Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 24, pp. 3007-3010, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00527-7

CYCLOPROCTOLIN AND [α-METHYL-L-TYR]-PROCTOLIN ARE POTENT ANTAGONISTS OF PROCTOLIN-INDUCED INOSITOL PHOSPHATE PRODUCTION IN LOCUST FOREGUT HOMOGENATES

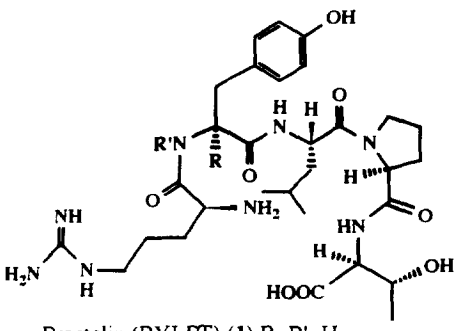
Jane M. Hinton[1], Richard H. Osborne[1] *, Barbara Odell[2], Stephen J. Hammond[2] and Ian S. Blagbrough[3]

[1]Department of Biology, University of the West of England, Frenchay Campus, Bristol BS16 1QY, [2]Shell Research Ltd, Sittingbourne ME9 8AG, [3]Department of Medicinal Chemistry, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

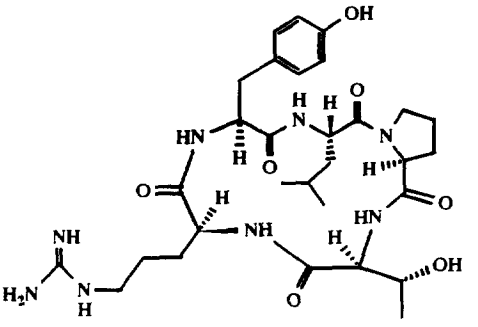
Abstract: Proctolin, an insect pentapeptide, causes dose-dependent production of inositol trisphosphate and inositol tetrakisphosphate from locust foregut homogenates which was antagonised by nM cycloproctolin and [α-Me-L-Tyr]-proctolin, but not by [*N*-Me-Tyr]-proctolin. We propose that cycloproctolin exists in a conformation which resembles a β-bulge loop and contains a *cis*-Pro.

The interactions of peptides and other small molecules with invertebrate neurotransmitter receptors are being studied, in detail, for the selective design of insecticides[1-3]. We are investigating the ligand-receptor interactions of the pentapeptide proctolin (H.Arg.Tyr.Leu.Pro.Thr.OH, RYLPT) (**1**) which is distributed widely in insect nerves[4,5]. Our on-going research into the structure-activity relationships (SAR) of proctolin receptors is focused upon the affinity and specificity of designed receptor-ligands as a function of modifications to the peptide backbone. The modified peptides of Konopinska and co-workers[6-8] and our own studies[9-11] demonstrate that proctolin (**1**) is a lead compound for the design of modified peptides and peptoids which may have metabolic stability. In this *Letter*, we report the results of our experiments to characterise pharmacologically and spectroscopically cycloproctolin (**2**) and [α-Me-Tyr]-proctolin (**3**) with biochemical techniques using tissue homogenates isolated from desert locust (*Schistocerca gregaria*) foregut. As a result of ^{1}H NMR solution spectroscopic analysis, we propose that proctolin receptor antagonists are active in a cyclic conformation which differs from that adopted by proctolin (**1**) when acting as an agonist at proctolin receptors.

