Drugs of the Future 1998, 23(12): 1305-1315

CCC: 0377-8282/98

Review Article

Bombesin receptor antagonists

Terry W. Moody[1*] and Robert T. Jensen[2]

[1]Cell & Cancer Biology Dept., Medicine Branch, Division of Clinical Sciences, National Cancer Institute, Bldg. KWC, Rm. 300, 9610 Medical Center Dr., Rockville, MD 20850 USA and [2]Digestive Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland USA.
[*]Correspondence:

CONTENTS

Introduction

Bombesin (BB) is a 14 amino acid peptide isolated from frog skin (1). It is biologically active in the periphery where it contracts smooth muscles, stimulates pancreatic enzyme (amylase) secretion (2), inhibits food intake (3) and stimulates pituitary hormone (growth hormone) secretion (4). Also, BB is biologically active in the central nervous system where it increases locomotor activity (5), causes hypothermia (6) and increases blood glucose levels (7). In the normal CNS and periphery, BB-like peptides function as neuromodulators where they are released from neurons and bind to receptors in adjacent cells (8). Perhaps the most important action of BB, however, is that it stimulates the growth of normal and malignant cells (9-11). In particular, high levels of BB-like peptides were detected in small cell lung cancer (SCLC), a neuroendocrine tumor which kills approximately 30,000 patients annually in the United States (12). Traditionally, SCLC is treated with chemo- and/or radiation therapy and initially the tumor regresses. Unfortunately, patient relapse occurs, the tumor proliferates and the median survival time of SCLC patients is less than 1 year. Therefore, new therapeutic approaches are needed for the treatment of SCLC. Subsequently, BB receptor antagonists were developed with the goal of inhibiting cancer proliferation.

BB agonists

BB contains 14 amino acid residues and has a blocked N-terminal (pyroglutamate) and C-terminal (amidated). Other frog skin peptides structurally related to BB include litorin, alytesin and ranatensin which have 9, 14 and 11 amino acids, respectively (13). Table I shows that these peptides have structural homology to BB at the C-terminal. Subsequently, the mammalian peptides gastrin releasing peptide (GRP) and neuromedin B (NMB) were isolated which contain 27 and 10 amino acids, respectively (14, 15). GRP and NMB have structural homology at the C-terminal octapeptide of BB. Substitution of D-amino acids for the natural L-amino acids at the 8(Trp), 10(Val), 12(His), 13(Leu) or 14(Met) of BB greatly reduced biological activity (16). A difference between BB and litorin, ranatensin and NMB is that the latter 3 peptides have a Phe at the penultimate position whereas GRP, BB and alytesin have a Leu. Subsequently, distinct GRP (384 amino acids) and NMB (390 amino acids) receptors were cloned (17-19). The order of peptide potency for GRP (BB_2) receptors is GRP = BB > NMB, whereas NMB (BB_1) receptors prefer NMB relative to BB or GRP (20, 21). Additional studies indicated that the Leu at position 3 of NMB was the most important for NMB receptor selectivity, followed by the Phe at position 9 of NMB (22). The genes for the BB_1 and BB_2 receptors are localized to chromosomes 6q2l and X, respectively (23). In addition, a third bombesin receptor was cloned (BRS-3) whose natural ligand is unknown (24).

BB-like peptides are expressed in SCLC cells in the form of high molecular weight precursor proteins. GRP and NMB are derived from 148 and 116 amino acid precursor proteins, respectively (25-27); the genes for preproGRP and proproNMB are on chromosomes 18 and 15q11, respectively. They are posttranslationally processed by signal proteases, trypsin-like enzymes, carboxypeptide B-like enzymes and peptidyl-α-monooxygenase enzymes to yield GRP and NMB which are amidated at the C-terminal. Because amidation of BB is essential for biological activity, the GRP and NMB

Table I: Structure of BB-like peptide agonists.

Peptide	Structure
GRP	Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
BB	Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Aly	Pyr-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
Litorin	Pyr-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2
Ranatensin	Pyr-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2
NMB	Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2

Sequence homologies relative to BB are underlined. Standard amino acid abbreviations are used; Pyr, pyroglutamate.

precursor proteins are likely inactive (28). GRP and NMB may be stored in the dense core neurosecretory granules associated with SCLC and the levels of immunoreactive GRP can be quite high [for example 18 pmol/mg protein in NCI-H209 cells (29)]. Addition of vasoactive intestinal peptide (VIP) elevates the intracellular cAMP in SCLC cell line NCI-H345 or NCI-H209, resulting in an increased secretion rate of GRP (30). When VIP binds to cell surface VIP_1 receptors, a stimulatory guanine nucleotide binding subunit (Gs) is activated increasing adenylyl cyclase activity (31). The stimulation caused by VIP is inhibited by somatostatin (SRIF), which binds to cell surface receptors activating an inhibitory guanine nucleotide binding subunit (Gi) (32). The GRP levels in SCLC conditioned media are approximately 1 nM and similar plasma levels are observed in patients with extensive SCLC (30). The secreted GRP binds to cell surface receptors and is rapidly internalized (33). The GRP receptor complex is rapidly internalized to lysozomes. The diphtheria toxin-GRP fusion protein is internalized by SCLC cells and after metabolism diphtheria toxin fragments inhibit protein synthesis and kill SCLC cells (34). These data indicate that the GRP receptor can be utilized to deliver cytotoxic agents into cancer cells. After receptor activation, GRP is degraded by endopeptidase 24.11 (35). The half-life of GRP in the blood is approximately 5 minutes.

In contrast to the GRP and NMB receptors, very little is known about the BRS-3 receptor. Its structure has been described in humans (24) and guinea pigs (36). Whereas NMB and GRP receptors have a widespread distribution in the CNS and peripheral tissues (37-39), the BRS-3 receptor has a pattern of expression limited to secondary spermatocytes (24), pregnant uterus (36), a few brain regions and the pituitary (36, 40), as well as human lung (24), breast (41) and epidermal cancer cell lines (41). In a recent study (40), BRS-3 receptor-deficient mice were generated by targeted disruption and the mice were obese, developed hypertension and had diabetes mellitus, suggesting the BRS-3 receptor is required for glucose metabolism, energy balance and maintenance of blood pressure. The involvement of BRS-3 receptor in satiety regulation is particularly interesting because numerous studies demonstrate both the GRP and NMB receptors are important regulators of satiety in animals (3, 37, 42-44) and man (45). Furthermore, a recent study (46) of mice which are GRP receptor-deficient due to targeted disruption confirms that the GRP receptor is a physiological regulator of satiety. These studies suggest each mammalian member of the bombesin receptor family plays an important role in the regulation of satiety. Recently, the pharmacology (47, 48) and cell biology (48, 49) of the BRS-3 receptor was described using a novel, synthetic ligand [D-Phe^6,β-Ala^{11},Phe^{13},Nle^{14}]BB(6-14), which has high affinity for all mammalian bombesin receptors (50). These studies show that the BRS-3 receptor has a unique pharmacology with high affinity for no known naturally occurring bombesin-related peptide and therefore its natural ligand is either not a bombesin peptide or is a bombesin peptide with unique structural features (47).

Because of the widespread actions of bombesin peptides in both physiological and pathologic processes, as well as an incomplete understanding of the involvement of these peptides in a number of processes, there has been considerable attention directed at identification of receptor antagonists (51-54). There have been four general classes of GRP receptor antagonists described: D-amino acid substituted substance P (SP) or SP-4-11 analogs, [D-Phe^{12}]BB analogs, reduced peptide bond analogs of BB and GRP, and [des-Met^{14}]BB or [des-Met^{27}]GRP analogs. There are three general classes of NMB receptor antagonists: D-amino substituted substance P, somatostatin octapeptide analogs and a nonpeptide antagonist (55-57). There are no specific receptor antagonists for the BRS-3 receptor, although a number of the NMB and GRP receptor antagonists function as low affinity antagonists at this receptor (49). Particular attention will be given to results of studies in small cell or non-small cell lung cancer cells in this review. This was done because of the known autocrine growth role of BB in these tumors (58) and, therefore, the potential for BB receptor antagonists to have a therapeutic role in this tumor.

