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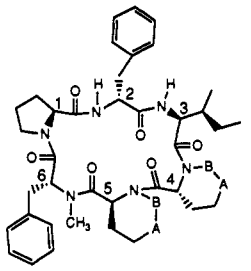
Communications to the Editor

Cyclic Hexapeptide Oxytocin Antagonists. Potency-, Selectivity-, and Solubility-Enhancing Modifications

Sir:

The neurohypophyseal hormone oxytocin (OT) plays an important role in parturition by contracting the uterine myometrium during labor and the mammary myoepithelium postpartum to elicit milk letdown.[1] The increased sensitivity of the human myometrium to oxytocin during uncomplicated preterm labor suggests that an oxytocin receptor antagonist could have potential utility as a selective agent for the prevention of premature birth.[2]

Historically, antagonists of oxytocin have been analogues of either oxytocin or the structurally related hormone arginine vasopressin (AVP).[3,4] Recently, a new structural class of cyclic hexapeptide oxytocin antagonists was isolated from *Streptomyces silvensis* and is typified by the chemically modified derivative L-365,209 (1).[5] While its



L-365,209 (1) A-B = CH=N

(2) A-B = CH_2-CH_2

potency and selectivity are attractive, the poor aqueous solubility of 1 (68 μg/mL) limits its utility, especially for intravenous (iv) administration. A principal goal of our efforts in this area has been to develop potent, selective antagonists based on this lead with aqueous solubility adequate for iv use. As reported separately, improved oxytocin receptor ligands can be obtained by direct chemical modification of L-365,209.[6] In this communication we report totally synthetic analogues with high potency, receptor selectivity, and substantially increased aqueous solubility.

Preparation of cyclic hexapeptides related to L-365,209 presents significant synthetic challenges due to the presence of four sequential secondary amino acids which include the unusual hydrazone-containing dehydropiperazic acids (Δ-Piz). The most difficult problems involve the preparation and incorporation of D- and L-Δ-Piz, and therefore their replacement with the commercially available D- and L-pipecolic acids (Pip) was an early target of our synthetic studies. The general synthetic route outlined in Scheme I was developed for synthesis of these analogues. The use of base-labile *N*-Fmoc protection was dictated by the acid lability of many of the intermediates.[7] Particular care is required in couplings to the dipeptide ester which demand sufficient activation of the acyl component to minimize diketopiperazine formation. Couplings at pipecolic acid are known to be particularly sluggish[8] and in this series required the use of acid chlorides as the acylating agent. In certain cases, the Fmoc-Ile coupling additionally required newly developed methodology involving catalysis by silver cyanide.[9] It has also proven possible to adapt the acid chloride approach to the solid-phase method. Analogues **2**, **3**, **5**, **6**, **12**, and **13** were prepared in solution by using either a linear or a fragment coupling approach, and the remaining peptides were prepared on

(1) Pritchard, J. A.; MacDonald, P. C.; Gant, N. F. *Williams Obstetrics*, 17th ed.; Appleton-Century-Crofts: Norwalk, 1985; p. 295.
(2) Fuchs, A.-R.; Fuchs, F.; Husslein, P.; Soloff, M. S.; Fernstrom, M. J. *Science* **1982**, *215*, 1396. Fuchs, A.-R.; Vangsted, A.; Ivanisevic, M.; Demarest, K. *Am. J. Perinatol.* **1989**, *6*, 205.
(3) Manning, M.; Sawyer, W. H. *J. Lab. Clin. Med.* **1989**, *114*, 617.
(4) (a) Melin, P.; Trojnar, J.; Johansson, B.; Vilhardt, H.; Akerlund, M. *J. Endocrinol.* **1986**, *111*, 125. (b) Chan, W. Y.; Rockway, T. W.; Hruby, V. J. *Proc. Soc. Exp. Biol. Med.* **1987**, *185*, 187.
(5) Pettibone, D. J.; Clineschmidt, B. V.; Anderson, P. S.; Freidinger, R. M.; Lundell, G. F.; Koupal, L. R.; Schwartz, C. D.; Williamson, J. M.; Goetz, M. A.; Hensens, O. D.; Liesch, J. M.; Springer, J. P. *Endocrinology* **1989**, *125*, 217.
(6) Bock, M. G.; DiPardo, R. M.; Williams, P. D.; Pettibone, D. J.; Clineschmidt, B. V.; Ball, R. G.; Veber, D. F.; Freidinger, R. M. *J. Med. Chem.*, in press.
(7) It has recently been shown that peptides having three or more *N*-alkyl amino acids in sequence can be sensitive to strong acid: Anteunis, M. J. O.; Van Der Auwera, C. *Int. J. Pept. Protein Res.* **1988**, *31*, 301.
(8) Nutt, R. F.; Holly, F. W.; Homnick, C.; Hirschmann, R.; Veber, D. F. *J. Med. Chem.* **1981**, *24*, 692.
(9) (a) Takimoto, S.; Inanaga, J.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2335. (b) Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350.

