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Position 4 analogues of [deamino-Cys[1]] arginine vasopressin exhibit striking species differences for human and rat V_2/V_{1b} receptor selectivity

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Abstract: Arginine vasopressin (AVP) mediates a wide variety of biological actions by acting on three distinct G-protein coupled receptors, termed V_{1a} (vascular), V_{1b} (pituitary) and V_2 (renal). It also binds to the oxytocin (OT) receptor. As part of a program aimed at the design of selective agonists for the human V_{1b} receptor, we recently reported the human V_{1b}, V_{1a}, V_2 and OT receptor affinities of the following position 4 substituted analogues of [deamino-Cys[1]] arginine vasopressin (dAVP) – (1) d[Leu[4]]AVP, (2) d[Orn[4]]AVP, (3) d[Lys[4]]AVP, (4) d[Har[4]]AVP, (5) d[Arg[4]]AVP, (6) d[Val[4]]AVP, (7) d[Ala[4]]AVP, (8) d[Abu[4]]AVP, (9) d[Nva[4]]AVP, (10) d[Nle[4]]AVP, (11) d[Ile[4]]AVP, (12) d[Phe[4]]AVP, (13) d[Asn[4]]AVP, (14) d[Thr[4]]AVP: (15) d[Dap[4]]AVP. With the exception of Nos. 7 and 12, all peptides exhibit very high affinities for the human V_{1b} receptor. Furthermore, peptides 1–4 exhibit high selectivities for the human V_{1b} receptor with respect to the V_{1a}, V_2 and OT receptors and, with d[Cha[4]]AVP, in functional tests, are the first high affinity selective agonists for the human V_{1b} receptor (Cheng LL *et al.*, *J. Med. Chem.* **47**: 2375–2388, 2004). We report here the pharmacological properties of peptides 1–4, 5 (from a resynthesis), 7, 9–13, 15 in rat bioassays (antidiuretic, vasopressor and oxytocic) (*in vitro*: no Mg^{++}) with those previously reported for peptides 5, 6, 8, 14. We also report the rat V_{1b}, V_{1a}, V_2 and OT receptor affinities of peptides 1–5 and the rat V_2 receptor affinities for peptides: 7–15.

The antidiuretic activities in units/mg of peptides 1–15, are: 1 = 378; 2 = 260; 3 = 35; 4 = 505; 5 = 748; 6 = 1150; 7 = 841; 8 = 1020; 9 = 877; 10 = 1141; 11 = 819, 12 = 110; 13 = 996; 14 = 758; 15 = 1053. Peptides 1–4 exhibit respectively the following rat and human (in brackets) V_2 receptor affinities: 1 = 3.1 nm (245 nm); 2 = 3.4 nm (1125 nm); 3 = 24.6 nm (11,170 nm); 4 = 0.6 nm (1386 nm). Their rat V_{1b} receptor affinities are 1 = 0.02 nm; 2 = 0.45 nm; 3 = 9.8 nm; 4 = 0.32 nm. Their rat V_{1a} receptor affinities are 1 = 1252 nm; 2 = 900 nm; 3 = 1478 nm; 4 = 32 nm. Their rat oxytocin (OT) receptor affinities are 1 = 481 nm; 2 = 997 nm; 3 = 5042 nm; 4 = 2996 nm. All four peptides have high affinities and selectivities for the rat V_{1b} receptor with respect to the rat V_{1a} and OT receptors. However, in contrast to their high selectivity for the human V_{1b} receptor with respect to the human V_2 receptor, they are not selective for the V_{1b} receptor with respect to the V_2 receptor in the rat. These findings confirm previous observations of profound species differences between the rat and human V_2 receptors. Peptides 1–4 are promising leads to the design of the first high affinity selective agonists for the rat V_{1b} receptor. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: vasopressin agonists; V_{1b}; V_{1a}; V_2; OT; receptors; selectivity

INTRODUCTION

Arginine vasopressin (AVP), a cyclic octapeptide, is synthesized in the hypothalamus and stored in the neurohypophysis. It elicits a variety of responses both centrally and peripherally by acting on three known G-protein coupled receptors: V_{1a}, V_{1b} (V_3) and V_2 and on the oxytocin (OT) receptor [1–4]. AVP V_{1a} receptors, present in many tissues including the central nervous system (CNS), mediate the vascular effects of AVP by causing vasoconstriction of the vascular smooth muscle cells [1,2,4]. V_{1b} receptors present in the pituitary, adrenals, pancreas and CNS mediate the release of the adrenocorticotropic hormone (ACTH) by AVP [2–13]. The AVP V_{1b} receptor has recently been shown to regulate anxiety and depression in rats [3,14–16] and also in humans [3,17–19]. V_2 receptors present in the kidney, mediate the well-known antidiuretic effects of AVP (for reviews see Refs 1,2,4). The V_{1a}, V_{1b}, V_2 and OT receptors from

