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COMMUNICATIONS

Two highly effective V_1/V_2 antagonists of vasopressin containing novel thioacids at position 1

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Abstract—In an attempt to develop more effective and selective V_1/V_2 antagonists of arginine vasopressin (AVP), two analogues have been designed and synthesized. In position 1 they contain thioacids that include a heteroatom in the cyclohexane ring. The 4-thio-4-tetrahydrothiopyraneacetic acid modification of position 1 used in one of them had advantages over the 1-mercaptocyclohexaneacetic acid substituted compound used as reference. The new compound was weaker at lower doses, but elicited a greater response at higher doses, whereas the reference compound reached its plateau earlier. Although the 4-thio-4-tetrahydropyraneacetic acid substitution used in the second compound resulted in a less potent analogue on a weight basis, this substitution also confers enhanced overall efficacy in terms of magnitude of effect.

The substitution of 1-mercaptocyclohexaneacetic acid (MCA) at position 1 is a modification that has been shown to be essential for antidiuretic antagonism of cyclic analogues of arginine vasopressin (AVP) (Manning & Sawyer 1985). However, evaluation in volunteers of a potent vasopressin V_2 receptor antagonist that has this residue at position 1, namely [1-(1-mercaptocyclohexaneacetic acid),2-(*O*-ethyl-D-tyrosine), 4-valine, 9-desglycine] -8-arginine vasopressin, revealed that this peptide behaved as a V_2 agonist rather than a V_2 antagonist (Huffman et al 1989). Recently we reported that even relatively minor modifications of the 1-mercaptocyclohexaneacetic acid residue are extremely important for either enhancing or decreasing the anti-antidiuretic activity of analogues (Lammek et al 1989). A recent publication from Huffman et al (1989) indicates that such subtle differences in the structure of the 1-mercaptocyclohexaneacetic acid occupying position 1 can result in major decrease of undesired agonistic activity. They discovered that the presence of a *cis*-4-methyl group on the 1-mercaptocyclohexaneacetic acid residue at position 1 of a V_2 vasopressin antagonist results in reduced agonist activity compared with the peptide with unsubstituted MCA residue. These findings emphasize the importance of the structure of the residue at position 1 for the V_2 activity and/or selectivity of cyclic AVP analogues.

As a continuation of our approach to develop more effective and selective V_1/V_2 antagonists of AVP, we designed and synthesized two new analogues: [1-(4-thio-4-tetrahydropyraneacetic acid),2-(*O*-ethyl-D-tyrosine),4-valine]-8-arginine vasopressin [OCADTyr(Et)VAVP] (I) and [1-(4-thio-4-tetrahydrothiopyraneacetic acid),2-(*O*-ethyl-D-tyrosine),4-valine]-8-arginine vasopressin [SCADTyr(Et)VAVP] (II).

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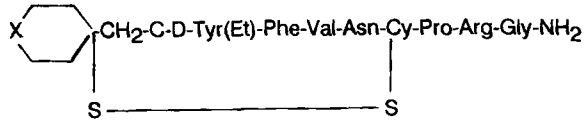

I X = O II X = S

We believe that the presence of the heteroatom in the cyclohexane ring may significantly influence the interaction of the peptide with the V_2 receptor and therefore increase the efficacy and/or selectivity of the analogue. Strong support for such hypothesis comes from the paper by Huffman et al (1989) in which the authors present a very interesting model for pharmacophore interaction about residue 1 in vasopressin agonists and antagonists.

Materials and methods

4-[(*p*-Methoxyphenylmethyl)thio]-4-tetrahydropyraneacetic acid and 4-[(p-methoxyphenylmethyl)thio]-4-tetrahydrothiopyraneacetic acid were obtained as previously described (Lammek et al 1990). BOC-*O*-ethyl-D-tyrosine was synthesized by a previously published procedure (Kolodziejczyk & Manning 1981). [1-(1-Mercaptocyclohexaneacetic acid), 2-(*O*-ethyl-D-tyrosine), 4-valine]-8-arginine vasopressin was synthesized as previously described (Manning & Sawyer 1985; Lammek et al 1988).

