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Bombesin receptor regulation of emotional memory

Abstract: Mammalian bombesin-like peptides neuromedin B (NMB) and gastrin-releasing peptide (GRP) act by activating NMB receptors (NMBR, BB1) and GRP receptors (GRPR, BB2), respectively. These two bombesin receptors are members of the G-protein-coupled receptor (GPCR) superfamily. In the brain, NMBR and GRPR are highly expressed in the brain areas involved in memory processing and emotional responses, such as the hippocampus and the amygdaloid nuclei. An increasing number of pharmacological and genetic studies in rodents indicate that NMBRs and GRPRs in brain regions including the dorsal hippocampus, the nucleus tractus solitarius, the basolateral amygdala, and cortical areas, regulate memory formation and expression, particularly for memories related to emotionally arousing tasks. GRPR signaling interacts with multiple protein kinase pathways as well as with other neurotransmitter, hormone, and growth factor systems in influencing memory formation. Together with evidence from human studies, the findings from rodent experiments suggest that bombesin receptors may be therapeutic targets in brain disorders involving memory dysfunction and anxiety.

Keywords: BB1 receptor; BB2 receptor; gastrin-releasing peptide receptor; memory modulation; neuromedin B receptor.

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Neuromodulatory systems regulating emotional memory formation

Memory formation and storage requires synaptic modifications that depend on gene expression and protein synthesis and is regulated by a range of signaling mechanisms triggered by several neurotransmitters, neuropeptides, and hormones. The strength of newly formed memories for novel, arousing, or stressful events ('emotional' memories) is particularly influenced by hormonal and neural modulatory systems, including adrenal stress hormones released by learning experiences (McGaugh, 2000).

Neuropeptides, which constitute an important class of signaling molecules in the mammalian brain, play a role in regulating emotional memory, often by facilitating its acquisition, consolidation, or expression. The neuropeptides shown to influence memory processes include opioids, vasopressin, cholecystokinin, oxytocin, neuropeptide Y, neuropeptide S, galanin, adrenocorticotropin, corticotrophin-releasing hormone, somatostatin, substance P, vasoactive intestinal peptide, and bombesin-like peptides (BLPs). Research on the role of neuropeptide signaling in memory has been stimulated by the increasing availability of experimental tools, including recombinant human and animal neuropeptides, synthetic peptides or small molecules that act on neuropeptide receptors, as well as neuropeptide receptor knockout mice, allowing the selective manipulation of components

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of neuropeptide signaling in rodents (for reviews, see Engelmann et al., 1996; Feany, 1996; de Wied, 1997; Fehm et al., 2000; Roesler and Schröder, 2011). This review will focus on the role of BLPs in influencing the formation and expression of emotional memories.

Bombesin family of neuropeptides

Bombesin is an amidated, 14-amino acid peptide isolated from the skin of the European frog *Bombina bombina* in 1970. The bombesin group of amphibian peptides was characterized as having the carboxyl terminus of Gly-His-Leu-Met-NH_2 (Erspamer et al., 1970). Bombesin was biologically active when administered in the mammalian central nervous system (CNS), as indicated by experiments showing that intracerebroventricular (i.c.v.) infusions of bombesin induced hypothermia and hyperglycemia in rats (Brown et al., 1977a,b). Subsequently, two mammalian BLPs, gastrin-releasing peptide (GRP) and neuromedin B (NMB), were identified (McDonald et al., 1979; Minamino et al., 1983). GRP, originally isolated from the porcine stomach, has 27 amino acids, which is synthesized as a 148-amino acid precursor (PreproGRP) and subsequently metabolized posttranslationally (Spindel et al., 1984, 1990; Lebacq-Verheyden et al., 1988). GRP and bombesin display similar biological activities and both peptides share the same seven carboxyl-terminal amino acids. NMB, the mammalian equivalent of the amphibian peptide ranatensin, is a decapeptide originally isolated from the porcine spinal cord (Table 1; Minamino et al., 1983). Another peptide originally named neuromedin C (Minamino et al., 1984) is in fact a decapeptide of GRP (GRP-10, $GRP_{18–27}$).

Studies investigating the presence of bombesin-binding sites in the mammalian CNS showed that bombesin could bind with high affinity to rat brain membranes, with high specific binding sites in brain areas including the hippocampus (Moody et al., 1978). The presence of endogenous BLPs in the rat brain was demonstrated by radioimmunoassay techniques, showing high concentrations in the nucleus tractus solitarius (NTS), the amygdala, and the hypothalamus (Moody and Pert, 1979; Moody et al., 1981). GRP and NMB mRNAs have differential distribution patterns across rat brain areas (Chronwall et al., 1985; Wada et al., 1990). NMB mRNA is most abundant in the olfactory bulb, the dentate gyrus, and the dorsal root ganglia, whereas GRP mRNA has the highest density in the forebrain areas and the hypothalamus (Wada et al., 1990; Battey and Wada, 1991; for reviews, see Moody and Merali, 2004; Roesler et al., 2006a; Jensen et al., 2008).

Bombesin *(B. bombina)*
QQRLGNQWAVGHLM-NH_2
GRP *(Homo sapiens)*
VPLPAGGGTVLTKMYPRGNHWAVGHLM-NH_2
NMB *(H. sapiens)*
GNLWATGHFM-NH_2

Table 1 Structures of bombesin, GRP, and NMB.
The amino acid sequence data are from as follows: bombesin: Anastasi et al., 1972; GRP: Sausville et al., 1986; Spindel et al., 1986; and NMB: Krane et al., 1988; for a review, see Jensen et al., 2008.