Abbreviations: Abbreviations are as in J. Peptide Science 9 : 1–8 (2003) and references there cited, and as follows. All amino acids are in the L-configuration unless otherwise noted. Other abbreviations used are: dAVP: [deamino-Cys[1]]-arginine vasopressin; [X[4]]-dAVP: position 4 substituted analogue of dAVP; [^{3}H]-AVP: Phe (3,4,5-^{3}H)-labeled AVP; CHO cells: Chinese Hamster Ovary cells; K_i: concentration of peptide leading to half-maximal specific binding deduced from competition experiments.

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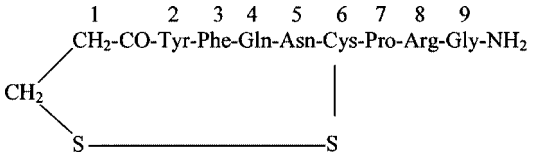
a variety of species, including the rat and human, have been cloned during the past 13 years [20–27]. The availability of reliable *in vitro* and *in vivo* rat bioassays for the antidiuretic [28], vasopressor [29] and oxytocic [30] activities of AVP and OT and their analogues led to the design of selective antidiuretic, vasopressor and oxytocic agonists and selective and nonselective antagonists [31–35]. Selective antidiuretic, vasopressor and oxytocic agonists and or nonselective antagonists played a major role in helping the pharmacological characterization of the V_{1a}, V_{1b}, V_2 and OT receptors long before they were cloned [2,4,5,10]. Progress in the design of selective agonists or antagonists for the rat V_{1b} receptor was greatly hampered by the lack of a routine bioassay for the ACTH releasing effect of AVP. The availability of cloned human and rat AVP V_{1b} receptors during the past decade led to recent breakthroughs in this area [36–40]. We recently reported that [deamino-4-cyclohexylalanine]arginine vasopressin (d[Cha^4]AVP) is the first selective agonist for the human V_{1b} receptor [37,39]. Almost simultaneously, the Sanofi-Synthelabo laboratory reported a selective nonpeptide antagonist for the human and rat V_{1b} receptors [36,38]. In a recent follow-up study [40], we reported the synthesis and the human V_{1a}, V_{1b}, V_2 and OT receptor affinities and selectivities of a broad series of 21 position 4 analogues of [deamino-Cys^1]-arginine vasopressin (dAVP) [41]. Remarkably, virtually all of these analogues exhibit very high affinities for the human V_{1b} receptor [40]. Furthermore, four of these analogues, d[Leu^4]AVP, d[Orn^4]AVP, d[Lys^4]AVP and d[Har^4]AVP have high affinities and selectivities for the human V_{1b} receptor and very low affinities for the human V_{1a}, V_2 and OT receptors [40]. In functional assays, all four peptides are highly selective for the human V_{1b} receptor with respect to the human V_{1a}, V_2 and OT receptors [40]. Thus, they and d[Cha^4]AVP [37] are the first high affinity selective agonists for the human V_{1b} receptor. It maybe recalled that Saito and colleagues had recently reported that the widely used highly selective antidiuretic agonist 1-desamino-8-D-arginine vasopressin (dDAVP) [4,42] is a potent human V_{1b} receptor agonist [43]. In humans, it is in fact more potent as a V_{1b} agonist than as a V_2 agonist [43]. dDAVP is thus a nonselective V_2/V_{1b} agonist in humans [43]. It exhibits partial V_{1b} agonism in rats [43]. d[Cha^4]AVP is a highly selective agonist for the rat V_{1b} receptor with respect to the rat V_{1a} and OT receptors. However, it is not selective with respect to the rat V_2 receptor. It exhibits significant antidiuretic activity (133.6 U/mg) in the rat antidiuretic assay [37]. Our discovery of the four additional d[X^4]AVP analogues (where X = Leu, Orn, Lys and Har), which are selective for the human V_{1b} receptor with respect to the human V_{1a}, V_2 and OT receptors [40], raised the question of whether these four new peptides would also exhibit a similar spectrum of receptor affinities in the rat and consequently possess high selectivity for the rat V_{1b} receptor with respect to the rat V_{1a}, V_2 and OT receptors. Utilizing both rat bioassays and rat receptor assays, the present study addresses this question.

