Journal of Peptide Science
J. Peptide Sci. **7**: 449–465 (2001)
DOI: 10.1002/psc.339

Design of Oxytocin Antagonists, which are more Selective than Atosiban

MAURICE MANNING[a,*], STOYTCHO STOEV[a], LING LING CHENG[a], NGA CHING WO[b] and W.Y. CHAN[b]

[a] Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH, USA
[b] Department of Pharmacology, Weill Medical College of Cornell University, New York, NY, USA

Received 5 April 2001
Accepted 15 April 2001

Abstract: We report the solid phase synthesis of four pairs of L- and D-thienylalanine (Thi/D-Thi) position two modified analogues of the following four oxytocin (OT) antagonists: des-9-glycinamide [1-(β-mercapto-β,β-pentamethylene propionic acid), 2-*O*-methyltyrosine, 4-threonine]ornithine-vasotocin (desGly$(NH_2)^9$,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT) (**A**); the Tyr-$(NH_2)^9$ analogue of (**A**), d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Tyr-$(NH_2)^9$]OVT (**B**); the Eda^9 analogue (where Eda = ethylenediamine) of (**A**), d$(CH_2)_5$[Tyr(Me)^2, Thr^4, Eda^9]OVT (**C**); and the retro Tyr^{10} modified analogue of (**C**), d$(CH_2)_5$[Tyr(Me)^2, Thr^4, Eda$^9\leftarrow$Tyr^{10}]OVT (**D**). The eight new analogues of **A**–**D** are (**1**) desGly(NH_2),d$(CH_2)_5$[Thi^2,Thr^4]OVT, (**2**) desGly(NH_2),d$(CH_2)_5$[D-Thi^2,Thr^4]OVT, (**3**) d$(CH_2)_5$[Thi^2, Thr^4,Tyr-$(NH_2)^9$]OVT, (**4**) d$(CH_2)_5$[D-Thi^2,Thr^4,Tyr-$(NH_2)^9$]OVT (**5**) d$(CH_2)_5$[Thi^2,Thr^{4}Eda^9]OVT, (**6**) d$(CH_2)_5$[D-Thi^2,Thr^4,Eda^9]OVT, (**7**) d$(CH_2)_5$[Thi^2,Thr^4,Eda$^9\leftarrow$Tyr^{10}]OVT, (**8**) d$(CH_2)_5$[D-Thi^2,Thr^4,Eda$^9\leftarrow$Tyr^{10}]OVT. We also report the synthesis of (**C**). Peptides **1**–**8** and **C** were evaluated for agonistic and antagonistic activities in *in vitro* and *in vivo* OT assays, in *in vivo* vasopressor (V_{1a} receptor) assays and in *in vivo* antidiuretic (V_2 receptor) assays. None of the eight peptides nor **C** exhibit oxytocic or vasopressor agonism. Peptides **1**–**8** are extremely weak V_2 agonists (antidiuretic activities range from <0.0005 to 0.20 U/mg). Peptide **C** is a weak mixed V_2 agonist/antagonist. Peptides **1**–**8** and **C** exhibit potent *in vitro* (no Mg^{2+}) OT antagonism (anti-OT pA_2 values range from 7.76 to 8.05). Peptides **1**–**8** are all OT antagonists *in vivo* (estimated *in vivo* anti-OT pA_2 values range from 6.54–7.19). With anti-V_{1a} pA_2 values of $\sim$5–5.80, peptides **1**–**8** exhibit marked reductions in anti-V_{1a} potencies relative to those of the parent peptides **A**–**D** (anti-V_{1a} pA_2 range from 6.48 to 7.10) and to 1-deamino[D-Tyr(Et)^2, Thr^4]OVT (Atosiban, trade name Tractocile) (anti-V_{1a} pA_2-6.14). Atosiban has recently been approved in Europe for clinical use for the prevention of premature labour (*Pharm. J.* **264**(7-100): 871). Peptides **1**–**8** exhibit striking gains in *in vitro* anti-OT/anti-V_{1a} selectivities with respect to the parent peptides **A**, **B**, **C** and **D** and to Atosiban. Peptides **1**–**8** exhibit anti-OT (*in vitro*)/anti-V_{1a} selectivities of 450, 525, 550, 450, $\sim$1080, 116, 355, 227 respectively. The corresponding values for **A**–**D** and Atosiban are 30, 4.2, 4.3, 2.6 and 37. With the exception of peptide **6**, the remaining seven peptides exhibit 3–18-fold gains in anti-OT (*in vivo)*/anti-V_{1a} selectivity with respect to Atosiban, peptides **1**–**8** exhibit anti-OT (*in vivo*)/anti-V_{1a} selectivities of 22, $\sim$82, $\sim$82, 147, $\sim$83, 11, 31 and 42. By comparison, Atosiban exhibits an anti-OT (*in vivo*)/anti-V_{1a} selectivity = 8. With an estimated *in vivo* anti-OT pA_2 value = 7.19 ± 0.06, peptide **4** is equipotent with Atosiban ($pA_2 = 7.05 \pm 0.05$). However, with its

Abbreviations: Symbols and abbreviations are in general accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1989; **180**, A9–A11) or the editorial of this journal (*J. Peptide Sci.* 1999; **5**: 465–471). All amino acids are in the L-configuration unless otherwise noted. Other abbreviations used are: OVT, ornithine vasotocin; D-Tyr(Et), *O*-ethyl-D-tyrosine; D-Tyr(Me), *O*-methyl-D-tyrosine; Eda, ethylendiamine; desGly(NH_2), desglycineamide; Eda$\leftarrow$Tyr, Eda retro-L-tyrosine; desGly$(NH_2)^9$, d$(CH_2)_5$[Tyr(Me)^2, Thr^4]OVT (**A**), des-9-glycinamide [1-(β-mercapto-β,β-pentamethylenepropionic acid), 2-*O*-methyl-tyrosine, 4-threonine] ornithine vasotocin; d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Tyr-$(NH_2)^9$]OVT (**B**), the Tyr-$(NH_2)^9$ analogue of (**A**); d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Eda^9]OVT (**C**); the Eda^9 analogue of (**A**); d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Eda$^9\leftarrow$Tyr^{10}]OVT (**D**), the retro-Tyr^{10} analogue of (**C**); d[D-Tyr(Et)^2,Thr^4]OVT, 1-deamino, 2-*O*-ethyl-D-tyrosine 4-threonine] ornithine vasotocin (generic name: Atosiban; trade name: Tractocile); TFMSA, trifluoromethane sulfonic acid; V_2, antidiuretic; V_{1a}, vasopressor; VP, vasopressin; 2-Cl-Z, 2-chlorobenzyloxycarbonyl; DIPEA, diisopropyl ethylamine; ESMS, electro-spray mass spectrometry.

* Correspondence to: Department of Biochemistry and Molecular Biology, Medical College of Ohio, 3035 Arlington Avenue, Toledo, OH 43614-5804, USA; e-mail: mmanning@mco.edu

significantly reduced anti-vasopressor potency, $pA_2 = \sim 5$, it is ~ 18 times more selective for OT receptors with respect to VP V_{1a} receptors than Atosiban. Since we have shown that V_{1a} antagonism could be an unwanted side-effect in tocolytics, peptide **4** and some of the OT antagonists reported here have advantages over Atosiban and thus may be suitable candidates for evaluation as potential tocolytic agents for the treatment of preterm labour. Copyright © 2001 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: antagonists; Atosiban; OT receptor; oxytocin; selectivity; Tractocile; vasopressin; V1a receptor

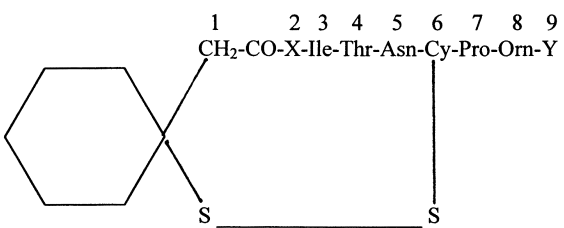
INTRODUCTION

Preterm birth is the largest cause of neonatal morbidity and death [1–4]. Although the mechanism that triggers preterm labour and the role of oxytocin (OT) in the initiation of labour have not been established [5–7], there is strong evidence implicating an autocrine or paracrine role of OT in labour [8–13]. The design and synthesis of antagonists of oxytocin as potential tocolytic agents for the prevention of preterm births has for many years been an area of intensive investigation [14–54]. For reviews see [55–61]. An OT antagonist [1-deamino, D-Tyr(Et)^2, Thr^{4}Orn^8] vasotocin (d[D-Tyr(Et)^2,Thr^4]OVT [27,48]; generic name Atosiban), following extensive clinical trials [10,61–66] has been shown to be an effective tocolytic for the treatment of preterm labour [67–70] and was approved (under the trade name Tractocile) on 20 January 2000 for clinical use in Europe (*Pharm. J.* **264**(7-100): 871). Atosiban is, however, far from being an ideal OT antagonist. It is highly non-selective for OT receptors versus VP V_{1a} vascular receptors [48,71,74,75]. For reviews of the known VPs V_{1a} (vascular), V_{1b} (pituitary) and V_2 (renal) receptors and OT uterine receptors see [72–75]. In the rat it is only eight times more potent as an OT antagonist than as a V_{1a} antagonist *in vivo* [48]. However, in humans its affinity for V_{1a} receptors is 100 times greater than for OT receptors [71,74,75]. It is thus a strikingly more potent V_{1a} antagonist than an OT antagonist in humans [75]. We have previously shown that V_{1a} antagonism is an undesirable side-effect in OT antagonists [76]. Thus, there is a pressing need for OT antagonists that have superior potency, selectivity and potential safety than Atosiban. As part of a longstanding programme aimed at the design and synthesis of potent and selective OT antagonists, we reported a series of OT antagonists that is more potent and selective than Atosiban in *in vivo* assays in the rat [48]. These were designed by replacing the Tyr(Me) residue at position 2 in one of our early selective OT antagonists: desGly-(NH_2),d(CH_2)$_5$[Tyr(Me)^2,Thr^4]-OVT [31] with D-Tyr(Me), D-Tyr, D-Phe and D-Trp.

In continuing to explore the effectiveness of other position 2 modifications in OT antagonist design, we selected L- and D-thienylanine (Thi and D-Thi) as replacements for L-Tyr(Me)^2 in four different OT antagonists. While thienylanine has been utilized as a replacement for Phe in the design of peptide agonists, such as bradykinin [77], angiotensin **II** [78], lysine vasopressin (LVP) [79], arginine VP [80] and VP analogues [81], to our knowledge, despite intensive investigation of position 2 in OT antagonists with a wide variety of substituents [17,18,21–23,25,28,31–36,41,43,46,48,51–54], it has not been previously utilized in OT antagonist design. Our choice of the Thi^2/D-Thi^2 modifications was also prompted by preliminary findings from a study of their effects alone and in combination with a Thi^3 substitution in the potent V_2 agonist: dVDAVP [82]. Whereas dVDAVP and d[Thi^3]VDAVP are weak OT agonists [81,82], their Thi^2 and D-Thi^2 analogues are potent *in vitro* OT antagonists (pA_2 7.12–7.91) [81]. Thus, these preliminary findings indicated that Thi^2 and D-Thi^2 modifications would be worth further exploration in OT antagonist design. We selected the following four OT antagonists **A**–**D**, all of which have a Tyr(Me)^2 residue at position 2, for this study on the effectiveness of Thi^2 and D-Thi^2 substitutions in OT antagonist design: (**A**) desGly-(NH_2),d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT[31]; (**B**) d(CH_2)$_5$-[Tyr(Me)^2,Thr^4,Tyr-(NH_2)^9]OVT [30,31]; (**C**) d(CH_2)$_5$-[Tyr(Me)^2,Thr^4,Eda^9]OVT (where Eda = ethylenediamine), (synthesis of **C** reported here); (**D**) d(CH_2)$_5$-[Tyr(Me)^2,Thr^4,Eda^9←Tyr^{10}]OVT [59]