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Table I. Inhibition of Binding of [^{3}H]Oxytocin to Rat Uterine Receptors and [^{3}H]Arginine Vasopressin to Rat Liver (V_1) and Kidney Medulla (V_2) Receptors by Synthetic Cyclic Hexapeptides

					K_i,[a] nM			
compd	W	X	Y	Z	OT	V_1	V_2	solubility[b]
				Pro^1--W^2--Ile^3 / Z^6---Y^5--X^4				
1	D-Phe	D-Δ-Piz	L-Δ-Piz	*N*-Me-D-Phe	7.3 ± 0.58	730 ± 180	540 ± 30	0.068 (7.0)
2	D-Phe	D-Pip	L-Pip	*N*-Me-D-Phe	83 (2)	890 (1)	1600 (1)	0.002 (7.4)
3	D-Phe	D-Pip	L-Pip	D-Phe	140 ± 0.18	1600 (1)	4500	nd[c]
4	D-Phe	D-Pro	L-Pro	*N*-Me-D-Phe	1400 (1)	9300 (1)	33000 (1)	nd
5	D-Phe	D-Δ-Piz	L-Pip	*N*-Me-D-Phe	5.9 ± 1.9	970 (2)	480 (2)	nd
6	D-Phe	D-Δ-Piz	L-Orn	*N*-Me-D-Phe	17 ± 0.86	240 ± 44	550 ± 35	1.5 (7.2)
7	D-Trp	D-Pip	L-Pip	*N*-Me-D-Phe	8.1 ± 0.81	3500 ± 330	260 ± 22	nd
8	D-Trp	D-Pip	L-Orn	*N*-Me-D-Phe	49 (1)	1000 (1)	500 (1)	nd
9	D-Trp	D-Pip	L-Ppz	*N*-Me-D-Phe	4.2 ± 0.39	9500 ± 500	240 ± 19	0.84 (7.0) 1.4 (6.5)
10	D-Trp	D-Pip	L-Pip	D-His	7.8 ± 0.66	2200 ± 250	1800 ± 79	2.0 (5.0) 0.11 (10.8)
11	D-2-Nal	D-Pip	L-Pip	D-His	1.6 ± 0.10	760 ± 100	320 ± 25	1.5 (5.0) 0.08 (6.6)
12	D-Trp	D-Δ-Piz	L-Pip	D-His	5.3 ± 0.53	1200 ± 86	370 ± 16	1.9 (5.6) 0.34 (8.0)
13	D-OEt-Tyr	D-Pip	L-Pip	*N*-Me-D-Phe	49 (2)	20000 ± 910	250 ± 30	nd

				K_i,[a] nM			
compd	W	T	Z	OT	V_1	V_2	solubility
			Pro^1--W^2--T^3 / Z^6--Pip^5--Pip^4				
14	D-Ala	Ile	D-Phe	0% inhibn at 10000	0% inhibn at 10000	4% inhibn at 10000	nd
15	D-Phe	Ala	*N*-Me-D-Phe	12000 (1)	>30000	>30000	nd
16	D-Phe	Ile	*N*-Me-D-Ala	490 (1)	>10000	>10000	nd

[a] K_i values are group means ± SEM for three or more replicate determinations unless otherwise noted in parentheses. K_i values were calculated from the IC_{50} values[15] determined by competition curves as reported previously.[5] [b] Aqueous solubility in mg/mL at 25 °C at the pH given in parentheses. [c] Nd = not determined.

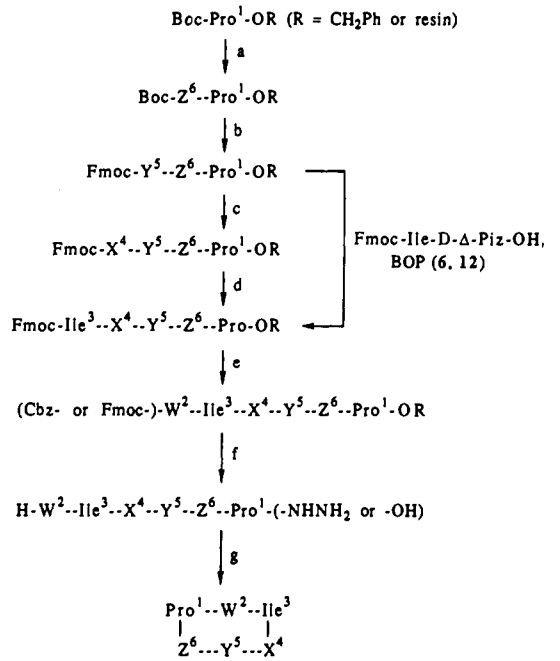
solid phase. In all cases, L-Pro^1 was the carboxy terminal amino acid of the linear hexapeptide intermediate, consistent with previous studies which predicted good cyclization at such a juncture.[10] Piperazic[11,12] and piperazine carboxylic acids[13] were synthesized and resolved according to literature procedures. The absolute configuration of the latter amino acid was established by chemical means which will be reported separately. Macrocyclizations were performed by either azide or DPPA procedures on the linear hexapeptides. Protecting groups were removed by using standard methods, and the purity of the final products was determined by HPLC. All compounds exhibited NMR, mass spectra, and elemental analyses consistent with the assigned structures.