Substance P antagonists

The COOH-terminal of BB is essential for biological activity. It came as quite a surprise when substance P (SP) antagonists, which have only a single amino acid

Table II: Structures of BB antagonists.

Peptide	Structure
(D-Phe^{12})BB	Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-D-Phe-Leu-Met-NH_2
($\psi^{13,14}$,Leu^{14})BB	Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-ψ-Leu-NH_2
BW2258U89	3-Phenylpropanoyl-His-Trp-Ala-Val-D-Ala-His-D-Pro-ψ-Phe-NH_2
RC-3095	Tpi-Gln-Trp-Ala-Val-Gly-His-Leu-ψ-Tpi-NH_2
BIM-26226	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-methyl ester
ICI-216140	Isobutyryl-His-Trp-Ala-Val-D-Ala-His-Leu-methyl amide
(RPWWL)SP	D-Arg-D-Pro-Lys-Pro-Glu-Glu-D-Trp-Phe-D-Trp-Gly-Leu-Leu-NH_2

Sequence homologies relative to BB are underlined. Standard amino acid abbreviations are used; ψ, reduced peptide bond (CH_2-NH) instead of (O=C-NH), the amide bond.

homology with BB, were discovered to antagonize BB receptors (Table II) (59). [D-Arg^1,D-pro^2,D-Trp^{7,9},Leu^{11}]SP (RPWWL)SP inhibited (^{125}I-Tyr^4)BB binding to pancreatic acinar cells with an IC_{50} value of 1 μM (59). Also, (RPWWL)SP had no effect on basal amylase secretion but antagonized the ability of 1 nM BB or SP to stimulate amylase release from acinar cells (59). Numerous other D-amino acid substituted SP analogs were also shown to function as BB receptor antagonists; however, none were more than 10-fold more potent than [D-Arg^1,D-Pro^2 D-Trp^{7,9},Leu^{11}]SP (60). (RPWWL)SP inhibited ^{125}I-GRP or (^{125}I-Tyr^0)NMB binding to SCLC cell line NCI-H345 with IC_{50} values of 1 μM (61, 62). Also, 10 μM (RPWWL)SP antagonized the ability of 10 nM BB to stimulate phosphatidyl inositol (PI) turnover. Activated GRP receptors stimulate phospholipase C to generate PI (Fig. 1). The resulting inositol-1,4,5-triphosphate (IP_3) causes the endoplasmic reticulum to release Ca^{2+} into the cytosol (63). (RPWWL)SP had no effect on basal cytosolic Ca^{2+} but antagonized the increase in cytosolic Ca^{2+} caused by 10 nM BB or 10 nM NMB. Subsequent studies demonstrated SP antagonists also interact with NMB receptors (21) and can inhibit NMB activation of the NMB receptor (21, 57). More recent studies demonstrate SP

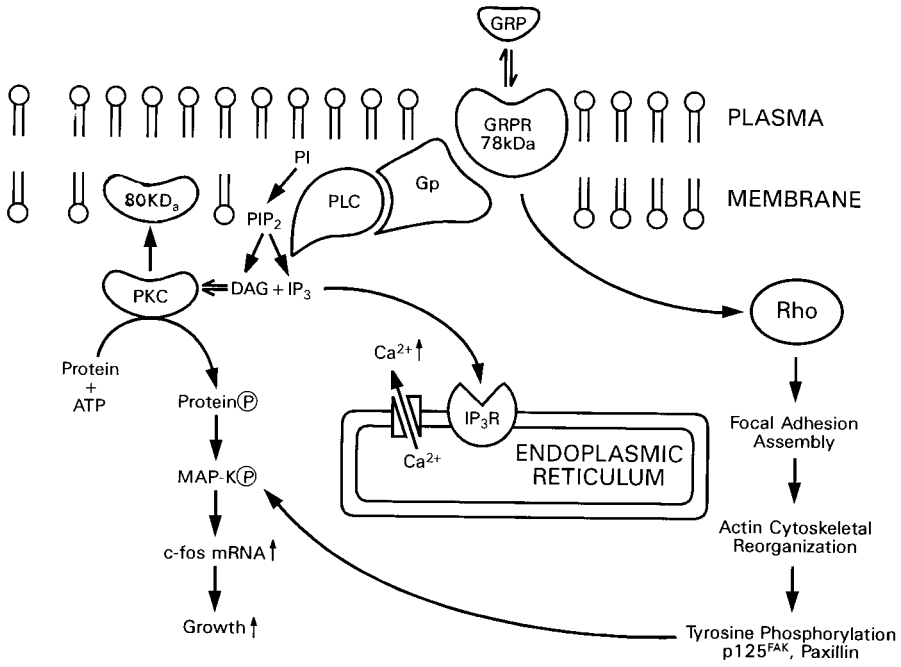

Fig. 1. Signal transduction mechanisms for the GRP receptor. GRP binds with high affinity to the 78 kD GRP receptor activating a G-protein (Gp) and Rho. Rho causes focal adhesion assembly, reorganization of the cytoskeleton and tyrosine phosphorylation of FAK kinase and paxillin. Phospholipase C activation causes metabolism of PIP_2 to IP_3 and diacylglycerol (DAG). IP_3 diffuses to the endoplasmic reticulum causing the release of Ca^{2+} into the cytosol. The DAG activates protein kinase C causing its translocation to the membrane. Activated PKC phosphorylates protein substrates, ultimately causing the phosphorylation of MAP kinase. Activated MAP-kinase ultimately leads to increased nuclear oncogene proliferation and growth.

receptor antagonists also inhibit BRS-3 receptor activation (49). These data indicate that SP antagonists block all three classes of mammalian BB receptors. Also, SP antagonists block the ability of BB or vasopressin to stimulate proliferation (64), inhibit the action of cholecystokinin in pancreatic acinar cells (60), inhibit the growth stimulatory effects of fetal bovine serum or bradykinin in human lung cancer cells (65), and inhibit binding to the receptor for interleukin-8 or the chemokine, CXCRI (66). These data indicate that SP antagonists have a broad antagonist spectrum inhibiting the action of SP, GRP, NMB, CCK and AVP. Recently, one such broad spectrum antagonist [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]SP has been reported (66) to function as a biased agonist rather than a pure receptor antagonist.

SP antagonists inhibit the growth of SCLC *in vitro* and *in vivo* causing SCLC cells to undergo apoptosis (67). Recently, [D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}]SP (antagonist D) and [D-Arg^{6},D-Trp^{7,9},MePhe^{8}]SP(6-11) (antagonist E) were synthesized and found to be approximately 1 order of magnitude more potent than (RPWWL)SP. Antagonist D, which causes apoptosis of SCLC cells, slows SCLC and other cancer xenografts in nude mice; however, high doses are required *in vivo* (20 mg/kg s.c.) (68). Antagonist D is metabolized by blood enzymes resulting in deamidation and removal of the C-terminal amino acid. Also, the C-terminal methionine can be oxidized. The half-life of antagonist D is 1 hour in the mouse blood. Because of favorable pharmacokinetic data, antagonist D is scheduled for phase I clinical trials in Europe.

[D-Phe^{12}]BB analogs

Due to the broad spectrum of the SP antagonists, more specific BB receptor antagonists were needed. The first of these identified was (D-Phe^{12})BB, in which an essential His was replaced with D-Phe (69). (D-Phe^{12})BB inhibited (^{125}I-Tyr^{4})BB binding to guinea pig pancreatic cells with an IC_{50} value of 1 μM (Table III) (69). Also, 10 μM (D-Phe^{12})BB antagonized the ability of 1 nM BB to stimulate amylase secretion from pancreatic acinar cells.

