Development of potent gastrin-releasing peptide antagonists having a d-Pro-Ψ(CH₂NH)-Phe-NH₂ C terminus

(Swiss 3T3 cell/mitogenesis/bombesin)

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ABSTRACT

Gastrin-releasing peptide (GRP) is a 27-amino acid neuroendocrine hormone that may play a role in the pathophysiology of small cell lung carcinoma. GRP and bombesin, a structurally related peptide, stimulate the growth of some cultured cell types. C-terminal GRP peptide analogs were developed that inhibited 6 nM bombesin-induced [³H]thymidine incorporation into quiescent murine Swiss 3T3 cells, which routinely produced a 6-fold stimulation over the basal extent of incorporation. The peptides were also analyzed for their capacity to inhibit the binding of 50 pM [¹²⁵I]labeled GRP to Swiss 3T3 cells. The combination of two chemical modifications, each antagonistic in itself, led to the creation of antagonists with orders of magnitude greater potency than either modification alone. (i) Antagonist analogs of the form -Leu²⁸-Ψ(CH₂NH)-Xaa²⁷-NH₂ [where Xaa is Leu, norleucine (Nle), or Phe; residues numbered after GRP], similar to those introduced by Coy and coworkers [for review, see Jensen, R. T. & Coy, D. H. (1991) Trends Pharmacol. Sci. 12, 13–19], were found to have nanomolar potencies. (ii) We found that an octapeptide C-terminal GRP analog having d-Pro adjacent to the C-terminal amino acid amide was antagonistic, with a potency of 40 nM. By combining both specific modifications, several analogs were found with potencies >1000-fold greater than our lead structure—[(4'-hydroxy)-3-phenylpropionyl]-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Ψ(CH₂NH)-Nle-NH₂ and greater than any antagonist previously reported. The analogs [(4'-hydroxy)-3-phenylpropionyl]-His-Trp-Ala-Val-d-Ala-His-d-Pro-Ψ(CH₂NH)-Phe-NH₂ and 1-naphthyl-His-Trp-Ala-Val-d-Ala-His-d-Pro-Ψ(CH₂NH)-Phe-NH₂ antagonized [³H]thymidine incorporation with IC₅₀ values of ~0.3 nM and inhibited the binding of [¹²⁵I]labeled GRP with IC₅₀ values of ~1 pM. These peptides may be of use in the study of the physiology of GRP.

Gastrin-releasing peptide (GRP) is a 27-amino acid neuroendocrine hormone and a member of a family of structurally related peptides that elicit a broad array of biologic activities. These activities include regulation of smooth muscle contraction, stimulation of secretion, modulation of neural activity, and growth regulation (for review, see refs. 1 and 2). Growth regulation has received special interest because a number of observations suggest that GRP may be involved as an autocrine factor in the pathophysiology of small cell lung carcinoma (3–8). GRP antagonists have been found to inhibit the growth of human small cell lung carcinoma cells in vitro (8) and in murine xenografts in vivo (9). Thus, GRP antagonists may have therapeutic potential. In other studies, GRP antagonists have helped to identify at least two subtypes of GRP receptors (10) that appear to be members of the large family of guanine nucleotide regulatory protein-coupled receptors.

The potential autocrine role of GRP in small cell lung carcinoma has stimulated substantial progress in the development of GRP antagonists (for review, see ref. 11). The receptor binding site on GRP is located in the C-terminal 8 amino acids of GRP where it is structurally identical to the homologous region of bombesin. Bombesin is a 14-residue amphibian peptide that reacts approximately equivalently to GRP in studies of mammalian responses. The C-terminal Met is naturally amidated and is required for full biologic activity, although it does not appear to be required for binding (12–18). Coy et al. (14, 15) replaced the terminal peptide bond with a reduced form, Ψ(CH₂NH), yielding a reasonably potent antagonist, [¹³⁵Ψ¹⁴,CH₂NH]bombesin, and have further explored the use of the Ψ bond to make more potent antagonists (11). Other antagonists have been made by replacing the terminal Met-NH₂ with either an alkyl ester (16) or an alkyl amide (17, 18). Specific members of these three classes of antagonists have potencies ≥1 nM, which is the potency of the natural hormone.