dAVP has the following structure:



The 15 position 4 analogues of dAVP examined in this study are:

1 d[Leu^4]AVP
2 d[Orn^4]AVP
3 d[Lys^4]AVP
4 d[Har^4]AVP
5 d[Arg^4]AVP
6 d[Val^4]AVP
7 d[Ala^4]AVP
8 d[Abu^4]AVP
9 d[Nva^4]AVP
10 d[Nle^4]AVP
11 d[Ile^4]AVP
12 d[Phe^4]AVP
13 d[Asn^4]AVP
14 d[Thr^4]AVP
15 d[Dap^4]AVP (Dap = diaminopropionic acid)

We now report the antidiuretic, vasopressor and oxytocic activities in rat bioassays and the binding affinities in rat V_{1a}, V_{1b}, V_2 and OT receptor assays of peptides 1–4: d[Leu^4]AVP, d[Orn^4]AVP, d[Lys^4]AVP and d[Har^4]AVP (Tables 1 and 2).

We also report the antidiuretic, vasopressor and oxytocic activities of the remaining 11 position 4 analogues of dAVP (peptides 5–15, Table 1), together with their rat V_2 receptor affinities (Table 2).

It should be noted that four of these 15 peptides – No. 8, d[Abu^4]AVP [49], No. 6, d[Val^4]AVP [48], No. 14, d[Thr^4]AVP [45] and No. 5, d[Arg^4]AVP [47] – had previously been reported to be potent antidiuretic agonists in the rat. We report here their rat V_2 receptor affinities. A preliminary report on these studies will be presented [53].

PEPTIDE SYNTHESIS

The previously reported peptides, AVP [44], dAVP [41], d[Val^4]AVP [48], d[Abu^4]AVP [49], d[Thr^4]AVP [45], d[Arg^4]AVP [47] and d[Cha^4]AVP [37] were synthesized by the solid phase method [54–56], as previously described. The remaining d[X^4]AVP analogues (where X = Leu, Orn, Lys, Har, Ala, Nva, Nle, Ile, Phe, Asn, Dap) were synthesized as recently described [40].

Table 1 Some pharmacological activities in rat bioassays of four substituted analogues of [deamino]arginine vasopressin (dAVP)

	Peptide	Antidiuretic Activity (Units/mg) (*A*)	Vasopressor activity (Units/mg) (*P*)	Oxytocic Activity (*in vitro*) no Mg^{++} (Units/mg)	Selectivity A/P	Selectivity A/OT
	AVP[a]	332 ± 20	376 ± 6	13.9 ± 0.5	0.9	24
	dAVP[b]	1745 ± 385	346 ± 13	63 ± 4	5	28
	d[Cha^4]AVP[c]	133.6 ± 5.61	0.067 ± 0.005	—	1994	
1	d[Leu^4]AVP[d,e]	378 ± 23.67	3.11 ± 0.11	0.67 ± 0.12	122	564
2	d[Orn^4]AVP[d,e]	260.32 ± 22.4	1.54 ± 0.13	6.49 ± 0.73	169	40
3	d[Lys^4]AVP[d,e]	34.83 ± 0.84	0.6 ± 0.03	0.027 ± 0.003	58	1290
4	d[Har^4]AVP[d,e]	504.77 ± 37.84	107.53 ± 5.65	0.09 ± 0.002	4.7	5609
5	d[Arg^4]AVP[d]	748.32 ± 12.96	159.93 ± 6.5	0.027 ± 0.03	4.7	27 715
		214[f]	128[f]	—	1.7	
6	d[Val^4]AVP[g]	1150 ± 110	51 ± 2	—	23	—
7	d[Ala^4]AVP[d,e]	840.58 ± 33.67	1.37 ± 0.1	1.21 ± 0.15	614	695
8	d[Abu^4]AVP[h]	1020 ± 67	11 ± 1	—	93	—
9	d[Nva^4]AVP[d,e]	877.19 ± 46.85	6.21 ± 0.24	3.23 ± 0.29	141	272
10	d[Nle^4]AVP[d,e]	1141.27 ± 86.2	3.64 ± 10.23	0.15 ± 0.01	314	7609
11	d[Ile^4]AVP[d,e]	819.23 ± 48.62	11.08 ± 0.36	2.53 ± 0.15	74	324
12	d[Phe^4]AVP[d,e]	11.47 ± 0.3	0.16 ± 0.013	0.08 ± 0.002	72	143
13	d[Asn^4]AVP[d,e]	996.39 ± 85.08	87.39 ± 3.05	8.29 ± 0.55	11	120
14	d[Thr^4]AVP[j]	758 ± 50	30 ± 1	10.2 ± 0.5	25	74
15	d[Dap^4]AVP[d,e]	1053.49 ± 176.67	0.45 ± 0.02	0.48 ± 0.03	2341	2194