Replacement of the Tyr(Me)^2 residue in these four peptides by Thi^2 and by D-Thi^2 residues resulted in the following eight new peptides: (**1**) des-9-glycinamide [1-(β-mercapto-β,β-cyclo-pentamethylene-propionic acid), 2-thienylalanine, 4-threonine] ornithine-vasotocin (desGly(NH_2), d(CH_2)$_5$[Thi^2, Thr^4]OVT); (**2**) des-9-glycinamide [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-thienylalanine, 4-threonine]ornithine-vasotocin (des-Gly(NH_2),d(CH_2)$_5$,D-Thi^2,Thr^4]OVT); (**3**) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-thienylalanine, 4-threonine, 9-tyrosinamide]-

ornithine-vasotocin (d(CH_2)$_5$[Thi^2,Thr^4,Tyr(NH_2)^9]-OVT); (**4**) [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid, 2-D-thienylalanine, 4-threonine, 9-tyrosinamide]ornithine-vasotocin (d(CH_2)$_5$[D-Thi^2, Thr^4,Tyr-(NH_2)^9]OVT); (**5**) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-thienylalanine, 4-threonine, 9-ethylenediamine]ornithine-vasotocin (d(CH_2)$_5$[Thi^2,Thr^4,Eda^9]OVT); (**6**) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid, 2-D-thienylalanine, 4-threonine, 9-ethylenediamine]ornithine-vasotocin (d(CH_2)$_5$[D-Thi^2,Thr^4,Eda^9]OVT); (**7**) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-thienylalanine, 4-threonine, 9-ethylendiamine, retro 10-tyrosine]ornithine-vasotocin (d(CH_2)$_5$Thi^2, Thr^4,Eda^9←Tyr^{10}] OVT); (**8**) [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid, 2-D-thienylalanine, 4-threonine, 9-ethylenediamine, retro 10-tyrosine]ornithine-vasotocin (d(CH_2)$_5$[D-Thi^2,Thr^4, Eda^9 ←Tyr^{10}]OVT). Peptides **A**–**D** and **1**–**8** have the general structure shown in below



Peptide	X^2	Y^9
A	Tyr(Me)	0
B	Tyr(Me)	Tyr-NH_2
C	Tyr(Me)	Eda
D	Tyr(Me)	Eda←Tyr
1	L-Thi	0
2	D-Thi	0
3	L-Thi	Tyr-NH_2
4	D-Thi	Tyr-NH_2
5	L-Thi	Eda
6	D-Thi	Eda
7	L-Thi	Eda←Tyr
8	D-Thi	Eda←Tyr

PEPTIDE SYNTHESIS

Starting from Boc-Tyr(2-C1-Z)-resin and Boc-Orn(Z)-resin, we synthesized all protected precursors **I**–**VIII** and **c** (Table 2) of the free peptides **1**–**8** and **C** (Tables 1 and 3) entirely by the Merrifield solid-phase method [83,84], with the modifications previously described [21,23,31,48]. The protected retromodified peptides **VII** and **VIII** (Table 2) were obtained by coupling the protected Eda peptides **V** or **VI** (Table 2) with *Z*-Tyr(Bzl) in DMF using the DCC/HOBt procedure [85], as described in [86]. In the SPPS, HCI (1 M)/AcOH was used in all the deprotection steps. Neutralizations were carried out with 10% Et_3N/CH_2Cl_2. Coupling reactions were mediated primarily by DCC/HOBt [85] in CH_2Cl_2/DMF, except for Boc-Asn, which was incorporated as its *p*-nitrophenyl ester [87] in DMF. The acylpeptide resins were cleaved using the following procedures: (i) acidolysis with HBr/TFA [83,84,88,89] to give the protected peptides **I** and **II**; (ii) ammonolysis in methanol [90,91] to give the protected peptide amides **III** and **IV**; (iii) aminolysis with Eda in methanol [86,92] to give the protected Eda peptides **c**, **V** and **VI**. All the protected precursors, including the retroprotected peptides **VII** and **VIII**, were purified by the same general method: extraction or dissolving with warm DMF followed by reprecipitations with H_2O or $EtOH/Et_2O$ until adjudged pure by TLC, as previously described [21,23,31,48,86,89], to give the required protected peptides **I**–**IV**, **c**, **V**–**VIII** (Table 2). The physicochemical properties of all protected peptides **I**–**IV**, **c**, **V**–**VIII** are given in Table 2. Deprotection of the peptide **c** was carried out with sodium in liquid ammonia [93,94] as previously described [20,21,23,31,48]. Peptides **I**–**VIII** were deprotected by TFMSA procedure [95,96]. All the resulting disulphydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$ using the normal procedure [97] or a modified reverse procedure [98]. The free peptides were desalted and purified by gel filtration on Sephadex G-15 and Sephadex LH-20 mainly in a two-step procedure [99] using 50% AcOH and 2 M AcOH as eluents, respectively, as previously described [20,21,23,31,48]. When necessary, an additional purification on Sephadex G-15 or/and Sephadex LH-20 with 0.2 M AcOH as eluent was carried out. The purity of the free peptides **1**–**8** and **C** (Tables 1 and 3) was checked by TLC, HPLC and ESMS.

Bioassays

Peptides 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. For agonists, the 4-point assay design [100] was used and for antagonists, the Schild's pA_2 method [101] was employed. The pA_2 is

Table 1 Pharmacological Properties of L- and D-Thi^2-Substituted Selective Oxytocin Antagonists (**1–8**), the Parent Tyr(Me)^2 Peptides (**A–D**) and Atosiban

No.	Peptide	Antioxytocic (Anti-OT)			Antivasopressor (Anti-V_{1a})		Antidiuretic activity (V_2)	Anti-OT selectivity	
		In vitro pA_2[a]	*In vivo*		ED[b]	pA_2[c]	Unit/mg	MD ratio[d]	ED ratio[e]
		No Mg^{2+}	ED[b]	pA_2[c]					
	Atosiban (d[D-Tyr(Et)^2, Thr^4]OVT)[f,g,h]	7.71 ± 0.05[i] $[M] = 1.94^{-8}$	5.95 ± 0.65	7.05 ± 0.05	48.5 ± 2.7 $[M] = 7.24^{-7}$	6.14 ± 0.02	Antagonist pA_2 5.9	37	8
A	desGly(NH$_2$),d(CH$_2$)$_5$ [Tyr(Me)^2,Thr^4]OVT[j]	7.89 ± 0.04 $[M] = 1.26^{-8}$	1.3 ± 0.2 $[M] = 2.0^{-8}$	7.69 ± 0.07	23 ± 4 $[M] = 3.74^{-7}$	6.48 ± 0.08	Antagonist pA_2 5.3	30	18
1	desGly(NH$_2$),d(CH$_2$)$_5$ [Thi^2,Thr^4]OVT[k]	7.87 ± 0.04 $[M] = 1.44^{-8} \pm 0.15$	19.9 ± 1.8 $[M] = 2.98^{-7} \pm 0.28$	6.54 ± 0.04	434 ± 34 $[M] = 6.48^{-6} \pm 0.50$	5.20 ± 0.03	0.20 ± 0.03	450	22
2	desGlyNH$_2$,d(CH$_2$)$_5$ [D-Thi^2,Thr^4]OVT[k]	7.76 ± 0.05 $[M] = 1.92^{-8} \pm 0.22$	8.16 ± 1.16 $[M] = 1.22^{-7} \pm 0.16$	6.95 ± 0.08	~ 700 $[M] \sim 10^{-6}$	~ 5	~ 0.04	~ 525	~ 82
B	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4, Tyr-(NH$_2$)^9]OVT[m]	7.63 ± 0.07 $[M] = 2.34^{-8}$	1.0 ± 0.1 $[M] = 1.5^{-8}$	7.83 ± 0.04	6.6 ± 0.9 $[M] = 9.8^{-8}$	7.02 ± 0.07	~ 0.015	4.2	6.6
3	d(CH$_2$)$_5$[Thi^2,Thr^4, Tyr-(NH$_2$)^9]OVT[k]	7.77 ± 0.05 $[M] = 1.82^{-9} \pm 0.19$	8.18 ± 0.57 $[M] = 1.22^{-7} \pm 0.08$	6.92 ± 0.03	~ 700 $[M] \sim 10^{-6}$	~ 5	< 0.01	~ 550	~ 82
4	d(CH$_2$)$_5$[D-Thi^2,Thr^4, Tyr-(NH$_2$)^9]OVT[k]	7.72 ± 0.07 $[M] = 2.21^{-8} \pm 0.35$	4.55 ± 0.56 $[M] = 6.78^{-8} \pm 0.84$	7.19 ± 0.06	~ 700 $[M] \sim 10^{-6}$	~ 5	~ 0.02	~ 450	~ 147
C	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4, Eda^9]OVT[k]	7.75 ± 0.04 $[M] = 1.85$	–	–	5.5 ± 0.51 $[M] = 7.9^{-8}$	7.10 ± 0.04	~ 0.03 Also antagonist $pA_2 \sim 6$	4.3	–
5	d(CH$_2$)$_5$[Thi^2,Thr^4, Eda^9]OVT[k]	8.05 ± 0.04 $[M] = 9.26^{-9} \pm 0.90$	8.41 ± 0.94 $[M] = 1.21^{-7} \pm 0.15$	6.94 ± 0.06	~ 700 $[M] \sim 10^{-6}$	~ 5	< 0.0005	$\sim 1{,}080$	~ 83
6	d(CH$_2$)$_5$[D-Thi^2,Thr^4, Eda^9]OVT[k]	7.85 ± 0.035 $[M] = 1.45^{-8} \pm 0.11$	10.3 ± 2.2 $[M] = 1.53^{-7} \pm 0.13$	6.90 ± 0.11	112.8 ± 16.2 $[M] = 1.68^{-6} \pm 0.24$	5.80 ± 0.06	~ 0.04	116	11
D	d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4, Eda$^9 \leftarrow$Tyr^{10}]OVT[m]	7.50 ± 0.04 $[M] = 3.24^{-8}$	–	–	6.56 ± 1.31 $[M] = 8.5^{-8}$	7.07 ± 0.09	~ 0.02 Also antagonist $pA_2 \sim 6$	2.6	–
7	d(CH$_2$)$_5$[Thi^2,Thr^4, Eda$^9 \leftarrow$Tyr^{10}]OVT[k]	7.84 ± 0.06 $[M] = 1.58^{-8} \pm 0.22$	12.1 ± 1.89 $[M] = 1.80^{-7} \pm 0.28$	6.78 ± 0.07	375.7 ± 54.8 $[M] = 5.61^{-6} \pm 0.82$	5.27 ± 0.06	< 0.0005	355	31
8	d(CH$_2$)$_5$[D-Thi^2,Thr^4, Eda$^9 \leftarrow$Tyr^{10}]OVT[k]	7.76 ± 0.03 $[M] = 1.77^{-8} \pm 0.16$	6.34 ± 0.81 $[M] = 9.45^{-8} \pm 1.21$	7.05 ± 0.06	269 ± 5.5 $[M] = 4.02^{-6} \pm 0.08$	5.40 ± 0.01	~ 0.006	227	42

[a] *In vitro* pA_2 values represent the negative logarithm to the base 10 of the average molar concentration [M] of antagonist, which reduces the response to 2*x* units of agonist to the response with *x* units of agonist.
[b] The effective dose (ED) is defined as the dose (in nmol/kg) of antagonist that reduces the response to 2*x* units of agonist to the response with *x* units of agonist administered in the absence of antagonist.
[c] Estimated *in vivo* pA_2 values represent the negative logarithms of the ED divided by the estimated volume of distribution (67 ml/kg).
[d] MD ratio = antivasopressor [M]/antioxytocic [M] (*in vitro*).
[e] ED ratio = antivasopressor ED/antioxytocic ED.
[f] Atosiban is the trade name for [1-deamino, 2-*O*-ethyl-D-tyrosine, 4-threonine]ornithine-vasotocin.
[g] Original synthesis is reported in [27].
[h] Pharmacological data here are from repeat synthesis: [48].
[i] Means ± S.E.
[j] Data from [31].
[k] This publication.
[l] Data from [30].
[m] Data from [59].

