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Original article

Influence of enantiomers of 1-naphthylalanine in position 2 of VAVP and dVAVP on their pharmacological properties

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Abstract

In this study, we described the synthesis and some pharmacological properties of four new analogues of arginine vasopressin (AVP). Two peptides are substituted in position 2 with L-1-naphthylalanine (L-1-Nal) or its D-enantiomer and in position 4 with valine. In the further two compounds, we combined the above modifications with placement into position 1 of 3-mercaptopropionic acid residue (Mpa). All new peptides were tested for vasopressor and antidiuretic activities. We also estimated the uterotonic activities of these compounds in vitro. Urine samples prior and after peptide administration were analyzed for electrolytes excretion. All analogues are potent oxytocin antagonists. One of them, namely [L-1-Nal^2,Val^4]AVP, which appears practically not to interact with V_{1a} and V_2 receptors, is exceptionally selective. Our results open new possibilities for the design of very potent and selective oxytocin antagonists in vitro.

Keywords: Arginine vasopressin; Naphthylalanine; Oxytocin antagonists

1. Introduction

Many analogues of the arginine vasopressin (AVP) have been synthesized in the course of extensive investigations into their structure and activity [1–4]. However, despite intensive efforts in many laboratories, attempts to design potent and truly selective agonists and antagonists of AVP receptors still remain a real challenge. To find a solution to the aforementioned problem, the discovery of new clues to the design of AVP analogues would be certainly helpful.

The most straightforward approach for peptide modification is to introduce changes into the side chains of chosen amino acids. This strategy allows, among others, by the incorporation of nonproteinogenic amino acid residues, to introduce bulky groups with the aim being to restrict the conformational flexibility of a peptide. Conformational restrictions are of particular value for peptide design targeted towards an increase of receptor selectivity, metabolic stability and the development of highly potent agonists and antagonists. Also, in our laboratory we have shown that such an approach could result in analogues with very interesting pharmacological properties [5,6]. In 1997, 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 [5]. One of the new peptides, [L-2-Nal^3,D-Arg^8]VP, was among the most potent and selective antagonists of V_{1a} receptors reported to date. Moreover, it was the first V_{1a} antagonist devoid of antiuterotonic activity, and its high antipressor potency arises without modification of position 1, which was previously thought to be essential for substantial pressor antagonism [5]. Two other peptides, [Mpa^1,L-1-Nal^3,D-Arg^8]VP and [Mpa^1,L-2-Nal^3,D-Arg^8]VP, were highly potent V_2 agonists. The second peptide was exceptionally selective. Our results suggested that position 3 in AVP and its analogues is important not only for

Abbreviations: AVP, arginine vasopressin; Mpa, 3-mercaptopropionic acid; Mob, 4-methoxybenzyl; L-1-Nal, L-1-naphthylalanine; D-1-Nal, D-1-naphthylalanine; L-2-Nal, L-2-naphthylalanine; D-2-Nal, D-2-naphthylalanine; NMP, 1-methyl-2-pyrrolidone; TBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.

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binding and recognition as previously thought, but also for pressor, antidiuretic and uterotonic activities [5]. We also assumed that the hindering effect caused by bulky naphthyl moiety has a significant impact on the bioactive conformations of molecules which contain Nal residue, and can thus influence their interaction with V_1, V_2 and oxytotic receptors. Subsequently, knowing that the tyrosine residue in position 2 seems to play a part in initiating the pressor response of AVP [7] we decided to place there, in AVP and its analogues, L-1-Nal or its D-enantiomer [6]. These modifications resulted in the removal of pressor activity, or conversion into moderate antagonists. All new peptides exhibited varying degrees of antiuterotonic activity. Two compounds, [Mpa^1,L-1-Nal^3]AVP and [Mpa^1,D-1-Nal^3]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.

Recently, continuing in this direction, we decided to replace the residues in positions 2 or 3 of AVP and some of its analogues with 1-aminocyclohexane-1-carboxylic acid (Acc) [8]. Again, we selected the Acc in order to reduce the flexibility of the peptides by imposing a sterically constrained residue limiting conformational freedom, thus forcing the peptide backbone and side chains to adopt specific orientations. Our results clearly demonstrated that Acc^3 modification was deleterious to interaction with V_1, V_2 and oxytocin receptors. In the case of Acc^2 substitution, the situation was different, as it selectively modified interaction with the aforementioned receptors. Two of the new analogues, [Acc^2]AVP and [Acc^2, D-Arg^8]VP, are potent antidiuretic agonists. The results described also provided useful information about structure–activity relationships and thus opened up new possibilities, particularly in the design of highly potent and selective V_2 agonists.