Table III: Binding to SCLC cells.

Peptide	^{125}I-GRP binding IC_{50} (nM)	(^{125}I-Tyr^{0})NMB binding IC_{50} (nM)
BB	3	50
GRP	2	100
NMB	100	2
(RPWWL)SP	1000	1000
(D-Phe^{12})BB	500	>3000
(ψ^{13,14},Leu^{14})BB	30	>3000
BIM-26226	5	>3000
BW2258U89	10	>3000

The IC_{50} to inhibit ^{125}I-GRP or (^{125}I-Tyr^{0})NMB using NCI-H345 cells is indicated. Structures of each of the antagonists are shown in Table II.

Because (D-Phe^{12})BB bound to GRP receptors with low affinity it was not very potent *in vivo*. Other substitutions in position 12 of BB, including D-*p*-chlorophenylalanine, or in other positions such as [D-Phe^{6,12}]BB only improved affinity 3-fold (70). Therefore, the utility of this class of antagonists was limited by their low affinity. Subsequently, other classes of peptides were developed which functioned as high affinity GRP receptor antagonists.

Reduced peptide bond GRP receptor antagonists

The first member of this class was (ψ^{13,14},Leu^{14})BB (71). In this peptide the amide bond (O=C-NH) is reduced (CH_2-NH) between the 13 and 14 positions of BB. (ψ^{13,14},Leu^{14})BB inhibited (^{125}I-Tyr^{4})BB binding to pancreatic acinar cells with an IC_{50} value of 500 nM (71). Also, it antagonized the ability of 1 nM BB to stimulate amylase release from pancreatic acini (71). Thus, the C-terminal of BB can be modified to generate GRP receptor antagonists.

(ψ^{13,14},Leu^{14})BB inhibited (^{125}I-Tyr^{4})BB binding to NCI-H345 lung cancer cells with an IC_{50} value of 30 nM (72). Also, 1 μM (ψ^{13,14},Leu^{14})BB antagonized the ability of 10 nM BB to stimulate PI turnover in small cell lung cancer cells (72). In these cells, GRP receptor activation natively causes increased cellular levels of IP_3 which stimulates calcium release from intracellular organelles such as the endoplasmic reticulum. It also stimulates the generation of diacylglycerol which activates protein kinase C (52) (Fig. 1). One μM (ψ^{13,14},Leu^{14})BB inhibits the translocation of PKC from the cytosol to the plasma membrane caused by BB. In turn, PKC phosphorylates protein substrates on serine and threonine amino acid residues such as raf. Activated raf may phosphorylate protein substrates such as MAP kinase kinase which in turn phosphorylates MAP kinase (74). Activated MAP kinase may enter the nucleus and alter phosphorylation of proteins such as elk-1 altering the expression of early oncogenes such as *c-fos* and *c-jun*. In small cell lung cancer cells BB stimulates *c-fos* and *c-jun* gene expression and the increases caused by 10 nM BB are blocked by 1 μM (ψ^{13,14},Leu^{14})BB (75).

Because the N-terminal of BB is not essential for high affinity binding, (ψ^{13,14},Leu^{14})BB fragments were developed which lacked the N-terminal (76-78). BW2258U89 [3-PhPr[D-Ala^{24},Pro^{26},ψ$^{26-27}$,Phe^{27}]GRP$^{20-27}$] and (Tpi^{6}, ψ^{13,14},Tpi^{14})BB$^{6-14}$ bound to the GRP receptor with higher affinity than (Psi^{13,14},Leu^{14})BB (Table III). BW2258U89 blocked the increase in cytosolic Ca^{2+} and proliferation caused by 10 nM BB in small cell lung cancer cells (79). BW2258U89 decreased SCLC cytosolic Ca^{2+} and colony formation caused by 10 nM BB in small cell lung cancer cells (79). In contrast, 1 μM BW2258U89 had no effect on cytosolic Ca^{2+} and proliferation caused by NMB (79). These data indicate that BW2258U89 is a GRP but not an NMB receptor antagonist.

Studies of various BB and GRP pseudopeptides, as well as [des-Met^{14}]BB receptor antagonists discussed in

the next section, demonstrate important species variation in agonist/antagonist activity (20, 51, 80). In general, the requirements for receptor activation are less stringent in the rat GRP receptor than in guinea pig or mouse. Therefore, a number of pseudopeptide analogs or [des-Met^{14}]BB analogs that function as full antagonists in the guinea pig or mouse had full or partial agonist activity in rat (20). These differences in observed responses in different species have led to the development of BB pseudopeptide analogs or [des-Met^{14}]BB analogs that function as pure antagonists in each species (20, 51, 78).

($\psi^{13,14}$,Leu^{14})BB and BW2258U89 (0.4 mg/kg) slowed SCLC xenograft formation by 50% and 75%, respectively, *in vivo* (81). When BW2258U89 was put into microspheres and slowly released over a 3-week period, it strongly inhibited SCLC xenograft growth by 90% (82). These data indicate that BB-like peptides are autocrine growth factors for SCLC. No toxicity was observed by BW2258U89 administration and the half-life of BW2258U89 in the serum was approximately 2 hours (83). SP antagonist D was less potent than BW2258U89 in that 20 mg/kg was required to inhibit SCLC xenograft proliferation. Similar to [D-Arg^{6},D-Trp^{7,9},Me-Phe^{8}]SP-(6-11), BW2258U89 is slowly deamidated by mouse blood enzymes to an inactive product. These data indicate that BW2258U89 has an appreciable half-life *in vivo*.

GRP receptor pseudopeptide antagonists may be used to inhibit the growth of cancers other than SCLC. RC-3095 slowed the growth of HT-29 human colon cancers, PC-82 human prostate cancers, NKN45 human gastric cancers, MXT breast cancers and human pancreatic cancers (84-86). These data indicate that the growth of many cancers may be slowed by GRP receptor pseudopeptide antagonists.

GRP receptor pseudopeptide antagonists may also function as chemopreventive agents. RC-3095 inhibited pancreatic carcinogenesis induced by nitrosamine in hamsters (87). Similarly, BW2258U89, a [des-Met^{14}]BB receptor antagonist, prevented lung carcinogenesis induced by urethane in A/J mice (83). These data suggest that BB-like peptides may promote lung carcinogenesis and that BB receptor antagonists are chemopreventive agents.

(Des-Met^{14})BB GRP receptor antagonists

Several (des-Met)BB analogs have been developed which function as potent GRP receptor antagonists, including BIM-26226 (Table II), Ac-GRP$^{20-26}$ ethyl ester and (isobutyryl-His^{20},D-Ala^{24})GRP$^{20-26}$ methylamide (88-90). BIM-26226 inhibited ^{125}I-GRP binding to the SCLC cell NCI-H345 cells with high affinity (IC_{50} = 5 nM). BIM-26226 inhibited the increase in cytosolic Ca^{2+} and proliferation caused by 10 nM BB. High doses of BIM-26226 (4 mg/kg day) were required to slow xenograft growth in nude mice. These data indicate that BIM-26226 is much better at inhibiting SCLC proliferation *in vitro* than *in vivo*, which may be because BIM-26226 is rapidly metabolized in and/or cleared from the blood. However, [des-Met^{14}]BB analogs are still being used *in vivo* (91) including in humans to address the importance of GRP in various physiologic responses (92, 93). For example, using the antagonist BIM-26226 by continuous infusion in humans, GRP has now been shown to be involved in physiological regulation of gastric emptying and acid secretion (92, 93).

