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Development and function of bombesin-like peptides and their receptors

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ABSTRACT Amphibian bombesin and its related peptides consist a family of neuropeptides in many vertebrate species. Bombesin and two major bombesin-like peptide in mammals, gastrin-releasing peptide (GRP) and neuromedin B (NMB), have been shown to elicit various physiological effects. These include inhibition of feeding, smooth muscle contraction, exocrine and endocrine secretions, thermoregulation, blood pressure and sucrose regulations and cell growth. Receptors for GRP and NMB (GRP-R and NMB-R), as well as third subtype of bombesin-like peptide receptor (BRS-3) have been cloned. These receptors are G-protein-coupled receptors and are expressed in various brain regions and in the digestive tract. In this paper, we will summarize studies on these peptides and their receptors, with special reference to research using gene-knockout mice. These studies clearly demonstrated the role of three receptors *in vivo* and *in vitro*. We will also discuss the phylogeny of these receptors.

KEY WORDS: *gastrin-releasing peptide, neuromedin B, bombesin-like peptide receptor subtype-3 (BRS-3)*

Discovery and purification of bombesin and related peptides

Bombesin is one of the active peptides purified from amphibian skin (Anastasi *et al.,* 1971). This peptide is also active in mammals and its pharmacological effect extends into various physiological aspect: hypertensive action, contractive effect on uterus, colon or ileum, stimulating action on the gastric secretion, hyperglycemic effect or increasing insulin secretion (Erspamer *et al.,* 1970). Many other peptides structurally related to bombesin are discovered from amphibian skin and divided into three groups: bombesin family that includes bombesin and alytesin, ranatensin family that includes ranatensin, litorin and their derivatives and phyllolitorin family (Erspamer *et al.,* 1984). The first mammalian bombesin-like peptide was isolated from porcine gastric tissue and named gastrin-releasing peptide (GRP) because of its potent gastrin releasing action (McDonald *et al.,* 1979). GRP has His-Leu-Met at its C-terminal region and shows high homology with bombesin and alytesin. Similarly, Neuromedin B (NMB) having His-Phe-Met at its C-terminal was identified from porcine spinal cord as a novel decapaptide that potently stimulates rat smooth muscle and classified into ranatensin family (Minamino *et al.,* 1983).

Immunochemical analysis using specific antibodies against bombesin, GRP or NMB revealed the existence of similar peptides in brain or gastric tissues of various species. Biochemical methods combined with radioimmunoassays or bioassays as well as molecular cloning techniques were employed for the isolation of bombesin-like peptides in mammals, birds, reptiles and fish. Only GRP or NMB homologues have been identified so far from these species and their peptide sequences were indicated in Figure 1.

Molecular cloning of *GRP* and *NMB*

Bombesin-like immunoreactivities are detected in brain, spinal cord, gastrointestinal tissues as mentioned above, but in addition, its localization in neuroendocrine cells in the lung is pointed out (Wharton *et al.,* 1978). Moreover, pulmonary carcinoid tumors as well as many small cell lung carcinomas are positive for bombesin immunoreactivity (Moody *et al.,* 1981; Yang *et al.,* 1983). By using human pulmonary carcinoid tumor rich in GRP-immunoreactivity as RNA source, Spindel et al. (1984) succeeded in cloning cDNA encoding human GRP. Human mature GRP is processed from a precursor form that consists of 148 amino acids

Abbreviations used in this paper: BRS-3, bombesin-like peptide receptor subtype 3; GRP, gastrin-releasing peptide; MCH, melanin-concentrating hormone; NMB, neuromedin B.

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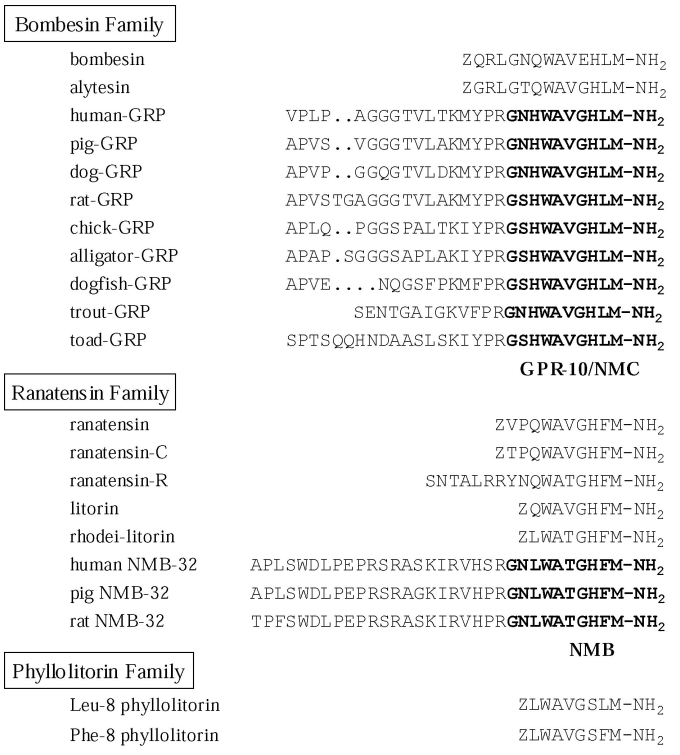

Fig. 1. Structure of bombesin and its related peptides.

of typical signal sequence, GRP sequence of 27 amino acids and a carboxyl-terminal extension peptide.

