Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 114-117

A GASTRIN-RELEASING PEPTIDE ANTAGONIST CONTAINING A $\psi(CH_2O)$ AMIDE BOND SURROGATE

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Received September 21, 1989

Summary: The [Leu^{26}-$\psi(CH_2O)Leu^{27}$] derivative of N-Ac-GRP20-27-peptide amide was prepared and evaluated as a gastrin-releasing peptide antagonist. This $\psi(CH_2O)$ derivative was found to be a more potent inhibitor of [^{3}H-Phe^{15}]GRP15-$27NH_2$ binding and N-Ac-GRP20-$27NH_2$ induced mitogenesis in Swiss 3T3 fibroblasts than the related nitrogen analog [Leu^{13}-$\psi(CH_2NH)Leu^{14}$]bombesin. Possible reasons for the improved activity of the (CH_2O) insert relative to the (CH_2NH) group include increased hydrophobicity and a reduced tendency of the oxygen derivative to form hydrogen bonds.

Gastrin-releasing peptide (GRP) is a 27 amino acid peptide hormone which stimulates a wide variety of biological responses including the release of gastrin into the systemic circulation (1-3). In addition, GRP and homologous peptides of the bombesin family have been reported to function as growth factors for human fetal lung (4) and human small cell lung cancer (SCLC) (5-7). The observation that anti-bombesin monoclonal antibodies which block binding of GRP to its receptor inhibit the growth of SCLC cells in vitro and in vivo (5,6) has stimulated the search for antagonists of this peptide which might have clinical utility as anticancer agents.

The strategy of peptide backbone modification through carbonyl reduction (8) has been applied to the design of potential antagonists of bombesin-like peptides. Replacement of selected peptide bonds in bombesin by the CH_2NH group led Coy and coworkers (9,10) to the synthesis of [Leu^{13}-$\psi(CH_2$-$NH)Leu^{14}$]bombesin, 1, a potent antagonist of this family of peptides.

In this report, we describe a synthesis of the related ether derivative [Leu^{26}-$\psi(CH_2O)Leu^{27}$],2, of N-Ac-GRP20-$27NH_2$ and its GRP antagonist properties.

MATERIALS AND METHODS

[Leu^{26}-$\psi(CH_2O)Leu^{27}$]N-Ac-GRP20-$27NH_2$, 2, was prepared from previously described Boc-(S,S)-Leu-$\psi(CH_2O)$LeuOH (11) by amidation and condensation with $(Boc)_2$-His using a mixed anhydride procedure. Deprotection with HCl in EtOAc

0006-291X/89 $1.50

afforded His-Leu-ψ(CH_2O)$LeuNH_2$ which was then coupled with Ac-His-Trp-Ala-Val-Gly-OH by a dicyclohexylcarbodiimide procedure. Satisfactory elemental analyses and PRM spectra were obtained for all intermediates. The final peptide was purified by reverse phase HPLC (98% pure). Amino acid composition data were obtained in duplicate and were consistent with the assigned structure. We thank D. Coy for providing a sample of [Leu^{13}-ψ(CH_2NH)Leu^{14}]bombesin.

Binding inhibition and mitogenic inhibition assays using Swiss 3T3 cells were performed as described previously (12). Receptor binding assays utilized [^{3}H-Phe^{15}]-GRP15-27-peptide amide as radioligand at a final concentration of 3nM. Mitogenesis inhibition studies were conducted by coadministration of the antagonist and 3nM N-Ac-GRP20-27-peptide amide.

