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Communications to the Editor

Dicarbavasopressin Antagonist Analogues Exhibit Reduced in Vivo Agonist Activity[1]

Sir:

Vasopressin V_2 receptor antagonists of the type first described by Manning et al.[2] are distinguished from agonists by, among other things, the requirement for a β,β-alkylidene-β-mercaptopropionic acid moiety at position 1.[3,4] This substitution introduces several features into the molecule, including increased steric bulk, increased lipophilicity, and conformational effects at the adjacent disulfide bond. These conformational effects have been postulated to be important in the generation of oxytocin antagonism in dPen[1] oxytocin analogues.[5] This is not entirely the case in vasopressin antagonists, since we have shown that the β,β-pentamethylene and and β,β-diethylmercaptopropionic acids produce potent antagonists, but the β,β-dimethyl (dPen) substitution produces a weak partial agonist.[6] Nevertheless, conformational effects on the adjacent disulfide bond may still be important to the production of a vasopressin antagonist response.

The disulfide bond is also important in determining the metabolic stability of vasopressin antagonists. The cyclic hexapeptide portion of the molecule is resistant to proteolytic clevage by enzymes such as chymotrypsin as long as the disulfide bond is intact. Once the disulfide bond is reduced, the resulting linear peptide becomes subject to proteolysis by endopeptidases such as papain (unpublished results).

In order to examine the role of the disulfide bond in vasopressin antagonists and in an attempt to prepare analogues with increased metabolic stability, we have prepared dicarba analogues, in which the disulfide bridge is replaced with two methylene groups. This structure preserves the 20-membered cyclic portion of the molecule but may alter the overall ring geometry.[7] Because the geometry of disulfide and dicarba bridges is different, the conformational effects of a β,β-disubstitution may be different as well. In addition, the ethylene bridge in the dicarba analogue would also be resistant to ring opening and thus should provide enhanced metabolic stability.

In order to prepare dicarbavasopressin antagonists, it was necessary to develop a synthesis for the unusual amino acid 2-amino-6,6-pentamethylenesuberic acid (Pas, **1**). The synthesis of optically pure Boc-L-Pas(OBzl) (**3**), suitable for solid-phase peptide synthesis, is shown in Figure 1.[8] Kolbe electrochemical coupling[9,10] of the monobenzyl ester of cyclohexanediacetic acid with Boc-L-glutamic acid α-benzyl ester gave Boc-L-Pas dibenzyl ester (**2**) in moderate yield. Selective hydrolysis of the less hindered α-benzyl ester gave the desired **3**. The linear peptide intermediates were prepared by solid-phase synthesis on benzhydrylamine resin followed by cleavage from the resin with anhydrous liquid HF. The linear peptides were converted to their hydrochloride salts and cyclized in good yield with diphenyl phosphorazidate and triethylamine.[11] Peptides were purified by preparative HPLC with a Hamilton PRP-1 polystyrene column, were homogeneous by HPLC and TLC, and had their structures confirmed by FAB mass spectrometry.

A comparison of antagonist potency of some dicarba analogues and their disulfide congeners is given in Table I. Each dicarba analogue is approximately equipotent with its corresponding disulfide congener in three different tail-modified analogues.[12,13] This indicates that, in spite of the slightly altered geometry of the ethylene bridge

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(7) The low-energy dihedral angle for a disulfide is approximately ±100° while the low-energy dihedral angles for an ethylene moiety are ±60° or 180°. The sulfur–sulfur bond length of 2.03 Å is also slightly longer than the carbon–carbon bond length of 1.54 Å. For a disulfide bond, the C–S–S–C distance can range from 2.85 to 4.55 Å as a function of the dihedral angle, with a distance of 3.93 Å at ±100°. The corresponding distance for an ethylene bridge ranges from 2.54 to 3.87 Å with a distance of 2.92 Å at ±60° and 3.87 Å at 180°.