Considering all this, as a continuation of our efforts to better understand the role of Tyr^2, we have now designed, synthesized and determined some pharmacological properties of two new peptides modified in positions 2 and 4 with L-1-Nal or D-1-Nal and valine, respectively. In the further two compounds, we combined the above substitutions with placement into position 1 of 3-mercaptopropionic acid residue (Mpa). The structures of these peptides are as follows:

X-Y-Phe-Val-Asn-Cys-Pro-Arg-Gly-NH_2			
where:	X = Cys	Y = L-1-Nal	(I)
	X = Cys	Y = D-1-Nal	(II)
	X = Mpa	Y = L-1-Nal	(III)
	X = Mpa	Y = D-1-Nal	(IV)

2. Materials and methods

2.1. General

All solvents were purified by conventional methods. Evaporation was carried out under reduced pressure. Melting points were determined on a capillary melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was carried out on silica plates (Merck). The spots being visualized by iodine or ninhydrin. The acetic acid/butan-1-ol/ethyl acetate/water (1:1:1:1) solvent system was used. High-performance liquid chromatography (HPLC) for peptides was carried out on a Gold System Beckman analytical chromatograph equipped with a UV detector. The purity of the peptides was determined on a Vydac C_{18} column (5 μm, 10 × 250 mm) with precolumn Ultrasphere ODS (5 μm, 4.6 × 45 mm). The following solvent systems were used: (1) 0.1% aqueous trifluoroacetic acid (TFA), (2) acetonitrile: 0.1% TFA (80:20 v/v); a linear gradient from 30–70% of (2) for 20 min at a flow rate of 1 ml/min (λ = 226 nm). FAB MS of peptides was recorded on a TRIO-3 mass spectrometer at 7 keV with argon as the bombarding gas.

All amino acid derivatives were purchased from Bachem AG. *S*-*p*-methoxybenzyl-3-mercaptopropionic acid, Mpa(Mob), was obtained according to Mpa(Bzl) [9] using *p*-methoxybenzyl chloride as follows:

200 mmol (4.6 g) of metallic natrium was added to mixture of 100 mmol (10.6 g, 8.7 ml) Mpa in 300 ml of anhydrous ethanol; and then 105 mmol (16.4 g, 14.5 ml, (98%)) of 4-methoxybenzoyl chloride (Mob-Cl) were add with stirring. The reaction mixture was heated and stirred for an additional 30 min, then the solvents were removed under reduced pressure. The oily residue was dissolved in water end extracted with diethyl ether (2×). The water layer was washed with ethyl acetate (2 × 30 ml), acidified with 6 N HCl to pH = 2 and extracted with ethyl acetate (3 × 50 ml). The organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude residue was crystallized from $CHCl_3$:*n*-hexane yielding 82% of white crystalline product. Mp. 73–75 °C.

2.2. Peptide synthesis

All peptides were synthesized manually by solid phase method, i.e. by the stepwise coupling of Boc-amino acids to the growing peptide chain on a 4-methylbenzhydrylamine resin (MBHA resin, Senn Chemicals AG, 1% DVB, 200–400 mesh, 0.67 mmol/g). Fully protected peptide resins or acylopeptide resins were synthesized according to the standard procedures involving (i) deprotection steps using 33% TFA in the presence of anisole (1%), 5 and 25 min; (ii) neutralization with 10% TEA/DCM, 3 and 7 min (iii), couplings in DCM/DMF (1:1, v/v) carried out using DCC and HOBT. The couplings of Boc-1-Nal, Boc-D-1-Nal, Boc-Cys(Mob) and Mpa(Mob) were mediated by TBTU and HOBt in the presence of DIEA in a mixture of DMF, NMP and DCM (1:1:1 v/v) containing 1% Triton. The completeness of each coupling reaction was monitored by the Kaiser et al. [10] or chloranil test [11]. Re-coupling was performed when the test was positive. After completion of the synthesis, the protected nonapeptidyl resins were treated with 10 ml of liquid hydrogen fluoride (HF) containing 0.5 ml of anisole at