[a] Original synthesis Ref. 44; data from Ref. 45.
[b] Original synthesis Ref. 41; data from Ref. 46.
[c] Data from Ref. 37.
[d] This publication.
[e] For original synthesis, see Ref. 40.
[f] Data from Ref. 47.
[g] Data from Ref. 48.
[h] Data from Ref. 49.
[j] Data from Ref. 45.

Bioassays

Peptides were assayed for agonistic activity in the rat antidiuretic assay, rat vasopressor assay and *in vitro* rat oxytocic assay using the four-point assay design [57]. All experimental procedures were approved by the Institutional Committee for the Care and Use of Animals at Weill Medical College of Cornell University. Synthetic arginine vasopressin and OT, which had been standardized in vasopressor and oxytocic units against the USP Posterior Pituitary Reference Standard, were used as working standards in all bioassays. Antidiuretic assays were on water-loaded rats under ethanol anesthesia as described in Sawyer [28]. Vasopressor assays were performed on urethane-anesthetized and phenoxybenzamine-treated rats as described by Dekanski [29]. Oxytocic assays were performed on isolated uteri from diethylstilbestrol-primed rats in a Mg^{2+}-free van Dyke-Hasting's solution [30]. When standard errors are presented in the Tables, the means reflect results from at least four independent assay groups.

Membrane Preparation

Membranes from the liver, kidney and anterior pituitary were prepared as previously described [37] from adult female rats purchased from Iffa credo (l'Arbrest France). All manipulations were performed according to the recommendations of the French Ethical committee and under the supervision of an authorized investigator.

Membranes from CHO cell lines stably transfected with the rat OT receptor were prepared as previously described [58].

Binding Assays and Data Analysis

Membrane incubations with [^{3}H] AVP were performed as previously described [8]. Briefly, membrane proteins (2 to 50 µg per assay) were incubated for 60 min at 30 °C (membranes from CHO cells) or 37 °C (membranes from native rat tissues) in a medium containing 50 mM Tris HCl pH 7.4, 3 mM $MgCl_2$, 1 mg/ml BSA, 0.01 mg/ml leupeptine. A concentration of 0.5 to 3 nM of [^{3}H] AVP was added in the incubation medium for V_2 or V_{1a}, V_{1b} and OT receptor measurements respectively with (nonspecific binding) or without (total binding) 1 µM unlabeled AVP or OT and increasing amounts of the analogue to be tested. Radioactivity found associated to the plasma membrane was determined by filtration through G/C filters. Specific binding was calculated for each experimental condition as the difference between total and nonspecific binding values. Data were analyzed by Graph Pad Software, Inc, Prism (GraphPad Software, Inc., San Diego, CA) as previously described [37]. Results are expressed as the mean of at least three distinct experiments.

Table 2 Binding properties of d[X^4]AVP analogues: comparisons of affinities and selectivities for rat and human vasopressin and oxytocin receptors

