

REVIEW ARTICLE

Oxytocin and Vasopressin Agonists and Antagonists as Research Tools and Potential Therapeutics

M. Manning*, A. Misicka*†, A. Olma*‡, K. Bankowski*S, S. Stoev*, B. Chini¶, T. Durroux**, B. Mouillac**, M. Corbani** and G. Guillon**

*Biochemistry and Cancer Biology, University of Toledo College of Medicine, Toledo, OH, USA.

†Faculty of Chemistry, University of Warsaw, Warsaw, Poland.

‡Faculty of Chemistry, Institute of Organic Chemistry, Technical University of Lodz, Lodz, Poland.

§Pharmaceutical Research Institute, Warsaw, Poland.

¶CNR Institute of Neuroscience, Milan, Italy.

**Institut de Genomique Fonctionnelle, UMR5203-CNRS, U661-INSERM, Univ. Montpellier I & II, Montpellier, Cedex, France.

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We recently reviewed the status of peptide and nonpeptide agonists and antagonists for the V_{1a} , V_{1b} and V_2 receptors for arginine vasopressin (AVP) and the oxytocin receptor for oxytocin (OT). In the present review, we update the status of peptides and nonpeptides as: (i) research tools and (ii) therapeutic agents. We also present our recent findings on the design of fluorescent ligands for V_{1b} receptor localisation and for OT receptor dimerisation. We note the exciting discoveries regarding two novel naturally occurring analogues of OT. Recent reports of a selective VP V_{1a} agonist and a selective OT agonist point to the continued therapeutic potential of peptides in this field. To date, only two nonpeptides, the V_2/V_{1a} antagonist, conivaptan and the V_2 antagonist tolvaptan have received Food and Drug Administration approval for clinical use. The development of nonpeptide AVP V_{1a} , V_{1b} and V_2 antagonists and OT agonists and antagonists has recently been abandoned by Merck, Sanofi and Pfizer. A promising OT antagonist, Retosiban, developed at Glaxo SmithKline is currently in a Phase II clinical trial for the prevention of premature labour. A number of the nonpeptide ligands that were not successful in clinical trials are proving to be valuable as research tools. Peptide agonists and antagonists continue to be very widely used as research tools in this field. In this regard, we present receptor data on some of the most widely used peptide and nonpeptide ligands, as a guide for their use, especially with regard to receptor selectivity and species differences.

Key words: vasopressin, oxytocin, agonists, antagonists, peptide, non-peptide.

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Correspondence to:

M. Manning, Biochemistry and Cancer Biology, University of Toledo College of Medicine, 3000 Arlington Avenue, Toledo, OH 43614-2598, USA (e-mail: maurice.manning@utoledo.edu).

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Introduction

Subsequent to the pioneering original synthesis of oxytocin (OT) (2) and arginine vasopressin (AVP) (3) by Vincent du Vigneaud and his associates, thousands of analogues of both of these neurohypophysial peptides have been synthesised in many laboratories throughout the world. The Merrifield solid phase method (4) has been of inestimable importance in facilitating the rapid and efficient synthesis of agonists and antagonists for the AVP V_{1a} , V_{1b} and V_2 receptors and for the OT uterine receptor (5). Many of these ligands have found widespread use as pharmacological tools for studies on the peripheral and central effects of OT and AVP. These design and synthetic studies have been the subject of numerous reviews (1, 6–20).

Structure activity and design studies carried out in other laboratories over the past five decades have laid the foundation for the design studies that we present here. These pivotal contributions by others have been fully documented (11, 14, 18, 20).

Oxytocin and AVP mediate their biological effects by acting on specific receptors (21–23). OT and AVP receptors belong to a G-protein coupled receptor family, characterised by seven putative trans-membrane helices. Reviews on AVP and OT receptors are available elsewhere (12, 22, 23). OT receptors are expressed in the uterus, the mammary gland, the ovary, the brain, the kidney, the heart, bone and in endothelial cells (23). In the uterus, OT receptors mediate the uterine contracting (oxytocic) effect of OT (23). The central effects of OT continue to be the focus of intense investigative scrutiny in

animals (24–28) and in humans (29–35), as a possible therapeutic agent for the treatment of autism and other anxiety disorders.

Arginine vasopressin mediates its actions through three known receptors: V_{1a} , V_{1b} and V_2 . V_{1a} receptors are expressed in the liver, vascular smooth muscle cells, brain and in many other tissues (12, 21, 22). In the vasculature, V_{1a} receptors mediate the pressor actions of AVP by a phospholipase C-mediated pathway. In the brain, V_{1a} receptors mediate the anxiety producing responses to AVP (27, 36). V_{1b} receptors, discovered long after the V_{1a} and V_2 receptors, present in the anterior pituitary, mediate the adrenocorticotrophic hormone-releasing effects of AVP, also by a phospholipase C-mediated pathway (22). Evidence for the presence of V_{1b} receptors in extra-pituitary tissues such as brain, the kidney and the adrenal medulla has also been reported (37). Recently, the V_{1b} receptor has been shown to mediate anxiety and stress in rats and in humans (45). V_2 receptors, present in the collecting duct of the kidney, mediate the antidiuretic action of AVP by an adenylate cyclase-mediated pathway (12, 21, 22).

Scope of the present review

We have previously reviewed the status of developments in the design and synthesis of peptide and nonpeptide AVP and OT agonists and antagonists (1). Here, we focus on the properties of the most widely used peptides requested from the Manning laboratory or purchased from suppliers, together with some recently reported potential clinically useful peptides from the Ferring Laboratory (38, 39). Space considerations preclude our being able to present or to discuss recent synthetic studies carried out in other laboratories (40–43). We also update the current status of the pre-clinical and clinical development of nonpeptide AVP and OT antagonists and of the pre-clinical development of nonpeptide OT agonists (44). The excellent reviews on nonpeptide AVP antagonists (45) and on nonpeptide OT antagonists (46) should be consulted for more in-depth presentations of their chemistry and pharmacology. We also review the merits of peptide versus nonpeptide AVP and OT agonists and antagonists as: (i) research tools and (ii) therapeutic agents. We present human and rat receptor data for a number of selective peptide agonists and for both peptide and nonpeptide antagonists. We illustrate the need to be aware of: (i) species differences, (ii) selectivity differences and (iii) *in vitro*–*in vivo* differences when using a specific ligand for receptor characterisation. Finally, we present the highlights of our recent studies aimed at: (i) the development of selective fluorescent ligands for the rat and human V_{1b} receptors (47) and (ii) the development of fluorescence based strategies that have been used to prove the existence of OT receptor dimers in native tissue (48).

Peptide synthesis

All the OT and AVP agonists, antagonists, radiolabelled and fluorescent ligands from our laboratories were synthesised using the Merrifield solid-phase method (4, 49). The synthetic strategy relies very heavily on methodology developed in the du Vigneaud laboratory for the original syntheses of OT and AVP (2, 3). The procedures used

are described in the original publications cited here. For other references, see Manning *et al.* (1).

Bioassays

All of the published peptides from our laboratories, presented in Tables 1, 3–8, were assayed for agonistic and antagonistic activities in *in vitro* and *in vivo* rat oxytocic assays, in the rat vasopressor assay and in the rat antidiuretic assay in the laboratories of our collaborators Drs Wilbur H. Sawyer, W. Y. Chan and Hazel Szeto. For agonists, the four-point assay design (50) was used and for antagonists, Schild's pA_2 method (51) was employed. The pA_2 is the negative logarithm of the molar concentration of the antagonist that requires a two-fold increase in agonist concentration to achieve the same effect as that found in the absence of antagonist. In practice, this concentration is estimated by finding concentrations above and below the pA_2 dose and interpolating on a logarithmic scale.

