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Insights into bombesin receptors and ligands: Highlighting recent advances

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ABSTRACT

This following article is written for Prof. Abba Kastin's Festschrift, to add to the tribute to his important role in the advancement of the role of peptides in physiological, as well as pathophysiological processes. There have been many advances during the 35 years of his prominent role in the Peptide field, not only as editor of the journal Peptides, but also as a scientific investigator and editor of two volumes of the Handbook of Biological Active Peptides [146,147]. Similar to the advances with many different peptides, during this 35 year period, there have been much progress made in the understanding of the pharmacology, cell biology and the role of (bombesin) Bn receptors and their ligands in various disease states, since the original isolation of bombesin from skin of the European frog *Bombina bombina* in 1970 [76]. This paper will briefly review some of these advances over the time period of Prof. Kastin 35 years in the peptide field concentrating on the advances since 2007 when many of the results from earlier studies were summarized [128,129]. It is appropriate to do this because there have been 280 articles published in Peptides during this time on bombesin-related peptides and it accounts for almost 5% of all publications. Furthermore, 22 Bn publications we have been involved in have been published in either Peptides [14,39,55,58,81,92,93,119,152,216,225,226,231,280,302,309,355,361,362] or in Prof. Kastin's Handbook of Biological Active Peptides [137,138,331].

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Discovery of Bn peptides

In addition to Bn, a large number of other Bn related peptides subsequently were isolated (1970–1990), primarily from other amphibian skins, mainly by Profs Espamer/Nakajima and colleagues, and these fell into three general groups [75,77,129,138]. One group was comprised of the Bn related peptides with COOH termini ending in Gly-His-Leu-Met-NH_2; a second group comprised of the litorin-ranatensin related peptides ending in Gly-His-Phe-Met-NH_2 and third group the phyllolitorin related peptides ending in: Gly-Ser-Phe (Leu)-Met-NH_2 [75,77,129,138]. It was not until 1980 that the mammalian member of the Bn peptide subgroup was isolated from porcine stomach, the 27 amino acid peptide, Gastrin Releasing Peptide (GRP) by McDonald and colleagues [213]. It was found to have a very high homology to Bn sharing the same 7 COOH terminal amino acids, which is the biologically active end of the peptide [38,179,213]. In 1983 Minamino isolated the decapeptide, neuromedin B (NMB) from porcine spinal cord [217] which he also later found to occur in larger forms of 30 and 32 amino acids [218]. Six of the 7 COOH terminal amino acids of NMB were identical to ranatensin. Subsequently in 1984 Minamino isolated the COOH terminal decapeptide of GRP from porcine spinal cord, and was called neuromedin C [GRP18-27] [219]. No mammalian equivalent of phyllolitorin has been described.

Abbreviations: βAla, βalanine; Bantag-1, selective BRS-3 peptide antagonist; Bn, bombesin; BnR, bombesin receptor; BRS-3(BB3), bombesin receptor subtype 3; COOH terminus, carboxyl terminus; CCK, cholecystokinin; CNS, central nervous system; Cpa, chlorophenylalanine; DAG, diacylglycerol; EC1, extracellular domain 1; ERK, extracellular regulated kinase; EGFR, epidermal growth factor receptor; fBB4, frog bombesin receptor subtype 4; GRP, gastrin-releasing peptide; GRPR, gastrin-releasing peptide receptor (BB2); GPCR, G protein-coupled receptor; 5-HT, serotonin, 5-hydroxytryptamine; IC1, intracellular domain 1; IR, immunoreactivity; MK-5046, selective nonpeptide BRS-3 agonist; NMB, neuromedin B; NMBR, neuromedin B receptor (BB1); NMC, neuromedin C; NSCLC, nonsmall cell lung cancer cell; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C; TM, transmembrane region; SP, substance P; ψbonds, pseudopeptide bonds; $p125^{FAK}$, p125 focal adhesion kinase; Stat, statine; TPA, 12-O-tetradecanoyl-phorbol-13-acetate.

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Molecular studies demonstrate that the various Bn peptides are derived from separate prohormones [331]. In the case of amphibians these studies show that many frog species in their skins contain multiple forms of Bn peptides with both Phe and Leu penultimate residues and that each is derived from separate genes [238,331]. For many years Bn was considered the mammalian equivalent of GRP, however, subsequently in the same frog was found different cDNA's encoding for both GRP and Bn with the frog GRP being much more analogous to mammalian GRP than frog Bn, leading to the conclusion that mammalian GRP is not the mammalian equivalent of frog Bn [239,331]. Therefore at present, no mammalian Bn peptide has been described that is equivalent to frog Bn [331].

Discovery of Bn peptide receptors

Early functional studies and binding studies suggested more than one subtype of Bn receptor existed in mammalian tissues both in peripheral tissues and in the central nervous system (CNS) [38,129,164–166,321,365,366].

Subsequent molecular studies have identified and characterized three different mammalian Bn receptor (BnR) members including: the gastrin-releasing peptide receptor (GRPR)(BB_2); the neuromedin B receptor (NMBR)(BB_1) and an orphan receptor, named bombesin receptor subtype 3 (BRS-3)(BB_3), that is also included in this family [21,80,96,332,369]. BRS-3 is included in the BnR family of receptors even though at present the native ligand is unknown, because the human BRS-3 has 51% amino acid identities with a the human GRPR, and 47% with the human NMBR, demonstrating close similarity to these receptors [80,95,96,129,194,331]. Each of the three BnRs is a member of the G-protein coupled heptahelical superfamily of membrane receptors.