Another bombesin-like peptide in mammals, NMB, is also expressed in brain and gastrointestinal tissue. cDNA encoding human NMB was isolated from human hypothalamic library (Krane *et al.,* 1988). Similar to GRP, NMB is encoded in a 76 amino acids precursor consists of a signal peptide, 32 amino-acid for large form of NMB and a carboxyl-terminal extension peptide. These two peptides share only 48% of identity at nucleotide level and localized to different chromosome (*GRP:* chromosome 18; *NMB:* chromosome 15). This fact indicates that these peptides might arise from a common ancestral gene but the divergence occurred rather early in evolutional event.

Distribution of *GRP* and *NMB* mRNA

Cloning of cDNA coding for GRP and NMB permits to describe and compare fine distribution of these peptides in the brain. To this aim, rat *GRP* and rat N*MB* genes were used to generate cRNA probe for in situ hybridization (Lebacq-Verheyden *et al.,* 1988; Wada *et al.,* 1990). Distributions of these two genes are rather distinct. Generally, *GRP* is expressed more widely and strongly compared to *NMB* in rat brain. Strong *GRP* mRNA expression was observed in hippocampal formation, notably entorhinal area, subiculum, Ammon's horn and dentate gyrus and also in several nuclei of amygdala. Isocortex, anterior olfactory nucleus, medial geniculate nucleus, suprachiasmatic nucleus, medial preoptic nucleus and parabrachial nucleus also express *GRP* moderately. In contrast, *NMB* mRNA expression is more restricted. Its expression is prominent in the main olfactory bulb, in the mitral cell and the external plexiform layers and the polymorph layer of the dentate gyrus. Central nucleus of amygdala, substantia nigra, ventral tegmentum area are also positive for *NMB* signal. In the brain stem, cells in somatosensory and motor nuclei and raphe express *NMB* mRNA moderately. In most of these regions, distributions of the *NMB* signals do not overlap with those of *GRP*. In consistent of this view, in the peripheral nervous system, strong hybridization signals for *NMB* are detected in dorsal root ganglion as well as trigeminal ganglion although these regions are negative for *GRP* mRNA signals.

The difference in distribution of these two peptides indicates that the function of GRP and NMB may be partly overlapped but rather distinct. Although in situ hybridization studies can tell us which cell may synthesize GRP or NMB, they cannot indicate where these peptides may act. The sites of action may be the axon terminals of nerve cells expressing *GRP* or *NMB* mRNA, where they form synaptic junctions with other cells and transmit the signal via specific receptors. Therefore, the precise study elucidating the individual role of these peptides required cloning and analysis of receptors for these peptides.

Cloning of GRP *(GRP-R)* and NMB receptors *(NMB-R)*

By using electrophysiological and luminometric Xenopus oocyte expression assays, Spindel et al. (1990) succeeded in cloning bombesin/GRP receptor from murine Swiss 3T3 cells that were known to express high levels of receptors. The cDNA clone encoded for the same receptor was also isolated by Battey et al. (1991) who constructed enriched cDNA library by subtracting Balb 3T3 mRNA from Swiss 3T3 cDNA pool and screened this library with an oligonucleotide probe designed to be specific for sequences encoding an fragment of GRP-R, isolated in advance from the purified GRP-R. Analysis of putative amino-acid sequences of this *GRP-R* predicted seven hydrophobic transmembrane domains indicating that this receptor is a member of the G-protein coupled receptor. Subsequently, *NMB-R* was cloned from a rat esophagus cDNA library by low-stringency cloning using a mouse *GRP-R* cDNA as a probe (Wada *et al.,* 1991). Properties of this receptor are different from those of GRP-R. NMB-R shows higher affinity binding to NMB than to GRP when expressed on Balb 3T3 cells. In the brain, *NMB-R* expression is prominent in the olfactory and central thalamic regions, while characteristic expression of *GRP-R* is observed in the hypothalamic region.