–70 °C and stirred for 60 min at 0 °C [12]. After the removal of HF and anisole in-vacuo, the mixture was washed with anhydrous diethyl ether, then with acetic acid and the solution diluted with methanol. The resulting dithiols were oxidatively cyclized with 0.1 M I_2 in methanol using the normal procedure. The solvents were evaporated under reduced pressure and the resulting materials dissolved in water and lyophilized. The crude products were desalted on a Sephadex G-15 column eluted with aqueous acetic acid (30%) at a flow rate of 4.0 ml/h, λ = 254 nm. The eluates were fractionated, and the fractions containing the major peak were pooled and lyophilized. The residue was then subjected to gel filtration on a Sephadex LH-20 column eluted with 10% aqueous acetic acid at a flow rate of 4.0 ml/h, λ = 254 nm. The peptides were eluted as single peaks. The purity and identity of each peptide were determined by HPLC and FAB mass spectrometry (molecular ion). The values of the molecular ions were as expected. The physicochemical properties of peptides are presented in Table 1.

2.3. 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.

The use of animals was reviewed and approved by the Local Ethics Committee for Animals Experiments in Katowice (Poland).

2.4. Vasopressor activity

Vasopressor assays of AVP analogues were performed in vivo according to the method of Dekanski [13] as described by Stürmer [14]. The rats were given 1.75 g/kg urethane intraperitoneally (ip.). Blood pressure stabilization was evoked by phenoxybenzamine (SmithKline Beecham, USA) in two or three repeated doses, each amounting to 1 mg/kg intravenously (iv.). 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 (type PL3JD, Gould-Statham, USA) fixed into the carotid artery.

2.5. Antidiuretic activity

Antidiuretic activity of peptides was estimated in rats in vivo according to Stürmer's modification [14] of Sawyer's method [15]. Anaethesia, however, was induced by injecting 150 mg/kg of thiobutabarbital (Inactin, RBI, USA) ip. [16,17]. Catheters were inserted into the jugular vein for peptide infusion. After laparatomy, a polyethylene (PE) cannula was introduced into the urinary bladder and ligated. The urine flow was collected directly from the urinary bladder up to urine flow rate stabilization (0.8–1.2 ml/10 min.).

From the beginning of the experiment, the animals were connected by PE catheter to a syringe infusion pump (Medipan, type 612, Poland). Water balance was kept constant for each rat by rehydrating with hypoosmotic water solution (180 mOsm/kg) containing 0.24% NaCl and 1.8% glucose, adding arbitrary excess (1–2 ml/h) to compensate for extrarenal water loss.

Peptides were injected into the jugular vein as a bolus in a total volume of ~0.3 ml (including wash) after achieving the urine flow stabilization in three consecutive 10 min periods at 40 min intervals. Anesthesia was maintained throughout the experiment.

Antidiuretic response was defined as: $V_p/V_o \times 100$, where V_o is the urine volume collected in the 10 min period preceding the injection of AVP or analogue; V_p is the volume of urine collected during the 10 min starting period immediately after injection. Agonistic activity was expressed in international units per milligram (IU/mg) of peptide through the comparison of regression lines for OT or AVP and related peptides [18].

Antagonistic activity of AVP analogues was measured by the method of Schild [24] and described as effective dose (ED) and pA_2 value.

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. The pA_2 values estimated in vivo represent the negative logarithms (to base 10) of EDs divided by an assumed volume of distribution in rats (67 ml/kg).

The AVP was used as a standard agonist in all assays. Its pressor and antidiuretic activities were accepted as 369 IU/mg and 323 IU/mg, respectively [19].

2.6. Uterotonic and antiuterotonic activity

Determination of uterotonic or antiuterotonic activity was assayed on isolated rat uterus using the procedure of Holton [20] as described previously with additional modifications [6,21]. Briefly, virgin female Wistar rats weighing 220–250 g were used in the proestrus/oestrus stage artificially induced 24 h earlier by injection of oestradiol benzoate (0.8 mg/kg, intramuscularly). Oestrus stages were determined by vaginal smears made 1–2 h before each experiment. The uterine horns were isolated about 2 cm, close to the ovarian end under Inactin (RBI, USA) anaesthesia (125 mg/kg ip.) [16] and immediately mounted in a 20 ml bathing chamber.

The bathing fluid was the Munsick's modification of van Dyke–Hasting's solution, supplemented with Mg^{2+} [22,23].