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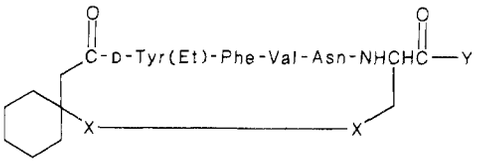
Table I. Antagonist Activity of Disulfide and Dicarba Analogues in Vitro



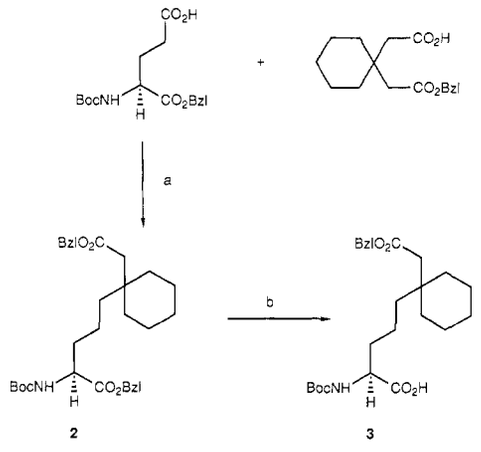
compd	X	Y	pig K_b,[a] nM	pig K_i,[b] nM	dog K_i,[b] mM	human K_i,[b] nM	rat ED_{300},[c] μg/mL
4	S	Pro-Arg-NH_2	11.8 (8)[d]	3.6 (7)	1.1 (4)	3.6 (17)	8.4 (17)
5	CH_2	Pro-Arg-NH_2	7.1 (2)	3.5 (3)	1.0 (4)	2.7 (6)	12.2 (3)
6	S	Arg-D-Arg-NH_2	5.4 (2)	1.6 (3)	0.5 (6)	2.4 (4)	13.5 (5)
7	CH_2	Arg-D-Arg-NH_2	5.1 (4)		0.4 (4)	3.9 (3)	9.0
8	S	Arg-NH_2	10.2 (2)	2.9 (2)		1.5 (3)	58.0 (3)
9	CH_2	Arg-NH_2	14.0			4.4 (3)	28.0

[a] Measured by inhibition of binding of [^{3}H]LVP to renal medullary membrane preparation. [b] Inhibition of vasopressin-stimulated adenylate cyclase in renal medullary preparation. [c] Dose required to lower urine osmolality to 300 mOsm/kg of H_2O. [d] Number in parentheses is number of determinations.

Table II. Agonist Activity of Disulfide and Dicarba Analogues

compd	X	Y	dog K_i, nM	dog U osm[a] (mOsm/kg)	dog vol[b] (mL/min)
4	S	Pro-Arg-NH_2	1.1 (4)[c]	1302 ± 162[d] (7)	0.07 ± 0.01 (7)
5	CH_2	Pro-Arg-NH_2	1.0 (4)	319 ± 75 (3)	0.69 ± 0.30 (3)
6	S	Arg-D-Arg-NH_2	0.5 (6)	429 ± 225 (7)	0.76 ± 0.23 (7)
7	CH_2	Arg-D-Arg-NH_2	0.4 (4)	56 ± 4 (4)	4.6 ± 0.6 (4)
control[e]				92 ± 12	2.8 ± 0.5
AVP (3 ng/kg)				939 ± 76 (3)	1.4 ± 0.3

[a] Maximum urine osmolality with a dose of 100 μg/kg peptide after pretreatment with indomethacin. [b] Maximum urine flow with a dose of 100 μg/kg peptide. [c] Number in parentheses is number of determinations. [d] Mean ± SEM. [e] Maximum effect of indomethacin treatment on urine osmolality and flow.



(a) e⁻, Na, pyridine, MeOH, 20-25 °C, 9 h, 18%; (b) 1N NaOH (1 equiv), dioxane; 1 N HCl.

Figure 1.

compared to the disulfide bridge, the 20-membered ring can present an equivalent antagonist pharmacophore.[14] A similar retention of potency has been observed in dicarba vasopressin agonist analogues[15] and oxytocin agonist analogues.[16]

The disulfide antagonist **4** has been shown to exhibit unexpected agonist activity in phase I clinical trials.[17] A model for examination of agonist activity in putative vasopressin antagonists has been developed.[18] Conscious, trained, water-loaded dogs are pretreated with indomethacin for 20 min prior to bolus administration of the antagonist. As can be seen in Table II, the disulfide antagonist **4** exhibits full agonist activity compared to AVP, although at a considerably higher dose. Unexpectedly, the dicarba congener **5** exhibits much reduced agonist activity. This same trend is seen in a second set of tail-modified analogues, **6** and **7**, where the dicarba analogue **7** does not exhibit any agonist activity at a dose of 100 μg/kg.

It is not clear at present whether the agonist effects seen in the clinical trials of **4** and in the water-loaded, indomethacin-treated dog protocols represent intrinsic agonist activity at the renal V_2 receptor or represent other vasopressin-related actions such as vascular V_1 receptor antagonism, changes in prostaglandin tone,[19] or some as yet uncharacterized activity. What is clear is that dicarba-vasopressin antagonists can be distinguished from their disulfide congeners by a reduced agonist activity with retention of full in vitro and in vivo antagonist potency. This

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more favorable pharmacological profile coupled with the expected increase in metabolic stability make dicarbavasopressin antagonists more viable potential therapeutic candidates than their disulfide congeners.