Mammalian bombesin receptors

The development of selective antagonists allowed the identification of two different mammalian receptors mediating the effects of BLPs: one with higher affinity for NMB than GRP (NMB receptor, NMBR) and the other binding preferentially to GRP with lower affinity for NMB (GRP receptor, GRPR; Jensen and Gardner, 1981; Moody et al., 1988, 1992; von Schrenck et al., 1989, 1990; Ladenheim et al., 1990, 1992; Wang et al., 1992). The first bombesin receptor to be cloned, from murine Swiss 3T3 cells, was GRPR in 1990 (Spindel et al., 1990; Battey et al., 1991). Subsequently, NMBR was cloned from a cDNA library made from the rat esophagus (Wada et al., 1991), and the structures of human NMBR and GRPR were described from a small-cell lung cancer cell line (Corjay et al., 1991). In addition to NMBR and GRPR, a third mammalian bombesin receptor was cloned in 1992, originally from guinea pig uterus (Gorbulev et al., 1992) and later from human (Fathi et al., 1993), mouse (Ohki-Hamazaki et al., 1997), rat (Liu et al., 2002), and sheep (Whitley et al., 1999). This receptor, named BRS-3, shows low affinity for all known BLPs and to date is considered an orphan receptor (Jensen et al., 2008). The current terminology adopted by several receptor classification guides uses the names BB1, BB2, and BB3 for NMBRs, GRPRs, and BRS-3 receptor, respectively. More recently, novel bombesin receptors with no mammalian equivalents have been identified in amphibians (BB4; Nagalla et al., 1995) and chickens (chBRS-3.5; Iwabuchi et al., 2003). Figure 1 illustrates the mammalian bombesin receptors and their endogenous ligands in mammals described to date.

All bombesin receptors are members of the G-protein-coupled receptor (GPCR) superfamily and exhibit the characteristic seven-transmembrane domain structure of GPCRs. Below we will present a brief overview of the structure and signaling of the three mammalian bombesin receptors (for a comprehensive review on bombesin receptor classification, nomenclature, expression, signaling,

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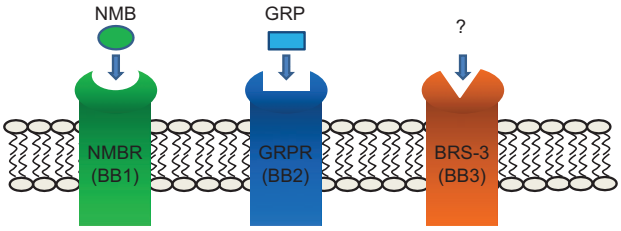

Figure 1 Mammalian bombesin receptors and their endogenous ligands.

and functions, see Jensen et al., 2008; see also Gonzalez et al., 2008 for an excellent review on bombesin receptor functions). Table 2 shows a summary of the molecular structures of NMBR, GRPR, and BRS-3 receptors.

NMBR (BB1)

The NMBR has 390 amino acids in humans, rats, and mice. The human NMBR shows 55% identity with human GRPR and 47% identity with human BRS-3 (Corjay et al., 1991). The human NMBR gene (*NMBR*) is localized at chromosome 6p21-qter, the mouse gene (*Nmbr*) on chromosome 10 A2, and the rat *Nmbr* on 1p13 (Table 2; Jensen et al., 2012). In rats and mice, NMBR is expressed in gastrointestinal and urogenital tissues and is widely spread within the brain, particularly in the olfactory tract (Wada et al., 1991; Ohki-Hamazaki et al., 1997). The NMBR is coupled to the G_q protein (G_q) and the phospholipase C (PLC)/protein kinase C (PKC) signaling pathway, and its activation leads also to stimulation of phospholipases A_2 and D and extracellular signal-regulated protein kinase (ERK)/mitogen-activated protein kinase (MAPK; Corjay et al., 1991; Wada et al., 1991; Moody et al., 1992, 1995; Wang et al., 1992; Dobrzanski et al., 1993; Benya et al., 1995; Hou et al., 1998). The physiological functions regulated by the NMBR in peripheral tissues include the contraction of gastrointestinal and urogenital smooth muscles and pituitary gland secretion (Severi et al., 1991; Rettori et al., 1992; Kilgore et al., 1993; Parkman et al., 1994; Milusheva et al., 1998).

GRPR (BB2)

The GRPR is a 384-amino acid protein in humans, mice, and rats. The human GRPR shows 51% identity with the human BRS-3 (Fathi et al., 1993). The chromosomal location for the GRPR gene (named *GRPR* in humans and *Grpr* in mice and rats) is localized at chromosome Xp22.2-p22.13 (human), X F4 (mouse), and Xq21 (rat; Table 2; Jensen et al., 2012). Peripheral tissues in which the GRPR is expressed in rodents under physiological conditions include the digestive tract and the developing lung (Spindel et al., 1990; Battey et al., 1991; Jensen et al., 2008). In the brain, autoradiographic studies indicated that the areas containing high densities of GRPRs include the olfactory bulb, the nucleus accumbens, the caudate putamen, the central amygdala, the dorsal hippocampal formation (CA3 area and dentate gyrus), as well as the paraventricular, central medial, and paracentral thalamic nuclei (Wolf et al., 1983; Wolf and Moody, 1985; Zarbin et al., 1985). The most detailed immunohistochemical characterization of GRPR expression in the mouse brain published so far (Kamichi et al., 2005) showed that GRPR immunoreactivity was widely distributed in the hippocampal formation, the hypothalamus, the brain stem, cortical areas, the amygdala [particularly in the basolateral (BLA) and central nuclei of the amygdala (CeA)], the NTS and with high densities also observed in the dorsal hippocampus (dHIP).

As with NMBR, GRPR is directly coupled to G_q and primarily associated with increased cellular Ca^{2+} and activation of the PLC/PKC (Hellmich et al., 1999; Stangelberger et al., 2005) but not the adenylyl cyclase (AC)/cyclic AMP (cAMP)/protein kinase A (PKA) pathway (although there is evidence of increased cAMP levels in response to GRPR activation and functional interactions between GRPR and cAMP/PKA; see, for example, Qin et al., 1995; Chan and Wong, 2005; de Farias et al., 2008). Cellular responses to GRPR activation also depend on MAPK signaling (Hellmich et al., 1999; Chen and Kroog, 2004; Stangelberger et al., 2005), and studies in normal tissues or cancer cells have indicated that GRPR signaling interacts with a range of other growth factor receptor systems (e.g., epidermal growth factor receptor, EGFR), signaling enzymes [e.g., tyrosine kinases, phosphatidylinositol 3-kinase (PI3K), and cyclooxygenase-2], and immediate-early genes (e.g., c-fos and c-jun; Szepeshazi et al., 1997; Chatzistamou et al., 2000; Thomas et al., 2005; Hohla et al., 2007; Ishola et al., 2007; Liu et al., 2007; Flores et al., 2008; de Farias et al., 2010; Czepielewski et al., 2012). The physiological functions of GRPRs in peripheral tissues likely include the regulation of gastrin and somatostatin release from cells in the gastric mucosa, gastric acid secretion, pancreatic secretion, gastrointestinal motility, lung development, and chemoattraction in immune system cells (Ruff et al., 1985; Schubert et al., 1991; Del Rio and De la Fuente, 1994; Niebergall-Roth and Singer, 2001; Ohki-Hamazaki et al., 2005; Jensen et al., 2008; Czepielewski et al., 2012).