We report structure–function studies reliant on incorporation of the reduced Ψ bond in the C-terminal region. We hoped to increase the potency of [¹³⁵Ψ¹⁴,CH₂NH]bombesin by applying principles developed by Folkers et al. (19, 20), who improved the activity of a substance P antagonist by introducing d-amino acids adjacent to the binding region. Our activity studies were performed using inhibition of bombesin-induced incorporation of [³H]thymidine into quiescent Swiss 3T3 cells. This approach contrasts with other antagonist development programs that have relied on measurements of competition receptor binding potency and/or amylase release. We also analyzed peptides for competitive binding. The Swiss 3T3 mitogenic response has been used to evaluate agonist and antagonist potencies (11), but it has not been employed as a "primary" screen. We envisioned that the mitogenic antagonist response might be a more stringent test of antiproliferative activity. In general, the growth promoting property of GRP had been studied extensively on Swiss 3T3 cells (21–23), murine colonic cancer cells (24), and normal bronchial epithelial cells (25, 26).

MATERIALS AND METHODS

Peptide Synthesis and Purification. The peptides were synthesized on an Applied Biosystems model 430A peptide synthesizer, using an improved version of Merrifield's solid-phase method (27, 28). For the synthesis of "pseudopeptides," the appropriate resin [e.g., Boc-d-Pro-Ψ(CH₂NH)-Phe-MBHA (where Boc is t-butoxycarbonyl and MBHA is p-methylbenzyldrylamine)] was loaded in the synthesizer.

Abbreviations: GRP, gastrin-releasing peptide; Nle, norleucine; FAB, fast atom bombardment.

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and a standard deprotection (trifluoroacetic acid/CH$_2$Cl$_2$–
neutralization (disopropylethylamine/CH$_2$Cl$_2$) program was
used as supplied by Applied Biosystems. The reduced bond
was introduced onto the resin in a variation to Sasaki’s
 technique (29). Boc-protected amino acids were coupled to
the resin using a modified program to suit the [benzotriazol-
1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophos-
phate] (BOP) coupling procedure (30). The coupling protocol
involved dissolving 1 mmol of Boc-protected amino acid, 1
mmol of BOP, and 1 ml of 1 M 1-methylimidazole in 7 ml of
dimethyl formamide. The mixture was added to 0.5 mmol of
resin, mixed for 1 h, and filtered. Afterwards, a series of
dimethyl formamide washes and CH$_2$Cl$_2$ rinses were
performed. After the peptide was assembled on the resin, it was
de-blocked and cleaved from the resin with liquid HF containing
10% (vol/vol) anisole in a variation of the method described
by Sakakibara et al. (31). The peptide and resin were next
washed with ethyl acetate and the peptide was extracted from
the resin with an aqueous 1% acetic acid solution and
lyophilized.

The peptides were then purified by reverse-phase HPLC
using a Vydac (Hesperia, CA) 218TP1022 column on a
Waters Delta-Prep 3000 system equipped with a Gilson model
116 ultraviolet detector. Purification was achieved by equil-
ibrating the column with 0.1% trifluoroacetic acid in H$_2$O
and developing with a linear gradient of acetonitrile from 10
to 100% in 20 min at a flow rate of 0.5 ml/min. Inhibin of bombesin-induced mitogenicity was performed similarly where test
peptide solutions, generally log$_2$ dilutions, were added in
the presence of 6 nM bombesin. One of the peptides (Table 1,
structure 3, see Results) served as an interassay control. The
monolayers were washed three times with ice-cold phosphate-
buffered saline, fixed with ice-cold 40% methanol/12% acetic
acid (vol/vol), rinsed with phosphate-buffered saline, solubi-
ized with detergent, and mixed with scintillant (Ready Safe,
Beckman), and radioactivity was determined by liquid scinti-
illation counting (LKB, 30% efficiency).

Binding was carried out similarly to published studies (32–
34). Peptides were serially diluted in maintenance medium and
overlayed in triplicate on quiescent monolayers in 24-well
trays to a total volume of 0.2 ml per well. Inhibition of bombesin-induced mitogenicity was performed similarly to test
peptide solutions, generally log$_2$ dilutions, were added in
the presence of 6 nM bombesin. One of the peptides (Table 1,
structure 3, see Results) served as an interassay control. The
monolayers were washed three times with chilled maintenance
medium, and solubilized with detergent, and radioactivity was determined (LKB, 55% efficiency).