No	Peptide	Rat[b] Antidiuretic Activity (Units/mg)	Rat receptors				Human receptors[a]		V_{1b}/V_2 selectivity indices	
			rV_2-R Ki(nM)	rV_{1a}-R Ki(nM)	rV_{1b}-R Ki(nM)	rOT-R Ki(nM)	hV_2-R Ki(nM)	hV_{1b}-R Ki(nM)	$hV_{1b}R/hV_2R$[a]	$rV_{1b}R/rV_2R$[h]
	AVP	332	0.45[c]	2.6[c]	3.3[c]	1.7[c]	1.2	0.68	1.8	0.14
	dAVP	1745	0.76[c]	1.23[c]	2.2[c]	1220	5.0	0.37	13	0.35
	dDAVP[d]	1200	0.3[e]	100[e]	9.29[m]		23.3	5.8	4.0	0.03
	dVDAVP[f]	1230	0.3[e]	316[e]	152[k]		0.8[k]	24.5[k]	0.03	0.002
	d[D-3Pal^2]AVP[g]	1	68[c]	450[c]	112[c]		9600	13.8	695	0.61
	d[Cha^4]AVP	133.6	12.7[c]	229[c]	1.40[c]	1430[c]	750	1.2	625	9.1
1	d[Leu^4]AVP	378	3.1[h]	1252[h]	0.02[h]	481.0[h]	245	0.23	1065	155
2	d[Orn^4]AVP	260.32	3.4[h]	900.0[h]	0.45[h]	997.0[h]	1125	0.49	2295	7.6
3	d[Lys^4]AVP	34.83	24.6[h]	1478.3[h]	9.8[h]	5042.0[h]	11 170	1.8	6206	2.5
4	d[Har^4]AVP	504.77	0.6[h]	32[h]	0.32[h]	2996[h]	1386	0.52	2665	1.9
5	d[Arg^4]AVP	748.32 214	0.2[h]	12.9[h]	0.13[h]	3552[h]	131	0.37	354	1.5
6	d[Val^4]AVP	1150	0.3[c]	60[c]	4.5[c]	nd	1.2	0.29	4.1	0.06
7	d[Ala^4]AVP	840.58	0.58[h]				13.7	6.5	2.1	
8	d[Abu^4]AVP	1020	0.46[h]				2.1	1.2	1.8	
9	d[Nva^4]AVP	877.19	1.36[h]				24.1	0.78	31	
10	d[Nle^4]AVP	1141.27	0.16[h]				117	0.43	273	
11	d[Ile^4]AVP	819.23	1.35[h]				9.9	0.42	24	
12	d[Phe^4]AVP	11.47	86[h]				1067	9.5	112	
13	d[Asn^4]AVP	996.39	0.85[h]				50	1.5	33	
14	d[Thr^4]AVP	758	0.30[h]				1.5	1.0	1.5	
15	d[Dap^4]AVP	1053.49	0.07[h]				36.8	3.4	11	

[a] Data from Ref. 40.
[b] Data from Table 1 and publications cited in footnotes d,f,g.
[c] Data from Ref. 37.
[d] For original synthesis see Ref. 42.
[e] Data from Ref. 50.
[f] For original synthesis see Ref. 51.
[g] For original synthesis see Ref. 52.
[h] This publication.
[k] Data reported in Ref. 42.
[m] Data reported in Ref. 43.

RESULTS AND DISCUSSION

The antidiuretic, vasopressor and oxytocic (*in vitro*) activities in rat bioassays for the position 4 analogues of dAVP [41] examined in this study, including the previously reported analogues, 5, 6, 8 and 14 are presented in Table 1. The rat V_2, V_{1a}, V_{1b} and OT receptor affinity data for the four d[X^4]AVP analogues (where X = Leu, Orn, Lys and Har) recently reported to be potent and selective human V_{1b} receptor agonists [40], together with rat V_2, V_{1a}, V_{1b} and OT receptor affinity data for the related d[X^4]AVP analogues (where X = Cha, Val, Arg), AVP [44], dAVP [41], dDAVP [42], dVDAVP [51] and d[D-3-Pal]AVP [52] are given in Table 2. Rat V_2 receptor affinity data for the d[X^4]AVP analogues (where X = Ala, Abu, Nva, Nle, Ile, Phe, Asn, Thr and Dap) are also given in Table 2.

Selective Human V_{1b} Agonists are Potent Antidiuretic Agonists in the Rat (Tables 1 and 2)