Table 1
Physicochemical properties of peptides I–IV

Analogue	$(M+H^+)$		RP-HPLC
	Calculated	Found	T_R (min)
I	1089.51	1091.0	11.60
II	1089.51	1090.6	11.82
III	1074.5	1075.3	13.63
IV	1074.5	1075.4	14.59

The purity of analogues determined on HPLC was between 97% and 99%.

The solution had the following composition: NaCl 114.0; KCl 6.2; $NaHCO_3$ 30.0; NaH_2PO_4 1.0; $CaCl_2$ 0.5; $MgCl_2$ 0.5 and glucose 2.8 (in millimolar concentration). The reservoir and the bath were gassed with a mixture of 95% oxygen and 5% carbon dioxide. The temperature of the bath solution was 37 °C.

Contractions were measured isometrically with a K-30 force displacement transducer (type 351), two-channel bridge amplifier (type 301, Hugo Sachs Electronics, Germany) and recorded on R-50 multi-pen recorder (model 83, Rikadenki, Japan). The tissue was subjected to a base-line tension of 1 g; if regular tension development was not maintained during the 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 (AUC) after the addition of different concentrations of OT ($10^{-10} \div 10^{-6}$ M/L) or AVP ($1 \times 10^{-9} \div 3 \times 10^{-6}$ M/L). The organ bath was rinsed out three times with bathing solution after each application of agonists.

The antiuterotonic potency of the AVP analogues was measured by the same procedure and the OT or AVP was used as the standards in noncumulative increasing concentrations 1 min after administration of the antagonist (10^{-7} M/L).

To express the antagonistic activity of AVP analogues as a pA_2 value, a similar procedure was used. The pA_2 value is defined as the negative logarithm (to base 10) of the molar concentration of an antagonist, which reduces the contractile response of agonist to the effect produced by one half of the administered agonist concentration. The mean values of concentration ratios for the OT or AVP pair were calculated by the method of Schild [24] and plotted in a diagram by regression analysis. The pA_2 value was obtained from the intercept of the regression line with the abcissa using PHARM/PCS 4.0 pharmacological calculation system [25]. All values of pA_2 were presented as the mean ± SEM.

2.7. Urinary electrolytes analysis

During the antidiuretic experiments, the urine volumes were collected in consecutive 10 min periods before and after peptide administration. Urinary sodium, potassium, calcium and chloride concentrations of all samples were determined using Synchrom EL-ISE Electrolite System (model 4410, Beckman, Instruments Inc., USA). The electrolytes excretion (mEq/10 min) were expressed as a percentage after vs. before peptide administration.

The results are presented as mean ± SEM of *n* samples. Statistical significance of differences of base-line values between experimental groups was determined by unpaired two-tailed *t*-test. A $P < 0.05$ value was considered significant.

3. Results

The four new analogues of AVP (**I-IV**) were synthesized by stepwise coupling of Boc-amino acids to the growing peptide chain on a *p*-methoxybenzhydrylamine resin. The couplings were mediated by the DCC/HOBT or TBTU/HOBT methods. For peptide **III** and **IV**, Mpa(Mob) was used in the final coupling step. On completion of the syntheses, the protected peptidyl or acylpeptidyl resins were treated with liquid HF in the presence of anisole at 0° and oxidized with I_2 in methanol. The crude peptides were purified on Sephadex G-15 and LH-20. Some of the pharmacological properties of the new analogues **I-IV**, also those of AVP, [Mpa^1]AVP and some related peptides, are presented in Table 2. None of these new analogues exhibited pressor, anti-antidiuretic or uterotonic activity.

The vasopressin antagonistic activity of analogues was measured according to the method of Schild [24] as described by Manning and Sawyer [19]. Peptides **II-IV** with pA_2 values ranging from 7.37–7.88 exhibited a moderate antivasopressor potency: peptide **I** was inactive.