Detailed study about distribution of these two receptors in brain was realized in rat by in situ hybridization (Wada *et al.,* 1992). They reported that moderate expressions of both receptors are detected in dentate gyrus and nucleus ambiguus, but in other brain area, expressions of these two receptors are rather complementary. Prominent expression of *GRP-R* is detected in hypothalamic region, whereas *NMB-R* is extensively expressed in olfactory and thalamic regions. In agreement with the distinct pattern of expression described for *GRP* and *NMB* as mentioned above, these results predict proper roles to GRP-R and NMB-R.

GRP-R and *NMB-R* are also cloned from human small cell lung carcinoma cell line (Corjay *et al.,* 1991). It is suggested that bombesin-like peptides may be involved in the pathogenesis and maintenance of some human lung carcinoma tumors and indeed, several human lung cancer cell lines express *GRP-R* and/or *NMB-R* at varying levels.

Cloning of bombesin-like peptide receptor subtype-3 *(BRS-3)*

In an attempt to search for novel G-protein-coupling receptors from guinea-pig uterus, Gorbulev et al. (1992) cloned a new subtype of bombesin-like peptide receptor. When searched in the database, this cDNA clone encodes a protein showing the highest amino acid similarity to GRP-R (52%) and NMB-R (47%) and designated as bombesin-like peptide receptors subtype-3 (*BRS-3;* Fig. 2). The gene encoded for human homologue of *BRS-3* was reported subsequently (Fathi *et al.,* 1993; Gorbulev *et al.,* 1994). As in the guinea-pig, *BRS-3* is expressed in human uterus. Its expression is also detected in human testis and several lines of lung carcinoma cells. Cloning of mouse *BRS-3* revealed that it is expressed in brain but rather restricted fashion (Ohki-Hamazaki *et al.,* 1997a). Its expression is limited in several nuclei of hypothalamic and hindbrain regions (Ohki-Hamazaki *et al.,* 1997a; Yamada *et al.,* 1999). Although expressions of *BRS-3* in mouse testis, pregnant or non-pregnant uteri are barely detected, all of these tissues express mouse *NMB-R.* In sheep, *BRS-3* is expressed in hypothalamus, pituitary, but not in testis or uteri (Whitley *et al.,* 1999). In the case of rat, *BRS-3* is detected in testis and brain including medial habenula nucleus and various hypothalamic nuclei (Liu *et al.,* 2002). To sum up, *BRS-3* is generally expressed in brain, notably in hypothalamic region, but its expression in uterus or testis varies between species. Interestingly, this absence of expression is often supplemented by other subtype(s) of bombesin-like peptide receptors.

The affinity of BRS-3 for bombesin is lower than that of GRP-R or NMB-R. Moreover, GRP and NMB only have a poor potency for BRS-3. Seek for endogenous high-affinity ligand for BRS-3 has not yet been succeeded, but instead, molecular genetic approach shed light on the role of this receptor.

Other subtypes of bombesin-like peptide receptors *(BB4* and *BRS-3.5)*

In search of receptors for bombesin-related peptides in amphibian, Nagalla et al. (1995) isolated two clones encoding fragments highly homologous to mammalian GRP-R and NMB-R and one clone encoded for a novel bombesin receptor subtype and named BB4. This receptor has higher affinity for bombesin than GRP and shared only 56%, 61% and 70% amino acid identity to the human GRP-R, human NMB-R and human BRS-3, respectively. mRNA expression of this receptor is detected only in brain.

Another subtype of bombesin-like peptide receptor was cloned from chick brain (Iwabuchi *et al.,* 2003). This subtype, called BRS-3.5, has moderate affinity for bombesin but low affinity for both GRP and NMB. Distinct from chick *GRP-R* that is expressed in brain and gastrointestinal tissues, *BRS-3.5* is expressed only in brain. Chick GRP-R has 82% identity to human GRP-R at the level of amino acid, but chick BRS-3.5 shows only 58% and 52% similarities to human GRP-R and NMB-R, respectively. This receptor has highest similarity for amphibian BB4 (74%) and human BRS-3 (69%) and this is the origin of its name.