Molecular basis of GRP receptor agonist and antagonist interactions

Extensive structure-activity studies have been performed on the GRP-related peptides (13, 16, 22, 94-100). For agonist activity the carboxyl terminal amide is essential for high affinity interaction (28). The N-terminal of either BB or GRP is not essential for high affinity interaction and GRP can be shortened to GRP$^{14-27}$ or BB to [D-Phe^{6}]BB(6-14) with almost no loss of agonist potency (20, 52). Substitutions of D- or L-amino acids at Trp^{8}, Val^{10} or His^{12} of BB result in a loss of high affinity for the GRP receptor (8, 16). The minimal fragment with biologic activity is the C-terminal heptapeptide of BB (22, 94).

The active conformation of BB or GRP for interacting with GRP receptors may be a β-sheet (71, 80). There is a β-turn at position 10-13 of BB and hydrogen bonds may exist between the COOH-terminal Leu^{14} amide NH_2 and the Trp^{8} carboxyl oxygen between the Leu^{13} carboxyl oxygen and the Val^{10} N-H, and between the Leu^{13} N-H and the Val^{10} carboxyl oxygen (Fig. 2, top). Support for this model by structure-activity studies and computer modeling has been reported in one study (101); however, an alternative model has been proposed involving five internal hydrogen bonds, with three consecutive inverse γ-turns, and a bend followed by two more inverse γ-turns (100).

The reduction in the carboxyl group between the Leu^{13} Met^{14} results in an antagonist (analog B, Fig. 2, bottom) by impairing the position 14 carboxamide group in the receptor-bound peptide (71). A similar result occurs when the position 14 carboxamide is removed such as in the [des-Met^{14}]BB (analog C, Fig. 2, bottom). Alkyl substituents on the position 13 NH_2 group dramatically improve binding affinity and antagonist as a result of electron-release (analog D, Fig. 2 bottom) (80). The much reduced antagonist potency of the position 13 free carboxy analog occurs because electrons are distributed over two CO groups (analog E, Fig. 2, bottom).

Recent GRP receptor structure-activity studies have begun to provide significant insights into the important determinants of high affinity agonist and antagonist interaction (51, 102, 103). In a recent study (104), the amino acid alignment of the frog bombesin receptor subtype 4, the NMB receptor (human, rat) and the GRP receptor (rat, human), all of which have high affinity for GRP and BB, was compared to that of the BRS-3 receptor (guinea pig, human) which has low affinity for these two naturally occurring agonists. Nine amino acids were identified

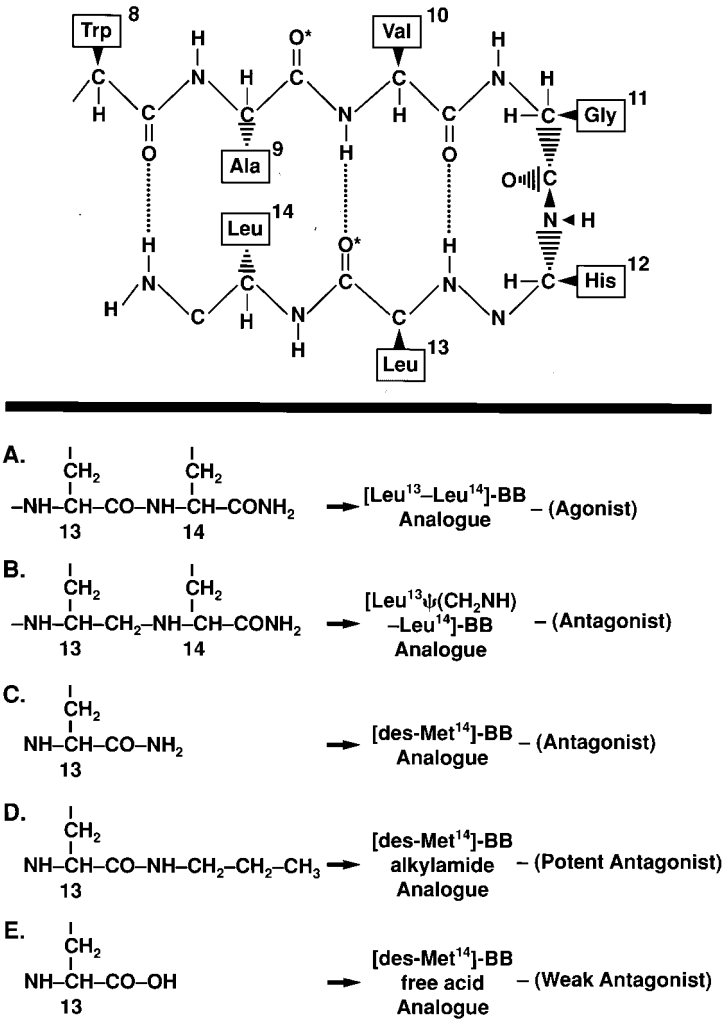

Fig. 2. Structure of the C-terminal of bombesin (top) and structural modifications altering agonist activity (bottom). *Top panel:* Possible conformation for the COOH-terminal octapeptide region of [Leu^{14}]bombesin with a type II' β-bend involving the Val-Gly-His-Leu tetrapeptide. Carbonyl groups (*) which produce antagonists when replaced by CH_2 and putative intramolecular hydrogen bonding interactions along the chain (dotted lines) are shown. *Bottom panel:* COOH-terminal structural modifications to bombesin that are responsible for either receptor agonist or antagonist activity. (A) Bombesin with Leu^{13} and Met^{14} (potent agonist); (B) [Leu^{13}ψ(CH_2NH)-Leu^{14}]BB with the position 13 CO replaced by CH_2 (antagonist); (C) des-Met^{14}-amidated analogs (antagonist); (D) des-Met^{14} alkylamide analogs (more potent antagonists); (E) des-Met^{14} free acid analog (very weak antagonist).

which differed between these two classes of receptors. The importance of each of these amino acids was investigated by sitedirected mutagenesis (104). Substitutions in position Glu^{121}, Pro^{199}, Ala^{308} and Arg^{288} (Fig. 3) resulted in a marked decrease in BB receptor affinity (104). By molecular modeling (104) it was predicted that side-chains of these amino acids form a pocket between transmembranes III, VI and VII.

In contrast, the preference of the antagonist [D-Phe^{6}]BB(6-13)methyl ester for the GRP receptor over the NMB receptor is due to the 4th extracellular loop (104). Thr^{297},Phe^{301} and Ser^{305} were the key amino acids in determining the selectivity of this antagonist for the GRP receptor (Fig. 3) (102, 103). The 4th extracellular loop was not important in determining the selectivity of the agonist GRP, demonstrating that different domains of the GRP receptor are responsible for antagonist and agonist selectivity.

Neuromedin B receptor antagonists

In addition to antagonizing GRP receptors, SP antagonists function as receptor antagonists of NMB receptors (21, 57). In fact, these antagonists have 3- to 10-fold selectivity for NMB over GRP receptors (21, 57). However, because their affinity remains relatively low (K_d >1 μM) for NMB receptors and they have low selectivity (as discussed previously under GRP receptors), D-amino