In 1995, Nagalla et al. [237] cloned a receptor from amphibian brain that had 61%, 56% and 70% identities to GRPR, NMBR and BRS-3, respectively. This receptor had a high affinity for [Phe^{13}] Bn, which was the most prevalent form in frog brain, and had a lower affinity for GRP and NMB [148,237]. This receptor was named BB4 for bombesin receptor subtype 4 [237]. At present no mammalian equivalent of the amphibian BB4 receptor (BB4R) has been described [129,331]. In 2003 Iwabuchi at al [124] described a BnR from chicken that had high amino acid identities to frog BBR4 (70%), human BRS-3 (69%), but lower for human GRPR (58%) and hNMBR (52%). Pharmacologically this receptor had low affinity for GRP and NMB, but high affinity [$DPhe^6$, βAla^{11}, Phe^{13}] Bn (6-14) [124], a synthetic Bn analog that has high affinity for human BRS-3 [201,275,299,301,355]. This receptor was called chBRS-3.5 because of its similarities to both frog BBR and human BRS-3 [124]. No mammalian equivalent of this receptor has been described [129].

Distribution of BnR and ligands

The distribution of the native ligands, GRP and NMB as well as their receptors, GRPR and NMBR has been extensively examined by both immunohistochemical studies, binding studies for the receptors and molecular assessments of the mRNA distribution [129,164,228,245,310,332,364,369]. Both the native ligands and their receptors are found widely distributed in the CNS and peripheral tissues including the gastrointestinal tract; pulmonary, urogenital and reproductive system; numerous endocrine glands (adrenal, pituitary, thyroid, islets); and the hematopoietic system (phagocytic cells, macrophages) and immune cells [120,129,139,245,277]. In the monkey CNS both NMBR and GRPR mRNA were found in the amygdala, caudate nucleus, cerebellum, hippocampus, hypothalamus, thalamus and spinal cord [310]. In rat CNS binding studies a particularly high density of NMBR was found in the olfactory regions and central thalamic nuclei and a highest density of GRPRs in the hypothalamus, particularly the suprachiasmatic and paraventricular nuclei, which both agree with the mRNA distribution studies of these two receptors in rat brain [368].

The distribution of the BRS-3 receptor is less well studied and because the native ligand is unknown there is no information available on it. No peptide binding studies on the distribution of the BRS-3 have been performed because until recently there were no high affinity ligands that bound specifically to the BRS-3 receptor [234]. The synthetic Bn peptide analog, [$DTyr^6$, βAla^{11}, Phe^{13}] Bn (6-14) can be radiolabeled and used for binding studies in human tissues, because it has high affinity for human BRS-3 [201,234,275,299,301,355], but is not useful in rodents, because it has a very low affinity for the mouse or rat BRS-3 [180]. Furthermore, its utility is limited because it has high affinity for GRPR and NMBR in all species examined [201,234,275,299,301,355]. In the monkey BRS-3 mRNA was found throughout the CNS with the highest amounts in the hypothamus and in low amounts in most peripheral tissues with the highest amount in the testis and lower amounts in the pancreas, thyroid, ovary and pituitary gland [310]. BRS-3 mRNA is found in the islets of mice, human, rhesus monkey and dog, but not in rat islets [82]. Immunohistochemical studies in the rat CNS [127] and gastrointestinal tract [273] demonstrated BRS-3 is strongly present in cerebral cortex, hippocampal formation, hypothalamus and thalamus in the CNS [127]. In the GI tract BRS-3 was found in all gut regions in nerves and non-neuronal tissues, including enteric and submucosal ganglia, myenteric ganglia and in cell bodies of c-KIT interstitial cells of Cajal, which are important in regulating GI motility [273]. Two recent studies have provided additional information. In one study using in situ hybridization the distribution of BRS-3 mRNA and its co-localization with various neurotransmitters was examined in rat and mouse brain [393]. BRS-3 was found in a variety of brain regions with the highest concentrations in the amygdala and hypothalamus [393]. Many of the BRS-3 expressing neurons were glutamatergic, a few cholingergic or GABAergic, and also a few partially co-localize with corticotropin-releasing factor (CRF) and growth hormone-releasing factor (GHRH) suggesting interactions of BRS-3 with stress- and growth hormone endocrine systems [393]. In a second study using a specific BRS-3 radiolabeled agonist ([^{3}H]Bag-2) [102] in mice brain, moderate to abundant BRS-3 binding was seen in various hypothalamic nuclei (PVN, DMH, ARC, VMH, PH, Pe, MPA, NHA), forebrain areas, caudal brain (PBN, NTS), the amygdala and the thalamus [102]. The strong binding in hypothalamic nuclei support the importance of BRS-3 in feed behavior and energy metabolism which was first reported in BRS-3 knockout mice which developed obesity, hypertension and impairment of glucose metabolism [249].

Pharmacology of Bn receptors/ligands

Pharmacology of Bn receptor agonists

Numerous studies primarily using cells containing rat/mouse BnRs demonstrate that the m, rGRPR has 24–148-fold higher affinity for GRP than NMB and that the rNMBR has 60–250-fold greater affinity for NMB than GRP [25,28,29,129,201,301,365,369,371]. Recently a detailed study on the human GRPR/NMBR in native and transfected cells has been reported [355] and this demonstrates that human GRPR has a 647- fold higher affinity for GRP than NMB and that the hNMBR has a 640-fold higher affinity for NMB than GRP (Table 1). In this study of the 12 natural occurring Bn related peptides tested, three had an equal high affinity to GRP for the hGRPR (IC_{50}, 0.12–0.5 nM) (Bn, alytesin, NMC) and none had and equal high affinity to NMB for hNMBR (IC_{50} 0.053 nM),