In the rat *in vivo* assays, the pA_2 (effective dose) is divided by an arbitrarily assumed volume of distribution of 67 ml/kg (52) in an attempt to derive the approximate molar concentration [M] of the pA_2 dose in the vicinity of the receptors. Thus, *in vivo* pA_2 values are very approximate estimates. The USP Posterior Pituitary Reference Standard or synthetic OT and AVP, which had been standardised in oxytocic and vasopressor units against this standard, were used as agonists for working standards in all bioassays. *In vitro* oxytocic assays were performed on isolated uteri from diethylstilbestrol-primed rats in a Mg^{2+} -free van Dyke Hasting's solution (53). *In vivo* anti-OT potencies were determined in urethane-anaesthetised diethylstilbestrol-primed rats as described previously (54, 55). Vasopressor assays were performed on urethane-anaesthetised and phenoxybenzamine-treated rats as described by Dekanski (55), and antidiuretic assays on water-loaded rats under ethanol anesthesia as described by Sawyer (56).

Receptor binding and functional assays

Membranes and/or cell lines that express the rat and human AVP V_{1a} , V_{1b} and V_2 receptors (57–64) and the human OT receptor (65) were used for binding and functional assays: inositol phosphate accumulation (66) for V_{1a} , V_{1b} and OT receptors and cyclic AMP accumulation (67) for V_2 receptors, as described previously (68–74). These receptor studies were carried out in Montpellier and Milan.

Selective oxytocin agonists (Table 1)

The pharmacological properties in rat bioassays for OT and the four analogues (peptides 1–4), which are more potent and/or more selective than OT (75), are given in Table 1. $[Thr^4, Gly^7]OT$ (peptide 3), also referred to as TGOT, has been widely used as a selective OT agonist. Its human and rat receptor affinities are given in Table 11. The recently reported (38) highly selective OT analogue FE 202767 (peptide 5) has not been evaluated in standard rat bioassays. It exhibits high affinity and selectivity for the human OT receptor. It thus offers promise as a potential new OT therapeutic (38).

Table 1. Potent and Selective Agonists for the Uterine Oxytocin Receptor in the Rat.

Number	Peptide	OT receptor oxytocic (O) (units/mg)	V _{1a} receptor vasopressor (P) (units/mg)	V ₂ receptor antidiuretic (A) (units/mg)	Ratios		Reference
					O/A	O/P	
	OT	520	4	4	130	130	75
1	[Thr ⁴]OT	923	0.4	0.9	1025	2307	75
2	HO[Thr ⁴]OT	4179	4.92	5.3	790	850	75
3	[Thr ⁴ , Gly ⁷]OT	166	<0.01	~0.002	83 000	>16 600	75
4	HO[Thr ⁴ , Gly ⁷]OT	218	<0.01	0.004	54 500	>21 800	75
5	Carba-1-[4-FBzlGly ⁷]dOT(FE 202767)*	ND	ND	ND			38

OT, oxytocin; HO, 1-hydroxy (hydroxyl group replaces α -amino group); FBzl, fluorobenzyl. **In vitro* potency EC₅₀^a (nM) hOT 0.08, hV₂ 330, hV_{1a}>10000 selectivity versus receptor hV₂ 4100, hV_{1b} > 120000. ^aEC₅₀ is the concentration of agonist leading to half-maximal activity. ND, Not determined.

Table 2. New Oxytocin (OT)-Related Peptides.

Number	Peptide	Reference
1	Oxytocin-Gly-Lys-Arg	77, 78
2	[Pro ⁸]OT	76

The two new OT-related analogues given in Table 2 (1, 2) have not yet been evaluated in standard rat bioassays. The discovery of [Pro⁸]OT in new world monkeys by Lee *et al.* (76), independently confirmed by Jeffrey French and colleague (E. B. Harrison and J. A. French, personal communication), is an exciting new development in this field. The novel C-terminal extended analogue of OT; oxytocin-Gly-Lys-Arg, reported by Gutkowska *et al.* (77) and Danalache *et al.* (78), opens up new possibilities for the design of potential new cardiomyogenic therapies.

Selective vasopressin V₂ receptor agonists (Table 3)

AVP is equipotent as an antidiuretic agonist and as a vasopressor agonist (79) (Table 3). Thus, it is totally nonselective. It is also not selective with respect to its oxytocic activity. The three analogues of AVP, peptides 1–3 in Table 3 namely; dDAVP, VDAVP and dVDAVP,

exhibit striking gains in antidiuretic/vasopressor selectivity. All three peptides have been widely used as selective V₂ agonists. dDAVP, first synthesised by the Zaoral *et al.* (80) in Prague and later licensed to Ferring, has long been the drug of choice for the treatment of diabetes insipidus. It has been marketed under the trademark Desmopressin (Minirin). The human receptor affinities for dDAVP and dVDAVP given in Table 11 shows clearly that dVDAVP has a ten-fold higher affinity for the human VP V₂ receptor than dDAVP. However, both peptides also exhibit high affinities for the human V_{1b} receptor and to somewhat lesser extent for the human OT receptor (Table 11). So clearly they are not selective V₂ agonists in humans with respect to both hV_{1b} or hV_{1a} receptors. The search for a V₂ agonist that is selective with respect to the V_{1a} and V_{1b} receptors in humans is still a challenging goal in this field. Yet, in the rat, dDAVP could be considered as a relatively good selective V₂ agonist (Table 11).

Selective vasopressin V_{1a} receptor agonists (Table 4)

In rat bioassays, [Phe²]OVT (peptide 2; Table 4) is a fairly potent vasopressor agonist (81). Its vasopressor (P) activity is 124 units/mg. In antidiuretic (A) assays, it exhibits only 0.55 units/mg. Its P/A ratio is 225 (81). Thus, for many years, it has been considered to be a selective V_{1a} agonist and has been widely used as a selective V_{1a} agonist. However, based on its rat V_{1a} receptor affinity

Table 3. Potent and Selective Agonists for the Vasopressin V₂ Receptor in the Rat^a.

Number	Peptide	OT receptor oxytocic (O) (units/mg)	V _{1a} receptor vasopressor (P) (units/mg)	V ₂ receptor antidiuretic (A) (units/mg)	Ratios		Reference
					A/P	A/O	
	AVP	14	373	320	0.9	22.8	79
1	dDAVP (desmopressin)*	1.5	0.39	1200	3000	800	79, 80
2	VDAVP	0.60	0.037	653	17 650	1 090	79
3	dVDAVP	8	Antagonist (pA ₂ = 7.03)	1230	Infinite		79

OT, oxytocin; AVP, arginine vasopressin; d, 1-deamino; DAVP, D-Arg⁸VP; V, Val⁴. *Desmopressin is the drug of choice for the treatment of diabetes insipidus. ^aThe data given are obtained from Sawyer *et al.* (79).

Table 4. Potent and Selective Agonists for the Vasopressin V_{1a} Receptor in the Rat.