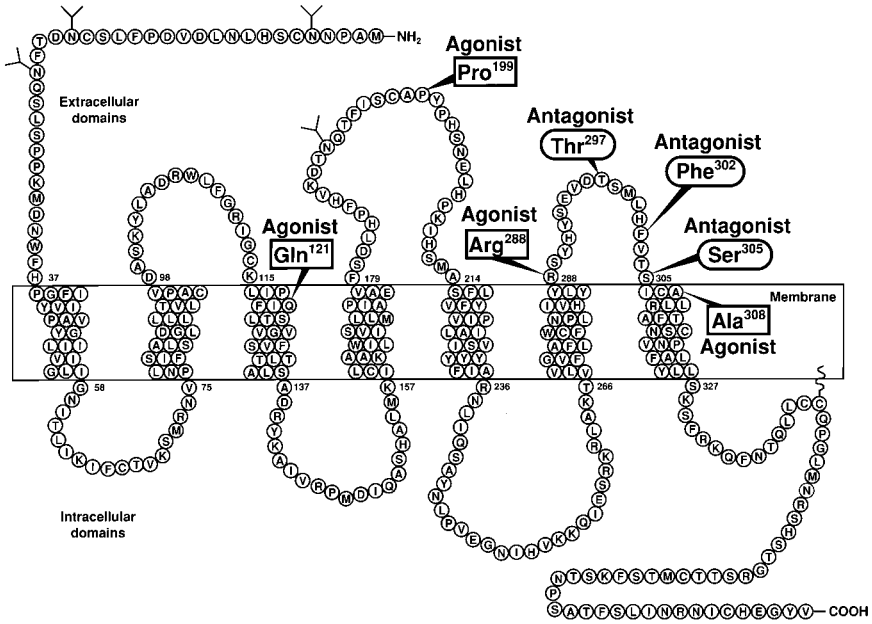

Fig. 3. Structure of the murine GRP receptor and important amino acids determining high affinity agonist activity or selectivity of the GRP receptor antagonist [D-Phe^6]Bn$^{6-13}$ methyl ester. The predicted 7-transmembrane spanning conformation of the GRP receptor is shown with the four extracellular domains and four putative intracellular domains. The four *N*-glycosylation sites are indicated by a Y and the putative palmitoylation site in the COOH terminus is shown. Four recently described important amino acids required for high affinity (104) agonist binding (Gln^{121},Pro^{199},Arg^{288},Ala^{308}) are shown by the dark squares and labeled "agonist". The location of the three amino acids recently demonstrated (104) to be important for high affinity interaction of the antagonist, [D-Phe^6]Bn$^{6-13}$ methyl ester [Thr^{297},Phe^{302},Ser^{305}] are shown by the dark circles labeled "antagonist" (102, 103).

acid-substituted SP analogs have not been widely used to inhibit NMB receptors. A surprising finding was that somatostatin (SRIF) agonists such as (Nal^{16},Thr^8)cyclo somatostatin analogs function as selective low affinity NMB receptor antagonists (56). D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Nal-NH_2 inhibited binding of (^{125}I-Tyr^0)NMB with an IC_{50} value of 100 nM and had >100-fold selectivity for the NMB over the GRP receptor (56). Also, D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Nal-NH_2 antagonized the ability of 10 nM NMB to elevate cytosolic Ca^{2+} (56). This D-amino acid substituted somatostatin octapeptide analog also functioned as a somatostatin receptor agonist and interacted with mu-opioid receptors (56). To reduce the affinity for μ-opioid receptors an ornithine analog, D-Nal-Cys-Tyr-D-Trp-Orn-Val-Cys-Nal-NH_2, was synthesized (105). This analog has recently been used to demonstrate that activation of NMB receptors regulate satiety in rats (105). Recently, nonpeptide antagonists such as PD165929 were developed (55). Upon substitution of Ala for amino acids in NMB, it was found that the Trp^4, Val^6 and Phe^9 were essential for activity. Subsequently, a small molecule template was developed which contains appended Trp, Val and Phe. The 2-pyridyl analog, PD165929, inhibited binding to NMB receptors with an IC_{50} value of 15 nM but had little effect on GRP receptor binding (IC_{50} >10,000 nM) (55). Also, PD165929 antagonized the ability of NMB to cause acidification in human NMB receptor transfected CHO cells and the ability of NMB to increase Cl^- currents in human NMB receptor containing xenopus oocytes (55).

Clinical studies

At present, clinical trials using BB antagonists have not begun. Phase I and phase II clinical trials have been conducted on 2A11, a murine monoclonal antibody which neutralizes BB (58). The antibody, similar to the receptor, recognizes the COOH-terminal of BB and has full cross-reactivity with GRP but does not recognize NMB. The antibody 2A11 inhibits (^{125}I-Tyr^4)BB interactions with the GRP receptor, and inhibited SCLC clonal growth and xenograft proliferation in nude mice. In a phase II trial, 12 human subjects with SCLC received a 4-week course of antibody 2A11. This resulted in a complete remission in 1 patient, stable disease in 4 patients and progressive disease in 6 others; 1 additional patient could not be evaluated (106). In the patient with remission, three 4-week courses of antibody 2A11 did not result in development of human anti-mouse antibodies. Despite initial remission, this patient suffered relapse and died from SCLC.

Summary

Three mammalian receptors have been cloned for the BB family of peptides. The best studied is the GRP receptor which binds BB and GRP with high affinity. The NMB receptor binds NMB with high affinity, whereas the BRS-3 receptor does not bind NMB, BB or GRP with high affinity.

Numerous peptide antagonists have been developed for the GRP receptor. (Des-Met^{14})BB analogs, such as BIM-26226, bind with high affinity and antagonize GRP receptors present on SCLC cells *in vitro*. Also, ($\psi^{13,14}$,Leu^{14})BB analogs have been developed which antagonize GRP receptors *in vitro* and *in vivo*. Currently, the pharmacokinetics and biodistribution of these antagonists are being studied. A general problem with peptide antagonists is their metabolism. The ($\psi^{13,14}$,Leu^{14})BB analog BW2258U89 (half-life of 2 h) is deamidated by blood enzymes to an inactive product. One potential way to avoid degradation problems is to develop nonpeptide antagonists.

While this has not been done for GRP receptors, a nonpeptide NMB receptor antagonist is available for *in vitro* studies. PD165929 inhibits binding to NMB receptors with high affinity but has little effect on GRP receptors. Further preclinical studies on the toxicity of PD165929 are required prior to clinical trials.

Recently, a new BB analog, [D-Phe^6,β-Ala^{11}, Phe^{13},Nle^{14}]BB(6-14) has been synthesized which binds with high affinity to BRS-3 receptors (67), as well as the GRP receptor, NMB receptor and the frog bombesin receptor subtype 4, BB_4 (50). Using [D-Phe^6,β-Ala^{11},Phe^{13},Nle^{14}]BB(6-14), the signal transduction cascade for the BRS-3 receptor has now been elucidated (49). It has been shown to be coupled to phospholipase C with activation causing increases in inositol phosphates, mobilization of calcium from cellular stores and stimulation of tyrosine phosphorylation of p125 focal adhesion kinase by a phospholipase C-dependent mechanism (49). Specific antagonists have yet to be developed for the BRS-3 receptor.

A fourth subtype of bombesin receptor, the BB_4 receptor, has been described in frog brain (107). It is unknown at present whether a mammalian equivalent of this receptor exists. Furthermore, little is known about its pharmacology or cell biology.

Acknowledgements

The authors wish to thank Drs. J. Battey, D. Coy, F. Cuttitta, T. Davis, S. Jakowlew, J. Leban, J. McDermed, J. Taylor and F. Zia for their assistance.

References

1. Anastasi, A., Erspamer, V., Bucci, M. *Isolation and amino acid sequences of alytesin and bombesin: Two analogous active tetradecapeptides from the skin of European discoglossid frogs.* Arch Biochem Biophys 1973, 148: 443-6.

2. Jensen, R.T., Moody, T.W., Pert, C.B. et al. *Interaction of bombesin and litorin with specific membrane receptors on pancreatic acinar cells.* Proc Natl Acad Sci USA 1978, 75: 6139-43.

3. Gibbs, J., Fauser, D., Rowe, E. et al. *Bombesin suppresses feeding in rats.* Nature 1979, 282: 208-10.

4. Westendorf, J., Schonbrun, A. *Characterization of bombesin receptors in a rat pituitary cell line.* J Biol Chem 1983, 258: 7527-35.