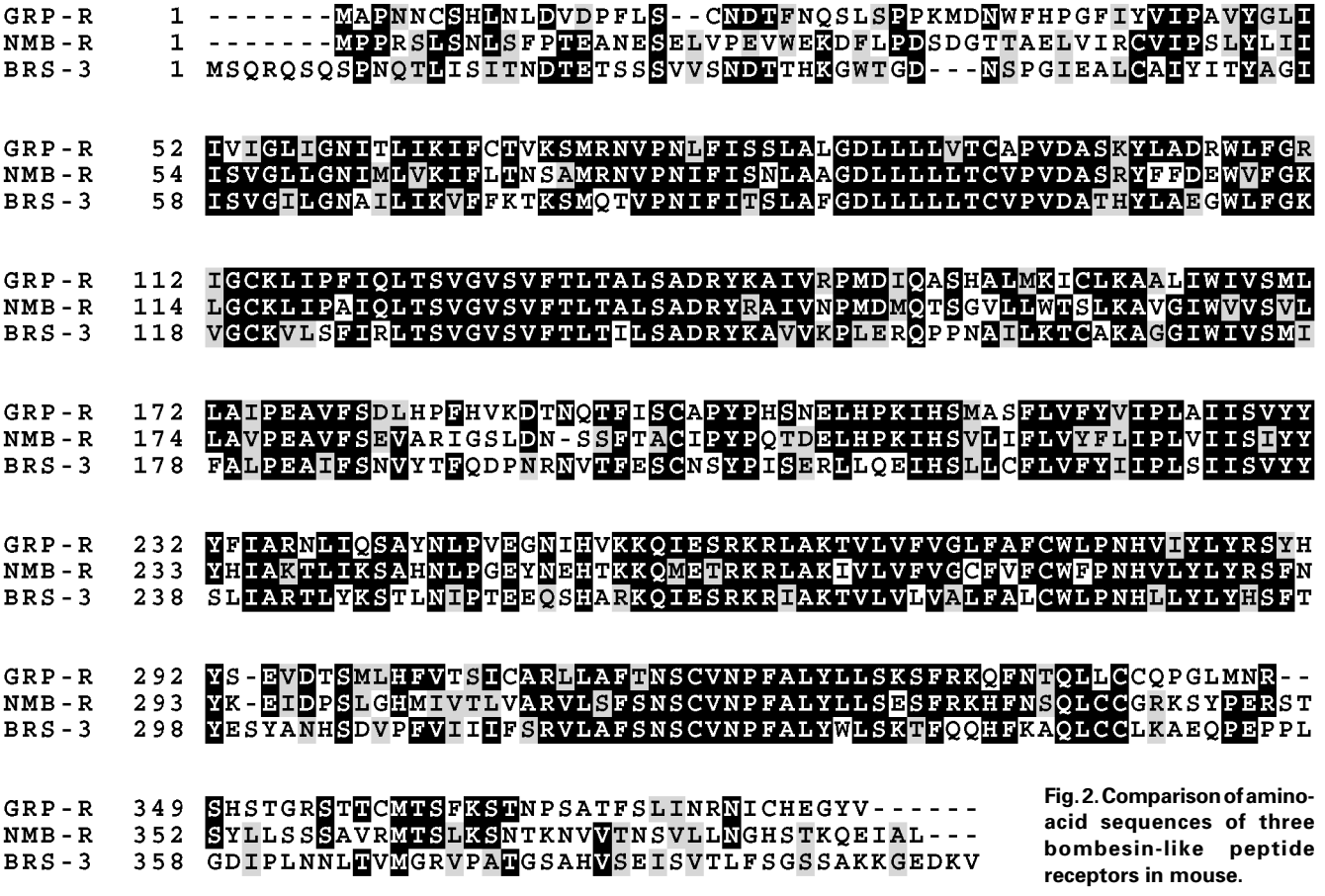

Fig. 2. Comparison of amino-acid sequences of three bombesin-like peptide receptors in mouse.