Binding affinities using rat uterine membranes[5] are given in Table I. Replacement of both dehydropiperazic acids in **1** with pipecolic acids resulted in a 10-fold loss in oxytocin receptor affinity (**2**). Removing the methyl group from *N*-Me-D-Phe produced a further 2-fold loss (**3**). The D-Pro,^{4}L-Pro^5 double-substitution analogue **4** reduced potency by another order of magnitude and this suggests that the larger six-membered rings are important for conformational reasons. L-Pip^5 analogue **5** proved to be equipotent to **1** and, in agreement with the structure–activity profile generated by the semisynthetic analogues,[6] demonstrates the importance of D-Δ-Piz^4 for achieving good receptor binding affinity. The better potency could result from a specific hydrogen-bonding interaction of the imine nitrogen or the influence of unsaturation in the ring on the bioactive conformation. The 5-position shows considerable flexibility for substitution and, in fact, the analogue which incorporates the *acyclic* amino acid L-Orn at the 5-position (**6**) retains about 50% of the antagonist potency of **1**. With its significantly increased water solubility, **6** represents one solution to our objective.

It was desirable to pursue simplifications and potency-enhancing modifications in other parts of the cyclic peptide system. A variety of cyclic and acyclic amino acids in place of L-Pro^1 uniformly led to loss of potency. Alanine point-substitution analogues of **2** (**14**–**16**) were prepared in order to assess the relative importance of the side chains at positions 2, 3, and 6. These analogues indicate that the D-Phe^2 and Ile^3 side chains play an important role in achieving good levels of receptor binding affinity, whereas the *N*-Me-D-Phe^6 side chain is much less critical. Increasing the size of the 2-position aromatic group as in D-Trp^2 analogue **7** was found to produce a 10-fold increase in oxytocin receptor affinity, a gain which offsets the replacement of D-Δ-Piz.^{4} Combining this modification with L-Orn^5 for aqueous solubility reduced potency (**8**), while the *cyclic* amino acid L-piperazine carboxylic acid (Ppz) at the 5-position in combination with D-Trp^2 provided aqueous solubility and a 2-fold enhancement in potency (**9**). Replacing the less critical aromatic amino acid at the 6-position with D-His provided another means for obtaining aqueous solubility and increasing receptor binding affinity (**10**). Oxytocin receptor affinity was optimized with an even larger aromatic amino acid, D-2-naphthylalanine (D-2-Nal), at the 2-position in conjunction with D-His^6 for aqueous solubility (**11**). Combination analogue **12** has both

(10) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A. Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101.
(11) Hassall, C. H.; Johnson, W. H.; Theobold, C. J. *J. Chem. Soc. Perkin Trans. 1* 1979, 1451.
(12) Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. J.; Pessolano, A. A.; Caldwell, C. G., manuscript in preparation.
(13) Felder, E.; Maffei, S.; Pietra, S.; Pitre, D. *Helv. Chim. Acta* **1960**, *43*, 888.

Scheme I.[a] Synthesis of Cyclic Hexapeptides



[a](a) HCl or TFA; Boz-Z^6-OH, BOP;[b] (b) HCl; Fmoc-Y^5-Cl or Fmoc-(N^δ-Boc)Orn-OH, BOP (6, 8); (c) piperidine or Et_2NH; Fmoc-X^4-Cl; (d) piperidine or Et_2NH; Fmoc-Ile-Cl or Fmoc-Ile-Cl/AgCN/toluene/80 °C (3, 5, 13) or Fmoc-Ala-Cl (15); (e) piperidine or Et_2NH; Fmoc-W^2-OH, BOP or Fmoc-D-Phe^2-Cl (2) or Cbz-D-Phe^2-Cl (3) or Boc-D-(O-Et)TyrOH, BOP (13); (f) piperidine or Et_2NH; NH_2NH_2 or H_2, $Pd(OH)_2$ (3) or HCO_2H (13); (g) *i*-$C_5H_{11}ONO$ or DPPA[c] (3, 4, 13); compounds 6 and 8 were obtained by treatment with HCO_2H and TFA, respectively, to remove the N^δ-Boc group on Orn^5; compound 9 was obtained by hydrogenolysis (H_2, $Pd(OH)_2$) to remove the N^δ-Cbz group on Ppz^5; compound 5 was obtained by hydrogenolysis (H_2, $Pd(OH)_2$) to remove the N^δ-Cbz group on D-Piz^4, followed by oxidation to D-Δ-Piz^4 with *t*-BuOCl. Compound 4 was prepared on solid phase using Boc protection, TFA deblocking, and symmetrical-anhydride couplings. The linear hexapeptide was cleaved from the resin with HF and cyclized with DPPA. [b]BOP = [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate. [c]DPPA = diphenyl phosphoroazidate.