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Species	TM	AA	Chromosomal location		Gene name
NMBR (BB1)					
Human	7	390	6q21-qter		*NMBR*
Rat	7	390	1p13		*Nmbr*
Mouse	7	390	10 A2		*Nmbr*
Amino acid sequence *(H. sapiens)*					
(1–60)	MPSKSLSNLS	VTTGANESGS	VPEGWERDFL	PASDGTTTEL	VIRCVIPSLY LLIITVGLLG
(61–120)	NIMLVKIFIT	NSAMRSVPNI	FISNLAAGDL	LLLLTCVPVD	ASRYFFDEWM FGKVGCKLIP
(121–180)	VIQLTSVGVS	VFTLTALSAD	RYRAIVNPMD	MQTSGALLRT	CVKAMGIWVV SVLLAVPEAV
(181–240)	FSEVARISSL	DNSSFTACIP	YPQTDELHPK	IHSVLIFLVY	FLIPLAIISI YYYHIAKTLI
(241–300)	KSAHNLPGEY	NEHTKKQMET	RKRLAKIVLV	FVGCFIFCWF	PNHILYMYRS FNYNEIDPSL
(301–360)	GHMIVTLVAR	VLSFGNSCVN	PFALYLLSES	FRRHFNSQLC	CGRKSYQERG TSYLLSSSAV
(361–390)	RMTSLKSNAK	NMVTNSVLLN	GHSMKQEMAL		
GRPR (BB2)					
Human	7	384	Xp22.2-p22.13		*GRPR*
Rat	7	384	Xq21		*Grpr*
Mouse	7	384	X F4		*Grpr*
Amino acid sequence *(H. sapiens)*					
(1–60)	MALNDCFLLN	LEVDHFMHCN	ISSHSADLPV	NDDWSHPGIL	YVIPAVYGVI ILIGLIGNIT
(61–120)	LIKIFCTVKS	MRNVPNLFIS	SLALGDLLLL	ITCAPVDASR	YLADRWLFGR IGCKLIPFIQ
(121–180)	LTSVGVSVFT	LTALSADRYK	AIVRPMDIQA	SHALMKICLK	AAFIWIISML LAIPEAVFSD
(181–240)	LHPFHEESTN	QTFISCAPYP	HSNELHPKIH	SMASFLVFYV	IPLSIISVYY YFIAKNLIQS
(241–300)	AYNLPVEGNI	HVKKQIESRK	RLAKTVLVFV	GLFAFCWLPN	HVIYLYRSYH YSEVDTSMLH
(301–360)	FVTSICARLL	AFTNSCVNPF	ALYLLSKSFR	KQFNTQLLCC	QPGLIIRSHS TGRSTTCMTS
(361–384)	LKSTNPSVAT	FSLINGNICH	ERYV		
BRS-3 (BB3)					
Human	7	399	Xq26-q28		*BRS3*
Rat	7	399	Xq36		*Brs3*
Mouse	7	399	X A7.1-A7.2		*Brs3*
Amino acid sequence *(H. sapiens)*					
(1–60)	MAQRQPHSPN	QTLISITNDT	ESSSSVVSND	NTNKGWSGDN	SPGIEALCAI YITYAVIISV
(61–120)	GILGNAILIK	VFFKTKSMQT	VPNIFITSLA	FGDLLLLLTC	VPVDATHYLA EGWLFGRIGC
(121–180)	KVLSFIRLTS	VGVSVFTLTI	LSADRYKAVV	KPLERQPSNA	ILKTCVKAGC VWIVSMIFAL
(181–240)	PEAIFSNVYT	FRDPNKNMTF	ESCTSYPVSK	KLLQEIHSLL	CFLVFYIIPL SIISVYYSLI
(241–300)	ARTLYKSTLN	IPTEEQSHAR	KQIESRKRIA	RTVLVLVALF	ALCWLPNHLL YLYHSFTSQT
(301–360)	YVDPSAMHFI	FTIFSRVLAF	SNSCVNPFAL	YWLSKSFQKH	FKAQLFCCKA ERPEPPVADT
(361–399)	SLTTLAVMGT	VPGTGSIQMS	EISVTSFTGC	SVKQAEDRF	

Table 2 Molecular structures of the human NMBR (BB1), GRPR (BB2), and BRS3 (BB3) receptor.
Structural data are from Spindel et al., 1990; Battey et al., 1991; Wada et al., 1991; Jensen et al. (2012); for a review, see Jensen et al., 2008.

BRS-3 receptor (BB3)

The BRS-3 in humans, rats, and mice has 399 amino acids (Fathi et al., 1993). The human *BRS3* gene is localized at chromosome Xq26-q28, whereas the locations of the mouse and rat *Brs3* genes are X A7.1-A7.2 and Xq36, respectively (Table 2; Jensen et al., 2012). Studies in rats, mice, and monkeys indicate that the BRS-3 is expressed in the testis, the CNS, and the enteric nervous system (Fathi et al., 1993; Ohki-Hamazaki et al., 1997; Sano et al., 2004), although its physiological role remains unclear and no naturally occurring ligand has been identified (Jensen et al., 2008). As with NMBR and GRPR, BRS-3 signaling may involve the activation of PLC, PKC, MAPK, and phospholipases (Fathi et al., 1993; Ryan et al., 1998; Weber et al., 2001; Sano et al., 2004). No involvement of BRS-3 in memory has been demonstrated to date; hence, in the next sections, we will focus on the NMBR and GRPR types of bombesin receptors.

Bombesin receptor regulation of synaptic plasticity and memory

The involvement of bombesin receptors in memory formation has been revealed mostly by studies using sys-

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temic or intracerebral administration of agonists and antagonists of NMBR and GRPR in rodents submitted to learning tasks. More recently, the use of genetic models, particularly GRPR knockout mice, has allowed the characterization of memory alterations associated with genetic ablation of bombesin receptors.