Number	Peptide	V _{1a} receptor vasopressor (P) (units/mg)	V ₂ receptor antidiuretic (A) (units/mg)	OT receptor oxytocic (O) (units/mg)	Ratios		Reference
					P/A	P/O	
	AVP	373	320	14	1.2	26.6	79, 84
	LVP ([Lys ⁸]VP)	270	284	10	0.95	27	85
1	[Phe ²]LVP (felypressin, octapressin)	57	21	0.3	2.7	190	17
2	[Phe ²]OVT, [Phe ² ,Orn ⁸]vasotocin	124	0.55	1	225	124	81
3	F-180	164	0.19		863		82
4	FE 202158*	ND	ND	ND			39, 83

OT, oxytocin; F180, Hmp-Phe-Ile-Hgn-Asn-Cys-Pro-Dab(Abu)-Gly-NH₂; where Hmp, 2-hydroxy-3-mercaptopropionic acid; Hgn, homoglutamine; Dab, 2,4-diaminobutyric acid; Abu, 2-aminobutyric acid; *FE 202158, [Phe²,Ile³,Hgn⁴,Orn(iPr)⁸]AVP, where Hgn is homoglutamine and iPr is isopropyl.

data in Table 11, it is not selective for the rat V_{1a} receptor in this assay. In this regard, the selective V_{1a} agonist F-180 (82), which is a highly selective V_{1a} agonist in rat bioassays (peptide 3; Table 4), is even more puzzling. In rat receptor assays (Table 11), it is clearly nonselective for V_{1a} receptors. By contrast, F-180 exhibits high affinity and selectivity for the human V_{1a} receptor. The exciting new V_{1a} agonist (peptide 4; Table 5), FE202158, recently reported by Ferring (39, 83) is currently undergoing clinical trials for the treatment of vasodilating hypotension. Compared to F180, this peptide exhibits better selectivity for the human V_{1a} receptor (Table 11). In the rat, this agonist is very specific for the V_{1a} receptor compared to the V₂ receptor (selectivity higher than 800), yet it has not been tested for the OT and V_{1b} receptors. This intriguing new V_{1a} agonist is not yet available to other scientists for use as a pharmacological research tool.

V_{1b} receptor agonists (Table 5)

AVP was synthesised in 1954 (3) (Table 5). The first 'selective' V_{1a} agonist [Phe²]OVT was synthesised 10 years later in 1964 (81). The first selective V₂ agonist dDAVP was reported in 1967 (80). Yet, it was not until 2002, almost 50 years after the synthesis of AVP, that the first selective agonist for the human V_{1b} receptor,

d[Cha⁴]AVP was synthesised (69). The reasons why this discovery took so long have been documented by Manning *et al.* (1).

Table 5 lists four analogues of dAVP (peptides 1–4) that exhibit high affinities for both the rat and human V_{1b} receptors. d[Cha⁴]AVP (peptide 1) was the first V_{1b} agonist that was shown to be selective for the human V_{1b} receptor (69). d[Leu⁴]AVP (peptide 3) has later been shown to be a selective agonist for the human V_{1b} receptor (72). Both (peptides 1 and 3) exhibit high affinities for the rat V_{1b} receptor. However, they also possess high *in vivo* antidiuretic activity. Thus, neither is a selective V_{1b} agonist in the rat.

Replacement of the Arg⁸ residue in (peptides 1 and 3) by a Lys⁸ residue to give d[Cha⁴,Lys⁸]VP (peptide 2) and d[Leu⁴,Lys⁸]VP (peptide 4), respectively, resulted in the first peptides that are selective V_{1b} agonists in the rat (73, 74). It was subsequently shown that both peptides 1 and 2 are also highly selective for human V_{1b} receptors (86). It bears noting that d[Leu⁴,Lys⁸]VP had been reported to be a weak antidiuretic V₂ agonist/weak vasopressor agonist in the rat (87), 30 years before the V_{1b} receptor was first predicted and/or cloned (88). The selective V_{1b} agonists d[Cha⁴]AVP and d[Leu⁴,Lys⁸]VP have been used as a research tool in a number of studies (1, 20, 62, 73, 89, 90). Furthermore, d[Leu⁴,Lys⁸]VP has been utilised in the design of a series of fluorescent ligands for the V_{1b} receptor (47).

Table 5. Lys⁸ Analogues of d[Cha⁴]AVP (1) and d[Leu⁴]AVP (3) exhibit High Affinities and Selectivities for both Rat and Human V_{1b} Receptors.

Number	Peptide	Rat antidiuretic activity (U/mg)	Affinity (K _i) (nM)								Reference
			rV _{1b} -R	hV _{1b} -R	rV ₂ -R	hV ₂ -R	rV _{1a} -R	hV _{1a} -R	rOT-R	hOT-R	
	AVP	323	0.29	0.68	0.45	1.2	2.6	1.1	1.7	1.7	74, 84
	dAVP	1745	0.20	0.37	0.76	5	10.8	3.8	0.97	–	74, 84
1	d[Cha ⁴]AVP	133.6	1.40	1.2	12.7	750	2297	151	1430	240	69, 72, 74
2	d[Cha ⁴ , Lys ⁸]VP	0.82	1.9	2.2	596	11 484	9093	283	586	141	85, 86
3	d[Leu ⁴]AVP	378	0.04	0.23	3.1	245	1252	44.1	481	211	72, 74
4	d[Leu ⁴ , Lys ⁸]VP	10.5	0.16	0.51	101	6713	3786	69.3	64	29	74, 86, 87

Table 6. Design of Highly Selective V_{1a} Antagonists.

Number	Peptide	Anti-OT (<i>in vitro</i>)	Anti-OT (<i>in vivo</i>)	Anti-V _{1a} (<i>in vivo</i>)	Anti-V _{1a} /anti-OT selectivity	Reference
		pA ₂ ^a	pA ₂ ^b	pA ₂ ^b		
1	d(CH ₂) ₅ [Tyr(Me) ²]AVP (Manning compound)	8.13	6.62	8.62	100	91
2	d(CH ₂) ₅ [Tyr(Me) ² , Dap ⁵]AVP	5.83	ND ^c	7.49	Infinite	92
3	d(CH ₂) ₅ [Tyr(Me) ² , Dab ⁵]AVP	ND ^c	ND ^c	6.71	Infinite	92

OT, oxytocin; d(CH₂)₅ = β-mercapto-β,β-cyclopentamethylenepropionyl. ^a*In vitro* pA₂ values represent the negative logarithm to the base 10 of the average molar concentration [M] of the antagonist that reduces the response to 2 × units of agonist to the equal the response seen with 1 × units of agonist administered in the absence of the antagonist. ^b*In vivo* pA₂ values are estimated because the molar concentration for the *in vivo* pA₂ is estimated by dividing the effective dose (ED) by the estimated volume of distribution of (67 ml/kg) (52). ED is defined as the dose (nmol/kg intravenously) of the antagonist that reduces the response to 2 × units of agonist to the response with 1 × units of agonist administered in the absence of the antagonist. ^cND, not detectable (weak agonist, <0.03 U/mg).

Selective V_{1a} antagonists (Table 6)

d(CH₂)₅[Tyr(Me)²]AVP, also known as Manning compound (peptide 1; Table 6) is a potent VP V_{1a} antagonist/weak VP V₂ agonist (91). It is thus highly selective for V_{1a} receptors versus V₂ receptors. It is, however, a potent *in vitro* OT antagonist and a fairly potent OT antagonist *in vivo* (91). It has found widespread use as a selective V_{1a} antagonist in a variety of studies on the peripheral and central effects of AVP. Indeed, it has become the most widely used V_{1a} antagonist reported to date. Isosteric modifications of d(CH₂)₅[Tyr(Me)²]AVP at position 5 with diaminopropionic acid (Dap) and diaminobutyric acid (Dab) led to d(CH₂)₅[Tyr(Me)²,Dap⁵]AVP (peptide 2; Table 6) and d(CH₂)₅[Tyr(Me)²,Dab⁵]AVP (peptide 3; Table 6), respectively. Both peptides are devoid of anti OT activity *in vivo* (92). Although both peptides are much less potent than

d(CH₂)₅[Tyr(Me)²]AVP as V_{1a} antagonists, because they lack anti OT potency *in vivo*, they are highly selective for V_{1a} receptors in the rat. Their use is recommended for *in vivo* studies that require discrimination between V_{1a} and OT receptors in the rat.

Nonselective and selective cyclic and linear V₂/V_{1a} antagonists for rat receptors

It was not until 1981, almost 30 years after the first laboratory synthesis of OT, that the first cyclic AVP V₂/V_{1a} antagonists were reported (8) (Table 7). Six years later, the unexpected discovery of the first linear V₂/V_{1a} antagonists was reported (93). The early cyclic and linear V₂/V_{1a} antagonists were nonselective for V₂ receptors. Further modifications of the early cyclic V₂/V_{1a} antagonists led to the discovery of selective cyclic V₂ antagonists (94). Some of the

Table 7. Nonselective and Selective Cyclic and Linear V₂/V_{1a} Antagonists for Rat Receptors.