Table 2 Physicochemical Properties of the Protected Peptides **I–VIII** and **c**[a]

No.	Peptide	Yield (%)[b]	m.p. (°C)	$[\alpha]_D^{25}$ (c = 1)DMF	TLC, R_f[c]			
					a	b	c	d
I	d$(CH_2)_5$(Mob)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(HBr)-COOH	47.3	162–163	−26.8	0.39	0.37	0.31	0.28
II	d$(CH_2)_5$(Mob)-D-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(HBr)-COOH	75.3	165–167	−12.6	0.27	0.35	0.29	
III	d$(CH_2)_5$(Mob)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Tyr(2-Cl-Bzl)-NH_2	49.8	230–232	−23.9	0.86	0.74	0.78	0.98
IV	d$(CH_2)_5$(Mob)-D-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Tyr(2-Cl-Bzl)-NH_2	43.8	172–174	−14.2	0.87	0.83	0.77	
V	d$(CH_2)_5$(Mob)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda	41.7	208–210	−22.8	0.47	0.54	0.67	
VI	d$(CH_2)_5$(Mob)-D-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda	90.0	198–200	−9.5	0.47	0.52	0.52	
VII	d$(CH_2)_5$(Mob)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda←Tyr(Bzl)-Z	64.6	203–205	−20.4	0.88	0.78	0.82	0.97
VIII	d$(CH_2)_5$(Mob)-D-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda←Tyr(Bzl)-Z	90.5	223–225	−12.4	0.94	0.76	0.87	
c	d$(CH_2)_5$(Bzl)-Tyr(Me)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Orn(Z)-Eda	78.3	204-205	−19.8	0.55	0.60	0.73	

[a] The protected peptides **I–VIII** and **c** are the immediate protected precursors for the peptides **1–8** and **C** given in Tables 1 and 3.
[b] Yields are based on the amino acid content of the resin except for **VII** and **VIII**, which were calculated on the theoretical yield expected from the solution coupling.
[c] Solvent systems are described in the Experimental section.

Table 3 Physicochemical Properties of Free Peptides **1**–**8** and **C**

No	Peptide	Yield[a] (%)	$[\alpha]_D^{25}$ ($c = 0.1$, 1N,AcOH)	TLC, R_f[b]				HPLC[c] t_R(min)	Formula	MW calc.	MS found (ESMS)
				a	b	c	d				
1	desGly-(NH_2),d(CH_2)$_5$[Thi^2,Thr^4]OVT	18.1	−77.0	0.30	0.24	0.23	0.43	32.3	$C_{42}H_{65}O_{11}N_9S_3$	968.2	968.3
2	desGly-(NH_2),d(CH_2)$_5$[D-Thi^2,Thr^4]OVT	31.5	−132.0	0.13	0.09	0.07		31.9	$C_{42}H_{65}O_{11}N_9S_3$	968.2	977.6
3	d(CH_2)$_5$[Thi^2,Thr^4,Tyr-(NH_2)^9]OVT	17.8	−66.0	0.33	0.22	0.26	0.17	32.3	$C_{51}H_{75}O_{12}N_{11}S_3$	1130.4	1130.3
4	d(CH_2)$_5$[D-Thi^2,Thr^4,Tyr-(NH_2)^9]OVT	21.8	−128.0	0.40	0.31	0.21		32.3	$C_{51}H_{75}O_{12}N_{11}S_3$	1130.4	1130.3
5	d(CH_2)$_5$[Thi^2,Thr^4,Eda^9]OVT	21.8	−72.0	0.25	0.02	0.07	0.24	28.3	$C_{44}H_{71}O_{10}N_{11}S_3$	1010.3	1010.3
6	d(CH_2)$_5$[D-Thi^2,Thr^4,Eda^9]OVT	12.0	−124.0	0.20	0.04	0.02		28.4	$C_{44}H_{71}O_{10}N_{11}S_3$	1010.3	1010.3
7	d(CH_2)$_5$[Thi^2,Thr^4,Eda^9 ← Tyr^{10}]OVT	24.8	−59.0	0.25	0.03	0.14	0.26	30.6	$C_{55}H_{80}O_{12}N_{12}S_3$	1173.5	1173.3
8	d(CH_2)$_5$[D-Thi^2,Thr^4,Eda^9 ← Tyr^{10}]OVT	26.7	−52.0	0.24	0.09	0.05		30.3	$C_{55}H_{80}O_{12}N_{12}S_3$	1173.5	1173.3
C	d(CH_2)$_5$[Thi^2,Thr^4,Eda^9]OVT	50.0	−73.0	0.14	0.07	0.32			$C_{47}H_{75}O_{11}N_{11}S_2$	1034.3	

[a] Yields are based on the amount of the protected peptide in the reduction-reoxidation step in each case and are uncorrected for acetic acid and water content.
[b] Solvent systems and conditions are given in the Experimental section.
[c] All peptides were at least 95% pure. For elutions a linear gradient 90:10 to 30:70 (0.05% aqueous TFA: 0.05% TFA in MeCN) over 60 min with flow rate 1.0 ml/min was applied.

the negative logarithm of the molar concentration of the antagonist that will reduce the response to $2x$ units of the agonist to equal the response to x unit of the agonist in the absence of antagonist. In practice, this dose is estimated by finding doses above and below the pA_2 dose and interpolating on a logarithmic scale. In the rat *in vivo* assays, the pA_2 dose (effective dose, ED) is divided by an arbitrarily assumed volume of distribution of 67 ml/kg to derive the molar concentration of the pA_2 dose [102]. Thus, *in vivo* pA_2 values are estimates. Synthetic oxytocin and arginine-vasopressin, which had been standardized in oxytocic and vasopressor units against the USP Posterior Pituitary Reference Standard, were used as 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 [103]. *In vivo* anti-OT potencies were determined in urethane anaesthetized diethylstibestrol-primed rats as previously described [104]. Vasopressor assays were performed on urethane-anaesthetized and phenoxybenzamine-treated rats as described by Dekanski [105]. Antidiuretic assays were on water-loaded rats under ethanol anaesthesia as described by Sawyer [106]. When standard errors are presented in Table 1, the means reflect results from at least four independent assay groups.

RESULTS AND DISCUSSION

The antioxytocic (anti-OT) (*in vitro*, no Mg^{2+} and *in vivo*) potencies, antivasopressor (anti-V_{1a}) potencies, antidiuretic (V_2 receptor) agonistic and/or antagonistic activities, together with *in vitro* and *in vivo* anti-OT/anti-V_{1a} selectivities, given as molar dose (MD) and effective dose (ED) ratios, respectively, of peptides **1**–**8**, their parent peptides **A**–**D** and Atosiban are presented in Table 1. None of the eight new peptides (nos. **1**–**8**) or peptide **C** exhibited oxytocic or vasopressor agonism. All eight Thi^2/D-Thi^2 substituted peptides are very weak antidiuretic agonists. Peptide **C** exhibits weak antidiuretic antagonism. All eight Thi^2/D-Thi^2 substituted peptides and peptide **C** are potent *in vitro* OT antagonists. All exhibit OT antagonism *in vivo*. All eight Thi^2/D-Thi^2 substituted peptides exhibit strikingly lower antivasopressor potencies relative to their respective parent peptides **A**–**D**. Thus, all eight peptides exhibit enhancements in anti-OT (*in vitro*)/anti-V_{1a} selectivities relative to their parent peptides. Two of these peptides (nos. **4** and **8**) are equipotent with Atosiban as OT antagonists *in vivo*. However, since their anti-V_{1a} potencies are much lower than the anti-V_{1a} potency of Atosiban, these two peptides and the remaining six peptides (**1**–**3**, **5**–**7**) all exhibit substantial gains in anti-OT (*in vivo*)/anti-V_{1a} selectivities compared to Atosiban.

Effects of Thi^2/D-Thi^2 Modifications in OT Antagonists A–D

Effects on in vitro (no Mg^{++})OT antagonism (Table 1). In general, both the Thi^2 and D-Thi^2 substitutions in the OT antagonists **A**–**D** were very well tolerated with complete retention of *in vitro* OT antagonism in nearly all cases. Replacement of the $Tyr(Me)^2$ residue in desGly-(NH_2),d$(CH_2)_5$[$Tyr(Me)^2$,Thr^4]OVT (**A**) (anti-OT $pA_2 = 7.89$) by Thi^2 and D-Thi^2 gave peptides **1** and **2** with anti-OT pA_2's = 7.87 and 7.76, respectively. Thus, while the L-Thi^2 analogue of **A** (peptide **1**) retains the full *in vitro* OT antagonism of **A**, the D-Thi^2 analogue of **A** (peptide **2**) exhibits slightly diminished *in vitro* OT antagonism. Replacement of the $Tyr(Me)^2$ residue in d$(CH_2)_5$[$Tyr(Me)^2$,Thr^4,Tyr-$(NH_2)^9$]OVT (**B**) (anti-OT $pA_2 = 7.63$) by Thi^2 and D-Thi^2 to give peptides **3** and **4** led to full retention of *in vitro* OT antagonism. With anti-OT pA_2s = 7.77 and 7.72, peptides **3** and **4** are equipotent with **B**. d$(CH_2)_5$[$Tyr(Me)^2$,Thr^4,Eda^9]OVT (**C**), reported here for the first time, was found to exhibit an anti-OT *in vitro* $pA_2 = 7.75$. The Thi^2 and D-Thi^2 analogues of **C** (Peptides **5** and **6**) with anti-OT *in vitro* pA_2 values of 8.05 and 7.85 respectively, both exhibit enhanced *in vitro* OT antagonism relative to **C**. d$(CH_2)_5$-[$Tyr(Me)^2$,Thr^4,$Eda^9 \leftarrow Tyr^{10}$]OVT (**D**) exhibits an anti-OT *in vitro* $pA_2 = 7.50$. The Thi^2 and D-Thi^2 analogues of **D** (peptides **7** and **8**), with anti-OT *in vitro* pA_2 values of 7.84 and 7.76, are significantly more potent than **D** as *in vitro* OT antagonists.

Thus, of the four pairs of Thi^2/D-Thi^2 peptides reported here, two pairs, **5** and **6** and **7** and **8** exhibit enhanced OT *in vitro* antagonistic potencies with respect to their parent peptides **C** and **D**. With the exception of peptide **2**, which exhibits slightly diminished OT antagonism with respect to its parent peptide **A**, the remaining three peptides, **1**, **3** and **4**, are virtually equipotent with their respective parents **A** and **B** as *in vitro* OT antagonists.

Effects on in vivo OT antagonism. Peptides **1**–**8** all exhibit *in vivo* OT antagonism. However, replacement of the $Tyr(Me)^2$ residue in the peptides **A** and **B** by Thi^2 and D-Thi^2 to give peptides **1**–**4** (Table 1) appears to have resulted in reductions of *in vivo* OT antagonistic potencies. Whereas, desGly-(NH_2),

d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**A**) exhibits an anti-OT *in vivo* pA$_2$ = 7.69, the corresponding pA$_2$ values for peptides **1** and **2** are 6.54 and 6.95 respectively. Likewise, d(CH$_2$)$_5$ [Tyr(Me)^2,Thr^4,Tyr-(NH$_2$)^9]OVT (**B**) possesses an anti-OT *in vivo* pA$_2$ = 7.83. The respective Thi^2 and D-Thi^2 analogues (peptides **3** and **4**) exhibit anti-OT pA$_2$ values of 6.92 and 7.19 respectively. However, it should be noted that since the *in vivo* anti-OT potencies of the parent peptides (**A**) and (**B**) were assayed by a slightly different quantitative method [30,31], these reductions in *in vivo* anti-OT potencies for peptides **1**–**4** may not be as substantial as they appear here. Since *in vivo* pA$_2$ values are not available for peptides **C** and **D**, we cannot evaluate whether the anti-OT *in vivo* pA$_2$ values of 6.94, 6.90, 6.78 and 7.05 exhibited by their corresponding Thi^2 and D-Thi^2 analogues (peptides **5**–**8**) are higher or lower than those of peptides **C** and **D**.

