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Bombesin

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Gene symbol: GRP

1. General Information

Peptides of the bombesin family were first isolated from frog skin in the early 1970s. Bombesin, a 14 amino acid peptide was isolated from the skin of the amphibian Bombina bombina **(Figure 1)**.

Figure 1. *Bombina bombina* also known as the European fire-bellied toad. Image from *en.wikipedia.org.*

Its amino acid sequence was determined and it was shown to contain blocked amino and carboxyl terminals (1). Related peptides termed alytensin, ranatensin and litorin were isolated from other frogs (10). Extracts from this and related frogs (Bombina variegate) had shown a number of actions on vascular smooth muscle, gall bladder contraction, gastric and pancreatic secretion, uterine smooth muscle and renal function. Bombesin is the most potent known stimulator of gastrin release in dogs and humans and thereby stimulates gastric acid secretion (4). It also stimulates CCK release (11).

Using cDNA cloning the bombesin precursor was shown to contain 107 amino acids (44). The precursor contained a signal peptide sequence and one copy of bombesin followed by a typical processing site. Antibodies against bombesin stained cells in the mammalian GI tract suggesting a counterpart (41). The first mammalian bombesin like peptide was isolated from pig gastric tissue and named gastrin-releasing peptide (GRP) because it had a potent action to release gastrin (34). GRP is a 27 amino acid peptide and results from a 148 amino acid precursor (46). Other subsequently identified related mammalian molecules include Neuromedin B isolated from porcine spinal cord (35). Neuromedin C was also isolated from pig spinal cord (36) but is now known to be GRP 18-27 (22). All share a common carboxyl terminal sequence as shown in **Table 1**.

Table 1

1										14	
pGlu—	Glu—	Arg—	Leu —	Gly—	Asn—	Glu	—Trp—	Ala—	Val—	Gly—	His—	Leu—	Met—NH_2	Bombesin
—Met—	Tyr—	Pro—	Arg—	Gly—	Asn—	His	—Trp—	Ala—	Val—	Gly—	His—	Leu—	Met—NH_2	GRP
					Gly—	Asn—	His	—Trp—	Ala—	Val—	Gly—	His—	Leu—	Met—NH_2	NMC

The entire structure is shown except for GRP which has 27 amino acids (the first 13 amino acids are not shown). The GRP sequence shown is for human, pig and dog. The other bombesin related peptides have similarity but not identity to the carboxl terminal sequence.

For a review of early work purifying mammalian bombesin-like peptides see (51).

The bombesin/GRP/NMC receptor was first characterized by the high affinity binding of radioiodinated bombesin. The receptor was cloned from murine Swiss 3T3 cells (3, 45) and a distinct NMB receptor from rat esophagus (50). Analysis of the predicted amino acid sequence revealed 7 putative transmembrane domains and the receptors were latter shown to be G-protein coupled. Subsequently two other receptors, BRS-3 and BBE were identified (22, 39). These four receptors are broadly distributed especially in the GI tract and brain. The major form in the pancreas is the GRP-R also known as the BB_2 receptor (39). A number of bombesin receptor antagonists have also been developed most of which are modified bombesin peptides some having a reduced peptide bond (8, 16, 49). One of the most potent is [D-Phe^6]BN-(6-13) ethyl ester with a K_i of 5 nM to inhibit pancreatic amylase secretion stimulated by bombesin. Bombesin peptide agonists and antagonists have also been proposed as imaging tools or to deliver chemotherapeutic agents to tumors with bombesin receptors.

2. Bombesin and the Pancreas

Early studies indicated that bombesin administration that stimulated pancreatic secretion, also stimulated gastrin, CCK, and acetylcholine release (2, 11, 33). The secretion induced was enzyme rich and poor in bicarbonate. Thus, an initial question was whether bombesin stimulated the pancreas directly or through a neural or hormonal intermediate. However, studies using antrectomized dogs to remove gastrin, CCK antagonists and atropine have shown in multiple species including humans that the effect of bombesin was primarily direct (17, 18, 29). This was confirmed by the finding of specific bombesin receptors on isolated pancreatic acini and pancreatic AR42J cells (23, 30). In vivo studies also reported a trophic effect on the exocrine pancreas although not as big as the response to caerulein (48).

Even after the discovery of mammalian GRP, bombesin continued in common use for the study of cellular mechanisms using isolated acini. Bombesin and related peptides showed a slight biphasic dose response with reduced ability to stimulate in vitro amylase release from isolated guinea pig acinar cells at supramaximal concentrations; maximal secretion was observed at 100 nM (32, 47). Similar results but with maximal secretion at 100 pM were seen in mouse acini (21). By contrast in rat acini, bombesin induced a monophasic dose response with a maximal response at 1 nM (31, 38). Bombesin activated IP3 and diacylglycerol production and intracellular calcium release (9, 31, 43). Thus bombesin activates signaling pathways through heterotrimeric $G_{q/11}$ protein similar to CCK although by different receptors. Other actions of

bombesin shared with CCK have included activation of protein tyrosine kinases, PKC, phospholipase A_2, p125FAK, ERK, JNK, p70 S6K and downregulation of c-Met (5, 7, 19, 24, 38). Other differences between bombesin and CCK on acini are that bombesin induces less damage and ER stress (27). In some studies bombesin is used as an alternative agonist to show that an event is not initiated by a single receptor (6). Other studies have looked at the properties of bombesin receptors to internalize bombesin (54), their ability to induce residual stimulation (21), to desensitize (31), and to be regulated by CCK (53).

Bombesin by itself does not induce experimental pancreatitis in vivo or acinar cell damage in vitro similar to what is produced by caerulein (42,52). Bombesin increases the activation of intracellular trypsin and the processing of procarboxypeptidase A1 in isolated acini (14). However, the activated enzyme was secreted from the cell following bombesin stimulation thus plausibly explaining the lack of cell damage (14). By contrast, when bombesin stimulation was combined with pancreatic duct obstruction, retention of active enzymes and pancreatitis resulted (40). Another difference from caerulein is that bombesin failed to activate NF-κB (15).

Endogenenous GRP, the mamammalian equivalent of bombesin in the pancreas is primarily located in neurons. Immunoflourescence has localized it to both pancreatic ganglia and in beaded neurons running between acini (13, 25, 37). The pig however, was the only species to have a high concentration of immunoreative GRP in the pancreas (37). Using rat pancreatic lobules, GRP was shown to stimulate acetylcholine release and about half of the effect on amylase release was blocked by neuronal or ganglionic blockers (12). This suggests GRP may act both on neurons and acinar cells. Studies in the pig have shown that electrical stimulation of the vagus releases GRP and its active fragment, GRP (18-27) both in vivo and in the perfused pancreas (25, 26). Furthermore bombesin receptor antagonists inhibited secretion induced by vagal stimulation by 33% (20). Thus in the pig, GRP plays a role in endogenous pancreatic secretion although the role in other species may be less.

3. Tools to study bombesin

a. Synthetic Peptide

Bombesin can be obtained from multiple sources including Sogma-Aldrich, Research Plus, Anaspec and Abbiotec.

b. Antibodies

Antibodies against bombesin are available from Genway and Phoenix Pharmaceuticals.

c. ELISA and RIA

An RIA kit for GRP is available from Phoenix Pharmaceuticals. An ELISA kit against Bombesin is available from MyBioSource and against mouse GRP from Antibodies-online.

d. Antagonists

Peptide antagonists against the Bombesin receptor are available from Sigma-Aldrich and Tocris

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