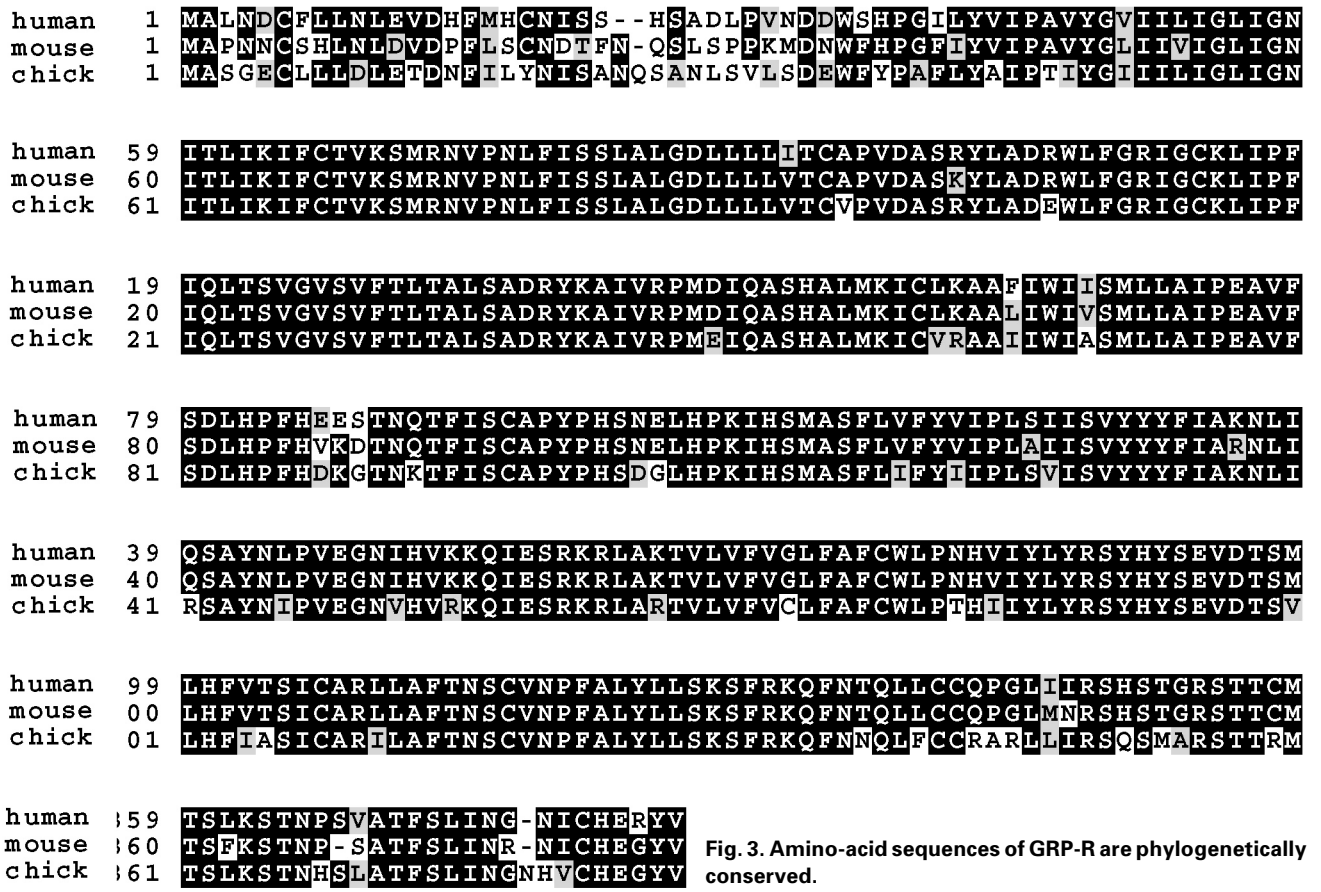

Fig. 3. Amino-acid sequences of GRP-R are phylogenetically conserved.

Bombesin-like peptide receptors are phylogenetically conserved

GRP-R and *NMB-R* are cloned from various species. When their amino acid sequences are compared, the sequence of GRP-R or NMB-R is well conserved among species. Homology is more than 90% among mammals and reaches 80% between mammal and birds (Fig. 3). Similarity of BRS-3 among mammals is lower than that of GRP-R, but is above 84%.

GRP-Rs cloned from various source share common pharmacological properties. GRP-R has high affinity for GRP and bombesin, low affinity for NMB. In contrast, all NMB-Rs have high affinity for NMB, moderate affinity for bombesin, but low affinity for GRP.

These facts indicated that these receptors are evolutionary conserved. In consistent with this view, *GRP-R* gene is localized on chromosone X in human (Xp22) and mouse and on chick chromosome 1 (1q23-q24), in the region where chicken homologs of other human X-linked genes have also been mapped (Iwabuchi *et al.,* 2003). Human *BRS-3* gene is mapped to chromosome X (Xq26-q28) and mouse *BRS-3* gene is also mapped to chromosome X. *NMB-R* gene is localized to human 6q21-qter and to mouse chromosome 10. Many human genes assigned to chromosome 6 were found to have orthologs on this region of mouse chromosome 10.

Concerning distribution pattern of the receptors in brain, *NMB-R* and *BRS-3* mRNA expressions in rat and mouse brain were reported and can be compared. In mouse brain, *NMB-R* is expressed abundantly in olfactory and thalamic regions as reported in rat brain (Wada *et al.,* 1992; Ohki-Hamazaki *et al.,* 1997a). Expressions of *BRS-3* mRNA are limited in both rat and mouse brain and were generally observed in several nuclei of the hypothalamus and lateral parabrachial nucleus of the brainstem (Yamada *et al.,* 1999; Liu *et al.,* 2002). However, several differences of expression between these two species were observed. Notably, in the medial habenula nucleus of the rat brain express *BRS-3* extensively but not of mouse brain. Recent cloning and characterization of chick *GRP-R* and *BRS-3.5* allow us to compare distribution of avian bombesin-like peptide receptors with those of rat and mouse (Maekawa *et al.,* 2004a). Chick *BRS-3.5* is widely distributed in the adult forebrain, whereas chick *GRP-R* mRNA is only detected in hypothalamus and medial striatum at early post-hatch period (Fig. 4). *BRS-3.5* expression pattern at late embryonic period (E16; 16 days of embryonic age) as well as one day after hatching is comparable to that of adult. As shown in Table 1, *BRS-3.5* expression is prominent in the hyperpallilum and nidopallium. These brain regions are considered to be homologous to layer I-III of mammalian cortex based on the comparison of connectivity pattern of visual pathways (Karten, 1969, see also http://www.avianbrain.org/). Indeed, layer II and III of rat cortex express one subtype of bombesin-like peptide receptor, *GRP-R*. Similarly, nucleus taeniae, homologous to mammalian amygdala, piriform cortex, hippocampus are positive for chick *BRS-3.5.* In addition, *GRP-R, NMB-R* and/or *BRS-3* mRNA are expressed in the