Relative Effects of L-Thi^2 and D-Thi^2 Substitutions on *in vivo* OT Antagonistic Potencies

The eight new analogues of peptides **A**–**D** constitute a series of four pairs of L-Thi^2/D-Thi^2 substituted OT antagonists. Examination of the ED and pA$_2$ values of each pair shows that for three of these pairs, the D-Thi^2 analogue is more potent than its L-Thi^2 counterpart. Thus, peptide **2**, the D-Thi^2 analogue of **A** with an anti-OT *in vivo* pA$_2$ of 6.95 is over twice as potent as peptide **1**, the L-Thi^2 analogue of **A**, pA$_2$ = 6.54. Likewise, with an anti-OT *in vivo* pA$_2$ = 7.19, peptide **4**, the D-Thi^2 analogue of **B** is almost twice as potent as peptide **3**, the L-Thi^2 analogue of **B**, pA$_2$ = 6.92. Similarly, peptide **8**, the D-Thi^2 analogue of **D**, with an anti-OT *in vivo* pA$_2$ = 7.05 is almost twice as potent as the L-Thi^2 analogue of **D**, pA$_2$ = 6.78. These findings recall those which we and others have reported for the relative effectiveness of D and L amino acid substitutions in OT antagonists [55–59]. Peptides **5** and **6**, the L-Thi^2 and D-Thi^2 analogues of **C** with anti-OT *in vivo* pA$_2$ of 6.94 and 6.90, respectively, are virtually equipotent OT antagonists *in vivo*. They represent a departure from the trend observed for the other 3 pairs of L-Thi^2/D-Thi^2 analogues, where in all three pairs, the D-Thi^2 analogue is clearly a more potent OT antagonist *in vivo*.

Effects of L-Thi^2 and D-Thi^2 Substitutions on Anti-Vasopressor Potencies

All eight Thi^2/D-Thi^2 peptides **1**–**8** exhibit drastic losses of vasopressor antagonism with respect to all four parent peptides **A**–**D**. The anti-V$_{1a}$ pA$_2$ values for peptides **A**–**D** are 6.48, 7.02, 7.10 and 7.07, respectively. The anti-V$_{1a}$ pA$_2$ values of peptides **1**–**8** ranges from a low of ~5 to a high of 5.80. In fact, four of these peptides, the D-Thi^2 analogue of **A** (peptide **2**), the Thi^2 and D-Thi^2 analogues of **B** (peptides **3** and **4**) and the Thi^2 analogue of **C** (peptide **5**) all exhibit anti-V$_{1a}$ pA$_2$ values of ~5. The remaining 4 peptides; peptide **1**, the Thi^2 analogue of **A**, peptide **6**, the D-Thi^2 analogue of **C** and peptides **7** and **8**, the Thi^2/D-Thi^2 analogues of **D**, with anti-V$_{1a}$ pA$_2$ = 5.20, 5.80, 5.27 and 5.40 are all also very weak V$_{1a}$ antagonists. With regard to the relative effectiveness of the Thi^2/D-Thi^2 substitutions in lowering anti-V$_{1a}$ potencies, no consistent pattern has emerged from this series. In one pair, peptides **3** and **4**, they are equally effective; for two pairs, peptides **5** and **6**, **7** and **8**, the Thi^2 analogues exhibit lower anti-V$_{1a}$ pA$_2$ values. Yet, for peptides **1** and **2** the D-Thi^2 analogue exhibits a lower anti-V$_{1a}$ pA$_2$ value.

Thi^2/D-Thi^2 Substitutions Enhance Anti-OT (*in vitro*)/Anti-V$_{1a}$ Selectivities

All eight Thi^2/D-Thi^2 containing peptides **1**–**8** exhibit significant increases in anti-OT (*in vitro*)/anti-V$_{1a}$ selectivities with respect to those exhibited by their parent peptides **A**–**D**. The increases exhibited by peptides **1**–**5** are particularly striking. Thus, desGly-(NH$_2$),d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**A**) has an anti-OT (*in vitro*)/anti-V$_{1a}$ selectivity = 30. The Thi^2 and D-Thi^2 analogues of **A** (peptides **1** and **2**, Table 1) exhibit anti-OT(*in vitro*)/anti-V$_{1a}$ selectivities of 450 and ~525 respectively. d(CH$_2$)$_5$[Tyr(Me)^2, Thr^4,Tyr-(NH$_2$)^9]OVT (**B**) exhibits an anti-OT (*in vitro*)/anti-V$_{1a}$ selectivity = 4.2. The Thi^2 and D-Thi^2 analogues of **B** (peptides **3** and **4**, Table 1) exhibit anti-OT(*in vitro*)/anti-V$_{1a}$ selectivities of ~550 and ~450, respectively.

The anti-OT (*in vitro*)/anti-V$_{1a}$ selectivity of d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Eda^9]OVT (**C**) = 4.3. The Thi^2 and D-Thi^2 analogues of **C** (peptides **5** and **6**; Table 1) exhibit corresponding selectivities of ~1080 and 116 respectively. In this instance, the increase brought about by the Thi^2 substitution is almost 10 times greater than that brought about by the D-Thi^2 substitution. The retro modified peptide, d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Eda^9←Tyr^{10}]OVT (**D**) exhibits an anti-OT (*in vitro*)/anti-V$_{1a}$ selectivity = 2.6. The Thi^2 and D-Thi^2 analogues of **D** (peptides **7** and **8**, Table 1) exhibit selectivities of 355 and 227

respectively. Peptides **1**–**5** with anti-OT selectivities ranging from 450 to ~1,080 are all more selective as *in vitro* OT antagonists than all but one of our previously reported position two modified analogues of **A**, namely the Tyr^2 analogue, anti-OT (*in vitro*)/anti-V_{1a} selectivity = 540) [48]. With an anti-OT (*in vitro*/anti-V_{1a} selectivity = ~1,080, d(CH$_2$)$_5$[Thi^2, Thr^4,Eda^9]OVT (peptide **5**, Table 1), is clearly the most potent and selective *in vitro* OT antagonist reported here. It may be recalled that replacement of the Asn residue with diaminopropionic acid (Dap) at position 5 in the potent and selective OT antagonist, desGly-(NH$_2$), d(CH$_2$)$_5$[D-Trp^2,Thr^4]OVT [48] resulted in the complete abolishment of detectable vasopressor antagonism exhibited by the resulting peptide, desGly-(NH$_2$),d(CH$_2$)$_5$[D-Trp^2,Thr^4,Dap^5]OVT [49]. This peptide is thus a potent, infinitely selective *in vitro* OT antagonist. A similar Dap^5 modification in some of the new peptides reported here, might be expected to further increase their anti-OT (*in vitro*)/anti-V_{1a} selectivities.

Thi^2/D-Thi^2 Substitutions Enhance Anti-OT (*in vivo*)/anti-V_{1a} Selectivities

Replacement of the Tyr(Me)^2 residue in desGly-(NH$_2$),d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**A**) by Thi^2 led to a modest increase from 18 to 22 in anti-OT (*in vivo*)/anti-V_{1a} selectivity exhibited by the resulting Thi^2 analogue of **A** (peptide **1**). However, the D-Thi^2 substitution effected a much greater enhancement in selectivity. With an anti-OT (*in vivo*)/anti-V_{1a} selectivity of ~82, the D-Thi^2 analogue of (**A**), peptide **2**, Table 1, exhibits almost a 4-fold increase in selectivity. Both the Thi^2 and D-Thi^2 substitutions in (**B**) led to striking gains in selectivity relative to the parent peptide (**B**). With anti-OT (*in vivo*)/anti-V_{1a} selectivities of ~82 and 147, the increases in anti-OT (*in vivo*)/anti-V_{1a} selectivity exhibited by peptides **3** and **4**, the Thi^2 and D-Thi^2 analogues of d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr-(NH$_2$)^9]OVT (**B**) (ED ratio = 6.6) are quite striking. With an anti-OT (*in vivo*) pA$_2$ = 7.19 and an anti-OT (*in vivo*)/anti-V_{1a} selectivity of ~147, d(CH$_2$)$_5$[D-Thi^2,Thr^4,Tyr-(NH$_2$)^9]OVT (Peptide **4**, Table 1) is clearly the most potent and selective *in vivo* OT antagonist of this series. The remaining four peptides (**5**–**8**, Table 1) exhibit anti-OT (*in vivo*)/anti-V_{1a} selectivities of 83, 11, 31 and 42. With an anti-OT (*in vivo*)/anti-V_{1a} selectivity = 147, peptide **4** is a more selective *in vivo* OT antagonist than those we previously reported [48]. It thus appears to be the most selective *in vivo* OT antagonist reported to date.

Comparison of Properties of New OT Antagonists 1–8 with those of Atosiban (Tractocile)

Atosiban (trade name: Tractocile): ([1-deamino-2-*O*-D-ethyl tyrosine, 4-threonine]ornithine vasotocin) [27] is the only oxytocin antagonist currently in clinical use for the treatment and prevention of premature labour [67–69]. However, as noted earlier, Atosiban, since it is a highly potent V_{1a} antagonist in humans [71,75], is far from ideal as an OT antagonist. We also found that a resynthesized version of Atosiban retains appreciable anti-V_{1a} potency (pA$_2$ = 6.14) in the rat [48]. We have shown that V_{1a} antagonism is an undesirable side effect in OT antagonists [76]. Thus, in searching for new OT antagonists that might exhibit a more favourable profile of anti-OT versus anti-V_{1a} properties, it is instructive to compare the properties of the eight new peptides we report here with those we previously reported for Atosiban [48]. In looking first at OT antagonism (*in vitro*), with anti-OT (*in vitro*) pA$_2$ values ranging from 7.76 to 8.05, all of Thi^2/D-Thi^2 substituted OT antagonists **1**–**8** are as potent or slightly more potent than Atosiban [anti-OT (*in vitro*) pA$_2$ = 7.71]. With anti-OT (*in vivo*) pA$_2$ values of 7.19 and 7.05, two of the eight peptides, d(CH$_2$)$_5$[D-Thi^2,Thr^4,Tyr-(NH$_2$)^9]OVT (peptide **4**) and d(CH$_2$)$_5$[D-Thr^2,Thr^4,Eda^9←Tyr^{10}]OVT (peptide **8**) are equipotent with Atosiban [anti-OT (*in vivo*) pA$_2$ = 7.05] as *in vivo* OT antagonists. The remaining six peptides (**1**–**3**, **5**–**7**) exhibit slightly diminished anti-OT (*in vivo*) potencies relative to Atosiban. However, since Atosiban possesses appreciable anti-vasopressor potency (anti-V_{1a} pA$_2$ = 6.14) [48], and since, by contrast, peptides **1**-**8** exhibit greatly diminished anti-vasopressor potencies, (pA$_2$ values range from ~5 to 5.40), Atosiban is much more potent as a vasopressor antagonist than all of the eight peptides **1**–**8**. Consequently, all eight peptides exhibit striking gains in both *in vitro* and *in vivo* anti-OT/anti-V_{1a} selectivities compared with Atosiban. Atosiban exhibits an anti-OT (*in vitro*)/anti-V_{1a} selectivity = 37, the corresponding selectivities for peptides **1**–**8** range from a low of 116 for peptide **6** to a high of ~1080 for peptide **5**. While the gains in *in vivo* selectivity are not as striking, nonetheless, with the exception of peptide **6** which exhibits an ED ratio of 11 in the same range as that of Atosiban (ED ratio = 8), all the remaining seven peptides exhibit 3-fold (peptide **1**) to 18-fold (peptide **4**) gains in *in vivo* anti-OT/anti-V_{1a} selectivities compared with Atosiban. Of the eight new peptides reported here, d(CH$_2$)$_5$[D-

Thi^2,Thr^4,Tyr-(NH$_2$)^9]OVT (peptide **4**) is clearly the most promising when compared with Atosiban. With an *in vivo* anti-OT pA$_2$ value = 7.19, it is equipotent with Atosiban as an OT antagonist. The corresponding pA$_2$ value for Atosiban is 7.05. However, because of its much lower anti-V$_{1a}$ potency (pA$_2$ = ~5), compared to that of Atosiban (pA$_2$ = 6.14), peptide **4** is 147 times more potent as an OT antagonist *in vivo* than as a V$_{1a}$ antagonist. This represents a big enhancement in anti-OT(*in vivo*)/anti-V$_{1a}$ selectivity compared to the value of 8 for Atosiban. It thus appears to have advantages over Atosiban as a safer tocolytic. Thus, peptide **4** is a potential candidate for development as a tocolytic for the prevention of preterm labour.

