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CHOLECYSTOKININ ANALOGUES: THE ERGOPEPTINE ALKALOIDS AS MODELS OF THE ACTIVE CONFORMATION OF CCK

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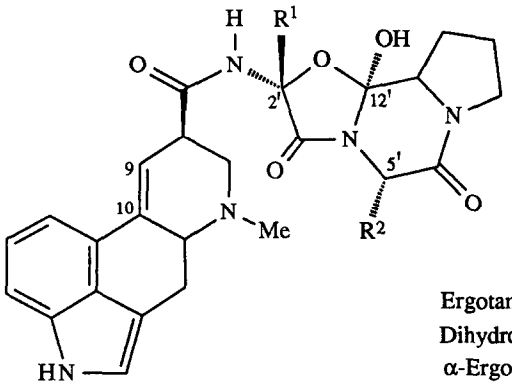
Abstract: Ergotamine was shown to inhibit the binding of radiolabelled CCK to both CCK-A and CCK-B receptors with a relatively modest potency (IC_{50} = 30 μM and 17 μM respectively).

The hormone Cholecystokinin (CCK) is currently the object of much interest. It plays an important role in the control of pancreatic and biliary secretion, and of intestinal motility. It is also widely distributed in the CNS, and CCK antagonists have been shown to be anxiolytic in several animal models.[1] A major advance in the development of non-peptide CCK ligands was the discovery of mould metabolite asperlicin, and its affinity for the CCK receptor.[2] This lead gave rise to a number of highly potent CCK antagonists based on the benzodiazepine skeleton[3] and, more recently, to a series of quinazolinone-based compounds.[4] A mould metabolite which has received far less attention in this context is ergotamine. It has been suggested that ergotamine mimics the active conformation of CCK.[5] This hypothesis was based on pharmacological similarities and the identification of some structural elements common to both compounds. No binding data was available at that time[6]. We felt that it was important to demonstrate a specific interaction between ergotamine and the CCK receptor as a first step in the exploitation of this potential lead, and we report here the results of this study.



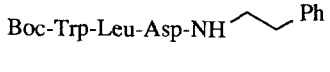
Ergotamine: R^1 = Me, R^2 = CH_2Ph
Dihydroergotamine: 9,10 reduced compound
α-Ergocryptine: R^1 = $CHMe_2$, R^2 = CH_2CHMe_2

Experimental Details

Ergotamine, dihydroergotamine and α-ergocryptine were purchased from Sigma (Poole, U.K.). The compounds 1 and 2 were synthesised by conventional solution techniques. Radioligand displacement experiments were carried out by Novascreen^R (Baltimore, Md) using mouse pancreatic membranes (CCK-A receptor) and mouse forebrain membranes (CCK-B receptor) according to a published method.[7] The results are summarised in the Table.

Compound	IC_{50} (μM)[a]	
	CCK-A	CCK-B
Ergotamine	34 ± 13	17 ± 1.5
Dihydroergotamine	>80	23 ± 5
α-Ergocryptine	>80	>80
1	3.5 ± 0.02	0.35 ± 0.02
2	12 ± 2	>80

(a) Concentration of compound required to inhibit binding of [^{125}I]-BH-CCK-8 by 50%. Mean ± S.E.M. of two determinations.



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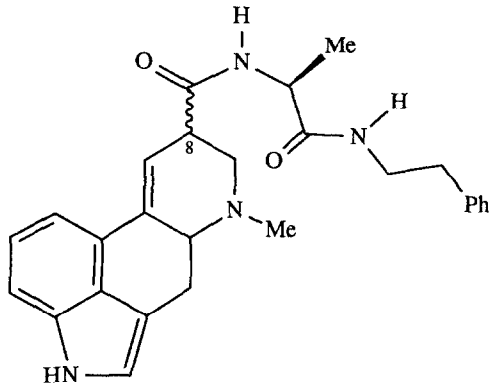
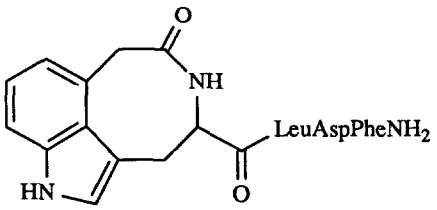


2 (60:40 mixture of epimers at C-8)

The results presented above demonstrate that ergotamine does have an affinity for both subtypes of CCK receptor. However, this affinity is 1 - 2 orders of magnitude lower than the tetrapeptide analogue 1[8] which is itself only a weak CCK antagonist,[9] and which is included in this study as a reference compound. Although these results suggest that ergotamine exerts its pharmacological effects at a site other than the CCK receptor they do not rule out the use of ergotamine as a model for CCK and as a potential lead into more potent compounds.

One or two points of interest arise from the results obtained with the other compounds studied. Reduction of the 9-10 double bond gives dehydroergotamine which retains its affinity for the CCK-B receptor, but loses affinity for the CCK-A receptor. The more radical structural change of α-ergocryptine gives a compound which is inactive. This might be due to the increased bulk at C-2', or the loss of the aromatic substituent at C-5' which in Hughes' and Andrews' model occupies the phenylalanine binding pocket.[5] Compound 2 retains the conformational restrictions of the lysergic acid-derived part of the alkaloid and not those of the peptide-derived part. The potential for receptor interactions with the hydroxyl group at C-12' (potentially an aspartic carboxyl mimic[5]) is also lost. The improved affinity for the CCK-A receptor indicates that this increased conformational freedom is beneficial, and hence that the restrictions imposed in ergotamine are sub-optimal. Conversely, the loss of affinity for the CCK-B receptor suggests that ergotamine might be a reasonable model for the active conformation of CCK at this receptor. Interestingly, a group from Abbott Laboratories have recently disclosed a series of CCK-B agonists (e.g. 3) in which an analogy to 2 can clearly be seen.[10]



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In conclusion, we have shown that ergotamine has a modest affinity for both subtypes of CCK receptor. Although this affinity might not be enough to explain the pharmacological actions of ergotamine there are indications that ergotamine can mimic the biologically important conformation of CCK.

Acknowledgement

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