Table 2
Pharmacological data on vasopressin analogues

Peptide		Uterotonic IU/mg	Vasopressor (IU/mg)	Antivasopressor pA_2	Antidiuretic IU/mg	Antiuterotonic pA_2	
						Oxytocin	AVP
[L-1-Nal^2,Val^4]AVP [a]	I	—	ND (6)	ND (7)	<0.001 (8)	7.82 ± 0.09 (9)	7.87 ± 0.01 (4)
[D-1-Nal^2,Val^4]AVP [a]	II	—	ND (6)	7.37 ± 0.2 (8)	<0.003 (8)	7.71 ± 0.08 (7)	8.00 ± 0.05 (4)
[Mpa^1,L-1-Nal^2,Val^4]AVP [a]	III	—	ND (6)	7.88±0.78 (8)	<0.001 (8)	8.02 ± 0.02 (8)	8.18 ± 0.08 (4)
[Mpa^1,D-1-Nal^2,Val^4]AVP [a]	IV	—	ND (6)	7.35 ± 0.59 (8)	<0.001 (8)	8.29 ± 0.05 (6)	8.32 ± 0.12 (4)
[L-1-Nal^2]AVP [b]	V	—	ND (4)	ND (4)	ND (4)	6.90 ± 0.12 (6)	7.10 ± 0.06 (4)
[D-1-Nal^2]AVP[b]	VI	—	ND (4)	ND (4)	ND (4)	7.46 ± 0.15 (8)	7.78 ± 0.09 (4)
[Mpa^1,L-1-Nal^2]AVP [b]	VII	—	ND (4)	7.1 ± 0.53 (8)	<0.02 (8)	8.02 ± 0.02 (8)	8.18 ± 0.08 (4)
[Mpa^1,D-1-Nal^2]AVP [b]	VIII	—	ND (4)	ND (4)	ND (4)	7.69 ± 0.07 (6)	7.94 ± 0.23 (4)
AVP [c]		25.5	369 ± 6	—	323 ± 16	—	—
OT [c]		486 ± 15					
[Mpa^1]AVP [d]		66 ± 3	346 ± 13	—	1745 ± 385	—	—
[Val^4]AVP [e]			32	—	738	—	

[a] Results are expressed as a mean ± SEM. The numbers of rats or rat uterine horns are shown in parentheses. ND, not detectable. None of the naphthylalanine analogues exhibited uterotonic activity or anti-antidiuretic activity up to 10^{-6} mol/l.

[b] Values taken from Ref. [6].

[c] Values taken from Ref. [26].

[d] Values taken from Ref. [30].

[e] Values taken from Ref. [31], estimated in Brattleboro rats.

The antidiuretic activity of the analogues was estimated according to Stürmer [14] and Sawyer [15]. From the results, it is clear that the modifications proposed resulted in almost complete removal of interaction of peptides with V_2 receptors as all new analogues had negligible antidiuretic activity. However, after administration of analogue I, we observed statistically significant decrease of concentration of Ca^{2+}, while peptide II diminished concentration of Na^+ and K^+ in urine (Fig. 1).

Antiuterotonic activity was assayed on an isolated rat uterus by the procedure of Holton [20]. In this assay, all new compounds **I–IV** are highly potent oxytocin antagonists (pA_2 7.82–8.29). They also effectively blocked the contraction of rat uterus caused by AVP (pA_2 7.87–8.32). It is worth pointing out that peptide **I**, which appears practically not to interact with V_{1a} and V_2 receptors, is exceptionally selective oxytocin antagonist in vitro. Moreover, the results showed that all new analogues of AVP had negligible antidiuretic activity (Table 2).

4. Discussion

Previously we reported that the hindering effect caused by the bulky naphthyl moiety of L-1-Nal or D-1-Nal in position 2 of AVP or [Mpa^1]AVP had a significant impact on bioactive conformations of molecules and thus influenced their interaction with V_{1a}, V_2 and oxytocic receptors [6]. The data for compounds **V–VIII**, described in the above paper and presented here in Table 2, demonstrate that the above-mentioned modifications 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 had only negligible agonistic V_2 potency. All analogues exhibited varying degrees of antiuterotonic potency (pA_2 6.9–7.69). Three compounds **V**, **VII**, and **VIII** were exceptionally selective, however moderately potent antagonists of oxytocin in vitro. Surprising activity of peptides **VII** and **VIII** is even more interesting when compared with [Mpa^1]AVP. This compound, which may be treated as a parent one for both analogues, is a potent pressor and antidiuretic agonist. Peptide **VII** differs from [Mpa^1]AVP only in the presence of different aromatic residues in position 2. We believe that, as in previous examples [5], the presence of a bulky naphthyl residue greatly influences the interaction of analogue with receptors. These, in our opinion interesting results, prompted us to search for additional modifications, which would improve the antioxytocic potency of the above analogues. Knowing that the valine in position 4 of AVP and its analogues in many cases improves antidiuretic potency and selectivity [26], but having also in mind unexpected results discussed above, in this study we decided to determine how Val^4 substitution of peptides **V–VIII** would change their pharmacological properties. Additional stimulus for such measure was another unexpected effect, when placement of D-Arg into position 8 of [L-1-Nal^3]AVP, instead of enhance antidiuretic activity of analogue resulted in highly potent and selective V_{1a} antagonist [5].

