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Inhibition of Bovine Brain Calmodulin-Dependent cGMP Phosphodiesterase By Peptide and Non-Peptide Angiotensin Receptor Ligands

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Inhibition of a purified 60 KDa bovine brain calmodulin-dependent cGMP phosphodiesterase (PDE) was investigated for a number of peptides and non-peptides which are known to bind to angiotensin (ANG) receptors. The peptide antagonists sarilesin and sarmesin had K_I = 120 and > 200 μM respectively, and the peptide agonists ANG II and ANG III had K_I = > 200 and 45 μM respectively. Non-peptide ANG receptor antagonists related to DuP 753 exhibited K_I values in the same range. For both peptide and non-peptide antagonists, inhibitory activities in the PDE assay reflected the order of antagonist potencies at ANG receptors in the rat isolated uterus assay and binding affinities at ANG receptors in rat uterine membranes, suggesting that molecular recognition factors are similar for both ANG receptors and cGMP PDE. The vasodilatory and blood pressure lowering effects of compounds related to DuP 753 may be due in part to inhibition of cGMP PDE. The differential effects of ANG II and ANG III at target tissues may relate in part to the marked differences in cGMP PDE inhibition associated with these two peptides hormones.

Sarilesin ([Sar^{1}Ile^8]ANG II) and Sarmesin ([Sar^{1}Tyr(Me)^4]ANG II) are analogues of ANG II which act as antagonists at ANG receptors in a variety of tissues (1). Recently a group of non-peptidic compounds, which are substituted imidazoles (Fig. 1), have been found to act as antagonists at ANG receptors and to have blood pressure lowering activity in rats (2); certain compounds of this class have also been shown to inhibit cGMP PDE from bovine renal artery (3). We thought it of interest to investigate the activities of both peptide and non-peptide ANG receptor ligands in our cGMP PDE inhibition assay, in order to investigate the possibility that the vasodilatory and blood pressure lowering effects of some of these ligands might be due in part to an increase in the intracellular levels of cGMP resulting from PDE inhibition.

Materials and Methods

Peptides use in this study were either purchased from Peninsula or were synthesized in this laboratory by methods described previously (4). Non-peptide ligands were synthesized essentially according to published procedures (2) and

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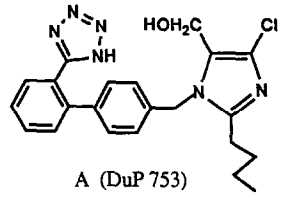
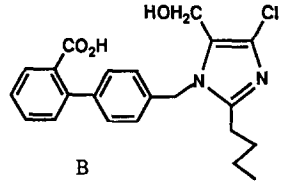
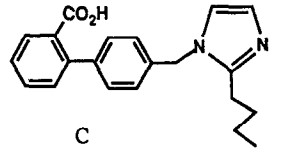


A (DuP 753)



B



C

FIGURE 1.

integrity and purity were established by NMR spectroscopy and HPLC (4). Rat isolated uterus bioassays were conducted as described previously (4). Displacement of ^{125}I-ANG II from rat uterine membranes was carried out according to described methods (5). Bovine brain calmodulin-dependent 60 KDa cyclic nucleotide phosphodiesterase isozyme was prepared as described by Sharma et al. (6); this enzyme has 10-fold higher affinity for cGMP than for cAMP. Assay of phosphodiesterase activity was measured as previously described (7).

Results

Figure 2 shows the concentration dependence for inhibition of PDE activity for a number of peptide and non-peptide ligands. Values for K_I derived from Figure 2 are shown in Table 1, together with the activities of these ligands in rat isolated uterus assays and in rat uterine membrane binding assays. In Table 1, the ligands have been divided into groups dependent on their molecular properties. Non-peptide ligands represent a class which can diffuse the cytoplasmic membrane. Peptide ligands on the other hand require a receptor-based internalization mechanism in order to gain access to intracellular compartments, which may only be applicable to agonists. The data in Table 1 show that non-peptide antagonists have the same order of potency at angiotensin receptors in rat uterus bioassays and binding assays as is observed for PDE inhibition. Similarly, the two peptide antagonists investigated in this study showed the same order of potency in all three assays. The agonist peptides ANG