**RESULTS**

Biological activity analyses for representative peptides are
depicted in Figs. 1–3. Fig. 1 demonstrates peptide-induced
$[^{3}H]$thymidine incorporation into quiescent Swiss 3T3 cells.
Bombesin produced a dose-related stimulation of incorpo-
ration that maximized at $\sim$10 nM bombesin. GRP was
$\approx$5-fold less potent (data not shown). In contrast, the other
indicated peptides failed to stimulate incorporation over
background and thus appeared to possess no intrinsic agonist
activity. As seen in Fig. 1, 6 nM bombesin stimulated a
near-maximal degree of $[^{3}H]$thymidine incorporation where
the extent of the incorporation was $\approx$6-fold greater than the
no-addition control.

Thus, 6 nM bombesin was selected as the concentration upon
which other peptides were evaluated for antagonist potency.
Typically, 6 nM bombesin-induced incorporation yielded $\approx$1800 cpm per microtiter well, and the no-addition
control gave 300 cpm. Representative antagonist screening
results are shown in Fig. 2. The results are expressed as
percent $[^{3}H]$thymidine incorporation, where 100% is the
value obtained for 6 nM bombesin. As can be seen in Fig. 2,
peptide 3 yielded an IC$_{50}$ value of 0.7 μM and peptide 13
yielded an IC$_{90}$ value of 0.2 nM.

All the structures were evaluated for their capacity to
compete for the binding of $^{125}$I-labeled GRP as exemplified in
Fig. 3.

Our complete analyses are summarized in Table 1. GRP
and bombesin demonstrated agonist activity comparable to
literature values (1). However, none of the remaining pep-
tides shown in Table 1 stimulated $[^{3}H]$thymidine incorpo-
ration (agonist activity) at concentrations $\leq$10 μM.

Peptide 3 served as the lead structure for our antagonist
program and was incorporated as an interassay control over
the course of our studies. As indicated in Table 1, the
interassay average IC$_{50}$ value for peptide 3 was 0.7 μM,
where the SEM for replicate trials ($n = 28$) was 0.2 nM. Thus,
the *in vitro* assay was not very precise probably due to the

**Biological Activity**

Swiss 3T3 cells (passage unknown) were a gift from E. Rozengurt
(Imperial Cancer Research Fund, London). Cells were maintained by serial passage at
37°C in a humidified atmosphere containing 5% CO$_2$/95% air
in Dulbecco’s modified Eagle’s medium/Ham’s nutrient mix-
ture F-12 (DMEM/F-12; Mediatech, Herndon, VA) supple-
mented with 10% (vol/vol) fetal bovine serum (lot 882050;
Biologics, Naperville, IL) on plasticware. Our passages 7–20
were employed in these studies. Cells were judged free of
mycoplasma by coculture on Vero cells followed by Hoechst
stain and examination by fluorescence microscopy. All studies
were performed on density-arrested quiescent cells. Cells were
seeded at 1.5 × 10$^4$ cells per cm$^2$ and cultured for 3 days.
The medium was replaced with DMEM/F-12 containing
0.75% fetal bovine serum (maintenance medium) and culture
was continued for 2 days, when peptide bioactivity analyses
progressed.
Table 1. Peptide structure and potentials for mitogenic antagonism and competitive binding