corresponding regions in mammals. In contrast, Entopallium, avian homologue of cortex layer IV and globus pallidus do not express any bombesin-like peptide receptors as in mammals. Chick *GRP-R* is detected in hypothalamus, medial striatum, thalamus and superficial layer of optic tectum at E16. In rat brain, *GRP-R* and *BRS-3* expressions are observed in the hypothalamic region and *NMB-R* in the thalamic region. Expression of these receptors in hypothalamic and thalamic regions suggest that they may have some effect in the development of these regions. In the early chick embryo, at E9, *BRS-3.5* expression is observed in the ventral part of the dorsal ventricular ridge. *GRP-R* expression is first detected in the pallidum of the forebrain at E5 and then in the subpallium at E9. Cobos et al. (2001) reported that this subpallial region is a source of inhibitory GABAergic interneurons. Cells originated within the subpallium migrated tangentially and finally invaded the pallium where they differentiated into interneurons. Thus, it would be possible that *GRP-R* expression is important for division and/or migration of these cells. Moreover, important role of GRP/GRP-R in the GABAergic interneurons in the amygdala in mouse is demonstrated and will be discussed later. *GRP-R* and *NMB-R* mRNAs are detected in rat caudate-putamen at early postnatal period, but are absent from adult caudate-putamen. In the avian homologous region, medial and lateral striatum, chick *BRS-3.5* is expressed in the embryo as well as in the adult. Search for the roles of these receptors in the development of caudate-putamen would be interesting.

Generation and analysis of mice lacking each receptor subtype reveal functional properties of receptors

Physiological functions of bombesin, GRP and NMB, when injected into animals or when applied to tissue and cultured cells, were well documented (for review, see Ohki-Hamazaki, 2000). After the cloning of receptors, the pathways by which these peptides exert their effects have been elucidated. Nonetheless, since GRP-R and NMB-R have substantial affinity for both GRP and NMB and since distribution of these receptors are overlapped in gastrointestinal tract as well as in many brain regions, the roles of each receptor in various biological effects have remained obscure. Concerning BRS-3, absence of information about high affinity endogenous ligand has disturbed us to explore the function of this receptor. Thus, cellular or molecular mechanism supporting the effect of these peptides has remained unknown. By using gene- targeting methods, three mouse lines lacking one of these three receptors are produced. Analyzing the phenotypes of these mice helped us to a great extent to elucidate the functions of three receptors.

One of the most well-known effect of bombesin, GRP and NMB, is an inhibition of food intake and the expressions of *GRP-R* and *NMB-R* in the hypothalamic feeding center predicted participation of these two receptors in the feeding regulation (Wada *et al.,* 1990; Wada *et al.,* 1992). We and other groups examined food intake and body weight of knock-out mice. Contrary to our expectations, the

TABLE 1

COMPARISON OF RECEPTOR DISTRIBUTION IN MURINE AND CHICK BRAINS

	Rat [a]GRP-R	Rat [a]NMB-R	Rat/Mouse [b]BRS-3		Chick [c]GRP-R	Chick [c]BRS-3.5
Anterior olfactory n.	+/-	+++	-	Anterior olfactory n.	-	++
Cortex I	-	-	-	[d]Hyperpallium	-	+++
II	++	-	-	Nidopallium	-	+++
III	++	-	-	Intermediate nidopallium	-	+++
				Caudal nidopallium	-	+++
IV	-	-	-	Entopallium	-	-
V	-	++	-	[e]Anterior arcopallium	-	-
VI	-	++	-	[e]Posterior arcopallium	-	+/-
				[e]Intermediate arcopallium	-	+/-
Piriform cortex	+/-	+++	-	Piriform cortex	-	++
[f]Hippocampus	++	+++	-	Hippocampus	-	+++
[g]Amygdala				Nucleus Taeniae	[h]-	+
Amygdalo-Hippocampal area	+/-	+++	-	Posterior arcopallium	-	+/-
Accessory amygdalar n.	-	-	-	Intermediate arcopallium	-	+/-
Hypothalamus	++	+	+	Hypothalamus	+	-
Thalamus	-	+++	-	Thalamus	[h]-	-
Caudate-putamen	[i]-	[i]-	-	Medial Striatum	+	+
				Lateral striatum	-	+
Dorsal pallidal complex (globus pallidus/ento-peduncular n.)	-	-	-	Globus pallidus	-	-
Bed n. of stria terminalis	+	+	+	Lateral part of the bed n. of stria terminalis	-	++