Number	Peptide	Antiantidiuretic (A) (anti-V ₂)		Antivaso pressor (P) (anti-V _{1a})		Antioxytocic <i>In vivo</i>	ED ratio A/P	Reference
		ED ^a	pA ₂ ^b	ED ^a	pA ₂ ^b	pA ₂ ^b		
1	d(CH ₂) ₅ [D-Tyr(Et) ²]VAVP	1.1	7.81		8.22	7.47*	0.4	98
2	desGly,d(CH ₂) ₅ [D-Tyr(Et) ² , Val ⁴]AVP	1.5	7.69	0.45	8.17	6.98*	0.3	99
3	d(CH ₂) ₅ [D-Ile ² , Ile ⁴]AVP	0.67	8.04	0.45	6.42	6.90*	39	94
4	desGly-NH ₂ ,d(CH ₂) ₅ [D-Ile ² , Ile ⁴]AVP	0.90	7.88	26	~5.2		~440	100
5	d(CH ₂) ₅ [D-Ile ² , Ile ⁴ , Ala-NH ₂ ⁹]AVP	0.46	8.16	~400	6.25		83	100
6	d(CH ₂) ₅ [D-Tyr(Et) ² , Ile ⁴ , Eda ⁹]AVP	0.77	8.00	38	8.33			101
7	Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu- Pro-Arg-Arg-NH ₂	0.53	8.11	0.32	7.75		2.3	48, 95
8	4-HO-Phaa-D-Tyr(Me)-Phe-Gln-Asn- Arg-Pro-Arg-NH ₂ (HO-LVA)	Agonist	0.056 U/mg	1.2	8.47			12, 96, 97

Aaa, adamantaneacetyl; Eda, ethylenediamine; 4-HO-Phaa, 4-hydroxyphenylacetyl. **In vivo* anti-oxytocin (OT) potencies were reported previously (10). ^aThe effective dose (ED) is defined as the dose (in nmol/kg) that reduces the response to 2 × units of agonist to equal the response to 1 × unit. ^b Estimated *in vivo* pA₂ values represent the negative logarithms of the EDs divided by the estimated volume of distribution (67ml/kg) (52).

most commonly used nonselective cyclic and linear V_2/V_1 antagonists (peptides **1**, **2**, **7**) and selective cyclic V_2 antagonists (peptides **3–6**) are given in Table 7. Peptide **6** (101) has been very useful for the design of a lanthanide cryptate-labelled ligand as a fluorescent probe for measuring receptor dimerisation (48) Peptide **8** (HO-LVA) (95), a potent linear V_{1a} antagonist, has served as a precursor for the radioiodinated V_{1a} ligand [125 I]HO-LVA (97). This radioligand has found widespread use as a selective probe for V_{1a} receptors. Its affinities for rat receptors are given in Table 12. A number of these V_2/V_{1a} antagonists (peptides **1–3**; Table 7) exhibit oxytocic antagonism *in vivo*. The remaining peptides **4–8** have not been evaluated in anti-OT assays. Caution should be exercised in using any of the peptides in Table 7 as selective V_2 ligands. The affinities of peptides **1** and **5** for the human and rat V_2 receptors are given in Table 12.

Some nonselective and selective oxytocin antagonists (Table 8)

The OT antagonists listed in Table 8 have all found widespread use as pharmacological tools. Under the tradename Tractocile, atosiban (peptide **1**) has been approved for clinical use in Europe for the prevention of premature labour (102). All of these OT antagonists exhibit varying degrees of anti- V_{1a} potency in the rat. Thus, they are far from being selective. Indeed, $d(CH_2)_5[Tyr(Me)^2]OVT$, one of our original OT antagonists (103), is five-fold more potent as a vasopressor antagonist than as an OT antagonist (103). Peptide **5**, desGly-NH₂, $d(CH_2)_5[D-Tyr^2,Thr^4]OVT$ (104) is the most selective OT antagonist in Table 8. It has been used in a variety of studies. These are listed under 'Research Uses'.

This OT receptor antagonist also exhibits a high affinity and selectivity for the human OT receptor (Table 12). Peptide **3**, desGly-

NH₂, $d(CH_2)_5[Tyr(Me)^2,Thr^4]OVT$, although much less selective than OTA No. 5, has proved to be very useful as a research tool in the Neumann laboratory for a wide variety of studies on the central effects of OT. Some examples of these studies are listed under 'Research Uses'. The ^{125}I derivative of peptide 4, $d(CH_2)_5[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT$ (105) has found widespread use as a probe of OT receptors.

Nonpeptide vasopressin antagonists as pharmacological tools and therapeutic agents (Table 9)

The search for nonpeptide antagonists for the AVP, V_{1a} , V_{1b} and V_2 receptors has been pursued with vigour by many pharmaceutical companies, most notably Otsuka, Sanofi, Azevan, Astellas, Wyeth-Ayerst, Johnson & Johnson, Yamanouchi and Pfizer (Table 9). In 1991, Otsuka reported the first nonpeptide V_{1a} antagonist OPC-21268 (No. **3**; Table 9) (107). During the subsequent 20 years, a number of promising nonpeptide V_1 , V_{1b} , and V_2 antagonists have been reported (1, 45). Table 9 lists a number of these, together with references to: (i) their original synthesis (45, 107, 108, 110, 116–119, 127, 164); (ii) some research uses (115, 117, 120, 124, 126, 147, 148, 150, 152); and (iii) their clinical uses (109, 111–115, 121–123, 128, 129). Most notable, are the Otsuka V_2 antagonist (Tolvaptan, No. **5**; Table 9) first reported in 1998 by Yamamura *et al.*, (108) and shown to be effective in the treatment of hyponatraemia (109) and the Astellas V_2/V_{1a} antagonist (Conivaptan, No. **8**; Table 9) first reported in 1997 by Tahara *et al.* (110). Both conivaptan and tolvaptan have been approved for clinical use by the Food and Drug Administration (111–115). The development of non-peptide V_{1a} , V_{1b} and V_2 antagonists at other companies has recently been abandoned as a result of failures in clinic trials. Thus, Sanofi, which had reported the V_{1a} antagonist SR-49059, Relcovaptan (No.

Table 8. Some Nonselective and Selective Oxytocin Antagonists in the Rat.

Number	Peptide	Antioxytocic (anti-OT)			Antivasopressor (anti- V_{1a})	Antidiuretic activity (V_2)	ED ratio ^d	Ref
		<i>In vitro</i> pA ₂ ^a	<i>In vivo</i>	pA ₂ ^c				
		No Mg ²⁺	ED ^a	pA ₂ ^c	ED ^b	pA ₂ ^c	Units/mg	
1	d[D-Tyr(Et) ² ,Thr ⁴]OVT (atosiban)	8.29	Antagonist		Agonist		Agonist	
		7.71	5.95	7.05	0.02 (IU/ μ mol)	6.14	Antagonist	8
2	$d(CH_2)_5[Tyr(Me)^2]OVT$	8.52	4.2	7.37	0.80	7.96	≈ 0.01	0.2
3	desGly-NH ₂ , $d(CH_2)_5[Tyr(Me)^2,Thr^4]OVT$	7.89	1.3	7.69	23	6.48	Antagonist (pA ₂ \approx 5.5)	17.7
4	$d(CH_2)_5[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT$	7.63	1.0	7.83	6.6	7.02	≈ 0.015	6.6
5	desGly-NH ₂ , $d(CH_2)_5[D-Tyr^2,Thr^4]OVT$	7.77	2.85	7.37	272	5.39	Antagonist (pA ₂ < 5.5)	95

^a*In vitro* pA₂ values represents the negative logarithm to the base 10 of the average molar concentration [M] of antagonist which reduces the response to 2 × units of agonist to the response with × units of agonist. ^bThe effective dose (ED) is defined as the dose (in nm/kg) of antagonist that reduces the response to 2 × units of agonist to the response with × units of agonist administered in the absence of antagonist. ^cEstimated *in vivo* pA₂ values represent the negative logarithms of the 'effective dose' divided by the estimated volume of distribution (67 ml/kg) (52). ^dED ratio = anti-vasopressor ED/antioxytocic ED.