Cyclic peptides/peptoids have important roles to play as ligands which help to define bioactive conformations at receptors because of their significantly restricted motion and the consequent ease of modelling their accessible conformations. Such peptoids have been designed recently and synthesized as specific ligands for enkephalin receptors[12,13] and for PI3-kinase p85 SH2 domains[14]. Merck Sharpe and Dohme have reported bicyclic hexapeptide analogues of somatostatin[15] and macrocyclic renin inhibitors[16]. Constrained cyclic peptides for dynorphin-A[17] and α-MSH (α-melanotropin)[18] receptors have recently been published and this approach has been reviewed comprehensively by Dean[19]



Proctolin (RYLPT) (**1**) R=R'=H
[α-Me-L-Tyr]-Proctolin (**3**) R=Me; R'=H
N-Me-Tyr-Proctolin (**4**) R=H; R'=Me



Cycloproctolin cRYLPT (**2**)

Following from ^{1}H NMR solution nOe studies, we propose that proctolin (**1**) possesses an inverse-γ-turn comprising an intramolecular hydrogen-bond near to the C-terminal, between LeuCO and NHThr. This γ-turn conformation leaves both the terminii accessible to solvent. In order to constrain further the geometry at this part of the molecule, a covalent bond was made between the *N*- and *C*-terminii resulting in cycloproctolin (cRYLPT) (**2**). The importance for binding/activity of the hydrogen atoms on the Tyr residue was also investigated using α-Me-L-Tyr- (**3**) and *N*-Me-L-Tyr-proctolin (**4**).

Five foreguts were isolated from locusts, placed in 10 mM ice-cold Tris buffer (3 ml, pH 7.0), containing 1 mM dithiothreitol (DTT) and 1 mM EDTA, and homogenised at 1000 rpm for 5 min. After addition of a further aliquot of Tris buffer (7 ml), the homogenate was centrifuged at 20000 g for 20 min. The supernatant was discarded, the pellet resuspended in 10 mM Tris-1 mM DTT buffer (10 ml, pH 7.0), recentrifuged as above and the resulting pellet resuspended in 100 mM Tris buffer (3 ml, pH 7.6, containing 12 mM $Mg(CH_3COO)_2$, 0.2 mM ATP, and 0.001 mM GTP). Aliquots (15 µl) were removed for protein determination by the Coomassie Blue method[20]. [^{3}H]-*myo*-Inositol (20 µCi/3 ml, Amersham, UK) was used to label this isolated foregut preparation as described by Baines and co-workers[21]. Tissue homogenates were incubated with proctolin (10 nM and 0.5 µM) for 2 min and the reaction was terminated by the addition of methanol-chloroform-5 M aq. HCl (200:100:1). For antagonist studies, homogenates were preincubated individually, at either 10 nM or 1 µM, with cycloproctolin (**2**), [α-Me-Tyr]- (**3**), and [*N*-Me-Tyr]-proctolin (**4**) for 20 min prior to retesting the effects of 0.5 µM proctolin (**1**). Tris buffer (100 mM, pH 7.6) was used as a control in lieu of proctolin. In all cases, the final extract was neutralized to pH 7.0 (with aq. NaOH), applied to an anion exhange column (DOWEX-1, X8, formate form, mesh 100-200, Bio-Rad) to separate water soluble inositol phosphates[22] and [^{3}H]-inositol phosphates eluted by the sequential addition of ammonium formate-formic acid mixtures of increasing ionic strength[23]. Anion exchange chromatography yielded peaks corresponding to the known elution profiles[23] of inositol 1,4,5-trisphosphate ($InsP_3$) and inositol 1,3,4,5-tetrakisphosphate ($InsP_4$). Radioactivity was quantified by liquid scintillation counting and the results, which have been normalised by subtracting the Tris buffer treated controls, are expressed as d.p.m. mg^{-1} protein. Data are expressed as the mean ± s.e. mean of 6 - 8 experiments. The data presented (Figure 1) are paired and pair differences were analysed by Student's "t" test.