In several of the experiments reviewed below, emotionally motivated memory was examined in rodents using Pavlovian fear conditioning paradigms, whereby an emotionally neutral stimulus (conditioned stimulus, CS), typically a sound (tone or white noise) in 'cued' fear conditioning or a particular environment (e.g., a chamber or box; context) in 'contextual' fear conditioning, is presented in conjunction with an innately aversive or unconditioned stimulus (e.g., electric footshock) such that, after repeated pairings, the CS alone acquires the capacity to elicit responses akin to fear. In other experiments, the fear-motivated conditioning model used was inhibitory avoidance, in which animals learn to associate a location in the training apparatus with an aversive stimulus (footshock). Experiments using these models have provided valuable insight into the roles of NMBR and GRPR in emotional memory.

Pharmacological studies

The first experiments directly addressing the effects of BLPs on memory were reported by Flood and Morley (1988). They showed that systemic or i.c.v. injections of either GRP or bombesin after training could modulate memory retention for a T-maze footshock avoidance task. However, the pattern of effects of GRPR agonists on memory formation was rather complex and dependent on specific experimental conditions. Thus, systemic GRP or bombesin produced memory enhancement when given at lower doses and when the animals were trained with a weak footshock but impaired memory when higher doses or a stronger training protocol were used (Flood and Morley, 1988, 1989). This characteristic inverted U-shaped dose-response pattern for the effects of GRPR agonists on memory is not surprising, given that it is often observed for the effects of neuropeptides and other modulatory drugs on memory consolidation (McGaugh, 1989). When infused i.c.v., both GRP and bombesin enhanced memory. In addition, the memory-enhancing effects of systemic GRP and bombesin were inhibited by vagotomy, indicating that the effects could be at least partially mediated by peripheral stimulation of ascending vagal pathways (Flood and Morley, 1988). The finding that systemic bombesin given posttraining could enhance memory retention was later confirmed in rats by another laboratory (Rashidy-Pour and Razvani, 1998). A few years later, Williams and McGaugh (1994) showed that bombesin could enhance memory for inhibitory avoidance and in a radial arm maze when infused directly into the NTS after training.

Studies using systemic injections of selective antagonists indicated that the pharmacological blockade of bombesin receptors could result in memory impairment for emotionally motivated tasks. Thus, pretraining injections of either the NMBR antagonist BIM23127 or the GRPR antagonist [Leu13-(psi-CH_2NH)-Leu14]BN impaired inhibitory avoidance retention in mice (Santo-Yamada et al., 2003). A series of experiments by Roesler et al. (2004b) showed that the pretraining injection of the selective GRPR antagonist RC-3095 in rats impaired memory for inhibitory avoidance but not for a task with less emotional content, novel object recognition (NOR), indicating that the GRPR is preferentially involved in regulating the formation of emotional, aversively motivated memories, although further studies using low arousing tasks are required to support that conclusion. Similar impairing effects of RC-3095 on inhibitory avoidance memory were obtained with posttraining injections given systemically (Roesler et al., 2004c), pretraining or posttraining infusions given directly into the dHIP (Roesler et al., 2003; Venturella et al., 2005; Dantas et al., 2006; Preissler et al., 2007), or posttraining infusions into the BLA (Roesler et al., 2004c). The effects of systemic or intra-dHIP injections of the GRPR antagonist on inhibitory avoidance memory often followed an inverted U-shaped dose-response pattern, in which intermediate doses resulted in memory impairment, whereas higher doses had no effect or produced memory enhancement (Roesler et al., 2003, 2004b; Dantas et al., 2006). Conversely, posttraining intra-dHIP infusion of bombesin resulted in the enhancement of inhibitory avoidance memory at intermediate doses and impairment at higher doses (Roesler et al., 2006b).

In addition to memory encoding and consolidation, GRPRs might influence the expression, extinction, and reconsolidation of emotional memories. Intra-dHIP infusion of RC-3095 blocked the consolidation of inhibitory avoidance extinction when given after extinction training (Luft et al., 2006) and produced a transient impairment of reconsolidation-like processes when given after retrieval (Luft et al., 2008). A series of studies by Merali et al. have examined the effects of pretest administration of bombesin receptor ligands on the expression of fear conditioning. Thus, the central administration of GRP, injected i.c.v. or localized at specific amygdaloid (CeA and BLA) or cortical sites, attenuated the expression of learned fear (as seen by reduced levels of freezing) in response to contextual

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cues (i.e., in the context in which animals had previously been exposed to shock) and to a tone that had previously been paired with a shock (Mountney et al., 2006, 2008; Merali et al., 2011). Conversely, the i.c.v. administration of RC-3095 increased freezing (Merali et al., 2011). The localized intracerebral microinjection of the GRPR antagonists, however, elicited a more complicated pattern of behavioral responses. Specifically, the administration of the GRPR antagonist, BW2258U89, into the infralimbic cortex (IL) attenuated the freezing response, whereas, at the CeA, the effects were biphasic: the high dose reducing freezing and the low dose increasing freezing (Mountney et al., 2006). The administration of RC-3095 to the BLA significantly reduced the freezing response to contextual cues but not to the conditioned tone (Mountney et al., 2008). At first blush, it would appear difficult to reconcile why, under certain conditions, the effects of GRPR antagonists were similar to those of GRP itself. However, it is noteworthy that some GRPR antagonists have intrinsic agonistic activity (De Castiglione and Gozzini, 1996), which may become evident at certain doses, in a site-specific manner. Furthermore, as noted above, and similar to other peptides, bombesin and GRP and GRPR antagonists have been shown to have an inverted U-shaped dose-response curve wherein low versus higher doses exhibit opposing effects on behavior (Flood and Morley, 1988; Dantas et al., 2006; Roesler et al., 2006b). Such differential effects may translate into modulation of distinct neuronal circuits and behavioral outcomes.