CONCLUSION

In attempts to design OT antagonists with lower anti-V$_{1a}$ properties than those of Atosiban (anti-V$_{1a}$ pA$_2$ = 6.14) we have explored the use of Thi^2/D-Thi^2 replacements for Tyr(Me)^2 in the OT antagonists: desGly(NH$_2$), d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4]OVT (**A**) [31]; d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr-(NH$_2$)^9]OVT (**B**) [30]; d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Eda^9]OVT (**C**) and (CH$_2$)$_5$-[Tyr(Me)^2, Thr^4, Eda^9 ← Tyr^{10}]OVT (**D**). The resulting eight Thi^2/D-Thi^2 peptides, **1**–**8**, retain the full *in vitro* OT antagonism of their respective parents **A**–**D**. While all eight peptides exhibit *in vivo* OT antagonism, somewhat diminished in the case of peptides **1**–**4** relative to the potencies of the parent peptides **A** and **B**, the anti-V$_{1a}$ potencies of peptides **1**–**8** (pA$_2$ = ~5–5.4) are drastically lower than those of the parent peptides **A**–**D** (pA$_2$ = 6.48–7.10) and also significantly lower than the anti-V$_{1a}$ potency of Atosiban (pA$_2$ = 6.14). With the exception of peptide **6**, the seven other peptides reported here all exhibit enhanced anti-OT (*in vivo*)/anti-V$_{1a}$ selectivities relative to Atosiban. A number of these peptides may be promising candidates for development as potential tocolytics for the prevention of premature labour. In this regard, of the new peptides reported here, d(CH$_2$)$_5$[D-Thi^2,Thr^4,Tyr-(NH$_2$)^9]OVT (peptide **4**) appears to be the most promising. It is equipotent with Atosiban as an OT antagonist *in vivo*, yet it possesses less than 6% of the anti-V$_{1a}$ potency of Atosiban. It thus clearly possesses a safer pharmacological profile than Atosiban for use as a tocolytic agent. With high anti-OT/anti-V$_{1a}$ selectivities = ~82, ~82, 147 and ~83 respectively, peptides **2**–**5** are promising new pharmacological tools for studies on the physiological roles of OT and for studies on OT receptors. The findings presented here also provide useful clues for the design of new selective OT antagonists for use as tocolytic agents for the prevention of premature labour and for possible therapeutic use in the treatment of chorion-derived OT receptor expressing tumours [109].

EXPERIMENTAL

The Merrifield resin was purchased from Eastman Chemical Co., Rochester, NY. The Boc-Tyr(2-Cl-*Z*)-resin was purchased from Chem-Impex International, Inc., USA. The Boc-Orn(*Z*)-resin was prepared by the caesium salt method [107]. The amino acid derivatives were purchased from Bachem Bioscience, Inc., Chem-Impex International, Inc., or from Synthetech, Inc., USA. The *β*-*S*-(4-methoxybenzylmercapto) - *β*,*β* - pentamethylenepropionic acid [108] was purchased from Bachem Bioscience, Inc, USA. TLC was run on precoated silica gel plates (60F-254, E. Merck) with the following solvent systems: (a) 1-butanol:AcOH:H$_2$O (4:1:5, upper phase); (b) 1-butanol:AcOH:H$_2$O (4:1:1); (c) 1-butanol:AcOH:H$_2$O:pyridine (15:3:3:10); (d) chloroform:methanol (7:3). Loads of 10–15 μg each were applied and chromatograms were developed at a minimal length of 10 cm. The chlorine gas procedure for the KI–starch reagent was used for detection [84]. Optical rotations were measured with a Rudolph Autopol III polarimeter. Analytical HPLC was performed on a Waters 810 instrument under the following conditions: 90:10 to 30:70 0.05% aqueous TFA:0.05% TFA in MeCN, linear gradient over 60 min at 1.0 ml/min (λ = 210 nm), on a Microsorb C$_{18}$ column (Rainin Instrument Co., Inc.). All peptides were at least 95% pure. Electron spray mass spectra (ESMS) were done by the University of Oklahoma Health Sciences Center Molecular Biology Resource Facility on a PE Sciex Q-STAR Ouadropole TOF Mass Spectrometer using 50:50 CH$_3$CN/H$_2$O with 0.5% AcOH as a solvent.

Solid Phase Synthesis Procedures

The protected precursors **I**–**VIII** and **c** (Table 2) of the free peptides **1**–**8** and **C** (Table 3) were synthesized entirely by the Merrifield solid-phase method [82,83], with the modifications previously described [21,23,31,48,90]. The protected retro-Tyr-modified peptide **VII** and **VIII** (Table 2) were prepared by retroaddition of *Z*-Tyr(Bzl) to the corresponding protected Eda peptides **V** or **VI** (Table 2) using

the DCC/HOBt procedure [85] as described in [86]. Boc-Tyr(2-Cl-*Z*)-resin and Boc-Orn(*Z*)-resin were prepared by esterification of Merrifield resin (chloromethylated polystyrene: 1% divinylbenzene copolymer beads, 200–400 mesh, 0.7 ± 0.10 meq/g) with either Boc-Tyr(2-Cl-*Z*) or Boc-Orn(*Z*) using the caesium salt method [107]. For the synthesis of protected peptidyl resins, seven or eight cycles of deprotection, neutralization and coupling were carried out starting as follows: for peptides **I**, **II**, **c**, **V** and **VI** from Boc-Orn(Z)-resin; for peptides **III** and **IV** from Boc-Tyr (2-Cl-Z)-resin. HCl (1 M)/AcOH was used in all the deprotection steps [83,84]. Neutralizations were carried out with 10% Et_3N/CH_2Cl_2. Boc amino acids (except Boc-Asn) were coupled by the DCC/HOBt procedure [85] in CH_2Cl_2:DMF (9:1, v/v). Boc-asparagine was coupled as its nitrophenyl ester [87] in DMF. The acylpeptide resin were cleaved using the following: procedures: (i) acidolysis with HBr/TFA [83,84,88,89] to give the protected peptides **I** and **II**; (ii) ammonolysis in methanol [90,91] to give the protected peptide amides **III** and **IV**; (iii) aminolysis with Eda in methanol [86,92] to give the protected Eda peptides **c**, **V** and **VI**. All of the protected precursors, including the retroprotected peptides **VII** and **VIII,** were purified by the same general method: extraction or dissolving with warm DMF followed by reprecipitations with H_2O and $EtOH/Et_2O$ until adjudged pure by TLC as previously described [20,21,31,48] to give the required protected peptides **I**–**IV**, **c**, **V**–**VIII** (Table 2). The physicochemical properties of all protected peptides **I**–**IV**, **c**, **V**–**VIII** are given in Table 2. Deprotection of the protected peptide **c** was carried out with sodium in liquid ammonia [93,94] as previously described [20,21,31,48,82]. Peptides **I**–**VIII** were deprotected by the TFMSA procedure [95,96]. The resulting disulphydryl compounds were oxidatively cyclized with $K_3[Fe(CN)_6]$ using the normal procedure [97] or a modified reverse procedure [98]. The free peptides were desalted and purified by a two-step gel filtration procedure [99] on Sephadex G-15 (eluent 50% AcOH) and LH-20 (eluent 2 M AcOH). For some peptides, an additional purification on Sephadex G-15 or/and on Sephadex LH-20 with 0.2 M AcOH as eluent was carried out. The purity of the free peptides **1**–**4**, **C**, **5**–**8** (Table 3) was checked by TLC and HPLC. Their structures were confirmed by ESMS. The TLC, HPLC, ESMS data and some other physicochemical properties of the free peptides **1**–**4**, **C**, **5**–**8** are presented in Table 3.

((β - S- (4 - methoxybenzylmercapto) - β,β - pentamethylenepropionyl) - Thi - Ile - Thr(Bzl) - Asn - Cys(Mob) - Pro - Orn(HBr) - OH (I, Table 2). Boc-Orn(*Z*)-resin (3.23g, 2 mmol) was converted to the protected acyl heptapeptidyl resin in seven cycles of deprotection, neutralization and coupling (mediated by DCC/HOBt or active ester) with Boc-Pro, Boc-Cys(Mob), Boc-Asn-ONp, Boc-Thr(Bzl), Boc-Ile, Boc-Thi and *β* - *S* - (4 - methoxybenzylmercapto) - *β*,*β* - pentamethylenepropionic acid, respectively by the manual method of solid-phase synthesis as previously described [20,21,31,48,82]. The resulting protected peptidyl resin was 5.02 g (98.3%). The protected peptide **I** was split from the resin by acidolytic cleavage [83,84,87,89] as follows. Hydrogen bromide was bubbled through a suspension of 2 g (0.76 mmol) of [(*β*-S-(4-methoxybenzylmercapto)-*β*,*β*-pentamethylenepropionyl]-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-resin in TFA (20 ml) and anisole (3 ml) in a glass funnel fitted with a fritted disk as described in [84]. After 30 min the filtrate was collected. The resin was resuspended in CH_2Cl_2 (10 ml), TFA (10 ml) and anisole (3 ml). HBr bubbling was resumed for a further 30 min, whereupon the filtrate was collected and the resin was washed with CH_2Cl_2:TFA (1:1, 20 ml × 3). The filtrates and washings were combined and evaporated to dryness on a rotary evaporator. Addition of ether (*ca.* 200 ml) to the residual anisole solution gave a precipitate, which following 3 h at 4°C was collected, washed with ether and dried over P_2O_5. This material was dissolved in warm DMF (*ca.* 5 ml), reprecipitated with water, collected, and dried in vacuo over P_2O_5 to give 0.5 g (47.3%) of the desired protected acylheptapeptide **I** (Table 2). The same procedure was used for preparation of the protected acylheptapeptide **II** (Table 2) using Boc-D-Thi in place of Boc-Thi for position 2.

((β - S- (4 - methoxybenzylmercapto) - β,β - pentamethylenepropionyl) - Thi - Ile - Thr(Bzl) - Asn - Cys(Mob) - ProOrn(Z) - Eda (V, Table 2). Starting from d$(CH_2)_5$(Mob) - Thi - Ile - Thr(Bzl) - Asn - Cys(Mob) - Pro-Orn(Z)-resin (3.02 g, 1.14 mmol), prepared as described above, the protected Eda-peptide **V** was obtained by aminolysis with Eda/MeOH and DMF extraction [86,92] as follows. The protected peptidyl resin was placed in 250-ml round bottomed flask, 75 ml of anhydrous methanol was added, the suspension was cooled at *ca.* 0°C and 30 ml of ethylenediamine (Eda, 99.5 + %, redistilled; Aldrich), was added under stirring. After 30 min, the cooling bath was removed and the suspension

stirred at room temperature for 2 days. The solvents were removed on a rotary evaporator and the protected peptide **V** (Table 2) was further extracted and purified as described above. The protected Eda peptides **c** and **VI** (Table 2) were prepared by the same procedure starting from $d(CH_2)_5$(Mob)-Tyr(Me)-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-resin and $d(CH_2)_5$(Mob)-D-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-resin, respectively.