Registry No. 2, 113084-42-7; 3, 113084-43-8; 4, 90332-82-4; 5, 114359-16-9; 6, 110500-78-2; 7, 114923-99-8; 8, 94497-37-7; 9, 114820-55-2; AVP, 113-79-1; LVP, 50-57-7; benzyl cyclohexanediacetic acid, 113009-25-9; BOC-L-glutamic acid α-benzyl ester, 30924-93-7; adenylate cyclase, 9012-42-4.

[†]Department of Pharmacology.
[‡]Department of Molecular Pharmacology.

Michael L. Moore,* Christine Albrightson[†] Bridget Brickson,[†] Heidemarie G. Bryan Nancy Caldwell,[†] James F. Callahan, Jonathan Foster Lewis B. Kinter,[†] Kenneth A. Newlander Dulcie B. Schmidt,[‡] Edmund Sorenson Frans L. Stassen,[†] Nelson C. F. Yim William F. Huffman

Departments of Peptide Chemistry, Pharmacology, and Molecular Pharmacology Smith Kline & French Laboratories King of Prussia, Pennsylvania 19406

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Articles

Dimeric 1,4-Dihydropyridines as Calcium Channel Antagonists

Alan F. Joslyn,[†] Elizabeth Luchowski, and David J. Triggle*

Department of Biochemical Pharmacology, School of Pharmacy, State University of New York, Buffalo, New York 14260.
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A series of 1,*n*-alkanediylbis(1,4-dihydropyridines) (*n* = 2, 4, 6, 8, 10, 12) bridged at C_3 of 2,6-dimethyl-3-carboxy-5-carbethoxy-4-(3-nitrophenyl)-1,4-dihydropyridine were synthesized and evaluated in a radioligand binding assay, [^{3}H]nitrendipine in intestinal smooth muscle, as Ca^{2+} channel ligands. Binding activity was comparable to that of nitrendipine itself but independent of chain length, suggesting the lack of a major binding contribution by the second 1,4-dihydropyridine group. Analogues lacking the second 1,4-dihydropyridine nucleus or possessing an inactive function (4-nitrophenyl) were no less active, confirming that this series of ligands likely does not bridge adjacent 1,4-dihydropyridine receptors of the Ca^{2+} channel.

The Ca^{2+} channel antagonist nifedipine has proved to be of value in a number of cardiovascular diseases, including angina and hypertension.[1,2] Nifedipine and other 1,4-dihydropyridines, both antagonist and activator, have proven to be valuable molecular probes for the delineation of the structural requirements of the 1,4-dihydropyridine receptor component of the Ca^{2+} channel.[3-5] Additionally, these agents have proved of value in protocols designed to isolate and reconstitute the 1,4-dihydropyridine-sensitive Ca^{2+} channel.[6,7] However, much remains to be learned of the relationship between the 1,4-dihydropyridine binding site and the functional machinery of the Ca^{2+} channel,[5,8] and nothing is known of the topographic relationship between 1,4-dihydropyridine binding sites. This relationship assumes increased importance because of recent reports that activator and antagonist 1,4-dihydropyridines may occupy discrete binding sites.[9,10]

Polyvalent ligands have been of value in probing the interbinding site distances at pharmacological receptors. Early examples include the bis(onium) neuromuscular- and ganglion-blocking agents.[11] More recently, dimeric enkephalins have been used to probe the different distributions of μ- and δ-opiate receptors,[12] and dimeric analogues of gonadotropin releasing hormone have been used to study the effects of receptor microaggregation on ligand-induced activity.[13] Successful bridging of two adjacent receptors by a divalent ligand may enhance affinity by a minimum of twofold and a maximum corresponding to the square of the affinity constant of the appropriate monovalent ligand. The lowest enhancement of activity may be difficult to detect.

As an initial effort to probe the distribution of 1,4-dihydropyridine binding sites associated with voltage-dependent Ca^{2+} channels, we have synthesized and evaluated a series of 1,*n*-alkanediylbis(1,4-dihydropyridines) in which two 1,4-dihydropyridine molecules are linked through C_3 ester substituents.

Chemistry

Ethyl acetoacetate and a substituted benzaldehyde were

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