Although a role for the GRPR in learned fear has been fairly well established, much less is known about the role of NMBRs in fear-related processes. Unlike GRPR, which appears to be more selectively involved in emotionally based learning and memory, NMBRs may play a more generalized role in both innate (unconditioned) anxiety and learned fear-type responses. Indeed, the i.c.v. administration of the NMBR receptor antagonist BIM23127 reduced anxiogenic behavior as reflected by increased open arm entries, time spent in the open arms, and decreased time spent in the closed arms on the elevated plus maze (Bédard et al., 2007). In addition, the pretreatment with BIM23127 or the mixed NMBR/GRPR antagonist PD176262 [administered i.c.v. or intraperitoneally (i.p.)] attenuated the fear-potentiated startle response (another model of Pavlovian fear conditioning; Merali et al., 2006a). Interestingly, the administration of the NMBR agonist, NMB, also reduced the fear-potentiated startle response, consistent to what was observed with GRP administration (Bédard et al., 2007). Also in keeping with the findings with GRP, it was recently shown that the i.c.v. infusion of NMB dose-dependently attenuated the expression of both contextual and cued (tone) learned fear responses (Figure 2). Note, however, that a recent study found no effects of intra-BLA infusions of GRP or the GRPR antagonist (D-Phe^{6},Leu-NHEt^{13},des-Met^{14})-bombesin(6–14) on the expression of contextual or cued fear conditioning in mice (Chaperon et al., 2012).

The GRPR might be relevant for CNS development, and studies using neonatal treatments with a GRPR antagonist have been performed in order to examine the long-lasting consequences of GRPR blockade during development. In studies performed by Schröder et al., rats given systemic

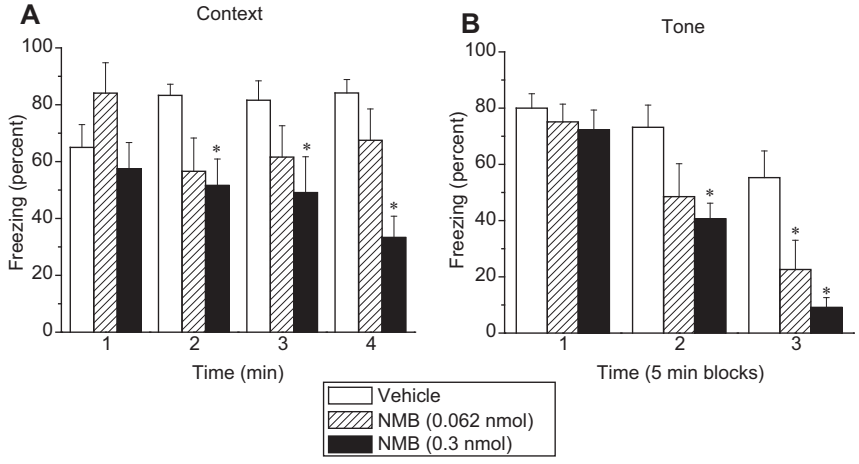

Figure 2 Mean±standard error of the mean (SEM) percentage of time engaged in freezing following injection of KRB (vehicle; open columns), NMB (0.062 nmol; hatched columns), or NMB (0.3 nmol; solid columns) into the third ventricle in the contextual task of the CER paradigm (A) and in response to the fear cue applied in a different environment (B).
*Significantly different from vehicle condition ($p<0.05$).

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injections of RC-3095 twice daily from postnatal days (PND) 1–10 showed, among other behavioral alterations, impaired long-term but not short-term memory for both inhibitory avoidance and NOR when trained and tested in the adulthood (Presti-Torres et al., 2007, 2012) and also impaired maternal odor preference when tested as infants (Garcia et al., 2010). The behavioral impairments induced by neonatal GRPR blockade were accompanied by decreases in the mRNA expression of GRPR, the *N*-methyl-D-aspartate (NMDA) receptor subunit NR1, and EGFR in the cortex and increases in all three receptor mRNAs in the hippocampus. These findings suggest that the disruption of GRPR signaling during the neonatal period can lead to long-lasting memory dysfunction, supporting a role for the GRPR in brain development and memory processes.

Genetic studies

Mice knockout for NMBR (Ohki-Hamazaki et al., 1999) and GRPR (Wada et al., 1997) have been generated and characterized. One study directly addressing memory in female mice knockout for the NMBR showed that they had impaired inhibitory avoidance memory when the rats were previously exposed to restraint stress, whereas non-stressed mice had normal memory (Yamada et al., 2003).

In a study using GRPR knockout mice, Shumyatsky et al. (2002) showed that contextual and cued fear conditioning was enhanced by the genetic deletion of GRPR. Consistent with the view that the GRPR is particularly involved in memory for emotionally motivated tasks, memory for a different type of task not involving explicit fear motivation (spatial memory assessed in the Morris water maze) was unaffected in mice lacking GRPR. Importantly, synaptic plasticity measured by long-term potentiation (LTP) in amygdala preparations was enhanced in GRPR knockout mice as well as by a GRPR antagonist in the amygdala from wild-type animals. GRPR was preferentially expressed in amygdalar inhibitory interneurons releasing γ-aminobutyric acid (GABA). The authors proposed that GRP might be released as a cotransmitter from glutamatergic neurons to activate preferentially GRPRs located on GABAergic interneurons. In this model, GRPR signaling would act to stimulate inhibitory transmission within the amygdala and represent an inhibitory constraint for the formation of fear-motivated memories.

Two recent studies further characterized emotional memory and synaptic plasticity in GRPR knockout mice. Martel et al. (2012) found impaired extinction of cued but not contextual fear accompanied by increased c-fos activity in the BLA and reduced c-fos in the prefrontal cortex. Chaperon et al. (2012) found enhanced cued fear conditioning, but unaltered contextual fear conditioning, multiple-trial cued fear conditioning, cued fear extinction, and conditioned taste aversion, in GRPR knockout mice. These findings, together with the lack of effect of intra-BLA GRP on fear conditioning expression (see above) and of either GRP or a GRPR antagonist on amygdalar LTP, led the authors to propose that GRPR signaling might play a limited role in regulating fear-motivated memory.