good potency and aqueous solubility but shows that the potency-enhancing modifications at the 2- and 4-positions are not strictly additive.

The importance of the D-Phe^2-Ile^3 dipeptide in the cyclic hexapeptide series suggests a possible structural homology with the identical dipeptide[14] or the D-(OEt)Tyr^2-Ile^3 dipeptide moiety[4] found in many oxytocin antagonists more closely related to the structure of the hormone. Consistent with this idea is the observation that D-(OEt)Tyr^2 analogue 13 possesses good binding affinity. This homology, however, has not offered fully predictive value for antagonist design. We continue to try to understand the true structural relationship between the two antagonist classes.

In summary, the structure–activity profile for cyclic hexapeptide analogues related to the natural product-derived lead 1 shows that high levels of oxytocin receptor affinity can be realized with certain amino acids at the 2- and 4-positions and that aqueous solubility can be increased substantially by introducing basic groups at the 5- and 6-positions. Several potent and selective oxytocin receptor ligands which have sufficient aqueous solubility for iv administration have been identified. All of the new high-potency analogues cited here have been characterized as functional oxytocin antagonists similar to L-365,209 (1) in the blockade of oxytocin-stimulated rat uterine contractions in vitro and in vivo.[5] Furthermore, these compounds behave as pure antagonists and have shown no oxytocin agonist activity in stimulating phosphatidylinositol turnover in vitro or rat uterine contractions in vitro or in vivo. These detailed studies will be reported separately. Such compounds may have utility as research tools and in certain therapeutic applications.

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(14) Manning, M.; Kruszynski, M.; Bankowski, K.; Olma, A.; Lammek, B.; Cheng, L. L.; Klis, W. A.; Seto, J.; Haldar, J.; Sawyer, W. H. *J. Med. Chem.* **1989**, *32*, 382.
(15) Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

Vinblastine and Vincristine Are Inhibitors of Monoamine Oxidase B

Sir:

Vinblastine (VBL) and vincristine (VCR) are widely used antitumor agents, and either VBL or VCR is an indispensable part of most curative and adjuvant chemotherapy regimens for metastatic malignancy.[1,2] The major mechanism of antitumor action attributed to these vinca alkaloids is cellular metaphase arrest, caused when the compounds disrupt cell microtubule assembly. VBL and VCR are structurally very similar, differing only in the state of oxidation of a single carbon atom attached to a nitrogen atom on the aspidosperma ring (Chart I). Despite this subtle structural difference, VBL and VCR exhibit different potencies, clinical applications, metabolic fates, and dose-limiting toxicities. Ample evidence indicates that the vinca alkaloids are extensively metabolized in mammals.[3-5] However, the possible role of drug metabolism in the mechanism(s) of action and/or dose limiting side-effects of the vinca alkaloids is unknown. Furthermore, the precise molecular basis for neurotoxicity[6] for this im-

(1) Gerzon, K. *Anticancer Agents Based on Natural Product Models, Dimeric Catharanthus Alkaloids*; Academic Press: New York, 1980; pp 271–317.
(2) Hellman, K.; Hutchinson, G.; Henry, K. *Cancer Chemother. Pharmacol.* **1987**, *20*, 21. Freireich, E. J.; Frei, E., III In *Progress in Hematology*; Moore, C. V., Brown, E. B., Eds.; Grune and Stratton: New York, 1964; pp 189–202.
(3) Houghton, J. A.; Torrance, P. M.; Houghton, P. J. *Anal. Biochem.* **1983**, *134*, 450.
(4) Beer, C. T.; Richards, J. F. *Lloydia* **1964**, *27*, 352.
(5) Owellen, R. J.; Harke, C. A.; Hains, F. O. *Cancer Res.* **1977**, *37*, 2597.
(6) *Chemistry and Pharmacology of Drugs, Volume 3*; *Antineoplastic Agents*; Remers, W. A., Lednicer, D., Eds.; John Wiley & Sons: New York, 1984; pp 210–212.

0022-2623/90/1833-1845$02.50/0