Table 9. Nonpeptide Vasopressin Antagonists as Pharmacological Tools and Therapeutic Agents.

Number	Receptor Type	Company	Code	Name	Supplier	Status	Reference: synthesis	Reference: pharmacological use	Reference: clinical use
1	V _{1a}	Sanofi	SR49059	Relcovaptan	Tocris	Phase II (terminated)	45, 116	117	
2	V _{1a}	Pfizer	PF-00738245	No name	Pfizer	New compound	118		
3	V _{1a}	Otsuka	OPC-21268	No name	Tocris Sigma-Aldrich	Phase II Japan stopped US/Europe	107		
4	V _{1b}	Sanofi	SSR149415	Nelivaptan	Axon Medchem	Preclinical (terminated)	45, 119, 164	120, 147, 148, 150	
5	V ₂	Otsuka	OPC-41061	Tolvaptan	Shanghai DND Pharm-Technology Co.,Inc.	Approved by US Food and Drug Administration oral use (Samsca)	108	115	111–115, 121–123
6	V ₂	Sanofi	SR121463(B)	Satavaptan	None	Phase III (terminated)	45	124–126	
7	V ₂	Otsuka	OPC-31260	Mozavaptan	Otsuka; Anhui Pharmaceutical Co., LTD	Phase II	127		
8	V ₂ /V _{1a}	Astellas	YM-087	Conivaptan	LGM Pharma, Beijing HuameiHuli Biochem Ltd	Approved by US Food and Drug Administration i.v. use (Vaprisol)	127	152	111, 112, 128, 129

Table 10. Non peptide Oxytocin Antagonists and a Nonpeptide Agonist.

Compound	Number	Code (name)	Company	Supplier	Status	Reference: original synthesis, structure and pharmacological properties)	Reference: use
OT antagonist	1	L-368,899	Merck	Tocris	Phase II Discontinued	46, 130	134–136
	2	L-371,257	Merck	Tocris	Phase II Discontinued	46	141
	3	WAY-162720	Wyeth-Ayerst (now Pfizer)	No	Failed in preclinical	131	141
	4	GSK2211149A (Retosiban)	Glaxo SmithKline	Simagchem; Manus Akteva Biopharma LLP	Phase II completed	132, 133	
OT agonist	1	WAY-267464	Wyeth-Ayerst (now Pfizer)	Tocris	Failed in preclinical	138, 140	137, 141

1; Table 9), the V_{1b} antagonist SSR-149415, Nelivaptan (No. 4; Table 9) and the V₂ antagonist SR-121463 (B) Satavaptan (No. 6; Table 9), all highly promising candidates for therapeutic development (45), has recently abandoned its entire AVP nonpeptide antagonist programme. It should be noted that these three Sanofi nonpeptide antagonists are highly useful pharmacological tools. The commercial availability of some of these nonpeptide VP antagonists is shown in Table 9. Human and rat receptor affinities for all three Sanofi V_{1a}, V_{1b} and V₂ nonpeptides are given in Table 12.

Nonpeptide oxytocin antagonists and a nonpeptide oxytocin agonist (Table 10)

A number of companies have been active in this area. In the mid-1990s, Merck reported a number of promising nonpeptide OT antagonists (46, 130) (Table 10). Most notable were L-368,899 (No. 1; Table 10) and L-371, 257 (No. 2; Table 10). Both of these failed in clinical development for the treatment of premature labour. Merck subsequently abandoned its nonpeptide OT antagonist programme.

Similarly, Wyeth-Ayerst (now Pfizer) reported that its nonpeptide OT antagonist WAY-1627720 (131) (No. 3; Table 10) failed in preclinical development. Glaxo SmithKline is now the only company pursuing the clinical development of a nonpeptide OT antagonist. Its promising nonpeptide OT antagonist GSK 221149A (Retosiban) (132, 133) is currently in a Phase II clinical trial. Its human and rat OT receptor affinities are given in Table 12. Its OT selectivities for human OT/VP receptors are excellent. Although the Merck and Wyeth-Ayerst nonpeptide OT antagonists have failed in clinical development, they are proving to be very useful as research tools (134–138) The Merck compounds are now available from TOCRIS. The nonpeptide OT agonist WAY-267464 reported by Pfizer (131) appeared to have promise as a therapeutic agent for the treatment of anxiety disorders such as autism spectrum disorders (139). However, its failure in preclinical development led to the abandonment of the nonpeptide OT agonist programme at Pfizer (44). WAY 267464 is now available from TOCRIS. Presently, there are no other companies pursuing the development of nonpeptide OT agonists.

The use of radiolabelled molecules, agonists and antagonists for characterising receptor affinities for OT and VP (Tables 11 and 12)

Some history

Initially in the 1960, the affinity, selectivity and potency of analogues for the different VP/OT receptors were deduced by *in vivo* bioassays such as oxytocic, antidiuretic and pressor tests (see

above), which reflected their activity through the OT receptor, V_2 and V_{1a} receptors, respectively. The characterisations performed at that time did not take into account the V_{1b} receptor, which was discovered only in the 1980s (142). As noted above, this led to a long delay in the discovery of selective ligands for the V_{1b} receptor. Nevertheless, the use of bioassays allowed the identification of key structure/function relationships of a large number of analogues and still represents a milestone in our understanding of OT/AVP selectivity (1, 7–18). Furthermore, because the data obtained using these *in vivo* tests integrated several ADME (Absorption, Distribution, Metabolism and Elimination) parameters, the values obtained reflect the *in vivo* physiological activity of the peptide being studied. These values sometimes differed from those obtained by *in vitro* pharmacological tests.

In the 1970s, the development of radiolabelled VP/OT analogues (143) and the discovery of second messenger cascades such as cAMP (67), calcium and inositol phosphate (144) made possible the determination of more reliable pharmacological parameters reflecting more precisely the interaction between analogues and their specific receptors. Binding assays with radiolabelled ligands conducted on plasma membrane preparations allowed the determination of the affinity (K_d) of a given molecule for a given VP/OT receptor subtype, a parameter which intrinsically characterises the analogue/receptor association (145). Second messenger measurements allowed the characterisation of its functional activity in order to measure precise functional effects. Comparison of the affinities of one analogue for the all receptors of the VP/OT family allowed the determination of its selectivity towards a given receptor iso-

Table 11. Common Agonists to Oxytocin (OT)/Arginine Vasopressin (AVP) Receptors^a.