The findings that mice lacking GRPR show enhanced fear conditioning and amygdalar LTP suggest that the GRPR plays an inhibitory role in fear memory formation and seem to contrast the conclusions from several pharmacological studies indicating that GRPR activation usually enhances, whereas its inhibition impairs, memory for fear-motivated tasks. However, as discussed above, the effects of GRPR ligands on memory are rather complex and usually show an inverted U-shaped dose-response pattern in which different drug doses display contrasting effects. It is possible that GRPRs in different brain areas can either stimulate or impair memory depending on the degree of pharmacological activation or inhibition through mechanisms yet to be characterized. Moreover, first-generation knockout mouse models have their own limitations in studies on memory. For example, the use of knockout mice does not allow the investigation of the role of the targeted gene in separate phases of memory (encoding, consolidation, and expression). In addition, it is possible that knockout mice have upregulation of compensatory pathways in response to the gene ablation, and behavioral alterations observed in knockout mice might be caused by abnormal CNS development rather than specific memory processes. These limitations may confound the interpretation of the findings on behavioral phenotypes of knockout mice. Table 3 summarizes the findings from studies examining the effects of pharmacological and genetic manipulation of bombesin receptors.

Brain systems mediating bombesin receptor regulation of memory

As seen above, the pharmacological manipulation of GRPRs specifically in discrete brain areas showing high GRPR expression, namely, the BLA, the dHIP, and the NTS, after training can modulate memory formation. Consistent with a crucial role of the BLA and NTS in mediating the effects of GRPR ligands on emotional memory consolidation, the memory-enhancing effect of posttraining systemic administration of bombesin was attenuated by unilateral lidocaine inactivation of either the amygdala

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Treatment	Species	Task	Effect on memory	References
Pharmacological manipulations				
GRP, i.p., posttraining	Mouse	T-maze footshock avoidance (weak training)	↑ (lower dose) ↓ (higher dose)	Flood and Morley, 1988 Flood and Morley, 1989
Bombesin, i.p., posttraining	Mouse	T-maze footshock avoidance (weak training)	↑ (lower dose) ↓ (higher dose)	Flood and Morley, 1988 Flood and Morley, 1989
GRP, i.p., posttraining	Mouse	T-maze footshock avoidance (strong training)	↓	Flood and Morley, 1988
Bombesin, i.p., posttraining	Mouse	T-maze footshock avoidance (strong training)	↓	Flood and Morley, 1988
GRP, i.c.v., posttraining	Mouse	T-maze footshock avoidance	↑	Flood and Morley, 1988
Bombesin, i.c.v., posttraining	Mouse	T-maze footshock avoidance	↑	Flood and Morley, 1988
Bombesin, intra-NTS, posttraining	Rat	Inhibitory avoidance	↑	Williams and McGaugh, 1994
Bombesin, intra-NTS, posttraining	Rat	Radial arm maze	↑	Williams and McGaugh, 1994
Bombesin, systemic, posttraining	Rat	Inhibitory avoidance	↑	Rashidy-Pour and Razvani, 1998
[Leu13-(psi-CH2NH)-Leu14]BN (GRPR antagonist), i.p., pretraining	Mouse	Inhibitory avoidance	↓	Santo-Yamada et al., 2003
BIM23127 (NMBR antagonist)	Mouse	Inhibitory avoidance	↓	Santo-Yamada et al., 2003
RC-3095 (GRPR antagonist), intra-dHIP, posttraining	Rat	Inhibitory avoidance	↓ (lower dose) ↑ (higher dose)	Roesler et al., 2003 Dantas et al., 2006 Preissler et al., 2007
RC-3095, i.p., pretraining	Rat	Inhibitory avoidance	↓	Roesler et al., 2004a
RC-3095, i.p., pretraining		NOR	No effect	Roesler et al., 2004a
RC-3095, i.p., posttraining	Rat	Inhibitory avoidance	↓	Roesler et al., 2004b
RC-3095, intra-BLA, posttraining	Rat	Inhibitory avoidance	↓	Roesler et al., 2004b
RC-3095, intra-dHIP, pretraining	Rat	Inhibitory avoidance	↓	Venturella et al., 2005
RC-3095, i.p., pretraining	Rat	Habituation	↓	Venturella et al., 2005
Bombesin, intra-dHIP, posttraining	Rat	Inhibitory avoidance	↑ (lower dose) ↓ (higher dose)	Roesler et al., 2006b Roesler et al., 2009
RC-3095, intra-dHIP, post-extinction training	Rat	Inhibitory avoidance extinction	↓	Luft et al., 2006
PD176252 (GRPR/NMBR antagonist), i.p., pretest	Rat	Fear-potentiated startle	↓	Merali et al., 2006a
PD176252, i.c.v., pretest	Rat	Fear-potentiated startle	↓	Merali et al., 2006a
GRP, intra-PrL, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2006
GRP, intra-IL, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2006
GRP, intra-IL, pretest	Rat	Cued fear conditioning	↓	Mountney et al., 2006
GRP, intra-CeA, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2006
BW2258U89 (GRPR antagonist), intra-PrL, pretest	Rat	Contextual fear conditioning	No effect	Mountney et al., 2006
BW2258U89, intra-PrL, pretest	Rat	Cued fear conditioning	No effect	Mountney et al., 2006
BW2258U89, intra-IL, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2006
BW2258U89, intra-IL, pretest	Rat	Cued fear conditioning	No effect	Mountney et al., 2006
BW2258U89, intra-CeA, pretest	Rat	Contextual fear conditioning	↑ (lower dose) ↓ (higher dose)	Mountney et al., 2006
NMB, i.c.v., pretest	Rat	Fear-potentiated startle	↓	Bédard et al., 2007
BIM23127, i.c.v., pretest	Rat	Fear-potentiated startle	↓	Bédard et al., 2007
GRP, i.c.v., pretest	Rat	Fear-potentiated startle	↓	Bédard et al., 2007
[Leu13-(psi-CH_2NH)-Leu14]BN, i.c.v., pretest	Rat	Fear-potentiated startle	No effect	Bédard et al., 2007
RC-3095, systemic from PND 1–10	Rat	Inhibitory avoidance	↓	Presti-Torres et al., 2007
RC-3095, systemic from PND 1–10	Rat	NOR	↓	Presti-Torres et al., 2007 Presti-Torres et al., 2012
RC-3095, intra-dHIP, postretrieval	Rat	Inhibitory avoidance	↓	Luft et al., 2008
GRP, intra-BLA, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2008
GRP, intra-BLA, pretest	Rat	Cued fear conditioning	No effect	Mountney et al., 2008
RC-3095, intra-BLA, pretest	Rat	Contextual fear conditioning	↓	Mountney et al., 2008
RC-3095, intra-BLA, pretest	Rat	Cued fear conditioning	No effect	Mountney et al., 2008