5. Merali, Z. Johnston, S., Zakman, S. *Bombesin induced behavioral changes: Antagonism by neuroleptics.* Peptides 1983, 4: 693-9.

6. Brown, M., Rivier, J., Vale, W. *Bombesin: Potent effects on thermoregulation in rat.* Science 1978, 196: 998-1000.

7. Brown, M.R., Rivier, J., Vale, W. *Bombesin affects the central nervous system to produce hyperglycemia in rats.* Life Sci 1978, 21: 1729-34.

8. Moody, T.W., Pert, C.B., Rivier, J. et al. *Bombesin: Specific binding to rat brain membranes.* Proc Natl Acad Sci USA 1978, 75: 5372-6.

9. Willey, J.C., Lechner, J.F., Harris, C.C. *Bombesin and the C-terminal tetradecapeptide of gastrin-releasing peptide are growth factors for normal human bronchial epithelial cells.* Exp Cell Res 1984, 153: 245-8.

10. Rozengurt, E., Sinett-Smith, J. *Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells.* Proc Natl Acad Sci USA 1983, 80: 2936-40.

11. Carney, D.N., Gazdar, A.F., Bepler, G. et al. *Establishment and identification of small cell lung cancer cell lines having classic and variant features.* Cancer Res 1985, 45: 2913-23.

12. Minna, J.D., Sekido, Y., Fong, K.M. et al. *Molecular biology of lung cancer.* In: Cancer: Principles & Practice of Oncology, 5th Edition. V.T. DeVita, S. Hellman, S.A. Rosenberg (Eds.). Lippincott-Raven: Philadelphia 1997, 849-57.

13. Falconieri, E.G., Severini, C., Erspamer, V. et al. *Parallel bioassay of 27 bombesin-like peptides on 9 smooth muscle preparations. Structure-activity relationships and bombesin receptor subtypes.* Regul Pept 1988, 21: 1-11.

14. Minamino, N., Kangawa, M., Matsuo, H. *Neuromedin B: A novel bombesin-like peptide identified in porcine spinal cord.* Biochem Biophys Res Commun 1983, 114: 541-8.

15. McDonald, T.J., Jornvall, J., Nilsson, G. et al. *Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue.* Biochem Biophys Res Commun 1979, 90: 227-33.

16. Rivier, J., Brown, M. *Bombesin, bombesin analogues and related peptides: Effects on thermoregulation.* Biochemistry 1978, 17: 1766-77.

17. Spindel, E.R., Giladi, E., Brehm, J. et al. *Cloning and functional characterization of a cDNA encoding the murine fibroblast bombesin/GRP receptor.* Mol Endocrinol 1990, 4: 1956-63.

18. Battey, J.F., Way, J., Corjay, M.H. et al. *Molecular cloning of the bombesin/GRP receptor from Swiss 3T3 cells.* Proc Natl Acad Sci USA 1991, 88: 395-9.

19. Wada, E., Way, J., Shapira, H. et al. *cDNA cloning, characterization and brain region-specific expression of a neuromedin-B preferring bombesin receptor.* Neuron 1991, 6: 421-30.

20. Mantey, S., Frucht, H., Coy, D.H., Jensen, R.T. *Characterization of bombesin receptors using a novel potent, radiolabeled antagonist that distinguishes bombesin receptor subtypes.* Mol Pharmacol 1993, 43: 762-74.

21. Von Schrenck, T., Wang, L.H., Coy, D.H. et al. *Potent bombesin receptor antagonists distinguish receptor subtypes.* Am J Physiol 1990, 256: G747-58.

22. Lin, J.T., Coy, D.H., Mantey, S.A., Jensen, R.T. *Comparison of the peptide structural requirements for high affinity interaction with bombesin receptors.* Eur J Pharmacol 1995, 294: 55-69.

23. Spindel, E.R., Giladi, E., Segerson, T.P. et al. *Bombesin-like peptides: Of ligands and receptors.* Recent Prog Horm Res 1993, 48: 365-91.

24. Fathi, Z., Corjay, M.H., Shapira, H. et al. *BRS-3: A novel bombesin receptor subtype selectively expressed in testis and lung carcinoma cells.* J Biol Chem 1993, 268: 5979-84.

25. Spindel, E.R., Chin, W.W., Price, J. et al. *Cloning and characterization of cDNAs encoding human gastrin releasing peptide.* Proc Natl Acad Sci USA 1984, 81: 5699-703.

26. Sausville, E., Lebacq-Verheyden, A.M., Spindel, E.R. et al. *Expression of the gastrin releasing peptide gene in human small cell lung cancer.* J Biol Chem 1986, 261: 2451-9.

27. Krane, I.M., Naylor, S.L., Helin-Davis, D. et al. *Molecular cloning of cDNAs encoding human bombesin-like peptide neuromedin B.* J Biol Chem 1988, 263: 13317-23.

28. Mervic, M., Moody, T.W., Komoriya, A. *A structure-function study of bombesin's C-terminal domain.* Peptides 1991, 12: 1315-9.

29. Moody, T.W., Pert, C.B., Gazdar, A.F. et al. *High levels of intracellular bombesin characterize human small cell lung carcinoma.* Science 1981, 214: 1246-8.

30. Korman, L.Y., Carney, D.N., Citron, M.L. et al. *Secretin/VIP stimulated secretion of bombesin-like peptides from human small cell lung cancer.* Cancer Res 1986, 46: 1214-8.

31. Moody, T.W., Leyton, J., Coelho, T. et al. *(Stearyl, Norleucine^{17})VIP hybrid is a potent nonsmall cell lung cancer VIP receptor antagonist.* Life Sci 1997, 53: 1657-66.

32. Kee, K.A., Finan, T.M., Korman, L.Y. et al. *Somatostatin inhibits the secretion of bombesin-like peptides from small cell lung cancer cells.* Peptides 1988, 9: 257-61.

33. Tsuda, T., Kusui, T., How, W. et al. *Effect of gastrin releasing peptide receptor number on receptor affinity, coupling, degradation and modulation.* J Pharmacol Exp Ther 1997, 51: 721-32.

34. Vanderspek, J.C., Sutherland, J.A., Zeng, H. et al. *Inhibition of protein synthesis in small cell lung cancer cells induced by the diptheria toxin-related fusion protein $DAB_{389}GRP$.* Cancer Res 1997, 57: 290-4.

35. Bunnett, N.W., Kobayashi, R., Orloff, M.S. et al. *Catabolism of gastrin releasing peptide and substance P by gastric membrane-bound peptidases.* Peptides 1986, 6: 277-83.

36. Gorbulev, V., Akhundova, A., Buchner, H. et al. *Molecular cloning of a new bombesin receptor subtype expressed in uterus during pregnancy.* Eur J Biochem 1992, 208(2): 405-10.

37. Tache, Y., Melchiorri, P., Negri, L. *Bombesin-like peptides in health and disease.* Ann NY Acad Sci 1988, 547: 1-541.

38. Battey, J., Wada, E. *Two distinct receptors for mammalian bombesin-like peptides.* Trends Neurosci 1991, 14(12): 524-8.

39. Vigna, S.R., Mantyh, C.R., Giraud, A.S. et al. *Specific binding sites for bombesin in the canine gastrointestinal tract.* Gastroenterology 1987, 93: 1287-95.

40. Ohki-Hamazaki, H., Watase, K., Yamamoto, K. et al. *Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity.* Nature 1997, 390(6656): 165-9.

41. Gorbulev, V., Akhundova, A., Grzeschik, K.H., Fahrenholz, F. *Organization and chromosomal localization of the gene for the human bombesin receptor subtype expressed in pregnant uterus.* FEBS Lett 1994, 340(3): 260-4.

42. Ladenheim, E.E., Wirth, I.K.E., Moran, T.H. *Receptor subtype mediation of feeding suppression by bombesin-like peptides.* Pharmacol Biochem Behav 1996, 54: 705-11.