The data in Tables 1 and 2 show clearly that, in addition to the previously published d[Cha^4]AVP [37], d[Leu^4]AVP, d[Orn^4]AVP, d[Lys^4]AVP and d[Har^4]AVP, all potent and selective agonists for the human AVP V_{1b} receptor [40] exhibit moderate to high potent antidiuretic activities in the rat. They exhibit the following antidiuretic activities: d[Leu^4]AVP = 378 units/mg, d[Orn^4]AVP = 260 units/mg, d[Lys^4]AVP = 35 units/mg and d[Har^4]AVP = 504 units/mg. Two of these peptides are more potent than AVP (antidiuretic activity = 332 units/mg) [45]. All four peptides exhibit much higher affinities for the rat V_2 receptor than for the human V_2 receptor (Table 2). Their corresponding rat and human (in brackets) V_2 receptor affinities are d[Leu^4]AVP = 3.1 nM (245 nM); d[Orn^4]AVP = 3.4 nM (1125 nM); d[Lys^4]AVP = 24.6 mM (11,170 nM);

d[Har^4]AVP = 0.6 nM (1386 nM). With the exception of d[Lys^4]AVP, the remaining three peptides exhibit high affinities for both the rat and the human V_{1b} receptors. Thus, while these three peptides have high affinities for the rat V_{1b} receptor, because they exhibit potent antidiuretic activities in the rat and have high affinities for the rat V_2 receptor, they are clearly not selective for the V_{1b} receptor with respect to the V_2 receptor in the rat. In this regard, the striking differences in the V_{1b}/V_2 selectivity indices of peptides 1–4 for human and rat receptors given in Table 2 is particularly striking. Since however, all four peptides exhibit low affinities for the rat and human V_{1a} and OT receptors they are highly selective for the rat and human V_{1b} receptors with respect to the rat and human V_{1a} and OT receptors. These findings show that these four peptides exhibit marked species differences for the rat and human V_2 receptors but not for their V_{1a}, V_{1b} or OT receptors.

Position 4 Analogues of dAVP Exhibit Potent Rat Antidiuretic Activities (Tables 1 and 2)

All of the new position 4 analogues of dAVP (Tables 1 and 2), with the exception of d[Phe^4]AVP, exhibit very high antidiuretic potencies, all in the same range as those for the previously reported analogues, d[Abu^4]AVP [49], d[Val^4]AVP [48] and d[Thr^4]AVP [45]. d[Arg^4]AVP was reported to exhibit antidiuretic activity equal to 214 units/mg [47]. We have obtained a substantially higher value equal to 748.32 units/mg for the resynthesized material [40] examined here. As noted below, all of the new position 4 analogues of dAVP examined in this study exhibit reductions in vasopressor potencies relative to dAVP (346 units/mg) [41,46] (Table 1). The most striking reduction in vasopressor potency was exhibited by d[Dap^4]AVP. Its vasopressor potency is 0.45 units/mg. With an antidiuretic potency equal to 1053 units/mg, this peptide is a highly potent and selective V_2 agonist in the rat. Its antidiuretic/pressor (A/P) ratio is 2341. These findings show that with the exception of the d[Lys^4]AVP and d[Phe^4]AVP analogues, the rat V_2 receptor tolerates a wide spectrum of structural variation at position 4 in dAVP analogues with the retention of potent antidiuretic activities.

Some Position 4 Analogues Exhibit Striking Species Difference for the Rat and Human V_2 Receptors (Table 2)

While all the position 4 analogues of dAVP, with the exception of d[Phe^4]AVP, exhibit potent antidiuretic activities in rat bioassays, only the three previously published peptides, d[Val^4]AVP [48], d[Abu^4]AVP [49] and d[Thr^4]AVP [45] exhibit high affinities for the human V_2 receptor [40]. Their human V_2 receptor affinities are respectively 1.2, 2.1 and 1.5 nM [40], all in the same range as AVP (1.2 nM) [37] and higher than that of dAVP (5.0 nM) [37]. The five d[X^4]AVP analogues (where X = Cha, Leu, Orn, Lys and Har) exhibit the greatest species differences between the rat and human V_2 receptors (Table 2). All are antidiuretic agonists in the rat, equipotent or more potent than AVP (Table 1), yet all exhibit very weak affinities for the human V_2 receptor (Table 2). These data show that the human V_2 receptor is much less tolerant of structural change at position 4 in dAVP than the rat V_2 receptor. They also illustrate the need for caution in using rat antidiuretic or rat V_2 receptor binding assays as guides for the design of potent selective V_2 agonists in humans.