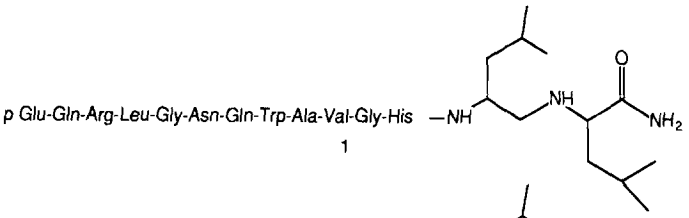
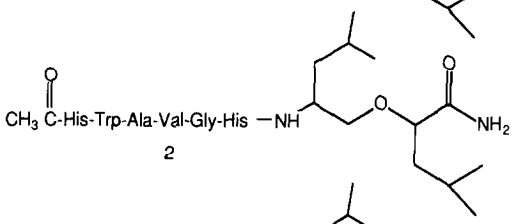
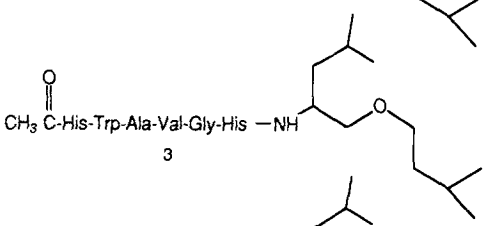
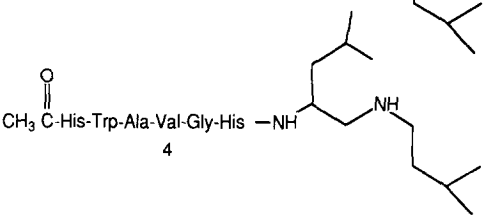
RESULTS AND DISCUSSION

The novel bombesin derived antagonist prepared by Coy and colleagues (9), [Leu^{13}-ψ(CH_2NH)Leu^{14}]bombesin, 1, was tested in our binding inhibition and mitogenic stimulation assays and was confirmed to be a GRP antagonist. However, in our binding inhibition assay, this compound exhibited an IC_{50} of 199 nM (Table 1), a value considerably higher than previously reported. This increased value for the binding inhibition IC_{50} of 1 compared to the published value is most likely due to the higher challenge dose of radioligand that was used in our studies.

We had previously observed that some ester derivatives of GRP were potent antagonists of this peptide (12). For example, the ethyl ester N-AcGRP20-26OEt was found to have IC_{50} = 4 nM in the competitive binding inhibition assay and to be more potent than the corresponding amide analog, N-Ac-GRP20-26NHEt, IC_{50} = 53 nM. The superiority of oxygen vs nitrogen in this series suggested that a similar substitution of oxygen for nitrogen in the reduced carbonyl derivatives might, by analogy, lead to more potent antagonists in this latter series.

Evaluation of the oxygen derivative 2 in the binding and mitogenic stimulation assays showed that it was a GRP antagonist and, as predicted, was more potent than the related nitrogen derivative 1 (Table 1). However, a quantitative assessment of the nitrogen to oxygen change is not possible in this case since the peptide chain of 2 is not exactly that of the larger bombesin derivative 1 (Table 1).

Table 1. Potency of GRP derivatives in Swiss 3T3 fibroblasts. *Numerical values represent the concentration of compound* which reduced binding of radioligand or mitogen action to 50% of the value observed in vehical treated controls.

	IC_{50}(nM)	
	Binding	Mitogenesis
 1	199	>300
 2	30	100
 3	9	32
 4	1100	Not Tested

Although bond angles and bond lengths for $\psi(CH_2O)$ modified bonds are actually closer to normal peptide bonds than the $\psi(CH_2NH)$ values (13), $\psi(CH_2O)$ substitution has been attempted in only a few cases with mixed results (13-16). One notable difference between the $\psi(CH_2O)$ and $\psi(CH_2NH)$ inserts is the hydrogen bonding capability of the more polar latter group. Although this structural feature would be expected to contribute favorably to binding of the nitrogen analog in polar areas of the receptor, it appears to be detrimental to binding in this case.

In addition, removal of the carbamoyl group of 2 to give the even less polar 3 (17, 18) further improves binding and mitogenesis inhibition. In contrast, the more basic and more polar derivative 4 (1-17, 18) exhibits much poorer binding to the GRP receptor when compared with 1, 2 and 3. These results demonstrate that hydrogen bonding ability of the carboxy terminal group of this series of GRP antagonists is not important for binding and further suggests that, in this antagonist series, this group lies in a hydrophobic area of the receptor.

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