43. Ladenheim, E.E., Taylor, J.E., Coy, D.H. et al. *Caudal hindbrain neuromedin B-preferring receptors participate in the control of food intake.* Am J Physiol 1997, 272: R433-7.

44. Ladenheim, E.E., Guss, J.L. *Bombesin receptor subtypes: Implications in bombesin satiety.* Appetite 1995, 24: 261.

45. Lieverse, R.J., Jansen, J.B., Vande Zwan, A. et al. *Bombesin reduces food intake in lean man by a cholecystokinin-independent mechanism.* J Clin Endocrinol Metab 1993, 76: 1495-8.

46. Hampton, L.L., Landenheim, E.E., Akeson, M. et al. *Loss of bombesin-induced feeding suppression in gastrin-releasing peptide receptor-deficient mice.* Proc Natl Acad Sci USA 1998, 95: 3188-92.

47. Mantey, S.A., Weber, H.C., Sainz, E. et al. *Discovery of a high affinity radioligand for the human orphan receptor, bombesin receptor subtype 3, which demonstrates that it has a unique pharmacology compared with other mammalian bombesin receptors.* J Biol Chem 1997, 272: 26062-71.

48. Wu, J.M., Nitecki, D.E., Biancalana, S., Feldman, R.I. *Discovery of high affinity bombesin receptor subtype 3 agonists.* Mol Pharmacol 1996, 50(5): 1355-63.

49. Ryan, R.R., Weber, H.C., Hou, W. et al. *Ability of various bombesin receptor agonists and antagonists to alter intracellular signaling of the human orphan receptor BRS-3.* J Biol Chem 1998, 273: 13613-24.

50. Pradhan, T.K., Katsuno, T., Taylor, J.E. et al. *Identification of a unique ligand which has high affinity for all four bombesin receptor subtypes.* Eur J Pharmacol 1998, 343: 275-87.

51. Jensen, R.T., Coy, D.H. *Progress in the development of potent bombesin receptor antagonists.* Trends Pharmacol Sci 1991, 12(1): 13-9.

52. Moody, T.W., Jensen, R.T. *Bombesin/GRP and vasoactive intestinal peptide/PACAP as growth factors.* In: Growth Factors and Cytokines in Health and Disease. D. LeRoith, C. Bondy (Eds.). JAI Press: Greenwich, CT 1996, 491-535.

53. De Castiglione, R., Gozzini, L. *Bombesin receptor antagonists.* Crit Rev Oncol Hematol 1996, 24: 117-51.

54. Jensen, R.T., Mrozinski, J.E. Jr., Coy, D.H. *Bombesin receptor antagonists: Different classes and cellular basis of action.* Recent Results Cancer Res 1993, 129: 87-113.

55. Eden, J.M., Hall, M.D., Higginbottom, M. et al. *PD165929 - The first high affinity non-peptide neuromedin B (NMB) receptor selective antagonist.* Bioorg Med Chem Lett 1996, 6: 2617-23.

56. Orbuch, M., Taylor, J.E., Coy, D.H. et al. *Discovery of a novel class of neuromedin B receptor antagonists, substituted somatostatin analogues.* Mol Pharmacol 1993, 44: 841-50.

57. Lin, J.T., Coy, D.H., Mantey, S.A., Jensen, R.T. *Peptide structural requirements for antagonism differ between the two mammalian bombesin receptor subtypes.* J Pharmacol Exp Ther 1995, 275: 285-95.

58. Cuttitta, F., Carney, D.N., Mulshine, J. et al. *Bombesin-like peptides can function as autocrine growth factors in human small cell lung cancer.* Nature 1985, 316: 823-5.

59. Jensen, R.T., Jones, S.W., Folkers, K. et al. *A synthetic peptide that is a bombesin receptor antagonist.* Nature 1984, 309: 61-3.

60. Jensen, R.T., Heinz-Erian, P., Mantey, S. et al. *Characterization of the ability of various substance P antagonists to inhibit action of bombesin.* Am J Physiol 1998, 254: G883-90.

61. Bepler, G., Zeymer, U., Mahmoud, S. et al. *Substance P analogues function as bombesin receptor antagonists and inhibit small cell lung cancer clonal growth.* Peptides 1988, 9: 1367-72.

62. Woll, P.J., Rozengurt, E. *(D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11}) Substance P, a potent bombesin antagonist in murine Swiss 3T3 cells, inhibits the growth of human small cell lung cancer.* Proc Natl Acad Sci USA 1988, 85: 1859-63.

63. Bunn, P.A., Dienhart, D.G., Chan, D. et al. *Neuropeptide stimulation of calcium flux in human lung cancer cells: Delineation of alternative pathways.* Proc Natl Acad Sci USA 1990, 87: 2162-6.

64. Sethi, T., Langdon, S., Smyth, J. et al. *Growth of small cell lung cancer cells: Stimulation by multiple neuropeptides and inhibition by broad spectrum antagonists in vitro and in vivo.* Cancer Res 1992, 52: 2737-42s.

65. Bunn, P.A. Jr., Chan, D., Stewart, J. et al. *Effects of neuropeptide analogues on calcium flux and proliferation in lung cancer cell lines.* Cancer Res 1994, 54: 3602-10.

66. Jarpe, M.B., Knail, C., Mitchell, F.M. et al. *(D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11})Substance P acts as a biased agonist toward neuropeptide and chemokine receptors.* J Biol Chem 1998, 273: 3097-104.

67. Reeve, J.G., Bleehen, N.M. *(D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11})-Substance P induces apoptosis in lung cancer cell lines in vitro.* Biochem Biophys Res Commun 1994, 199: 1313-9.

68. Jones, D.A., Cummings, J., Langdon, S.P. et al. *Metabolism of the broad spectrum neuropeptide growth factor antagonist (D-Arg^{1},D-Phe^{5},D-Trp^{7,9},Leu^{11})substance P.* Br J Cancer 1996, 73: 715-20.

69. Heinz-Erian, P., Coy, D.H., Tamura, M. et al. *(D-Phe^{12})bombesin analogues: A new class of bombesin receptor antagonists.* Am J Physiol 1987, 254: G883-90.

70. Saeed, Z.A., Huang, S.C., Coy, D.H. et al. *Effect of substitutions in position 12 of bombesin on antagonist activity.* Peptides 1989, 10(3): 597-603.

71. Coy, D.H., Heinz-Erian, P., Jiang, N.Y. et al. *Probing peptide backbone function in bombesin: A reduced peptide bond analogue with potent and specific receptor antagonist activity.* J Biol Chem 1988, 263: 5056-60.

72. Mahmoud, S.M., Palaszynski, E., Fiskum, G. et al. *Small cell lung cancer bombesin receptors are antagonized by reduced peptide bond analogues.* Life Sci 1989, 44: 367-73.

73. Trepel, J.B., Moyer, J.D., Cuttitta, F. et al. *A novel bombesin receptor antagonist inhibits autocrine signals in a small cell lung carcinoma cell line.* Biochem Biophys Res Commun 1988, 156: 1383-9.

74. Seufferlein, T., Rozengurt, E. *Galanin, neurotensin and phorbol esters rapidly stimulate activation of mitogen-activated protein kinase in small cell lung cancer cells.* Cancer Res 1996, 56: 5758-64.

75. Draoui, M., Chung, P., Park, M. et al. *Bombesin stimulates c-fos and c-jun mRNAs in small cell lung cancer cells.* Peptides 1995, 16: 289-92.

76. Leban, J.J., Kull, F.C. Jr., Landavazo, A. et al. *Development of potent gastrin-releasing peptide antagonists having a D-Pro-Psi-(CH_2NH)-Phe-NH_2C terminus.* Proc Natl Acad Sci USA 1993, 90: 1922-6.