Some Position 4 Analogues of dAVP Exhibit Highly Diminished Reductions in Rat Vasopressor Activities (Table 1)

It has previously been shown that the replacement of the Gln residue at position 4 in dAVP by Abu, Val and Thr, to give respectively d[Abu^4]AVP [49], d[Val^4]AVP [48] and d[Thr^4]AVP [45] brought about substantial reductions in vasopressor activity. dAVP exhibits vasopressor activity equal to 346 units/mg [46]. The vasopressor activities of these three analogues are respectively 11 units/mg [49], 51 units/mg [48] and 30 units/mg [45]. All of the new position 4 analogues of dAVP examined in this study (Table 1) exhibit reductions in vasopressor activity relative to that of dAVP. Nonetheless, with vasopressor activities of 107 units/mg, 160 units/mg and 97 units/mg respectively, the Har^4, Arg^4 and Asn^4 analogues retain substantially high vasopressor potencies compared to all the remaining position 4 analogues of dAVP in Table 1. In addition to the recently reported d[Cha^4]AVP, which exhibits very weak vasopressor activity equal to 0.067 units/mg [37], three other analogues exhibit drastic reductions in vasopressor activity. The vasopressor activities of d[Lys^4]AVP, d[Phe^4]AVP and d[Dap^4]AVP are 0.6 units/mg, 0.16 units/mg and 0.45 units/mg respectively. The differences in vasopressor potencies of the Arg^4 and Har^4 analogues on one hand and the Lys^4 and Dap^4 analogues on the other are particularly striking.

All the New Position 4 Analogues of dAVP Exhibit Moderate to Drastic Reductions in Oxytocic Activities (Table 2)

dAVP exhibits *in vitro* oxytocic potency equal to 63 units/mg [46]. All the new position 4 analogues of dAVP in Table 2 exhibit significant reductions in oxytocic activities compared to dAVP. Replacement of the Gln^4 residue in dAVP by Leu, Lys, Har, Arg, Nle, Phe and Dap resulted in analogues that exhibited drastic reductions in oxytocic potencies. Their oxytocic activities range from 0.027 units/mg for d[Arg^4]AVP to 0.48 units/mg for d[Dap^4]AVP. The

replacement of the Gln^4 residue in dAVP by Orn, Ala, Nva, Ile, Asn and by Thr resulted in analogues that exhibit less drastic losses in oxytocic potencies. Nonetheless, their oxytocic potencies range from a low of 1.21 units/mg for d[Ala^4]AVP to a high of 10.2 units/mg for d[Thr^4]AVP [45]. On the basis of these findings and on their rat V_{1b} and rat OT receptor affinities reported in Table 2, d[Leu^4]AVP, d[Orn^4]AVP, d[Har^4]AVP, d[Arg^4]AVP exhibit high rat V_{1b}/OT receptor selectivity.

Human and Rat V_{1b} Receptors are Highly Tolerant of Structural Changes at Position 4 in dAVP

We previously reported that the human V_{1b} receptor is highly tolerant of structural change at position 4 in a broad series of d[X^4]AVP analogues [40]. We report here that the d[X^4]AVP analogues where X = Cha, Leu, Orn, Har, Arg and Val all exhibit a high affinity for the rat V_{1b} receptor. Thus, in contrast to the species differences exhibited by rat and human V_2 receptors, the rat and human V_{1b} receptors appear to be highly tolerant of structural changes at position 4 in dAVP analogues.