((β-S-(4-methoxybenzylmercapto)-β,β-pentamethylenepropionyl)-Thi-Ile-Thr(BzL)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda← Z-Tyr(Bzl) (VII, Table 2). The retromodified peptide **VII** was synthesized by DCC/HOBt mediated coupling [85,86] of protected peptide **V** and *Z*-Tyr(Bzl) as follows. To a cooled (0°C) solution of *Z*-Tyr(Bzl) (0.24 g, 0.6 mmol) and HOBt (0.138 g, 0.9 mmol) in 3 ml of anhydrous DMF was added 0.45 ml (0.9 mmol) of 2 M solution of DCC in DMF. The reaction mixture was stirred for 1 h, whereupon the dicyclohexylurea (DCU) was removed by filtration. The filtrate was added to a solution of $d(CH_2)_5$(Mob)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Eda (**V**) (0.22 g, 0.15 mmol) in 2 ml anhydrous DMF. DIPEA was added to give a pH ~7.5. After the mixture was stirred for 18 h at room temperature (TLC monitoring), MeOH (20 ml) was added followed by Et_2O (250 ml). The precipitated product was collected following overnight storage at 4°C. Washing with warm MeOH gave the required protected peptide **VII**, 0.18 g, yield 64.6% (Table 2). The protected retromodified peptide **VIII** (Table 2) was prepared by the same procedure utilizing the protected Eda peptide **VI**.

((β-S-(4-methoxybenzylmercapto)-β,β-pentamethylenepropionyl)-Thi-Ile-Thr(Bzl)-Asn-Cys(Mob)-Pro-Orn(Z)-Tyr(2-Cl-Z)-NH$_2$ (III, Table 2). Boc-Tyr(2-Cl-*Z*)-resin (0.3 g, 0.25 mmol) was converted to the protected acyl octatapeptidyl resin in eight cycles of deprotection, neutralization and coupling (mediated by DCC/HOBt or active ester) with Boc-Orn(Z), Boc-Pro, Boc-Cys(Mob), Boc-Asn-ONp, Boc-Thr(Bzl), Boc-Ile, Boc-Thi and *β*-*S*-(4-methoxybenzylmercapto)-*β*,*β*-pentamethylenepropionic acid, respectively, by the manual method of solid-phase synthesis, as previously described [21,23,31, 48,83,84]. The protected peptide **III** was cleaved from the resin by ammonolysis with DMF extraction [90,91] as follows. The resin was suspended in anhydrous methanol (50 ml) at *ca.* −70°C in a 250-ml round bottom flask and NH_3 (*ca.* 30 ml) was bubbled through the suspension for a *ca.* 30-min period. The tightly stoppered flask was kept at room temperature in the hood for 2 days. The suspension was recooled, the stopper removed and the NH_3 and methanol allowed to evaporate at room temperature. The protected peptide was extracted with warm (*ca.* 50°C) DMF (*ca.* 30 ml) and precipitated with warm (*ca.* 50°C) water (*ca.* 500 ml). Following overnight storage at 4°C, the product was collected, dried *in vacuo* over P_2O_5 reprecipitated from warm (*ca.* 50°C) DMF (*ca.* 5 ml) with ethyl ether (*ca.* 300 ml), collected and dried in vacuo over P_2O_5 to give 0.34 g (79.8%) of the protected acyloctapeptide amide **III** (Table 2). This procedure was used for the preparation of the protected peptide amide **IV** (Table 2) by replacing Boc-Thi with Boc-D-Thi in the penultimate coupling step.

(β-Mercapto)-β,β-pentamethylenepropionyl)-Thi-Ile-Thr-Asn-Cys-Pro-Orn-OH (desGly-(NH$_2$),dCH$_2$)$_5$ (Thi^2, Thr^4) OVT) (1, Table 3). The deprotection of the acylheptapeptide **I** (Table 2) was performed by the standard TFMSA procedure [96,97] as follows. The protected peptide **I** (150 mg) was placed in a round bottom flask with a stirring bar and 0.5 ml of thioanisole and 0.25 ml of 1,2-ethanedithiol was added, the mixture was chilled with an ice bath and 5 ml of TFA was added. After stirring for 5 min at 0°C, 0.5 ml of TFMSA was added slowly dropwise with vigorous stirring. Following additional stirring for 30 min at room temperature, 50 ml of anhydrous ether was added dropwise. The precipitated peptide was filtered, washed with ether and dried overnight *in vacuo* over P_2O_5 and NaOH. Oxidation of the resulting residue of the deblocked disulfydryl precursor of peptide **I** was performed by the modified reverse procedure [98] as follows. The residue (120 mg) was dissolved in degassed 50% AcOH (25 ml) and the solution diluted with 50 ml of H_2O. The peptide solution was added dropwise with stirring over a period of 15–30 min to an 800-ml aqueous solution which contained 20 ml of a 0.01 M solution of potassium ferricyanide. Meanwhile, the pH was adjusted to *ca.* 7.0 with concentrated ammonium hydroxide. The yellow solution was stirred for an additional 20 min. Following oxidation, the free peptide (**1**, Table 3) was isolated and purified as follows: after acidification with AcOH to pH 4.5 and stirring for 20 min with an anion exchange resin (Bio-Rad, AG 3x4, Cl^- form, 5 g damp weight), the suspension was filtered and washed with 0.2 M AcOH (3 × 30 ml), the combined filtrate and washings were lyophilized. The resulting powder was desalted on a Sephadex G-15 column (110 × 2.7 cm) eluting with aqueous acetic acid (50%) with a flow rate of 5 ml/h

[99]. The eluate was fractionated and monitored for absorbance at 254 nm. The fractions making up the major peak were checked by TLC, pooled and lyophilized. The residue was further subjected to gel filtration on a Sephadex LH-20 column (100 × 1.5 cm) eluting with 2 M aqueous acetic acid with a flow rate of 4 ml/min. The peptide was eluted in a single peak (absorbance at 254 nm). Lyophilization of the pertinent fractions gave the desired OVT analogue (**1**, Table 3). With minor modifications, the same procedure was utilized for the deprotection, oxidation and purification of the protected peptides **II**–**VIII** (Table 2) to give the free peptides (**2**–**8**, Table 3). The desalting and the purification of the free peptides usualy requires the 2-step gel filtration procedure [99] on Sephadex G-15 and Sephadex LH-20 using 50% AcOH and 2 M AcOH as eluents, respectively, as previously described [20,21,23, 31,48]. Only when necessary, an additional purification on Sephadex G-15 and/or Sephadex LH-20 with 0.2 M AcOH as eluent was carried out.

(β-Mercapto)-β,β-pentamethylenepropionyl)-Tyr-(Me)-Ile-Thr-Asn-Cys-Pro-Orn-Eda (d(CH$_2$)$_5$(Tyr-(Me)^2,Thr^4,Eda^9) OVT) (C, Table 3). The sodium/liquid NH_3 procedure [93,94] with modifications as previously described [20,21,23,31,48] was used for deprotection of the protected peptide **c**, Table 2. A solution of the protected Eda-peptide **c** (110 mg) in sodium-dried ammonia (*ca*. 400 ml) was treated at the boiling point with stirring with sodium from a stick of metal contained in a small-bore glass tube until a light blue color persisted in the solution for *ca*. 30 s. NH_4Cl was added to discharge the color. The reoxidation of the deblocked disulphydryl precursor of peptide **C** was performed by the normal procedure [97] as follows. The ammonia was evaporated and nitrogen was passed through the flask. After 5 min the residue was dissolved in degassed aqueous acetic acid (50%, 30 ml) and quickly poured into ice-cold water (*ca*. 700 ml). The pH was adjusted to approximately 7 with concentrated ammonium hydroxide. Following neutralization, an excess of a solution of potassium ferricyanide (0.01 M, 15 ml) was added gradually with stirring. The yellow solution was stirred for an additional 20 min. The isolation and purification steps (the treatment with anion-exchange resin, lyophilization, and the gel filtration on Sephadex G-15 [99] and Sephadex LH-20) were performed as described above to give 39 mg (50.0%) of the free peptide **C**. The physicochemical properties of all free peptides **1**–**4**, **C** and **5**–**8** are given in Table 3.

Acknowledgements

This work was supported in part by a research grant from the National Institute of General Medical Sciences (No. GM-25280). We thank Ms Suzanne Payne for her expert assistance in the preparation of the manuscript.

REFERENCES

1. McCormick MC. The contribution of low birth weight to infant mortality and morbidity. *N. Engl. J. Med.* 1985; **312**: 82–90.
2. Adams MM. The continuing challenge of preterm delivery. *JAMA* 1995; **273**: 730–740.
3. Creasy RK. Preterm birth prevention: Where we are? *Am. J. Obstet. Gynecol.* 1993; **168**: 1223–1230.
4. Radetsky P. Stopping premature births before it's too late. *Science* 1995; **266**: 1486–1488.
5. Norwitz ER, Robinson JM, Challis JRG. The control of labor. *N. Engl. J. Med.* 1999; **341**: 660–666.
6. Challis JRG, Matthews SG, Gibb W, Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. *Endocrine Reviews* 2000; **21**(5): 514–550.
7. Weiss G. Endocrinology of parturition. *J. Clin. Endocrinol. Metabol.* 2000; **85**: 4421–4425.
8. Soloff MS, Alexandrova M, Fernstrom MJ. Oxytocin receptors: triggers for parturition and lactation? *Science* 1979; **204**: 1313–1315.
9. Fuchs AR, Fuchs F, Husslein P, Solloff MS, Fernstrom MJ. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* 1982; **215**: 1396–1398.
10. Anderson LF, Lyndrup J, Akerlund M, Melin P. Oxytocin receptor blockade: A new principle in the treatment of preterm labor? *Am. J. Perinatol.* 1989; **6**: 196–199.
11. Chan WY, Chen DL. Myometrial oxytocin receptors and prostaglandin in the parturition process in the rat. *Biol. Reprod.* 1992; **46**: 58–64.
12. Lefebvre DL, Giaid A, Benett H, Lariviere R, Zingg HH. Oxytocin gene expression in rat uterus. *Science* 1992; **256**: 1553–1555.
13. Antonijevic IA, Douglas AJ, Dye S, Bicknell RJ, Leng G, Russell JA. Oxytocin antagonists delay the initiation of parturition and prolong its active phase in rats. *J. Endocrinol.* 1995; **145**: 97–103.
14. Berankova Z, Rychlik I, Jost K, Rudinger J, Sorm F. Inhibition of the uterus-contracting effect of oxytocin by O-methyl oxytocin. *Collect. Czech. Chem. Commun.* 1961; **26**: 2673–2675.
15. Schulz H, du Vigneaud V. Synthesis of 1-L-penicillamine-oxytocin, 1-D-penicillamine-oxytocin and 1-deaminopenicillamine-oxytocin, potent inhibitors of the oxytocic response of oxytocin. *J. Med. Chem.* 1966; **9**: 647–650.