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Table 3 (Continued)

Treatment	Species	Task	Effect on memory	References
RC-3095, systemic from PND 1–10	Rat (infants)	Odor-shock conditioning	No effect	Garcia et al., 2010
GRP, i.c.v., pretest	Rat	Contextual fear conditioning	↓	Merali et al., 2011
GRP, i.c.v., pretest	Rat	Cued fear conditioning	↓	Merali et al., 2011
GRP, i.c.v., pretest	Rat	Fear-potentiated startle	↓	Merali et al., 2011
RC-3095, i.c.v., pretest	Rat	Contextual fear conditioning	↑	Merali et al., 2011
RC-3095, i.c.v., pretest	Rat	Cued fear conditioning	↑	Merali et al., 2011
GRP, intra-BLA, pretest	Mouse	Contextual fear conditioning	No effect	Chaperon et al., 2012
GRP, intra-BLA, pretest	Mouse	Cued fear conditioning	No effect	Chaperon et al., 2012
(D-Phe^{6},Leu-NHEt^{13},des-Met^{14})-bombesin(6–14) (GRPR antagonist), intra-BLA, pretest	Mouse	Contextual fear conditioning	No effect	Chaperon et al., 2012
Genetic manipulations				
GRPR knockout	Mouse	Contextual fear conditioning	↑	Shumyatsky et al., 2002
GRPR knockout	Mouse	Cued fear conditioning	↑	Shumyatsky et al., 2002 Chaperon et al., 2012
GRPR knockout	Mouse	Morris water maze	No effect	Shumyatsky et al., 2002
NMBR knockout	Mouse	Inhibitory avoidance	↓ (when combined with stress)	Yamada et al., 2003
GRPR knockout	Mouse	Cued fear extinction	↓	Martel et al., 2012
GRPR knockout	Mouse	Contextual fear extinction	No effect	Martel et al., 2012
GRPR knockout	Mouse	Contextual fear conditioning	No effect	Chaperon et al., 2012
GRPR knockout	Mouse	Multiple-trial cued fear conditioning	No effect	Chaperon et al., 2012
GRPR knockout	Mouse	Multiple-trial cued fear conditioning extinction	No effect	Chaperon et al., 2012
GRPR knockout	Mouse	Conditioned taste aversion	No effect	Chaperon et al., 2012

Table 3 Memory modulatory effects of pharmacological and genetic manipulation of bombesin receptors.
BLA, basolateral amygdala; CeA, central amygdala; dHIP, dorsal hippocampus; GRP, gastrin-releasing peptide; GRPR, gastrin-releasing peptide receptor; i.c.v., intracerebroventricular; IL, infralimbic cortex; i.p., intraperitoneal; NMB, neuromedin B; NMBR, neuromedin B receptor; NTS, nucleus tractus solitarius; PN, postnatal Day; PrL, prelimbic cortex.

or the NTS (Rashidy-Pour and Razvani, 1998), and the memory-impairing effect of systemic RC-3095 was blocked by muscimol inactivation of the BLA (Roesler et al., 2006b). Importantly, Mountney et al. (2011) have recently shown that the levels of GRP measured with *in vivo* microdialysis are increased in the BLA after fear conditioning, and GRP release correlates with freezing levels. Extensive evidence indicates that the BLA is critical for enabling the modulatory actions of a variety of drugs and hormones on memory consolidation as well as to regulate synaptic plasticity processes involved in memory formation in other brain areas, including the hippocampus (McGaugh, 2002, 2004). The dHIP is a site for LTP and activation of a range of neurochemical cascades, protein synthesis, and gene expression mediating memory consolidation for inhibitory avoidance (Izquierdo and Medina, 1997). Thus, the expression levels of the GRPR in the BLA and dHIP, as well as the effects of intra-BLA and intra-dHIP infusions of GRPR ligands, and the requirement of the BLA in enabling memory modulation by drugs acting on the GRPR, are strongly consistent with the view that the GRPR acts as a relevant molecular regulator of emotional memory formation. In addition, the fear conditioning studies reviewed above indicate that bombesin receptors in several brain areas, including the PrL, IL, CeA, and BLA, might also modulate the expression of fear-motivated memories (Mountney et al., 2006, 2008).

Signaling mechanisms mediating bombesin receptor regulation of memory

Experiments using combined or sequential infusions of memory-enhancing doses of bombesin and inhibitors or activators of different intracellular signaling pathways into the dHIP were the first used to investigate the molecular mechanisms mediating the memory modulatory activities of bombesin receptors. The enhancement of inhibitory avoidance memory produced by posttraining intra-dHIP bombesin was prevented by RC-3095 but not

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by BIM23127, indicating that it depends on GRPR but not NMBR activation. Bombesin-induced memory enhancement was also prevented by inhibitors of PKC, MAPK, PKA, and PI3K (Roesler et al., 2006b, 2009). Conversely, memory enhancement by bombesin was potentiated by the coinfusion of different activators of the dopamine D1/D5 receptor (D1R) pathway, namely, the D1R agonist SKF 38393, the AC activator forskolin, and the cAMP analog 8-Br-cAMP (Roesler et al., 2006b). The same stimulators of cAMP/PKA signaling block the memory-impairing effect of GRPR blockade by RC-3095 in the dHIP. These findings indicate that the PKC, MAPK, PI3K, and PKA pathways are critical in mediating memory modulation by hippocampal GRPRs and that GRPR activation can interact with cAMP/PKA signaling in enhancing memory formation in the hippocampus. Because GRPR signaling through G_q is not directly coupled with the AC/cAMP/PKA pathway, Roesler et al. (2006b) have hypothesized that increases in Ca^{2+} associated with GRPR activation might activate Ca^{2+}-responsive hippocampal AC (Wong et al., 1999; Chan and Wong, 2005), leading to PKA activation. Figure 3 illustrates a model of intracellular signaling associated with GRPR modulation of memory formation in the hippocampus.