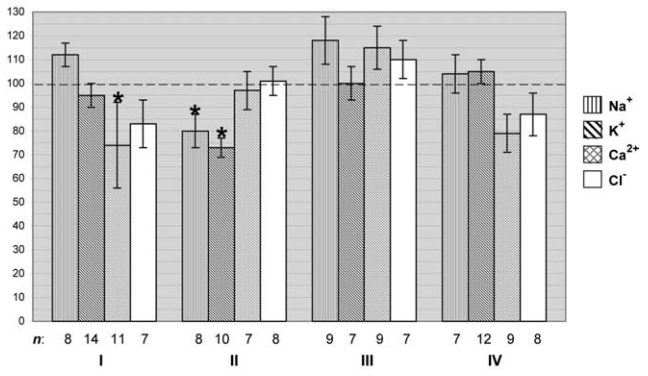

Fig. 1. Changes in electrolyte levels in rats' urine following administration of argininevasopressin analogues I–IV (expressed in percent relative to the levels before administration). Results are expressed as mean ± S.D. Number of rats *n* are shown under the bars. Sampling was made at 10 min intervals. Electrolytes excretion was expressed in percent relative to the levels before peptide administration (100%), which were determined for Na^+ 19 ± 7.6; K^+ 18 ± 5.7; Ca^{2+} 0.59 ± 0.11 and Cl^- 44.5 ± 15.8 (μEq/10 min). * $P < 0.05$; statistically significant differences, when compared with value before peptide administration.

The present work is a part of our studies aimed at clarifying the impact of sterical restrictions in the N-terminal part of the AVP analogues on pharmacological properties. The pharmacological data presented in Table 2 showed that our modification, commonly used to improve V_2 agonism, leads in all cases to substantial enhancement of antiuterotonic potency. This improvement is very impressive especially when comparing a pair of peptides: **I, V,** as it is approximately in the range of one order of magnitude (~10-fold enhancement). Passing to antipressor activity, it is clear that Val^4 substitution converted inactive in this respect compounds **VII** and **VIII** into moderately potent V_{1a} antagonists, thus diminishing their selectivity. Therefore, it is even more interesting to note that peptide **I** which appears practically not to interact with V_{1a} and V_2 receptors is a potent and exceptionally selective blocker of oxytocin receptor in vitro and thus promising candidate for the development of potential tocolytic agents for the prevention of pre-term labour.

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. In 1995, Manning described a series of moderately potent and fairly selective antagonists of oxytocin [27]. More recently, Chan designed a specific antagonist for the oxytocin receptors [28]. This analogue however, still possesses some anti-V_{1a} activity. Other studies described the synthesis and pharmacological evaluation of analogues with L- or D-Nal 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 [29]. This and previous studies [5,6] clearly demonstrated the new possibilities opened up by the use of Nal for modification of neurohypopheseal hormones. The results of this study seem to strongly support these findings.

It is well known that AVP plays a major role in the regulation of water balance and participates in cardiovascular homeostasis. The vasopressor effects of AVP are mediated by V_{1a} receptors, whereas the V_2 type receptors, located almost exclusively in the renal collecting duct, promote water permeability and sodium, potassium, calcium, magnesium and chloride reabsorption in the ascending limb [32,33]. Although the aquaretic effect is in general linked with V_2 antagonistic action [34–36], nevertheless also the effect of V_{1a} receptor antagonists on urine and electrolytes excretion was investigated [37,38]. Our results (Fig. 1) seem to support the hypothesis that also V_{1a} and oxytocic blocker could influence the concentration of Ca^{2+}, Na^+ and K^+.

5. Conclusion

Our studies resulted in four analogues with interesting pharmacological properties. Peptide **I** is a potent and exceptionally selective oxytocin antagonist which has potential as a tocolytic agent. All new analogues may constitute valuable tools for studies on the physiological roles of oxytocin. Moreover, our results offer new possibilities in the design of new potent and selective oxytocin blocers.

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