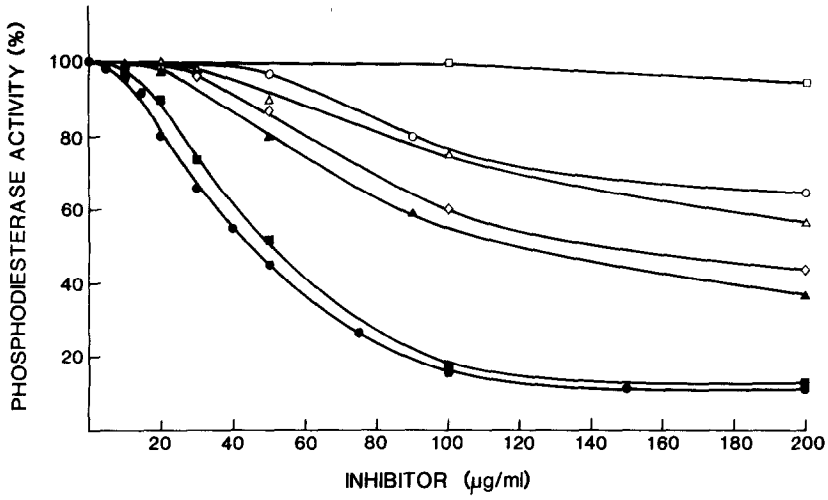


FIGURE 2. Inhibition of phosphodiestease activity by peptide and non-peptide ligands.
● Compound A, ◇ Compound B, □ Compound C, ▲ Sarilesin, ○ Sarmesin, △ ANG II, ■ ANG III

II and ANG III, on the other hand, had opposite effects in the PDE assay compared to the two uterus assays.

Discussion

The present findings illustrate that ANG receptor antagonists may elicit their effects in vitro and in vivo by a mechanism which involves cGMP PDE inhibition in addition to displacement of ANG II from its receptors. However, it should be noted that ANG receptor ligands have much higher affinity for ANG receptors than for PDE (Table 1). This difference in affinity of several orders of magnitude may become less significant if receptor-transduction compartmentalization considerations are taken into account, particularly if the ligands are internalized. Thus a ligand-receptor complex which is internalized by the cell may present an artificially high concentration of sequestered ligand if the complex is thereafter directly coupled to PDE. Non-peptide ligands will presumably be able to traverse the cell membrane by passive diffusion and influence intracellular events without the need for a receptor internalization mechanism.

The order of potency for the non-peptide antagonists is the same in cGMP PDE and ANG receptor assays. The absence of activity for compound C in the PDE assay emphasizes the importance of the hydroxymethyl and/or chloro substituents (Fig. 1) on the imidazole ring for PDE inhibition. The order of potency of the peptide antagonists sarilesin and sarmesin also suggests common recognition

Table 1. Activities of peptide and non-peptide ligands in rat isolated uterus, rat uterine membrane and brain cGMP phosphodiesterase assays

Ligand[1]	Bioassay[2] (pA_2)	Binding Assay[3] (ID_{50}, nM)	cGMP phosphodiesterase[4] (K_I, μM)
A	7.9	20	100
B	6.5	100	150
C	5.6	>200	inactive
Sarilesin	8.5	1	120
Sarmesin	7.5	30	>200
ANG II	Agonist (100%)	1	>200
ANG III	Agonist (10%)	6	45

[1] See Fig. 1 and text for structural details.

[2] Rat isolated uterus contraction assay; the pA_2 is the negative logarithm of the dose of antagonist required to reduce the response to an ED50 dose of ANG II to that of an ED50/2 dose of ANG II; in the absence of factors relating to uptake and metabolism, $pA_2 = -\log K_D$.

[3] Displacement of specifically bound ^{125}I-ANG II from rat uterine membranes; ID_{50} = concentration of ligand causing 50% displacement of radioligand.

[4] See Materials and Methods section and Fig. 2 for details.

features for PDE and ANG receptor binding. The finding that the affinities of peptide and non-peptide antagonists for ANG receptors and PDE correlate within each group suggests that molecular recognition properties operate similarly at both ANG and PDE. This unexpected finding appears to establish a relationship between a receptor and a signal transduction element which may equate the receptor-transduction process to a level previously unrecognized. Possibly the binding sites of the ANG receptor and PDE are related, perhaps as the result of evolutionary divergence. The PDE assay used in these studies included imidazole as an enzyme activator (7). The level of inhibition was not affected when imidazole was removed from the assay buffer illustrating that activator and inhibitor binding sites of brain PDE operate independently of one another.

Another interesting finding deriving from the present study was the observation that the most potent inhibitor of PDE observed herein was the agonist ANG III. It would seem, therefore, that ANG III could have contradictory actions by causing smooth muscle contraction due to its action at ANG receptors, on the one hand, and smooth muscle relaxation due to elevation of cellular cGMP levels by inhibiting PDE after internalization, on the other hand. This might explain why ANG III has 17% of the binding affinity of ANG II at rat uterine receptors but has only 10% of the potency of ANG II in the rat uterus bioassay (Table 1).

This discrepancy between affinity and potency may also relate to previous work which has suggested that ANG III could inhibit ANG II effects in certain vascular smooth muscle tissues (8). The ANG III analogue [Sar^1 Ile^7]ANG III, which is a more potent inhibitor of ANG III than ANG II (9), does not inhibit our brain PDE (data not shown). This is consistent with [Sar^1 Ile^7]ANG III having opposing actions to ANG III at receptors [as well as on PDE]; it also suggests that the Arg residue of angiotensin peptides is an important element of PDE inhibitory action. Finally, it has been suggested that ANG III, rather than ANG II, might be the primary mediator of ANG effects in brain (10, 11). In this regard, it may be relevant that the brain cGMP PDE used in these studies is inhibited more readily by ANG III than ANG II.

Acknowledgments

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