77. Coy, D.H., Taylor, J.E., Jiang, N.Y. et al. *Short-chain pseudopeptide bombesin receptor antagonists with enhanced binding affinities for pancreatic acinar and Swiss 3T3 cells display strong antimitotic activity.* J Biol Chem 1989, 264: 14691-7.

78. Coy, D., Wang, L.H., Jiang, N.Y., Jensen, R. *Short chain bombesin pseudopeptides with potent bombesin receptor antagonistic activity in rat and guinea pig pancreatic acinar cells.* Eur J Pharmacol 1990, 190: 31-8.

79. Moody, T.W., Venugopal, R., Gozes, Y. et al. *BW1023U90: A new GRP receptor probe for small cell lung cancer cells.* Peptides 1996, 17: 1337-43.

80. Wang, L.H., Coy, D.H., Taylor, J.E. et al. *Desmethionine alkylamide bombesin analogues: A new class of bombesin receptor antagonists with a potent antisecretory activity in pancreatic acini and antimitotic activity in Swiss 3T3 cells.* Biochemistry 1990, 29(3): 616-22.

81. Mahmoud, S., Staley, J., Taylor, J. et al. *($\psi^{13,14}$)Bombesin analogues inhibit the growth of small cell lung cancer in vitro and in vivo.* Cancer Res 1991, 51: 1798-802.

82. Moody, T.W., Venugopal, R., Zia, F. et al. *BW2258U89: A GRP receptor antagonist which inhibits small cell lung cancer growth.* Life Sci 1995, 50: 521-9.

83. Marquez, C., Treston, A., You, L. et al. *Chemoprevention of lung adenomas by BW2258U89.* Proc Am Assoc Cancer Res 1996, 37: 270.

84. Thomas, F., Arvelo, F., Antoine, E. et al. *Antitumoral activity of bombesin analogues on small cell lung cancer xenografts: Relationship with bombesin receptor expression.* Cancer Res 1992, 52: 4872-7.

85. Pinski, J., Halmos, G., Yano, T. et al. *Inhibition of growth of MKN human gastric adenocarcinoma xenografts in nude mice by treatment with gastrin releasing peptide antagonist (RC-3095) and somatostatin analogue RC-160.* Int J Cancer 1994, 57: 574-80.

86. Radulovic, S., Miller, G., Schally, A.V. *Inhibition of growth of HT-29 human colon cancer xenografts in nude mice by treatment with bombesin/gastrin releasing peptide antagonist (RC-3095).* Cancer Res 1991, 51: 6006-9.

87. Szepeshazi, K., Schally, A.V., Groot, K. et al. *Effect of bombesin, gastrin releasing peptide (GRP(14-27)) and bombesin/GRP receptor antagonist RC-3095 on growth of nitrosamine induced pancreatic cancers in hamsters.* Int J Cancer 1993, 54: 282-9.

88. Wang, L.H., Coy, D.H., Taylor, J.E. et al. *Des-Met carboxyl-terminally modified analogues of bombesin function as potent bombesin receptor antagonists, partial agonists or agonists.* J Biol Chem 1990, 265: 15695-703.

89. Heimbrook, D., Saari, W.S., Balishin, N.L. et al. *Carboxyl-terminal modification of a gastrin releasing peptide derivative generates potent antagonists.* J Biol Chem 1989, 2640: 11258-62.

90. Camble, R., Cotton, R., Dutta, et al. *N-Isobutyryl-His-Trp-Ala-Val-D-Ala-His-Leu-NHMe (ICI 216140), a potent in vivo*

antagonist analogue of bombesin/gastrin releasing peptide (BN/GRP) derived from the C-terminal sequence lacking the final methionine residue. Life Sci 1989, 45: 1521-7.

91. Varga, G., Reidelberger, R.D., Liehr, R.M. et al. *Effects of potent bombesin antagonist on exocrine pancreatic secretion in the rat.* Peptides 1991, 12: 493-7.

92. Hildebrand, P., Rossi, L., Meyer, M. et al. *Effect of BIM26226, a potent and specific bombesin/GRP receptor antagonist, on gastrointestinal functions in man.* Gastroenterology 1993, 104: A308.

93. Hildebrand, P., Ketterer, S., Stringelin, T. et al. *GRP receptor antagonist inhibits gastric acid secretion in man without altering plasma gastrin release.* Gastroenterology 1996, 110: A1080.

94. Broccardo, M., Erspamer, G.F., Melchiorri, P. et al. *Relative potency of peptides.* Br J Pharmacol 1976, 55: 221-7.

95. Girard, F., Aube, C., St.-Pierre, S., Jolicoeur, F.B. *Structure-activity studies on neurobehavioral effects of bombesin (BB) and gastrin releasing peptide (GRP).* Neuropeptides 1983, 3: 443-52.

96. Girard, F., Bachelard, H., St.-Pierre, S., Rioux, F. *The contractile effect of bombesin, gastrin releasing peptide and various fragments in the rat stomach strip.* Eur J Pharmacol 1984, 102: 489-97.

97. Guard, S., Watling, K.J., Howson, W. *Structure-activity requirements of bombesin for gastrin-releasing peptide- and neuromedin B-preferring bombesin receptors in rat brain.* Eur J Pharmacol 1993, 240: 177-84.

98. Marki, W., Brown, M., Rivier, J.E. *Bombesin analogs: Effects on thermoregulation and glucose metabolism.* Peptides 1981, 2: 169-77.

99. Mukai, H., Tanaka, H., Goto, K. et al. *Structure-activity relationships of mammalian bombesin-like neuropeptides in the contraction of rat uterus.* Neuropeptides 1991, 19: 243-50.

100. Horwell, D.C., Howson, W., Naylor, D. et al. *Alanine scan and N-methyl amide derivatives of Ac-bombesin[7-14]. Development of a proposed binding conformation at the neuromedin B (NMB) and gastrin releasing peptide (GRP) receptors.* Int J Peptide Protein Res 1996, 48: 522-31.

101. Kull, F.C. Jr., Leban, J.J., Landaazo, K.D. et al. *Conveyance of partial agonism/antagonism to bombesin/gastrin-releasing peptide analogues on Swiss 3T3 cells by a carboxyl-terminal leucine insertion.* J Biol Chem 1992, 267: 21132-9.

102. Katsuno, T., Donohue, P.J., Akeson, M.A. et al. *Different extracellular domains of the gastrin-releasing peptide receptor (GRP-R) are crucial for determining affinity for peptide agonists and peptide antagonists.* Gastroenterology 1997, 112: A1161.

103. Katsuno, T., Pradhan, T.K., Mantey, S.A. et al. *Elucidation of receptor domains of gastrin-releasing peptide receptor (GRPR) determining high selectivity for peptide antagonists.* Gastroenterology 1998, 114: A1153.

104. Akeson, M., Sainz, E., Mantey, S.A. et al. *Identification of four amino acids in the gastrin releasing peptide receptor that are required for high affinity agonist binding.* J Biol Chem 1997, 272: 17405-9.

105. Ladenheim, E.E., Taylor, J.E., Coy, D.H. et al. *Blockade of feeding inhibition by neuromedin B using a selective receptor antagonist.* Eur J Pharmacol 1994, 271: R7-9.

106. Kelly, M.J., Linnoila, R.I., Avis, I.L. et al. *Antitumor activity of a monoclonal antibody directed against gastrin-releasing peptide in patients with small cell lung cancer.* Chest 1997, 112: 256-61.

107. Nagalla, S.R., Barry, B.J., Creswick, K.C. et al. *Cloning of a receptor for amphibian [Phe^{13}]bombesin distinct from the receptor for gastrin-releasing peptide: Identification of a fourth bombesin receptor subtype (BB_4).* Proc Natl Acad Sci USA 1995, 92: 6205-9.