Compound	Receptor subtype												Reference
	hOTR	hV _{1a}	hV _{1b}	hV ₂	rOTR	rV _{1a}	rV _{1b}	rV ₂	mOTR	mV _{1a}	mV _{1b}	mV ₂	
OT	0.8	120	> 1000	3500	1.0	71	294	89	0.6 ^c	46.1 ^c	494		71, 146–149, 153
AVP	1.7	1.1	0.7	1.2	1.7	2.6	0.3	0.4	1.8	1.3	0.3	0.3	1, 69 154
[Thr ⁴ ,Gly ⁷]OT	6.6 ^a	305	> 10 000	> 10 000	0.8	> 10 000	8000 ^d						153, 155, 156
WAY-267464	58.4								51.6				138
[Phe ² ,Orn ⁸]VT = [Phe ² ,Ile ³]OVT	21.9	9.57 ^d				65.2 ^e							149, 151
F180	520	11.7	2100	> 10 000		480	750	2000					68
FE202158	> 840	4.4 ^a	> 5200	> 7700									39
d[Cha ⁴]AVP	240	151	1.2 ^a	750	1430	2297	1.4	12.7			5.1 ^b		1, 69 147
d[Leu ⁴ ,Lys ⁹]VP	29	69.3	0.51	6713	64	3786	0.16 ^a	101		1933 ^c	1.4 ^e	61 ^e	1, 74
dDAVP	203	62.4	5.8	23.3	31 ^c	100	9.3	0.3			192		148, 152
VDAVP						501		0.2					63
dVDAVP	630 ^b	20.7	5.8	2.2		316	152	0.3					63, 159
d[Thi ³]VDAVP	460 ^b	50 ^b	20 ^b	4.3 ^b	34 ^c	1100 ^b	680 ^b	0.3 ^b					159

^aAffinities values (in nM) were obtained on cells expressing the oxytocin receptor (OTR), V_{1a} , V_{1b} and V_2 receptor subtypes. Light grey boxes report affinities of selective ligands for a particular receptor subtype as defined by the selectivity criteria: to be selective a ligand must have a K or K_d for that receptor subtypes two orders of magnitude lower than for the other three receptor subtypes in the same species, ^bRounded off or representative values from all the data available; ^cB. Chini B, unpublished data; ^dB. Mouillac, unpublished data; ^eG. Guillon, unpublished data.

Table 12. Common Antagonists to Oxytocin (OT)/Arginine Vasopressin (AVP) Receptors^a.

Compound	Receptor subtype										Reference	
	Affinity K _i (nM)											
	hOTR	hV _{1a}	hV _{1b}	hV ₂	rOTR	rV _{1a}	rV _{1b}	rV ₂	mV _{1a}	mV _{1b}		
d(CH ₂) ₅ [Tyr(Me) ²]OVT	3.5	32.7										1, 103
d(CH ₂) ₅ [Tyr(Me) ² ,Thr ⁴ , Tyr-NH ₂ ⁹]OVT (OTA)	0.6	3.9	>10 000 ^b	929	0.2	25.6 ^b	> 10 000	15.1 ^b				12, 104, 105
desGly-NH ₂ ,d(CH ₂) ₅ [D-Tyr ² ,Thr ⁴]OVT	0.9 ^b	100	> 1000									104
d[D-Tyr(Et) ² ,Thr ⁴]OVT (Atosiban)	76.4	5.1	256	3195	215	1059	241	> 1000				160, 161
Phaa-D-Tyr(Me)-Phe- Gln-Asn-Arg-Pro- Arg-Tyr-NH ₂					1.4	0.18	92	62				162
L-368,899	7.6				570	370	8.9					163
GSK 221149A	0.6 ^{a,b}	>12 000	>10 000	950	4.1							132
SSR126768A	1.5 ^a	143	223 ^b	> 1000	1.6 ^a	99	60	> 1000		29		147, 161
d(CH ₂) ₅ [Tyr(Me) ²]AVP (Manning Compound)	3	1.6 ^b	359	82	218	0.7	23	313				148
SR49059 (K _i measured with a tritiated ligand)	100 ^b	6.3	265 ^b	275	76 ^b	2.2	671			158		45, 152
SR49059 (K _i measured with a iodinated ligand)	130 ^b				1080 ^b							164
OPC-21268	170	8800	> 10 000	>10 000	> 10 000	350			510			12, 107
SSR149415 (K _i measured with a tritiated ligand)	1.5 ^b	91	1.6 ^{a,b}	1412	28 ^b	1050	1.3	2897	1446	1.6 ^b		90, 147
SSR149415 (K _i measured with a iodinated ligand)	174 ^b				270 ^b							89, 147
d(CH ₂) ₅ [D-Tyr(Et) ²]VAVP				4.4		1.1	2000	1.1				89
d(CH ₂) ₅ [D-Ile ² ,Ile ⁴ , Ala-NH ₂]AVP		53	541	88								148
SR121463(A)	1213	460	>10 000	4.1	3220	> 10 000	> 10 000	1.4		> 10 000		124
OPC-31260	1077	860	>10 000	25.4	2096	748		21.7				124

^aAffinities values (in nM) were obtained on cells expressing the oxytocin receptor (OTR), V_{1a}, V_{1b} and V₂ receptor subtypes. Light grey boxes report affinities of selective ligands for a particular receptor subtype as defined by the following selectivity criteria: to be selective a ligand must have a K or K_d for that receptor subtypes two orders of magnitude lower than for the other three receptor subtypes in the same species, ^bRounded off or representative values from all the data available.

form. Moreover, the ability of a given analogue to activate, inhibit or leave unaffected second messenger production in cell cultures, provided important insights into its pharmacological status (agonist, partial agonist, pure antagonist, inverse agonist). Such classical pharmacological assays have efficiently served the scientific community for the last three decades and allowed the characterisation of the numerous VP/OT analogues designed and synthesised during this period (1).

Conundrums posed by pharmacological data

From all the *in vitro* and *in vivo* pharmacological studies carried out on OT and VP agonists and antagonists, three intriguing fea-

tures have emerged; namely: (i) lack of receptor selectivity; (ii) species differences; and (iii) *in vitro in vivo* difference. In Tables 11 and 12, we have listed the most commonly used agonists and antagonists available for each VP/OT receptor isoform in three mammalian species: human, rat and mouse.

The affinities (K_i) of these isoforms have been measured using classical pharmacological tests and their agonist or antagonists properties determined using classical second messenger assays. As proposed in previous reviews (156, 157), receptor subtype selectivity can be defined, within a single species, on the basis of the ability of a compound to bind to a single VP/OT receptor isoform with a nanomolar affinity, at the same time displaying, for the three other receptor isoforms, an affinity at least two orders of magnitude

lower. The compounds that fulfill these requirements are highlighted with light grey in Tables 11 and 12. It immediately appears from this criterion that only a very few analogues are selective. A major problem is also that selectivity is not conserved among species as a result of subtle but nevertheless crucial differences in receptor pharmacology. Despite these limitations, the use of selective compounds still represents the best experimental strategy to unambiguously characterise VP/OT receptors in a given biological sample, keeping in mind that receptor selectivity for any given compound is: (i) strictly dependent upon the receptor species considered; (ii) usually lost if high doses (100-fold the K_i) of a selective compound are used; and (iii) dependent upon the biological models tested. Experiments performed on membrane preparations or on cell cultures generally need lower concentrations of selective analogue compared to experiments performed on organ slices, where drugs need to diffuse within the tissues and may be rapidly degraded.

It should also be noted that the pharmacological profile of any given compound determined by classical tests on membranes or cell models cannot be directly translated *in vivo* without adequate controls. Adsorption, distribution in different biological compartments, and metabolism greatly interfere with the biological activity of drugs, sometimes completely altering their pharmacological properties.

For example, [Phe²Orn⁸]VT (also known as [Phe²]OVT), which does not display any V_{1a} selectivity in classical binding experiments (Table 11), has been characterised as a selective V_{1a} agonist *in vivo* in rats (Table 4) (81).

Concerning selective agonists, it should also be noted that a major difference exists between the two natural hormones, OT and VP. Although OT is selective for the human OT receptor, VP is not, because it binds with similar affinities to V_{1a} , V_{1b} , V_2 and OT receptors. This may explain why VP may trigger physiological functions *in vivo* via OT receptors, as described previously (158). However, fully characterised selective agonists for human V_{1a} receptor (F 180, FE202158), human V_{1b} receptor (d[Cha⁴]AVP); rat V_{1b} receptor (d[Leu⁴,Lys⁸]AVP), rat OT receptor [Thr⁴,Gly⁷]OT and rat V_2 receptor (dDAVP) are now available (Table 11). For the rat, V_2 receptor d[Thi³]VDAVP (15, 159) appears to be the best selective agonist as a result of its good V_2 versus V_{1b} selectivity.