<table>
<thead>
<tr>
<th>No.</th>
<th>Sequence</th>
<th>Mitogenic antagonism, M</th>
<th>Binding, M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$] (GRP)</td>
<td>—</td>
<td>$2 \times 10^{-10}$</td>
</tr>
<tr>
<td>2</td>
<td>pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH$_2$ (bombesin)</td>
<td>—</td>
<td>$5 \times 10^{-10}$</td>
</tr>
<tr>
<td>3</td>
<td>[(4'-OH)-3-PrP]-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Nle-NH$_2$</td>
<td>7.2 $\pm 2.0 \times 10^{-7}$ (n = 28)</td>
<td>$4 \times 10^{-8}$</td>
</tr>
<tr>
<td>4</td>
<td>[(4'-OH)-3-PrP]-Pro-Arg-D-Ala-D-Phe-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Nle-NH$_2$</td>
<td>9.8 $\pm 1.3 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>[(4'-OH)-3-PrP]-Pro-D-Ala-Gly-D-Phe-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Nle-NH$_2$</td>
<td>3.2 $\pm 1.1 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>[(4'-OH)-3-PrP]-d-Pro-Arg-Gly-D-Phe-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Nle-NH$_2$</td>
<td>4.0 $\pm 1.7 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(3-PrP)-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Leu-NH$_2$</td>
<td>1.6 $\pm 0.5 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(3-PrP)-His-Trp-Ala-Val-D-Ala-His-Leu-$^{\psi}$-Nle-NH$_2$</td>
<td>2.4 $\pm 0.6 \times 10^{-9}$</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>(3-PrP)-His-Trp-Ala-Val-d-Ala-His-Pro-Phe-NH$_2$</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>(3-PrP)-His-Trp-Ala-Val-d-Ala-His-d-Pro-Phe-NH$_2$</td>
<td>3.7 $\pm 2.0 \times 10^{-8}$</td>
<td>$5 \times 10^{-8}$</td>
</tr>
<tr>
<td>11</td>
<td>(3-PrP)-His-Trp-Ala-Val-d-Ala-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>1.0 $\pm 0.3 \times 10^{-9}$</td>
<td>$1 \times 10^{-12}$</td>
</tr>
<tr>
<td>12</td>
<td>(3-PrP)-His-Trp-Ala-Val-d-Ala-His-$^{\psi}$-Phe-NH$_2$</td>
<td>1.1 $\pm 0.4 \times 10^{-8}$</td>
<td>$1 \times 10^{-12}$</td>
</tr>
<tr>
<td>13</td>
<td>[(4'-OH)-3-PrP]-His-Trp-Ala-Val-d-Ala-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>2.5 $\pm 0.8 \times 10^{-10}$ (n = 3)</td>
<td>$5 \times 10^{-11}$</td>
</tr>
<tr>
<td>14</td>
<td>(3-PrP)-His-Trp-Ala-Val-Sar-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>5.0 $\pm 1.0 \times 10^{-9}$</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>15</td>
<td>[3',4',5'-(OMe)$_3$-3-PrP]-His-Trp-Ala-Val-d-Ala-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>3.0 $\pm 0.8 \times 10^{-10}$</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1-Naphthoyl-His-Trp-Ala-Val-d-Ala-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>3.0 $\pm 1.0 \times 10^{-10}$</td>
<td>$1 \times 10^{-12}$</td>
</tr>
<tr>
<td>17</td>
<td>(2-Me-2-PrP)-His-Trp-Ala-Val-d-Ala-His-d-Pro-$^{\psi}$-Phe-NH$_2$</td>
<td>3.4 $\pm 0.5 \times 10^{-10}$ (n = 3)</td>
<td>$3 \times 10^{-12}$</td>
</tr>
<tr>
<td>18</td>
<td>[3-(Naphthyl)-d-Ala]-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Phe-NH$_2$</td>
<td>1.0 $\pm 6.7 \times 10^{-7}$ (n = 3)</td>
<td>$1 \times 10^{-8}$</td>
</tr>
<tr>
<td>19</td>
<td>d-Phe-His-Trp-Ala-Val-Gly-His-Leu-$^{\psi}$-Phe-NH$_2$</td>
<td>1.1 $\pm 4.1 \times 10^{-8}$</td>
<td>$3 \times 10^{-9}$</td>
</tr>
<tr>
<td>20</td>
<td>d-Phe-His-Trp-Ala-d-Ala-His-Leu-$^{\psi}$-Phe-NH$_2$</td>
<td>8.4 $\pm 1.1 \times 10^{-8}$ (n = 3)</td>
<td>$3 \times 10^{-9}$</td>
</tr>
<tr>
<td>21</td>
<td>d-Phe-His-Trp-Ala-Val-Gly-His-d-Leu-$^{\psi}$-Phe-NH$_2$</td>
<td>NT</td>
<td>3 $\times 10^{-7}$</td>
</tr>
<tr>
<td>22</td>
<td>d-Phe-His-Trp-Ala-Val-d-Ala-His-Leu-$^{\psi}$-Phe-NH$_2$</td>
<td>NT</td>
<td>$4 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