Peptide synthesis. The protected peptide precursors required for the synthesis of both peptides (I and II) 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 stepwise coupling of BOC-amino acids to the growing peptide chain on a Merrifield resin. 4-[(*p*-Methoxyphenylmethyl)thio]-4-tetrahydropyraneacetic acid and 4-[(*p*-methoxyphenylmethyl)thiol]-4-tetrahydrothiopyraneacetic acid were used in the final coupling steps. After completion of the synthesis the protected acyloctapeptidyl resins were ammonolysed in methanol. Following evaporation of the solvent, the products were extracted into hot 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 MeOH/Et_2O (1:3). Precursors were deblocked with sodium in liquid ammonia and the resulting disulphhydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$. The analogues were desalted on Sephadex G-15 (50% AcOH) and purified on Sephadex LH-20 (30% acetic acid). The purity and identity of each peptide was

ascertained by thin-layer chromatography in two different solvent systems, by HPLC, and by amino acid analysis.

Bioassay methods. The analogues I and II were assayed in conscious intact male Wistar rats, 300–500 g (unpublished data). The anti-antidiuretic potency of the analogues was evaluated by their ability to inhibit the antidiuretic effect of endogenous AVP. The rats were individually housed in metabolic cages on the day preceding the experiment, with free access to food and water. On the day of the experiment, the animals were weighed and received an intraperitoneal injection of sterile 5% dextrose (1 mL kg^{-1}). Urine was collected continuously over two 1·5 h periods as a baseline. Upon completion of this collection, one dose of analogue was injected intraperitoneally. Urine was collected continuously at 1·5 h intervals until the urinary output returned to baseline. This procedure was repeated every other day with a different dose of the same analogue in the same rat. At least three and sometimes as many as five different doses of each analogue were tested. The peak response to each dose was used for evaluation. The V_2 antagonistic activity is expressed as the effective dose (ED6, in nmol kg^{-1}) which increases urine volume from a baseline average of 1·2 ± 0·05 mL/1·5 h (n = 169) to 6 mL/1·5 h (Kinter et al 1988). ED6 was estimated by interpolation on a log(dose)-response curve for the response in the interval of 1·5–3 h.

The antivasopressor potency of analogues was assessed by their ability to inhibit the pressor response to exogenous vasopressin. The right iliac artery and jugular vein were catheterized in rats on the day preceding the experiment. On the day of the experiment, the rats were maintained conscious and unrestrained in plastic cages. Fig. 1 shows the dose-pressor response curve of 8-arginine vasopressin acetate salt (AVP, Sigma). The pressor response to 2·5 and 5·0 m units AVP is located on the linear part of the curve. Accordingly, these two doses were selected for the following pressor assay: first, the two doses of AVP, 2·5 and 5·0 m units, were injected into the jugular vein. The averages of two pressor responses to each of these two doses of AVP were taken respectively as the control values. Next, one dose of analogue was injected via the iliac catheter. Then 5 m units of AVP was injected serially at 15, 30, 60, 120, 180, up to 240 min after the administration of the analogue, until the pressor response to 5 m units of AVP returned to the control level. On the second day, the same rat was tested in the same way with another dose of the same analogue. The anti-V_1 receptor potency of each analogue was expressed as the ED (effective dose) and pA_2 calculated according to the principle of Schild (1947) (for definition see footnotes to Table 1).

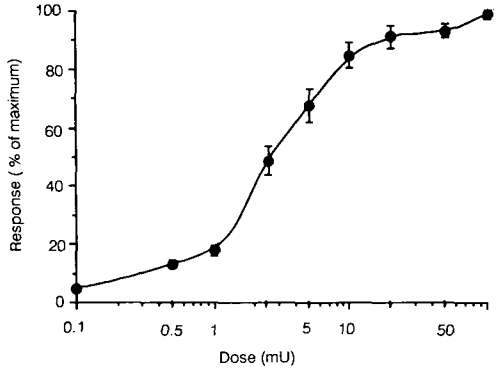

FIG. 1. Dose-pressor response curve of arginine-vasopressin (AVP). The two doses of 2·5 and 5 m units (which fall on the steepest part of the curve) were selected for assaying the anti-pressor effects of the AVP antagonist.