16. Vavrek RJ, Ferger MF, Allen GA, Rich DH, Blomquist AT, du Vigneaud V. Synthesis of three oxytocin analogs related to [1-deaminopenicillamine]-oxytocin possessing antioxytocic activity. *J. Med. Chem.* 1972; **15**: 123–126.
17. Bisset GV, Clark BJ, Krejci I, Polacek I, Rudinger J. Some pharmacological properties of a synthetic oxytocin analogue 1-*N*-carbamoyl-hemicystine-2-*O*-methyltyrosine (carbamoyl-methyloxytocin), an antagonist to the neurohypophysial hormones. *Br. J. Pharmacol.* 1970; **40**: 342–360.
18. Kaurov OA, Martynov VF, Mikhailov Yu D, Auna ZP. Synthesis of new oxytocin analogs modified in the 2-position. *Z. Obshchei Khimii* 1972; **42**: 1654.
19. Nestor JJ Jr, Ferger MF, du Vigneaud V. [1-β-mercapto-β,β-pentamethylenepropionic acid]oxytocin, a potent inhibitor of oxytocin. *J. Med. Chem.* 1975; **18**: 284–287.
20. Manning M, Lowbridge J, Seto J, Haldar J, Sawyer WH. [1-Deaminopenicillamine, 4-threonine]-oxytocin, a potent inhibitor of oxytocin. *J. Med. Chem.* 1978; **21**: 179–182.
21. Lowbridge J, Manning M, Seto J, Haldar J, Sawyer WH. Synthetic antagonists of *in vivo* responses by the rat uterus to oxytocin. *J. Med. Chem.* 1979; **22**: 565–569.
22. Sawyer WH, Haldar J, Gazis D, Seto J, Bankowski K, Lowbridge J, Turan A, Manning M. The design of effective in vivo antagonists of rat uterus and milk ejection response to oxytocin. *Endocrinology* 1980; **106**: 81–91.
23. Bankowski K, Manning M, Seto J, Haldar J, Sawyer WH. Design and synthesis of potent *in vivo* antagonists of oxytocin. *Int. J. Peptide Protein Res.* 1980; **16**: 382–391.
24. Hruby VJ, Mosberg HI, Hadley M, Chan WY, Powell AM. Synthesis, pharmacological, conformational, and dynamic studies of the potent hormone antagonists [1-penicillamine, 4-threonine]-oxytocin. *Int. J. Peptide Protein Res.* 1980; **16**: 372–381.
25. Lebl M, Barth T, Servitova L, Slaninova J, Jost K. Oxytocin analogues with inhibitory properties, containing in position 2 a hydrophobic amino acid of D-configuration. *Collect. Czech. Chem. Commun.* 1985; **50**: 132–145.
26. Chan WY, Hruby VJ, Rockway TW, Hlavacek J. Design of oxytocin antagonists with prolonged action: Potential tocolytic agents for the treatment of preterm labor. *J. Pharmacol. Exp. Ther.* 1986; **239**: 84–87.
27. Melin P, Trojnar J, Johansson B, Vilhardt H, Akerlund M. Synthetic antagonists of the myometrial response to vasopressin and oxytocin. *J. Endocrinol.* 1986; **111**: 125–131.
28. Chan WY, Rockway TW, Hruby VJ. Long-acting oxytocin antagonists: effects of 2-D-stereoisomerism substitution on antagonistic potency and duration of action. *Proc. Soc. Exptl. Biol. Med.* 1987; **185**: 187–192.
29. Rekowski P, Melin P, Lammek B. Synthesis and some pharmacological properties of 2 new antagonists of oxytocin. *Pol. J. Pharmacol. Phar.* 1987; **39**: 303–307.
30. Elands J, Barberis C, Jard S, Tribollet E, Dreifuss JJ, Bankowski K, Manning M, Sawyer WH. ^{125}I-labeled d$(CH_2)_5$[Tyr(Me)^2, Thr^4, Tyr-$(NH_2)^9$]OVT: a selective oxytocin receptor ligand. *Eur. J. Pharmacol.* 1988; **147**: 197–207.
31. Manning M, Kruszynski M, Bankowski K, Olma A, Lammek B, Cheng LL, Klis WA, Seto J, Haldar J, Sawyer WH. Solid phase synthesis of sixteen potent (selective and nonselective) *in vivo* antagonists of oxytocin. *J. Med. Chem.* 1989; **32**: 382–391.
32. Lankiewicz L, Glanz D, Grzonka Z, Slaninova J, Barth T, Fahrenholz F. Synthesis and biological-activity of oxytocin analogs with beta-amino acids in position-2. *B. Pol. Acad. Sci. Chem.* 1989; **37**: 45–51.
33. Wilson Jr L, Parsons MT, Ouano L, Flouret G. A new tocolytic agent: development of an oxytocin antagonist for inhibiting uterine contractions. *Am. J. Obstetr. Gynecol.* 1990; **163**: 195–202.
34. Flouret G, Brieher W, Majewski T, Mahan K, Wilson Jr L. Some pharmacological properties of cyclic and linear analogs obtained by substituting each residue of an oxytocin antagonist with D-tryptophan. *Int. J. Peptide Protein Res.* 1991; **38**: 169–175.
35. Flouret G, Brieher W, Mahan K, Wilson Jr L. Design of potent oxytocin antagonists featuring D-tryptophan at position 2. *J. Med. Chem.* 1991; **34**: 642–646.
36. Flouret G, Brieher W, Majewski T, Mahan K, Wilson Jr L. Improvement in potency of an oxytocin antagonist after systematic substitutions with L-tryptophan. *J. Med. Chem.* 1991; **34**: 2089–2094.
37. Smith DD, Slaninova J, Hruby VJ. Structure-activity studies of a novel bicyclic oxytocin antagonist. *J. Med. Chem.* 1992; **35**: 1558–1563.
38. Prohazka Z, Slaninova J, Barth T, Stierandova A, Trojnar J, Melin P, Lebl M. Analogs of deamino carba oxytocin with inhibitory properties-synthesis and biological-activities. *Collect. Czech. Chem. Commun.* 1992; **57**: 1335–1344.
39. Pal BC, Slaninova J, Barth T, Trojnar J, Lebl M. Synthesis and properties of hormonogen and inhibitorogen type oxytocin analogs. *Collect. Czech. Chem. Commun.* 1992; **57**: 1345–1351.
40. Lebl M, Toth G, Slaninova J, Hruby VJ. Conformationally biased analogs of oxytocin. *Int. J. Peptide Protein Res.* 1992; **40**: 148–151.
41. Flouret G, Majewski T, Brieher W, Wilson Jr L. Systematic substitution of an oxytocin antagonist with D-amino acids: unexpected high antagonistic potency of the D-Cys^6-substituted analogue. *J. Med. Chem.* 1993; **36**: 747–749.
42. Williams PD, Anderson PS, Ball RG, Bock MG, Carroll LA, Chiu S-HL, Clineschmidt BV, Culberson JC, Erb

JM, Evans BE, Fitzpatrick SL, Freidinger RM, Kaufman MJ, Lundell GF, Murphy JS, Pawluczyk JM, Perlow DS, Pettibone DJ, Pitzenberg SM, Thompson KL, Veber DF. 1-(((7,7-Dimethyl-2(S)-(2(S)-amino-4-(methylsulfonyl) butyramido)bicyclo[2.2.1]-heptan-1(S)-yl)methyl)sulfonyl)-4-(2-methylphenyl)piperazine (L-368,899): an orally bioavailable, non-peptide oxytocin antagonist with potential utility for managing pre-term labor. *J. Med. Chem.* 1994; **37**: 565–571.

43. Jezek R, Zertova M, Slaninova J, Majer P, Prohazka Z. Antagonistic analogs of oxytocin with substituted phenylalanine or tyrosine in position 2. *Collect. Czech. Chem. Commun.* 1994; **59**: 1430–1438.
44. Mayer P, Slaninova J, Lebl M. Synthesis of methylated phenylalanines via hydrogenolysis of corresponding 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids. Synthesis and biological activity of oxytocin analogs with methylated phenylalanines in position 2. *Int. J. Peptide Protein Res.* 1994; **43**: 62–68.
45. Pavo I, Slaninova J, Klein U, Fahrenzolz F. Enhanced selectivity of oxytocin antagonists containing sarcosine in position-7. *J. Med. Chem.* 1994; **37**: 255–259.
46. Prohazka Z, Slaninova J. The 1- and 2-naphthylalanine analogs of oxytocin and vasopressin. *Collect. Czech. Chem. Commun.* 1995; **60**: 2170–2177.
47. Manning M, Cheng LL, Balaspiri L, Klis WA, Olma A, Sawyer WH, Wo NC, Chan WY. Effects of a D-Cys^6/L-Cys^6 interchange in selective and non-selective antagonists of vasopressin and oxytocin. *J. Med. Chem.* 1995; **38**: 1762–1769.
48. Manning M, Miteva K, Pancheva S, Stoev S, Wo NC, Chan WY. Design and synthesis of highly selective *in vitro* and *in vivo* uterine receptor antagonists of oxytocin: Comparisons with Atosiban. *Int. J. Peptide Protein Res.* 1995; **46**: 244–252.
49. Chan WY, Wo NC, Cheng LL, Manning M. Isosteric substitution of Asn^5 in antagonists of oxytocin and vasopressin leads to highly selective and potent OT and V_{1a} receptor antagonists. New approaches for the design of potential tocolytics for preterm labor. *J. Pharmacol. Exptl. Ther.* 1996; **277**: 999–1003.
50. Williams PD, Clineschmidt BV, Erb JM, Freidinger RM, Guidotti MT, Lis EV, Pawluczyk JM, Pettibone DJ, Reiss DR, Veber DF, Woyden CJ. 1-(1-[4-[*N*-acetyl - 4 - piperidinyl]oxy] - 2 - methoxybenzoyl]piperidin - 4-yl)-4H-3,1-benzoxazin-2(1H)-one (L-371,257): a new, orally bioavailable, non-peptide oxytocin antagonist. *J. Med. Chem.* 1995; **38**: 4634–4636.
51. Lian SB, Shenderovich MD, Zhang ZG, Maletinska L, Slaninova J, Hruby VJ. Substitution of the side-chain-constrained amino acids beta-methyl-2′, 6-dimethyl-4′-methoxytyrosine in position 2 of a bicyclic oxytocin analogue provides unique insights into the bioactive topography of oxytocin antagonists. *J. Am. Chem. Soc.* 1998; **120**: 7393–7394.
52. Wisniewski K, Trojnar J, Riviere P, Haigh R, Yea C, Ashworth D, Melin P, Nilsson A. The synthesis of new class of oxytocin antagonists. *Bioorg. Med. Chem. Lett.* 1999; **9**: 2801–2804.
53. Prohazka Z, Zertova M, Slaninova J. The analogs of oxytocin and D-homoarginine vasopressin with bulky substituted phenylalanine in position 2. *Lett. Pept. Sci.* 2000; **7**: 179–185.
54. Sobocinska M, Lempicka E, Konieczna E, Derdowska I, Lammek B, Melhem S, Kozik W, Janecka J, Janecki M, Trzeciak HI. Analogues of arginine vasopressin modified in position 2 or 3 with naphthylalanine: selective antagonists of oxytocin *in-vitro*. *J. Pharm. Pharmacol.* 2000; **52**: 1105–1112.
55. Hruby VJ, Smith CJ. Structure-activity relationships of neurohypophyseal peptides. In *The Peptides* Udenfriend S, Meienhofer J (eds), vol. 8, *Chemistry, Biology and Medicine of Neurohypophyseal Hormones and their Analogs*, Smith CW (ed.). Academic Press: Orlando, FL, 1987; 177–207.
56. Lebl. M. Analogs with inhibitory properties. In *Handbook of Neurohypophyseal Hormone Analogs*, vol. II Part 1, Jost K, Lebl M, Brtnik F (eds). CRC Press: Boca Raton, FL, 1987; 17–74.
57. Manning M, Sawyer WH. Discovery, development and some uses of vasopressin and oxytocin antagonists. *J. Lab. Clin. Med.* 1989; **114**: 617–632 [Published erratum appears in *J. Lab. Clin. Med.* 1990; **115**: 530].
58. Manning M, Sawyer WH. Design, synthesis and some uses of receptor specific agonists and antagonists of vasopressin and oxytocin. *J. Receptor Research* 1993; **13**: 195–214.
59. Manning M, Cheng LL, Klis WA, Stoev S, Przybylski J, Bankowski K, Sawyer WH, Barberis C, Chan WY. Advances in the design of selective antagonists, potential tocolytics and radioiodinated ligands for oxytocin receptors. In *Oxytocin: Cellular and Molecular Approaches in Medicine and Research*, Ivell R, Russell J (eds). Plenum Press: New York, 1995; 559–583.
60. Freidinger RM, Pettibone DJ. Small molecule ligands for oxytocin and vasopressin receptors. *Med. Res. Revs.* 1997; **17**: 1–16.
61. Goodwin TM, Zograbyan A. Oxytocin receptor antagonists update. *Emerg. Concepts Perinatal Endocrinol.* 1998; **25**: 859–871.
62. Bossmar T, Akerlund M, Fantoni G, Szamatowicz J, Melin P, Maggi M. Receptor for and myometrial responses to oxytocin and vasopressin in preterm and term human pregnancy: Effects of the oxytocin antagonist Atosiban. *Am. J. Obstet. Gynecol.* 1994; **171**: 1634–1642.
63. Goodwin TM, Paul R, Silver H, Spellacy W, Parsons M, Chez R, Hayashi R, Valenzuela G, Creasy GW, Merriman R. The effect of the oxytocin antagonist Atosiban on preterm uterine activity in the human. *Am. J. Obstet. Gynecol.* 1994; **170**: 475–478.