Activities were assessed in triplicate on quiescent Swiss 3T3 cells as described in Figs. 1–3; mitogenic antagonism, the capacity to inhibit 6 nM bombesin-induced [H]thymidine incorporation; competitive binding, the capacity to inhibit the binding of 50 pM $^{35}$S-labeled GRP. Values are the IC$_{50}$ (mean ± SEM) and are representative of replicate trials. For binding, the SEMs were <8% of the mean; for mitogenicity, n is the number of trials and data are the mean ± SEM of the replicate trials. $\psi$(CH$_2$/NH); NA, no activity observed at 10$^{-4}$ M; NT, not tested; pGlu (2), pyroglutamyl-; PrPr, phenylpropanoyl; Nle, norleucine; Sar, sarcosine.

piping error introduced by serial dilutions. However, the assay led to reproducible enhancement in antagonist potency as discussed in greater detail below.

**DISCUSSION**

Structure 3 is a GRP C-terminal dodecapeptide fragment with a reduced bond between residues 11 and 12 that is blocked at the N-terminal end with a (4'-hydroxy)-3-phenylpropanoic acid residue, des-NH$_2$-Tyr. In addition, the C-terminal end contains Nle-NH$_2$, which may be more metabolically stable than the Met-NH$_2$ in GRP (35). Peptide 3 was comparable in antagonist potency to $^{13}$H,C$_2$NH-bombesin and $^{26}$H,C$_2$NH)GRP (15–27) when analyzed by similar methods as reported elsewhere (34). Analogs 4–6 were derived from structure 3, but each had different D-amino acid substitutions in positions 1, 2, and 4 and yielded an ≈10-fold increased activity in our binding and inhibition assays. The

**FIG. 1.** Agonist activity: Induction of [H]thymidine incorporation into quiescent Swiss 3T3 cells. Bombesin (Table 1, structure 2) and structures 3, 10, and 11 (Table 1) were solubilized in maintenance medium, serially diluted, and overlayed in triplicate on quiescent Swiss 3T3 cell monolayers in microtiter culture as described in the text. The cells were then cultured for 18 h and labeled for 2 h by the addition of 0.5 µCi of [H]thymidine per 200 µl per well. The monolayers were washed with ice-cold phosphate-buffered saline, fixed with 40% methanol/12% acetic acid (vol/vol), and solubilized with detergent for determination of radioactivity by liquid scintillation counting (30% efficiency). Values are expressed as the percent maximum incorporation [mean ± SEM (bars) minus control (no additions), where the maximum is the value obtained for 60 nM bombesin. •, Bombesin; ○, peptide 3; □, peptide 10; △, peptide 11.

**FIG. 2.** Antagonist activity: Representative inhibition of bombesin-stimulated [H]thymidine incorporation. Peptides 3 and 13 (Table 1) were solubilized in maintenance medium, diluted, and overlayed on quiescent Swiss 3T3 cell monolayers in the presence of 6 nM bombesin. Cultures were incubated for 18 h, labeled for 2 h with [H]thymidine, and harvested as described in Fig. 1. Points depict percent maximum incorporation (mean ± SEM), where the maximum is the value obtained for 6 nM bombesin alone minus control (no additions). •, Peptide 13 (six replicates per point); ○, peptide 3 (three replicates per point).
increased binding activity of these peptides was probably due to the stabilization of a putative type II β-turn as the active conformation in the adjacent binding region. [A model proposing the molecular features that convey agonism/antagonism has been described (15).] Analogs 4–6 had an Asn→D-Phe replacement. The increased activity suggested the possibility of terminating the peptide at this position with 3-phenylpropanoic acid, D-Phe, or other aromatic acids. Such changes led toward the highly active short analogs 7–22 but did not, in themselves, yield the most potent analogs. Analogs terminating with D-Phe or 3-(2-naphthyl)-D-Ala have been reported previously with binding potencies in the nanomolar range (11). Gly11 in bombesin has also been replaced with D-Ala without loss of activity in an in vivo assay (36). Applying this modification to our analog 7, yielding analog 8, gave no substantial change in activity.