Among the several antagonists reported and currently employed, only a few have been fully characterised and have been demonstrated to be selective within a species (Table 12).

Among the OT receptor antagonists, SSR126768A has been shown to be a very selective antagonist for both human and rat OTR and GSK 221149A for human OT receptor. Manning compound is relatively selective for the rat V_{1a} receptor (but not for the human V_{1a} receptor) for which SSR49059 should be preferred. Finally, SSR149415 (147) is selective for both the human and the rat V_{1b} receptor isoforms, whereas SSR121463(A) is highly selective for the human V_2 receptor. Concerning the SSR149415 and SSR49059, it should be noted that different laboratories have obtained different values for their affinities, probably depending on the binding assay employed (i.e. competition against a radioactive agonist or antagonist) (89). In our opinion, the values obtained in competition experiments using radiolabelled agonists will better

correlate with biological antagonistic activity *in vitro* and *in vivo* and should be preferred.

Until now, other analogues commonly employed as 'selective' have not been fully characterised and, when they are used at high doses, could lead to ambiguous results in species in which their pharmacological properties have not been assessed. It should be noted that the pharmacology of OT/VP analogues on mouse receptors is still very limited, representing a gap that needs to be filled; in particular, for the relevance that genetically-modified mouse models have acquired in translational medicine.

The lack of selective analogues for some rat, mouse and human receptor isoforms makes the design and synthesis of new molecules very necessary. The restriction of radioactivity approaches in laboratory practice and the need to easily test a large number of molecules led to the development of new assays using the gene reporters. Such tests using the measurement of reporter gene activities allows an easy screening of a large number of molecules and rapid identification of 'lead molecules' (83).

Yet, these '*in vitro* methodologies' also have some limitations. First, they can be used only in transfected cells and not in native models. Moreover, according to the second messenger cascade associated with the receptor being considered (cAMP for the V_2 , InsP3 for the V_{1a} , V_{1b} and OT receptor isoforms), such assays require the use of different reporter genes. This may introduce a bias in the determination of receptor selectivity. It is also well known that assays using luciferase gene expression and luciferase activity measurements involve a strong amplification of the initial receptor-mediated second messenger accumulation. This prevents the good determination of the agonist or partial agonist properties of the analogue tested. One needs to be aware of the limitations of these recent '*in vitro* methodologies' and to verify, using classical pharmacological tests, the selectivity, affinity and functional potencies of the lead compounds characterised by this approach. Obviously, to move to clinical development, the best approach would be to test the compounds of interest by *in vivo* technologies similar to those used to evaluate virtually all of the peptides in Tables 1, 3–8.

New technologies for screening more selective VP/OT analogues

Recently, new physical techniques involving label-free biosensors have been proposed for pharmacological screening of muscarinic and corticotrophic analogues (165). These methods are based on the measurement of cell shape changes induced by ligand-receptor interactions. Such techniques have the advantage of being performed on native cells and do not require the use of radioactive molecules. Their efficiency for testing new VP/OT molecules may represent another alternative for screening new analogues.

Finally, a new bioluminescence or fluorescence resonance energy transfer (BRET or FRET) approach in which analogues could be screened for their capability to promote receptor coupling and activation of single G-protein isoforms has been recently applied to the human OT receptor (166). This technique allows the precise characterisation of which G protein is associated with which recep-

tor isoform. Thus, for example, $d(\text{CH}_2)_5[\text{D}-2\text{-NaI}^2, \text{Thr}^4, \text{Tyr-NH}_2^9]\text{OVT}$ (OTA) and atosiban (160) (Table 12) were found to be entirely biased respectively toward Gi1 or Gi3 activation (166). However, this technique cannot be used on native tissues or primary cultures.

The recent development of fluorescent ligands for a better knowledge of central and peripheral VP/OT receptors

Design and use of classical fluorophores

Receptors of the AVP and OT family are important in the regulation of the stress processes (167). Centrally, the V_{1a} , V_{1b} and OT receptors have been involved in stress and especially in learning and memory processes. Important data have been obtained by the use of knockout animals but, after a period of cloning and pharmacological characterisation in the last decade, it became necessary to elucidate the distribution of these receptors to better understand their central functions *in vivo*.

Although several publications describe the AVP V_{1a} , V_2 and OT receptor distribution by using autoradiography (105, 168, 169) or immunodetection (170), the lack of selective V_{1b} radio-labelled VP analogues or of receptor antibodies has hindered progress in the detection of receptor distribution in native tissues. Results obtained by molecular approaches such as reverse transcriptase-polymerase chain reaction (62, 88) or mRNA detection by *in situ* hybridisation (171–173), although more accurate, did not provide clear information regarding the brain regions detected by immunostaining. Thus, developing fluorescent ligands to decipher AVP receptor distribution in the brain and at the periphery, and to study molecular interactions such as receptor dimerisation, appeared as an absolute necessity.

Various fluorescent analogues of AVP and OT have been synthesised for several receptors of the VP/OT family (174). Thus, good fluorescent V_{1a} and OT ligands have been produced, although no good fluorescent specific ligand was available to selectively detect central and peripheral V_{1b} receptors.

In our previous work (69, 73, 74), by replacing the glutamine⁴ of the natural AVP with a cyclohexylalanine or a leucine, the arginine⁸ by a lysine and by removing the NH_2 of the cysteine¹ to increase stability towards aminopeptidases, we produced analogues showing an increased selectivity for the V_{1b} receptors (Table 5). $d[\text{Leu}^4, \text{Lys}^8]\text{VP}$ (Peptide 4; Table 5) was found to be selective for the rat V_{1b} receptors and, to a lesser extent, for the human hV_{1b} receptors, conserving a nanomolar affinity for these receptor isoforms (73, 74). We have taken advantage of the Lys⁸ residue in $d[\text{Leu}^4, \text{Lys}^8]\text{VP}$ with its epsilon NH_2 group to introduce fluorophores on its side chain. This allowed us to create fluorescent tools that would conserve the pharmacology of the $d[\text{Leu}^4, \text{Lys}^8]\text{VP}$, to resist degradation and to selectively decorate the plasma membrane of Chinese hamster ovary cells expressing V_{1b} and/or OT receptors with an excellent resolution (47).

Different fluorophores were attached to the $d[\text{Leu}^4, \text{Lys}^8]\text{VP}$: First, the antraniloyl group (Atn), a small fluorescent molecule of 97 Da highly sensitive to microenvironmental changes (175) may also be a good donor in FRET experiments to identify V_{1b} receptor homodi-

mers *in vivo*. We have also selected the Alexas (Molecular Probes) for their brightness and their resistance to photobleaching (176). We have used Alexa 488 and Alexa 647, with the latter being one of the brightest fluorescent molecules reported so far (177).

The pharmacological properties (binding, coupling to phospholipase C) of fluorescent analogues of $d[\text{Leu}^4, \text{Lys}^8]\text{VP}$ indicate that they conserved a very good selectivity for V_{1b} versus V_{1a} and V_2 receptors, and remained full agonists. These properties allow receptor labelling and measurement of biological activity at the cellular level. Thus, these new fluorescent analogues are promising tools for the detection of functional V_{1b} or OT receptors in human (47) and in rat native tissues.

Use of long life fluorophores

However, it should be noted that, except for very recent ones, all the ligands previously reported were designed with classical fluorophores, exhibiting short-lived fluorescence properties (fluorescence half-time life in the 10 ns range). Most of them were essentially used to follow internalisation in cell lines (146, 178) or to label receptors in a native context (179). Interestingly, a first nonpeptide antagonist with a nanomolar affinity for the human V_2 receptor has been developed (180). This ligand will find application in fluorescence polarisation-based binding assays aiming to screen for small organic molecule libraries.