64. Thornton S, Smith SK. The physiological basis for administration of oxytocin antagonists in preterm labour. *J. Royal Soc. Med.* 1995; **88**: 166P–170P.
65. Goodwin TM, Valenzuela GJ, Silver H, Creasy G. Dose ranging study of the oxytocin antagonist Atosiban in the treatment of preterm labor. *Obstet. Gynecol.* 1996; **88**: 331–336.
66. Goodwin TM, Valenzuela GJ, Silver H, Hayashi R, Creasy GV, Lane R. The treatment of preterm labor with the oxytocin antagonist Atosiban. *Am. J. Perinatol.* 1996; **13**: 143–146.
67. Romero R, Sibai BM, Sanches-Ramos L, Valenzuela GJ, Veille J-C, Tabor B, Perry KG, Varner M, Murphy G, Lane R, Smith J, Shangold G, Creasy GW. An oxytocin receptor antagonist (Atosiban) in the treatment of preterm labor: A randomised, double-blind, placebo-controlled trial with tocolytic rescue. *Am. J. Obstet. Gynecol.* 2000; **182**: 1173–1183.
68. Valenzuela GJ, Sanches-Ramos L, Romero R, Silver HM, Koltun WD, Millar L, Hobbins J, Rayburn W, Shangold G, Wang J, Smith J, Creasy GW. Maintenance treatment of preterm labor with the oxytocin antagonist Atosiban. *Am. J. Obstet. Gynecol.* 2000; **182**: 1184–1190.
69. Moutquin J-M, Sherman D, Cohen H, Mohide PT, Hochner-Celnikier D, Fejgin M, Liston RM, Dansereau J, Mazor M, Shalev E, Bouchner M, Glezerman M, Zimmer E, Rabinovici J. Double-blind, randomized, controlled trial of Atosiban and ritodrine in the treatment of preterm labor: A multicenter effectiveness and safety study. *Am. J. Obstet. Gynecol.* 2000; **182**: 1191–1199.
70. Grigsby PL, Poore KR, Hirst JJ, Jenkin G. Inhibition of premature labor in sheep by a combined treatment of nimesulide, a prostaglandin synthase type 2 inhibitor, and Atosiban, an oxytocin receptor antagonist. *Am. J. Obstet. Gynecol.* 2000; **183**: 649–657.
71. Pettibone DJ, Kishel MT, Woyden CJ, Clineschmidt BV, Bock MG, Freidinger RM, Veber DF, Williams PD. Radioligand binding studies reveal marked species differences in the vasopressin V1 receptor of rat, rhesus and human tissues. *Life Sci.* 1992; **50**: 1953–1958.
72. Howl J, Wheatley M. Molecular pharmacology of V_{1a} vasopressin receptors. *Gen. Pharmac.* 1995; **26**(6): 1143–1152.
73. Barberis C, Mouillac B, Durroux T. Structural bases of vasopressin/oxytocin receptor function. *J. Endocrinol.* 1998; **156**: 223–229.
74. Tahara A, Tsukada J, Tomura Y, Wada K-i, Kusayama T, Ishii N, Yatsu T, Uchida W, Tanaka A. Pharmacologic characterization of the oxytocin receptor in human uterine smooth muscle cells. *Br. J. Pharmacol.* 2000; **129**: 131–139.
75. Barberis C, Morin D, Durroux T, Mouillac B, Guillon G, Hibert M, Tribollet E, Manning M. Molecular pharmacology of vasopressin and oxytocin receptors and therapeutic potential. *Drug News Perspect.* 1999; **12**: 279–292.
76. Chan WY, Wo NC, Manning M. The role of oxytocin receptors and vasopressin V_{1a} receptors in uterine contractions in rats; Implication for tocolytic therapy with oxytocin antagonists. *Am. J. Obstet. Gynecol* 1996; **175**: 1331–1335.
77. Dunn FW, Stewart JM. Analogs of bradykinin containing β-2-thienyl-L-alanine. *J. Med. Chem.* 1971; **14**: 779–781.
78. Khosla MC, Hall MM, Smeby RR, Bumpus FM. Agonist and antagonist relationships in 1- and 8-substituted analogs of angiotensin II. *J. Med. Chem.* 1974; **17**: 1156–1160.
79. Smith CW, Ferger MF. Synthesis and some pharmacological properties of [3-β-(2-thienyl)-L-alanine]-8-lysine-vasopressin. *J. Med. Chem.* 1975; **18**: 822–825.
80. Stoev S, Cheng LL, Olma A, Klis WA, Manning M, Sawyer WH, Wo NC, Chan WY. An investigation of position three in arginine vasopressin with aliphatic, aromatic, conformationally restricted, polar and charged amino acids. *J. Peptide Sci.* 1999; **5**: 141–153.
81. Chan WY, Wo NC, Stoev S, Cheng LL, Manning M. Discovery and design of novel and selective vasopressin and oxytocin agonists and antagonists: The role of bioassys. *Exptl. Physiol.* 2000; **85S**: 7S–18S.
82. Manning M, Balaspiri L, Acosta M, Sawyer WH. Solid phase synthesis of [deamino-4-valine]-8-D-arginine vasopressin (dVDAVP). *J. Med. Chem.* 1973; **16**: 975–978.
83. Merrifield RB, Solid phase peptide synthesis. III. An improved synthesis of bradykinin. *Biochemistry* 1964; **3**: 1385–1390.
84. Stewart JM, Young JD. *Solid Phase Peptide Synthesis*. Pierce Chemical Company: Rockford, IL, 1984.
85. Koenig W, Geiger R. New method for the synthesis of peptides: Activation of the carboxyl group with dicyclohexylcarbodiimide by using 1-hydroxy-benzotriazole as additives. *Chem. Ber.* 1970; **103**: 788–798.
86. Manning M, Przybylski J, Grzonka Z, Nawrocka E, Lammek B, Miscka A, Cheng L-L, Chan WY, Wo NC, Sawyer WH. Vasopressin antagonists with C-terminal ethylenediamine-linked retro amino acids. *J. Med. Chem.* 1992; **35**: 3895–3904.
87. Bodanszky M, du Vigneaud V. A method of synthesis of long peptide chains using a synthesis of oxytocin as an example. *J. Am. Chem. Soc.* 1959; **81**: 5688–5691.
88. Walter R, Havran RT, Schwartz IL. Synthetic metabolites of neurohypophyseal hormones [des-9-glycinamide]oxytocin and [des-9-glycinamide, des-8-leucine]oxytocin. *J. Med. Chem.* 1976; **19**: 328–330.
89. Manning M, Miscka A, Olma A, Klis WA, Bankowsi K, Nawrocka E, Kruszynski M, Kolodziejczyk A, Cheng

L-L, Seto J, Wo NC, Sawyer WH. C-terminal deletions in agonistic and antagonistic analogs of vasopressin that improve their specificities for antidiuretic (V_2) and vasopressor (V_1) receptors. *J. Med. Chem.* 1987; **30**: 2245–2252.

90. Manning M. Synthesis by the Merrifield method of a protected nonapeptide amide with the amino acid sequence of oxytocin. *J. Am. Chem. Soc.* 1968; **90**: 1348–1349.
91. Bodanszky M, Sheehan JT. Active esters and resins in peptide synthesis. *Chem. Ind.* 1964: 1423–1424.
92. Glass JD, du Vigneaud V. Solid-phase synthesis and pressor potency of [1-deamino-9-ethylenediamine]-lysine-vasopressin. *J. Med. Chem.* 1973; **16**: 160–161.
93. du Vigneaud V, Gish DT, Katsoyannis PG. A synthetic preparation possessing biological properties associated with arginine-vasopressin. *J. Am. Chem. Soc.* 1954; **76**: 4751–4752.
94. du Vigneaud V, Ressler C, Swan JM, Roberts CW, Katsoyannis PG. The synthesis of oxytocin. *J. Am. Chem. Soc.* 1954; **76**: 3115–3121.
95. Yajima H, Fujii N, Ogawa H, Kawatani H. Trifluoromethanesulfonic acid as a deprotecting reagent in peptide chemistry. *J. Chem. Soc. Chem. Commun.* 1974: 107–108.
96. Yajima H, Fujii N. Acidolytic deprotecting procedures in peptide synthesis. In *The Peptides*, vol. 5, Gross E, Meienhofer J (eds). Academic Press: New York, 1983; 65–109.
97. Hope DV, Murti VVS, du Vigneaud V. A highly potent analogue of oxytocin, desamino-oxytocin. *J. Biol. Chem.* 1962; **237**: 1563–1566.
98. Rivier J, Kaiser R, Galyean R. Solid phase synthesis of somatostatin and glucagon-selective analogs in gram quantities. *Biopolymers* 1978; **17**: 1927–1938.
99. Manning M, Wuu TC, Baxter JWM. The purification of synthetic oxytocin and analogues by gel filtration on Sephadex G-15. *J. Chromatogr.* 1968; **38**: 396–398.
100. Holton P. A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Br. J. Pharmacol.* 1948; **3**: 328–334.
101. Schild HO. pA, a new scale of the measurement of drug antagonism. *Br. J. Pharmacol. Chemother.* 1958; **2**: 189–206.
102. Dyckes DF, Nestor JJ Jr, Ferger MF, du Vigneaud V. [1-β-mercapto-β,β-diethyl-propionic acid]-8-lysine-vasopressin, a potent inhibitor of 8-lysine-vasopresssin and of oxytocin. *J. Med. Chem.* 1974; **17**: 250–252.
103. Munsick RA. Effect of magnesium ion on the response of the rat uterus to neurohypophysical hormones and analogues. *Endocrinology* 1960; **66**: 451–457.
104. Chan WY, Kelly N. A pharmacologic analysis on the significance of the chemical fuctional groups of oxytocin to its oxytocic activity and on the effect of magnesium on the in vitro and in vivo oxytocic activity of neurohypophysial hormones. *Pharmacol. Expl. Thers.* 1967; **156**: 150–158.
105. Dekanski J. The quantitative assay of vasopressn. *Br. J. Pharmacol.* 1952; **7**: 567–572.
106. Sawyer WH. Biologic assays for oxytocin and vasopressin. *Methods Med. Res.* 1961; **9**: 210–219.
107. Gisin BF. The preparation of Merrifield-resins through total esterification with caesium salts. *Helv. Chim. Acta* 1973; **56**: 1476–1482.
108. Yim NCF, Huffman WF. A facile synthesis of β-(S-benzylmercapto)-β,β-cyclopentamethylene-propionic acid. *Int. J. Peptide Protein Res.* 1983; **21**: 568–570.
109. Cassoni P, Sapino A, Munaron L, Deaglio S, Chini B, Grazini A, Ahmed A, Bussolati G. Activation of functional oxytocin receptors stimulates cell proliferation in human trophoblast and choriocarcinoma cell lines. *Endocrinology* 2001; **142**: 1130–1136.