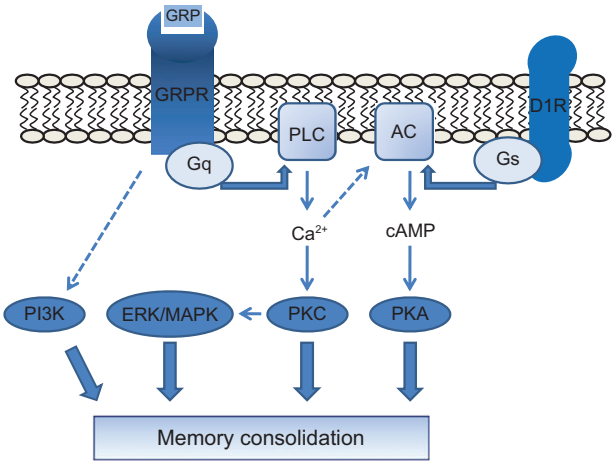

Figure 3 A proposed model of intracellular signaling mechanisms mediating the regulatory influence of GRPRs on memory consolidation.
Bombesin (BB)-induced enhancement of emotional memory in the rat dHIP requires PKC, MAPK, PKA, and PI3K and is potentiated by the activation of the D1R/cAMP/PKA pathway (Roesler et al., 2006a, 2009). Bombesin or GRP can activate G_q protein (G_q)-coupled GRPRs at the postsynaptic membrane, leading to an increase in Ca^{2+} and stimulation of the PLC/PKC and ERK/MAPK pathways. D1R is coupled to G_s protein (G_s) and AC activation. The D1R-induced cAMP signal might be potentiated by Ca^{2+}-induced stimulation of Ca^{2+}-responsive types of AC (Wong et al., 1999; Chan and Wong, 2005), providing a possible mechanism for the requirement of cAMP/PKA signaling for GRPR influences on memory. Modified from Roesler et al. (2006b).

Bombesin receptor interactions with other neurotransmitter, hormone, and growth factor systems in regulating memory

The GRP/GRPR system in the brain may interact with signaling triggered by other neurotransmitters, hormones, and neuronal receptors involved in regulating memory consolidation. The enhancement of inhibitory avoidance retention produced by posttraining intra-dHIP infusion of a high dose of RC-3095 was prevented by an otherwise ineffective dose of the $GABA_A$ receptor agonist muscimol, suggesting that the memory-enhancing effect of high doses of GRPR antagonists might be mediated by inhibition of hippocampal GABAergic transmission (Dantas et al., 2006). The memory-impairing effect of a lower dose of intra-dHIP was prevented by a coinfusion of an otherwise infective dose of basic fibroblast growth factor/fibroblast growth factor-2 as well as by a systemic injection of the glucocorticoid receptor agonist dexamethasone (Venturella et al., 2005).

Therapeutic implications of bombesin receptors in memory and anxiety disorders

As reviewed above, bombesin receptors in the mammalian brain are likely activated by endogenous BLPs released from neurons as cotransmitters. Moreover, bombesin receptors are highly expressed in brain areas involved in memory formation and expression and regulating emotional responses, and a range of pharmacological and genetic studies in rodents indicates that both NMBR and GRPR can act as molecular regulators of memories associated with emotional arousal. In addition, the presence of BLPs in brain regions known to be activated by stressors, together with findings that exposure to stressors alters the release of BLPs, suggests that bombesin receptors may play a more general role in mediating and integrating stress responses (Merali et al., 2002). This evidence is consistent with a possible role of deregulated bombesin receptor signaling in memory dysfunction and anxiety disorders. In fact, some human studies suggest that alterations in bombesin receptor function are associated with neurological and psychiatric disorders affecting memory and emotional responses. Thus, bombesin receptor density and bombesin-induced

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calcium signaling are altered in fibroblasts from patients with Alzheimer's disease (AD; Ito et al., 1994). In fibroblasts from patients with familial AD presenting the Swedish APP670/671 mutation, elevations in calcium induced by bombesin were reduced by 40% (Gibson et al., 1997). In addition, GRPR has emerged as a candidate gene in autism spectrum disorders (ASD). A translocation breakpoint on the X chromosome in the first intron of the *GRPR* gene was described in a patient with autism accompanied by mental retardation and epilepsy (Ishikawa-Brush et al., 1997). A subsequent study investigating two polymorphic sites in the second exon of the *GRPR* gene in patients with autism did not support that the *GRPR* is a candidate locus for autism (Marui et al., 2004). However, a possible role of C6S and L181F mutations of the *GRPR* gene in GRPR function and ASD has been identified in two patients (Seidita et al., 2008). Evidence from preclinical experiments showing impaired social behavior in rats given neonatal GRPR blockade supports a role for GRPR in ASD (Presti-Torres et al., 2007, 2012).

Merali et al. (2006b) described discrete alterations in the levels of NMB and GRP analyzed postmortem in some areas relevant for stress responses in the brains of suicide cases compared to control subjects. Subsequently, an association and linkage analysis of GRP and GRPR as candidate genes in panic disorders did not find a relevant linkage (Hodges et al., 2009). Further studies are required to establish what, if any, is the significance of alterations in the *GRPR* gene or receptor-triggered signaling for the pathogenesis of brain disorders. Taking together the evidence from preclinical and human studies, we have proposed (Roesler et al., 2004a, 2006a, 2007; Merali et al., 2006a; Presti-Torres et al., 2007) that bombesin receptors should be investigated as novel therapeutic targets in neurological and psychiatric disorders, particularly those affecting memory and anxiety. Recent preclinical experiments have indicated that, as with central administration, systemically administered GRP is effective at reducing both the expression and the reconsolidation of learned fear, supporting the view that GRP has the potential of alleviating fear memories. The possible relative selectivity of neuronal GRPRs in regulating primarily emotionally influenced aspects of memory might make GRPR signaling a particularly promising target among neuropeptide systems. Human studies examining the effects of GRP administration on satiety and eating behavior (Gutzwiller et al., 1994) and the effects of RC-3095 in patients with cancer (Schwartsmann et al., 2006) have provided preliminary evidence indicating that neither GRP nor a GRPR antagonist induce overt side effects when given intravenously in human subjects, suggesting that the potential therapeutic effect of GRPR ligands could be investigated in patients with CNS disorders involving memory dysfunction and anxiety.

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