[a]Adult rat, Wada *et al.,* 1992.
[b]Adult rat, Liu *et al.,* 2002; adult mouse, Yamada *et al.,* 1999.
[c]One day post-hatching, Maekawa *et al.,* 2004a.
[d]Hyperpallium is considered homologous to the mammalian dorsal cortex, but the details of this homology are not clearly defined. See http://www.avianbrain.org/ for details.
[e]According to the cortical-layered hypothesis, mammalian cortical layers V/VI are similar to avian arcopallium, but the avian arcopallium has more features in common with the mammalian amygdala than with either the motor cortex or layer V of the cortex.
[f]In the rat hippocampal formation, entorhinal area, presubiculum, parasubiculum, subiculum, CA1/CA3 and dentate gyrus are positive for both GRP-R and NMB-R mRNAs.
[g]In the medial nucleus, GRP-R, NMB-R and BRS-3 mRNAs are expressed. Expressions of these receptors are also detected in several other nuclei.
[h]Expression is detected in E16 chick embryo.
[i]mRNA is observed in the early postnatal period, but not in adult animals.

amount of food consumed and body weight were unchanged in GRP-R-deficient or in NMB-R-deficient mice compared with wild-type littermates, although old GRP-R-deficient mice showed slight increase in body weight (Wada *et al.,* 1997; Hampton *et al.,* 1998; Ohki-Hamazaki *et al.,* 1999; Ladenheim *et al.,* 2002; Maekawa *et al.,* 2004b). It is demonstrated that this change was due to the increase of pellet consumed at each meal, but not the alteration of total amount of food consumed. It may be possible that GRP/GRP-R is important for the termination of meals in mice. When GRP-R- or NMB-R-deficient mice are injected intraperitoneally with bombesin, GRP or NMB, bombesin and GRP inhibit glucose intake in wild-type mice, but not in GRP-R-deficient mice (Ladenheim *et al.,* 2002). GRP inhibit glucose intake in NMB-R-deficient and wild-type mice equally (Ohki-Hamazaki *et al.,* 1999). NMB was not effective in feeding suppression in mice. Central administration of bombesin also inhibit food intake in wild-type mice, but low dose was not effective in GRP-R-deficient mice (Maekawa *et al.,* 2004b). Thus, for the regulation of food intake, GRP/GRP-R seems to be more important than NMB/NMB-R.

Surprisingly, BRS-3-deficient mice showed mild obesity associated with hypertension, impairment of glucose tolerance and insulin resistance (Ohki-Hamazaki *et al.,* 1997b). Reduced metabolic rate, increased feeding efficiency and hyperphagia were found in these mice and were attributed to a cause of obesity. These mice have elevated level of circulating leptin and we demonstrated that they were resistant to leptin administration. When leptin was applied intracerebroventricularly, food intake was inhibited in wild-type mice, but this effect was attenuated in BRS-3-deficient mice (Maekawa *et al.,* 2004b). To further investigate the mechanism of leptin resistance, we treated these mice with various orexigenic or anorexigenic peptides. Although most of these peptides were similarly effective in wild-type and BRS-3-deficient mice, orexigenic response to melanin-concentrating hormone (MCH) was enhanced in BRS-3 deficient mice (Fig. 5). In addition, *MCH* as well as *MCH-R* mRNA expressions in hypothalamus were significantly elevated in BRS-3-deficient mice. These results demonstrated that BRS-3 constitutes a member of appetite-regulating network in the hypothalamus.

Recently, it has been shown that GRP/GRP-R signaling in the amygdala is important for inhibiting memory specifically related to learned fear (Shumyatsky *et al.,* 2002). GRP-R-deficient mice showed decreased inhibition of principal neurons by the interneurons in the lateral nucleus of the amygdala, enhanced long-term potentiation and greater and more persistent long-term fear memory. The roles of GRP and GRP-R expressed in the amygdala are thus clearly shown.

It has been demonstrated that *GRP* and *GRP-R* mRNAs are detected abundantly in suprachiasmatic nucleus (SCN) (Wada *et al.,* 1990; Wada *et al.,* 1992). The light-induced phase shift in behavior and the induction of mPer mRNA in the dorsal SCN were attenuated in GRP-R-deficient mice, demonstrating that GRP is important in conveying the photic entrainable signals in the SCN (Aida *et al.,* 2002).