The Challenge of Selective V_2 Agonist Design

Since the original synthesis of arginine vasopressin in 1954 [44], structure activity studies on this peptide have relied on the rat vasopressor [29], rat antidiuretic [28] and rat oxytocic [30] assays to measure the pharmacological properties of all synthetic analogues of AVP [31–35]. Utilization of these three assays led to the pharmacological discovery of the respective V_{1a}, V_2 and OT receptors [1,2,4] and to the design of selective agonists [31–35] and, over time, antagonists for all three receptors [32–35]. Although it was known that AVP and a wide variety of analogues, such as dDAVP [42], [Val^4] AVP [48] and dVDAVP [51] could cause the release of ACTH [59] (and references therein) [60], there was no routine bioassay that could be utilized to measure this activity for AVP analogues. Also, for a long time, it was not known which vasopressin receptor triggers the ACTH releasing activity of AVP [59,60]. During the mid-1980s, 30 years after the original synthesis of AVP [44], pharmacological studies with AVP antagonists provided evidence for the existence of a separate AVP ACTH releasing receptor, termed the V_{1b} (or V3) receptor [2,5,10]. This discovery led to the cloning of the V_{1b} receptor in 1994 [21,25,26]. Subsequently, pharmacological and functional studies showed that AVP analogues such as dDAVP [42], dVDAVP [51] and [Val^4]AVP [48] which had heretofore been described as selective V_2 agonists [31–35] were shown to mediate their ACTH releasing effects by their direct actions on the AVP V_{1b} receptor [4,37,43]. dDAVP [42], widely used for over three decades as a selective antidiuretic agonist [4], within the past decade, has been shown to be a partial V_{1b} agonist in rats and a full V_{1b} agonist in humans [43]. All the potent rat V_2 receptor agonists, examined in this study, have high affinities for the rat V_{1b} receptor. In all likelihood, they will either be partial or full V_{1b} agonists in the rat. V_{1b} agonism is an unwanted side effect in clinically used V_2 agonists such as dDAVP [4,11,61]. These findings show that the design of selective peptide V_2 receptor agonists, devoid of V_{1b} receptor agonism in rats and in humans is now a formidable challenge. These findings also raise another highly daunting and intriguing challenge: how to retrospectively pharmacologically characterize the V_{1b} receptor agonistic or antagonistic properties of the hundreds of published AVP and OT V_2, V_{1a} and OT agonists and antagonists [31–35,62–68]. This will require the development of a cell line that stably expresses the rat V_{1b} receptor known to be tightly coupled to phospholipase C [69], to provide a routine bioassay for measuring V_{1b} receptor activity. The development of an *in vivo* bioassay for measuring AVP-stimulated ACTH secretion will be much more difficult.

The Challenge of Selective Rat V_{1b} Receptor Agonist Design

We have previously shown that dAVP can be modified at position 4 with Cha, Leu, Lys, Orn and Har to give peptides that are high-affinity selective agonists for the human V_{1b} receptor [37,40]. The studies reported here show that these peptides have high affinities for the rat V_{1b} receptor. However, because they also exhibit moderate to highly potent antidiuretic activities in the rat they are not selective for the V_{1b} receptor with respect to the V_2 receptor in rats. The challenge now is to modify one or more of these peptides, d[Cha^4]AVP, d[Leu^4]AVP, d[Orn^4]AVP, d[Lys^4]AVP, and [Har^4]AVP, to give peptides that will exhibit selective V_{1b} receptor agonism with respect to the V_{1a}, V_2 and OT receptors in the rats. Preliminary studies along these lines are highly promising [70] and will be the subject of future publications.

CONCLUSION

The studies presented here show that the four d[X^4]AVP analogues (where X = Leu, Orn, Lys and Har), previously shown to be potent and selective agonists for the human V_{1b} receptor [40], also exhibit high affinities for the rat V_{1b} receptor. They exhibit low affinities for the rat V_{1a} and OT receptors. Thus, in the rat they are selective for the V_{1b} receptor with respect to both the V_{1a} and OT receptors. However, in rat bioassays all four peptides exhibit antidiuretic activities of 378, 260, 34 and 504 units/mg respectively and affinities for the rat V_2 receptor of 0.02, 0.45, 9.8 and 0.32 nM respectively.

These four peptides are thus not selective for the V_{1b} receptor with respect to the V_2 receptor in the rat. Of the remaining d[X^4]AVP analogues, those with X = Arg, Ala, Nva, Nle, Ile, Asn, Dap exhibit very high antidiuretic potencies, ranging from 748 to 1141 units/mg. These findings show that in contrast to the human V_2 receptor, the rat V_2 receptor is very tolerant of structural modifications at position 4 in dAVP. These findings present more evidence for profound species differences between the rat and human V_2 receptors [4,8,43,50,71,72]. Because all the position 4 analogues examined here also exhibit high affinities for the rat and human V_{1b} receptors, these findings, together with the recently uncovered V_{1b} agonism [37,43] of previously reported selective V_2 agonists, such as dDAVP [42], [Val^4]AVP [48] and dVDAVP [51], clearly show that the design of selective V_2 agonists, devoid of V_{1b} agonism is a formidable challenge in this field. Finally, the finding that d[Leu^4]AVP, d[Orn^4]AVP, d[Lys^4]AVP and d[Har^4]AVP exhibit high affinities for the rat V_{1b} and V_2 receptors, while exhibiting very low affinities for the rat V_{1a} and OT receptors, has provided very promising new leads to the design of the first truly selective agonists for the rat V_{1b} receptor [70]. Selective agonists for the human and rat V_{1b} receptors could be very useful pharmacological tools for studies on the role of the AVP V_{1b} receptor in the etiology of stress and depression [3,9,14–19,38].

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