Our most potent analogs appeared to emerge from the incorporation of two approaches, use of the Ψ bond as introduced by Coy et al. (14, 15) and the application of our hypothesis. Our hypothesis involved the introduction of Ψ-Pro as a scaffold at the opposite site of the isobutyryl side chain of [Leu13]bombesin. No antagonism with the D-amino acid replacement of Leu at the C-terminal of GRP or bombesin had been reported, to our knowledge. We thought that conformational restriction, induced by Pro in this position, might lead to antagonistic activity. In fact, when trying to introduce such an amino acid (isobutyryl-DL-Pro), we were unable to couple it due to steric hindrance. We settled for the introduction of D-Pro instead, assuming that the Leu side chain may not be required for binding but that the D-Pro would bend the benzyl side chain of the C-terminal Phe out of an active binding mode.

Comparison of L-Pro and D-Pro in this position (structures 9 and 10) showed only modest antagonist activity in the D-Pro analog and no intrinsic agonist activity (Fig. 1). Structure 10, which lacked the Ψ bond, was nevertheless more potent than the lead structure 3. Surprisingly, a gain in potency was observed when D-Pro was placed adjacent to the reduced bond (Ψ structure 11). More potent antagonist activity was gained when N-terminal aromatic blocking groups were substituted (structures 13–17). As a group, an =10-fold increase in potency was observed among structures 13–17. The mitogenic antagonist potencies of structures 13 and 15–17 were 0.3 nM and indicated a 2500-fold increase over our lead structure 3. Structures 11, 12, and 15–17 had competitive binding potencies that were =100-fold more potent than the natural hormones GRP and bombesin.

Not just any D-amino acid produced either antagonist activity or the significant enhancement in potency. It has been reported (36) that [D-Leu13]bombesin was inactive compared to a L-Leu13 analog in an in vivo assay for agonist activity. The analogous L-Leu-Ψ(CH2NH2) peptides (structures 19 and 20) were less potent, and the D-Leu-Ψ(CH2NH2) substitutions (structures 21 and 22) led to still 10-fold lower potencies.

Mitogenic antagonism, to our knowledge, has not been used previously as a primary screen from which to establish a structure–function relationship among GRP antagonists. Mitogenic antagonism, which consisted of an overnight exposure to cells and low serum concentration, may have been a more stringent test of potency relative to competitive binding, which was a 30-min exposure under otherwise similar conditions. Peptides were generally 10–100-fold less potent for mitogenic antagonism than competitive binding. Nevertheless, competitive binding potency correlated well with antagonist potency (Table 1). However, structure 12 was an exception, where mitogenic antagonism was considerably less potent than competitive binding. Structure 12, an L-Pro-Ψ(CH2NH2)-Phe analogue, may be less stable than its homologous D-Pro-Ψ(CH2NH2)-Phe analogue (structure 11). The increased potencies of structures 11–17 may be due to peculiarities in the assay conditions, that is, to resistance to specific cellular or serum esterases or to nuances of the murine Swiss 3T3 cell GRP receptor. The relative potencies of our structures have not been examined in other species. However, structure 11 has been recognized for its potency to antagonize bombesin-stimulated gastrin release in rats and dogs (37).

Our structure–function relationship supports the model proposed by Coy et al. (15) that describes molecular features conveying agonism/antagonism. The model proposes a type II β-bend stabilized by hydrogen bonding. A D-Pro, with or without the Ψ bond (structures 10 and 11), would destabilize such a conformation by eliminating one hydrogen bond since the NH of D-Pro is not available for hydrogen bonding and still retains a similar type II β-bend conformation. The L-Pro analogue would have a different conformation that does not bind to the receptor (structure 9). A D-ProΨ analogue would have an even more destabilized type II β-turn, thus becoming a better antagonist (structures 11 and 13–17). Our NMR analysis of structure 6 indicates the presence of a type I or type III β-turn in methanol; data comparing it with an agonist will be published later (A. Aulabaugh, J.J.L., A.L., and J.D.McD., unpublished data).

We thank Dr. Lester Taylor and his associates for amino acid analysis and FAB–mass spectroscopy.

6. Maruno, K., Yamaguchi, K., Abe, K., Suzuki, M., Saijo, N.,