Table 1. Pharmacological data on AVP analogues.

	Vasopressor (V_1)			Antidiuretic (V_2)	
Analogue	n	ED[a]	pA_2[a]	n	ED6[b]
I	5	2·45 ± 0·57	7·57 ± 0·22	5	20·19 ± 5·11
II	5	5·17 ± 1·17	7·21 ± 0·18	6	14·23 ± 2·35
Reference peptide[c]	5	3·88 ± 0·51	7·24 ± 0·055	5	12·65

n = number of rats tested with each compound. [a] The "effective dose" (ED) is defined as the dose (in nmol kg^{-1}) that reduces the response to two units of agonist to the level obtained previously by one unit of agonist. The pA_2 values represent the negative logarithms of the ED values divided by the estimated volume of distribution (67 mL kg^{-1}) (Schild 1947). [b] The effective dose (ED6) is the dose (in nmol kg^{-1}) of analogue which increases urine volume from baseline to 6 mL/1·5 h (Kinter et al 1988). [c] This compound was previously synthesized by Manning & Sawyer (1985) and was tested in our system as a reference peptide.

Results

The antivasopressor and anti-antidiuretic potencies of the new analogues I and II and of [1-(-mercaptocyclohexaneacetic acid),2-*O*-ethyl-D-tyrosine),4-valine]-8-arginine vasopressin or $d(CH_2)_5$D-Tyr(Et)VAVP (8) which was used for comparison are presented in Table 1. Both analogues are antidiuretic and vasopressor antagonists. Comparison of the ED6 of these peptides indicates that both substitutions result in decreased potency (increased values of ED6) compared with the reference peptide. The increases in urinary flow rate elicited by the two new analogues and the reference peptide are shown in Table 2. The maximal response to the reference peptide was achieved with a 50 $\mu g\ kg^{-1}$ dose and higher doses produced no greater response. The urinary flow rate caused by SCADTyr(Et)VAVP (II) at the same concentration was similar; however, the latter peptide produced a further increase in flow rate at a higher dose and the maximum effect was accomplished when the dose reached 100 $\mu g\ kg^{-1}$. The urinary flow rate caused by OCADTyr(Et)VAVP (I) in doses up to 100 $\mu g\ kg^{-1}$ was lower compared with the reference peptide; however, the maximum effect was accomplished with a dose of 300 $\mu g\ kg^{-1}$ and was greater than that of the reference peptide. The maximum effect of all peptides appeared at 3 h. The recovery time for both new analogues was shorter compared with the reference peptide and depended on the concentration used. In another group of rats (n = 5), 1 mL kg^{-1} of 5% dextrose (vehicle) was given. There was no significant change in urinary output within 4·5 h (Table 2).

Analogue II exhibited antivasopressor potency similar to that of the reference peptide while the antivasopressor potency of analogue I was higher (Tables 1, 3).

Discussion

We report here the synthesis and evaluation in rats of two new AVP analogues with dual V_1/V_2 antagonistic properties. As already mentioned, position 1 of V_2 antagonists of AVP appears to be extremely important for the peptide-receptor interaction (Huffman et al 1989; Lammek et al 1989). This finding prompted us to design and synthesize new analogues which have at position 1, a novel thioacid with a heteroatom in the cyclohexane ring (Lammek et al 1990). We reasoned that modification in the steric structure of the substituent at position 1 may lead to peptides which will interact differently with the V_2 receptor and thus may have higher V_2 antagonistic efficacy and/or selectivity.

Based on the data presented here for analogues I and II it is clear that the 4-thio-4-tetrahydrothiopyraneacetic acid modification of position 1 of AVP antagonists (analogue II) is

Table 2. Aquaretic effects of the reference peptide (R) and new analogues of AVP, [OCADTyr(Et)VAVP (I) and SCADTyr(Et)VAVP (II)].