Recently, fluorescence-based strategies have been extensively used to study molecular interactions. Thus, the FRET approach was used to demonstrate G protein-coupled receptors oligomerisation (181, 182). Regarding AVP and OT receptors, various experimental approaches based on chimeric receptor expression in cell lines have been developed to analyse receptor oligomers. The studies have used receptors fused either to small tags recognised by fluorescent antibodies (183, 184), or to bioluminescent or fluorescent proteins (185, 186), or to suicide enzymes (48). However, these strategies were not relevant for proving the existence of such receptor complexes in native tissues. Therefore, a FRET strategy based on the

Table 13. Food and Drug Administration Approved Peptide and Nonpeptide Drugs from the Oxytocin (OT)/Arginine Vasopressin (AVP) Field.

OT/AVP field	
Peptides ^a	Nonpeptides ^b
Carbetocin (Duratocin, Depotocin, Sofla, Pabal)	Conivaptan hydrochloride (Vaprisol)
Desmopressin (Minirin, DDAVP)	Tolvaptan
Ornithine vasopressin (Ornipressin, POR-8)	
Lypressin (Diapid, LVP)	
Oxytocin	
Terlipressin (glypressin)	
Vasopressin (Pitressin)	
Atosiban (in Europe)	

^aSee Reichert (194). ^bSee Ferguson-Myrthil (115).

indirect labelling of receptors with fluorescent donor and acceptor ligands has been recently developed (48). Unfortunately, because of the overlap between excitation and emission spectra of the donor and acceptor fluorophores on one hand, and of the high autofluorescence of the biological preparation on the other hand, specific FRET could hardly be detected. To improve the signal-to-noise ratio, lanthanide cryptate-labelled ligands were designed and characterised. Despite the size of the cage, these ligands still display very good affinities for the V_{1a} and OT receptors (48). Lanthanide cryptates display interesting fluorescent properties because they have a fluorescent half-time life of approximately 1 ms (i.e. 100 000-fold greater than classical fluorophores), allowing time-resolved FRET experiments to be set up (187). Experiments using these new probes have been performed not only on AVP V_{1a} and V_2 receptors and on OT receptors expressed in cell lines, but also on OT receptors naturally expressed in lactating rat mammary gland. The sensitivity is such that it has been possible to prove the existence of OT receptor dimers in this latter native tissue (48).

These newly-synthesised ligands and those that exhibit high quantum yield have also been used to develop original binding assays. These assays, based on time-resolved FRET between compatible fluorophores carried by tagged receptors and ligands, display very good sensitivities and are safer than radioactive-based assays (188–190).

Therapeutics uses of peptide and nonpeptide oxytocin and vasopressin agonists and antagonists (Table 13)

Table 13 lists the seven peptides and two nonpeptide drugs in the OT and AVP field that have been approved for therapeutic use. Numerous recent studies point to the use of OT as a potential new therapy for the treatment of a broad range of psychiatric disorders (24, 33, 34). Also, a very recent report suggests the exciting prospect that OT may have potential for the treatment of human obesity and type 2 diabetes (191).

To date, only two nonpeptides, the VP V_2/V_{1a} antagonist conivaptan and the VP V_2 antagonist tolvaptan have been approved for clinical use (112–115, 123, 192). The nonpeptide OT antagonist retosiban (132, 133) is currently in a Phase II clinical trial. Clinical trials with other nonpeptide VP, V_{1a} and V_{1b} antagonists shown in Table 9 and with the other OT antagonists and the OT agonist shown in Table 10 have been terminated (44). No new nonpeptides in this field are currently in clinical trial. Thus, the early promise of nonpeptides as therapeutic agents in this field (45, 46) has clearly not been realised. This should be a cautionary tale for those in pharmaceutical companies (138), in granting agencies (139) and on study sections (44) who have strongly promoted the development of nonpeptides over peptides as therapeutic agents. This philosophy led to the abandonment of peptides as potential drugs by Big Pharma almost two decades ago. Clearly, it is now time for Big Pharma to reassess the value of peptides as therapeutic drugs (44). In this regard, recent progress in the development of peptide drugs (193, 194) provides very compelling support for the thesis that peptides are clearly superior to nonpeptides as therapeutic agents, thus bolstering the case for continued support for the design and syn-

thesis of peptides as potential therapies. In this regard, in the OT field, there is an urgent need for functionally selective OT ligands (166) and for a long lasting OT agonist as a potential therapy for the treatment of autism and other anxiety disorders (139).

Research uses of peptides

During the period 1980–2012, over 3000 samples of OT and AVP agonists and antagonists from the Manning laboratory have been and continue to be donated as research tools to over 700 investigators (some multiple times) in the USA and worldwide for their own independent studies. Studies carried out with these donated peptides and with those purchased from commercial suppliers, such as Sigma, Bachem and Peninsula, have resulted in more than 2000 publications by these and other investigators during this period. Examples of studies carried out since 2008 with some of these peptides are available (90, 117, 195–246).

Research uses of nonpeptides

With the exception of the Sanofi nonpeptide V_2 antagonist satavaptan, all of the nonpeptide AVP antagonists listed in Table 9 are now available from Tocris or other suppliers. To date, a small number of studies that have utilised the Sanofi V_{1a} antagonist relcovaptan, the Sanofi V_{1b} antagonist nelivaptan and the Sanofi V_2 antagonist satavaptan have been reported (117–120, 125, 126) (Table 9).

The two Merck nonpeptide OT antagonists L-368,899 and L-371,257 and the Pfizer nonpeptide OT agonist Way-267464 shown in Table 10 are all available from Tocris. The Glaxo SmithKline nonpeptide OT antagonist GSK-221149A (Retosiban) is also available from a number of suppliers. The Pfizer nonpeptide OT antagonist WAY-162720 is not yet commercially available. A number of studies that have utilised some of these nonpeptide ligands as research tools have been reported (138–141, 145) (Table 10).

Conclusions

In our 2008 review (1), we examined the status (as of 2007) of both peptide and nonpeptide agonists and antagonists of the OT receptor and of the VP V_{1a} , V_{1b} and V_2 receptors as: (i) research tools and (ii) therapeutic agents. Although the research uses of both peptide and nonpeptide ligands have continued to grow during the intervening 4 years, by contrast, the therapeutic development of nonpeptide AVP and OT antagonists has been drastically curtailed. Merck, Pfizer and Sanofi have all abandoned their nonpeptide programmes. The nonpeptide V_2/V_{1a} the antagonist, conivaptan and the nonpeptide V_2 antagonist tolvaptan, which have been approved by the Food and Drug Administration, have not as yet found widespread acceptance in the clinic (113, 192). Pfizer has also abandoned its nonpeptide OT agonist programme (44). It remains to be seen how the Glaxo SmithKline nonpeptide OT antagonist retosiban will fare in its current Phase II clinical trial. All in all, since our 2008 review (1), interest in the development of nonpeptides as therapeutics has greatly diminished. On the other hand, as noted above, Ferring has a promising V_{1a} agonist (Table 4) and a

promising OT agonist (Table 1), awaiting clinical development. The design and synthesis of: (i) functionally selective OT peptides and (ii) of a long lasting OT analog as a potential therapy for autism spectrum disorders and other anxiety disorders remain as pressing needs in this field. Both OT and VP peptides and nonpeptides are continuing to be very valuable research tools. In this regard, we have addressed here the issues of the: (i) lack of receptor selectivity, (ii) species differences and (iii) *in vitro*–*in vivo* differences, all of which need to be taken into account when using a given peptide or nonpeptide ligand as a research tool. Finally, the development of new fluorescent ligands as powerful new tools for localising and characterising OT and VP receptors, which we have presented here, points to the continued usefulness of OT and AVP peptide ligands (agonists, antagonists and fluorescent derivatives) as incisive molecular pharmacological probes.

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