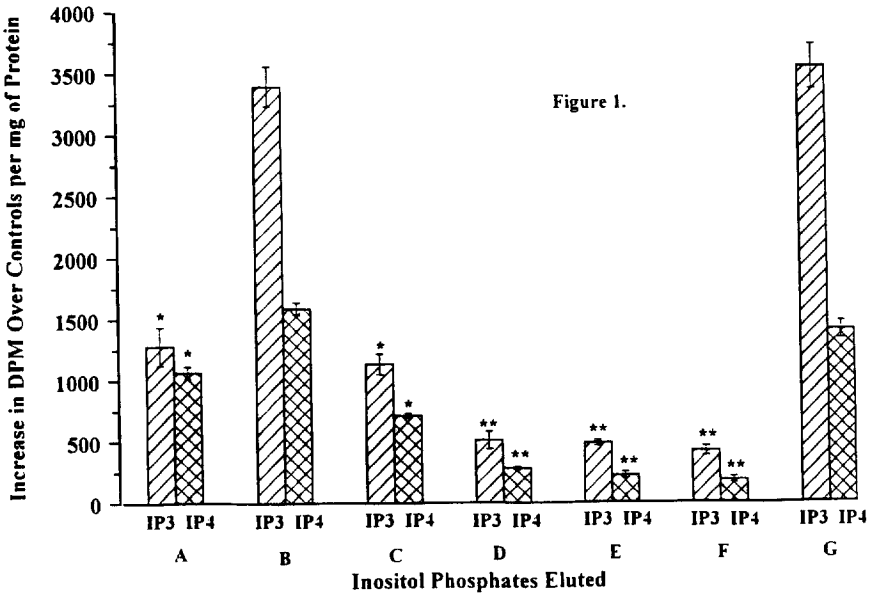


Figure 1.

Proctolin (**1**) caused dose-dependent release of $InsP_3$ and $InsP_4$ at 10 nM (A) and 0.5 μM (B), which was reduced significantly ($P<0.0005$, **) in the presence of cycloproctolin (**2**) (10 nM, C; 1 μM, D) and [α-Me-Tyr]-proctolin (**3**) (10 nM, E; 1 μM, F). [*N*-Me-Tyr]-Proctolin (**4**) was without antagonist action and co-incubation of (**1**) (0.5 μM) with (**4**) (1 μM) gave results (G) statistically indistinguishable from (B).

The solution conformations of cycloproctolin (**2**) and [α-methyl-Tyr]-proctolin (**3**) were investigated using variable temperature ^{1}H NMR and nOe experiments, in d^6-DMSO. We propose that each of the two peptides shows a preference for a conformation different from the inverse γ-turn found for proctolin (**1**)[11], in the same solvent, even though there are some significant similarities between them. The Leu CHα..Pro CHα nOe is indicative of a *cis*-proline geometry and there is an intramolecular hydrogen bond Tyr NH..Thr CO. Overall, these data are consistent with a β-bulge loop for cycloproctolin (**2**). [*N*-Me-Tyr]-Proctolin (**4**), neither agonist[11] nor antagonist, displayed similar strong nOes to (**1**), together with temperature coefficient data which support the presence of a salt bridge between the terminii. As *N*-methylation of [Tyr] (**4**) caused abolition of the biochemical and myogenic[11] activities displayed by the parent pentapeptide (**1**), the presence of a hydrogen atom on this amide bond may therefore be significant in eliciting the affinity of the ligand at this receptor(s).

In this *Letter*, we have shown that cycloproctolin (**2**) and [α-Me-Tyr]-proctolin (**3**) are potent antagonists of proctolin-induced production of the second messenger molecules $InsP_3$ and $InsP_4$. These studies provide further evidence[4-11] that proctolin receptors are targets for insecticide design as proctolin (**1**) is a powerful stimulant of insect muscle as well as occurring in the insect CNS. Furthermore, proctolin shows no significant effects in vertebrates and, consequently, proctolin analogues may disrupt insect physiology and biochemistry without causing toxic effects in mammals; such studies are in progress in our laboratories.

Acknowledgements: RHO acknowledges financial support from Shell Research Ltd and a bursary from the Department of Biology, UWE (to JMH). ISB is the recipient of a Nuffield Foundation Science Lecturer's award.