Dose	(μg kg^{-1})	n	−1·5 h	1·5 h	3 h	4·5 h	6 h	7·5 h
R	10	5	*1·14±0·10	4·42±1·51	4·6±0·94	1·12±0·14	0·85±0·1	—
	50	5	1·25±0·19	6·40±0·79	12·9±0·92	7·74±1·24	1·62±0·36	0·93±0·07
	100	5	0·93±0·13	6·48±1·12	12·66±1·53	11·98±2·91	4·90±1·92	1·90±0·43
	300	5	1·03±0·24	4·40±0·42	9·16±1·74	12·4±2·88	6·32±2·29	2·95±1·02
I	10	5	0·98±0·21	4·14±0·68	1·78±0·16	1·03±0·27	—	—
	50	5	1·24±0·13	7·88±0·91	9·00±1·91	2·10±0·50		
	100	5	1·25±0·18	7·08±1·65	8·80±1·63	5·40±1·0		
	300	5	1·19±0·20	8·46±1·39	15·62±0·54	14·74±1·7	4·24±0·89	1·5±0·5
II	10	5	1·21±0·20	4·28±0·49	4·12±2·33			
	50	5	0·96±0·08	7·40±2·1	11·78±1·13	4·38±0·68	1·60±0·3	
	100	5	1·0±0·22	8·96±1·04	18·02±1·88	8·80±1·31	2·20±0·9	
	300	5	0·92±0·21	7·28±0·84	16·76±1·05	2·80±0·30	0·92±0·08	
5% Dextrose		5	1·60±0·29	1·16±0·20	1·33±0·52	0·97±0·23		

* The values in the table are volumes of urine in mL/1·5 h (mean ± s.e.m.) collected at intervals before and after injection of the peptide. n = number of rats tested with each dose.

Table 3. Antivasopressor effect of the reference peptide (R) and the new analogues of AVP, OCADTyr (Et) VAVP (I) and SCADTyr (Et) VAVP (II).

Compound	Dose (μg kg^{-1})	n	Time after administration of AVPA (min) 15	30	60	120	180	240	300
R	2·5	5	58·48±7·98	60·15±4·69	59·08±3·00	70·93±9·53	82·90±7·75	79·49±15·38	—
	5	4	53·64±9·74	64·35±11·73	59·88±9·43	56·90±9·84	66·42±13·60	52·35±17·97	86·36±8·87
I	5	5	80·46±7·39	75·62±11·02	95·78±11·64	102·63±22·13	112·72±13·66	—	—
	10	5	37·04±4·57	38·38±10·86	52·29±10·44	64·16±9·47	76·16±6·47	77·50±4·34	—
II	2·5	5	65·64±4·28	74·73±5·91	83·58±1·71	83·58±9·95	68·69±11·99	77·07±19·84	—
	5	5	63·24±6·28	50·11±7·23	69·57±3·70	70·73±9·67	75·52±6·72	90·76±7·11	—

The values in the table are the percent of the pressor response induced by 5 m units of AVP after administration of the cited dose of each AVP antagonist compared with the response to the same dose of AVP before the antagonist. n = number of rats tested with each dose.

advantageous in comparison with the 1-mercaptocyclohexane-acetic acid substituted compound, which is considered as one of the most effective compounds described so far. Although the ED6 value of analogue II is slightly higher than that of the reference peptide, when the overall efficacy of the two compounds is compared analogue II appears to be superior.

Analogue I, which differs from analogue II only in the presence of oxygen instead of sulphur in the cyclohexane ring of the substituent occupying position 1, clearly shows much weaker antagonistic potency; however, its efficacy also appears to be greater than that of the reference peptide.

In summary besides providing new information on structural requirements for residues occupying position 1 of AVP analogues for V_2 antagonism, these studies yielded two novel effective V_1/V_2 antagonists, which may have potential as pharmacological tools in the study of the actions of endogenous AVP or as useful therapeutic agents.

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