amino acid residues, for example, Tyr^2 or Phe^3 in AVP. As $[(D\text{-}1\text{-}Nal)^2]$AVP is a moderately potent V_{1a} and uterotonic antagonist, it demonstrates the importance of appropriate localization of D-1-Nal in the analogue for its biological activity.

Finally, the combination of $(L\text{-}1\text{-}Nal)^2$ or $(D\text{-}1\text{-}Nal)^2$ modifications with deamination of Cys residue in position 1 gave compounds **2** and **4**, which seemed not to interact with V_{1a} and V_2 receptors. However, the peptides surprisingly turned out to be weak (**2**) and moderately potent (**4**), uterotonic antagonists in-vitro. These compounds are among the first examples of selective blockers of oxytocic

receptors. All antagonists known to date, also display varying degrees of antipressor activity. The activity of the new peptides is even more interesting when compared with [Mpa[1]]AVP. This peptide, which may be treated as a parent for both analogues, is a potent pressor and antidiuretic agonist. Peptide **2** differs from [Mpa[1]]AVP only in the presence of different aromatic residues in position 2. We believe that, as in previous examples (Lammek et al 1997), the presence of a bulky naphthyl residue has a significant influence on the conformation of the cyclic part of analogues and thus greatly influences the interaction with receptors.

In recent years there has been increasing interest in oxytocin antagonists, as it is thought that oxytocin mechanisms are involved in the initiation of term and pre-term labour. Manning et al (1995) have described a series of moderately potent and fairly selective antagonists of oxytocin. These were designed by modifying desGly-NH_2, $d(CH_2)_5$[Tyr(Me)[2],Thr[4]]OVT at position two with D-Tyr(Me), L-Tyr(Et), D-Tyr(Et), D-Tyr, D-Phe and D-Trp where $d(CH_2)_5$ is 1-mercaptocyclohexaneacetic acid. Recently, Chan et al (2000) designed and synthesized a specific antagonist for the oxitocin receptor, desGly-NH_2 , $d(CH_2)_5$ (D-Trp[2], Thr[4], Dap[5])OVT where Dap is diaminopropionic acid. This analogue however, still possesses some anti-V_{1a} activity ($pA_2 = 5{\cdot}35$). Other studies carried out by Prochazka & Slaninova (1995) described the synthesis and pharmacological evaluation of analogues with L- or D-naphthylalanine in position 2 of oxytocin or AVP. In the case of oxytocin analogues, the peptides obtained were selective and moderately potent antagonists of this hormone. This and our previous study (Lammek et al 1997) clearly demonstrate the new possibilities opened up by the use of Nal for modification of both oxytocin and AVP in the design of analogues of neurohypophyseal hormones. It has been shown that single substitution with Nal residue results in a very selective and quite potent analogue of both hormones (Prochazka & Slaninova 1995; Lammek et al 1997). The results of this study seem to strongly support these findings.

In summary, the modifications proposed resulted in the removal of pressor activity, or conversion into moderate antagonists. As regards antidiuretic activity, the modifications gave peptides which were either devoid of or exhibited only negligible agonistic V_2 potency. Five peptides exhibited varying degrees of anti-uterotonic activity while one was a weak agonist. Two compounds, [Mpa[1],(L-1-Nal)[2]]AVP and [Mpa[1],(D-1-Nal)[2]]AVP were exceptionally selective antagonists of oxytocin in-vitro and thus are promising candidates for the development of potential tocolytic agents for the prevention of pre-term labour. They also may constitute valuable tools for studies on the physiological roles of oxytocin.

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