References

1. Pickett J.A. *Chemistry in Britain* **1988**, *24*, 137-141.
2. Blagbrough, I.S.; Brackley, P.T.H.; Bruce, M.; Bycroft, B.W.; Mather, A.J.; Millington, S.; Sudan, H.L.; Usherwood, P.N.R. *Toxicon* **1992**, *30*, 303-322.
3. Copping L.G. CRAC Insect Control, RSC, **1995**, in press.
4. Brown, B.E. *Life Sci.* **1977**, *23*, 861-864.
5. Osborne, R.H. *Pharmacol. Therap.* **1995**, in press.
6. Konopinska, D.; Sobotka, W.; Lesicki, A.; Rosinski, G.; Sujak, P. *Int. J. Peptide Protein Res.* **1986**, *27*, 597-603.
7. Konopinska, D.; Rosinski, G.; Lesicki, A.; Sujak, P.; Sobotka, W.; Bartosz-Bechowski, H. *Int. J. Peptide Protein Res.* **1988**, *31*, 463-467.
8. Konopinska, D.; Rosinski, G.; Sobotka, W. *Int. J. Peptide Protein Res.* **1992**, *39*, 1-11.
9. Gray, A.S.; Osborne, R.H.; Jewess, P.J. *J. Insect Physiol.* **1994**, *40*, 595-600.
10. Gray, A.S.; Osborne, R.H. *Brit. J. Pharmacol.* **1994**, *112*, 128P.
11. Osborne, R.H.; Odell, B.; Blagbrough, I.S. *Bioorg. Med. Chem. Letters* **1995**, *5*, 2085-2088.
12. Tourwe, D.; Couder, J.; Ceusters, M.; Meert, D. *Int. J. Peptide Protein Res.* **1992**, *39*, 131-136.
13. Chandrakumar, N.S.; Stapelfeld, A.; Beardsley, P.M.; Lopez, O.T.; Drury, B.; Anthony, E.; Savage, M.A.; Williamson, L.N.; Reichman, M. *J. Med. Chem.* **1992**, *35*, 2928-2938.
14. Roller, P.P.; Otaka, A.; Nomizu, M.; Smythe, M.S.; Barchi, J.J.; Burke, T.R.; Case, R.D.; Wolf, G.; Shoelson, S.E. *Bioorg. Med. Chem. Letters* **1994**, *4*, 1879-1882.
15. Brady, S.F.; Paleveda, W.J.; Arison, B.H.; Saperstein, R.; Brady, E.J.; Raynor, K.; Reisine, T.; Veber, D.F., Freidinger, R.M. *Tetrahedron* **1993**, *49*, 3449-3466.
16. Dhanda, D.S.; Parsons, W.H.; Greenlee, W.J.; Patchet, A.A. *Tetrahedron Letters* **1992**, *33*, 1725-1728.
17. Meyer, J.P.; Collins, N.; Lung, F.D.; Davis, P.; Zalewska, T.; Porreca, F.; Yamamura, H.I.; Hruby, V.J. *J. Med. Chem.* **1994**, *37*, 3910-3917.
18. Haskell-Luevano, C.; Shenderovich, M.D.; Sharma, S.D.; Nikiforovich, G.V.; Hadley, M.E.; Hruby, V.J. *J. Med. Chem.* **1995**, *38*, 1736-1750.
19. Dean, P.M. *Molecular Foundations of Drug-Receptor Interaction*, CUP, **1987**.
20. Bradford, M.M. *Anal. Biochem.* **1976**, *72*, 248-254.
21. Baines, R.A.; Lange, A.B.; Downer, R.G.H. *J. Comp. Neurol.* **1990**, *297*, 479-486.
22. Berridge, M.J.; Dawson, R.M.S.; Downes, C.P.; Heslop, J.P.; Irvine, R.F. *Biochem. J.* **1983**, *212*, 473-482.
23. Godfrey, P.P. *Signal Transduction: a Practical Approach*, ed. Milligan, G., OUP, **1992**, pp. 105-121.

(*Received in Belgium* 12 *September* 1995; *accepted* 9 *November* 1995)