Finally, the role of NMB/NMB-R is still indistinct. Although this system may take part in modulation of serotonergic (5-HT) system and stress response, the machinery of this modulation has not yet clarified (Yamano *et al.,* 2002). Instead, by comparing responses of NMB-R-deficient mice to NMB or GRP, functional segregation of these two receptors has been investigated (Ohki-Hamazaki *et al.,* 1999). Smooth muscle strip was prepared from stomach of these mice and then contraction elicited by GRP or NMB was observed. Deficiency in NMB-R did not affect contractile responses

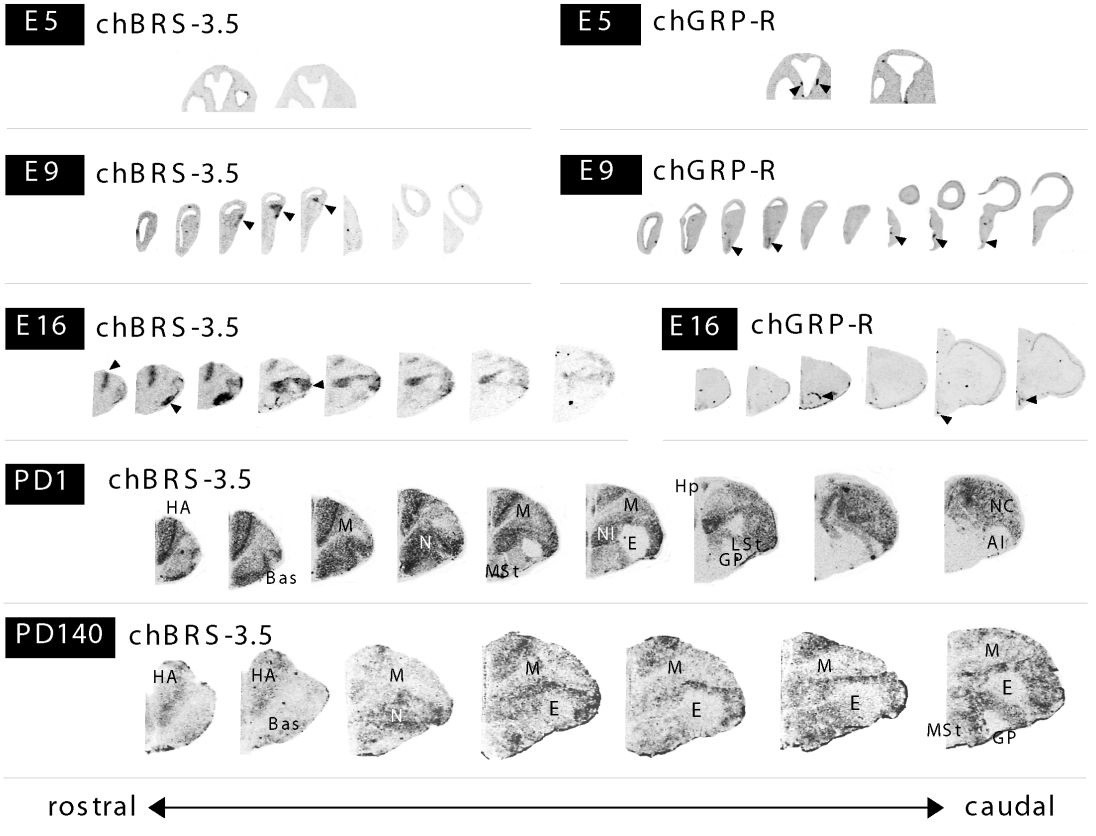

Fig. 4. Distributions of BRS-3.5 and GRP-R mRNAs in chick brain. *BRS-3.5 expression is detected at E9 in the DVR (dorsal ventricular ridge; arrowheads) and GRP-R signal is observed at E5 in the pallidum (arrowheads) and in the subpallium at E9 (arrowheads). At E16 and PD1, BRS-3.5 is strongly expressed in the HA, Bas and N, whereas GRP-R expression is restricted in the superficial layer of optic tectum, thalamic and hypothalamic regions at E16. Arrowheads in chGRP-R at E16 indicate signals in medial portion of pallial-subpallial lamina, suprachiasmatic nucleus and dorsal hypothalamic area. Abbreviations: HA, accessory part of the hyperpallium; Bas, the basorostral pallial nucleus; M, mesopallium; N, nidopallium; MSt, medial striatum; NI, intermediate nidopallium; E, entopallium; Hp, hippocampus; LSt, lateral striatum; GP, globus pallidus; NC, caudal nidopallium; AI, intermediate arcopallium.*

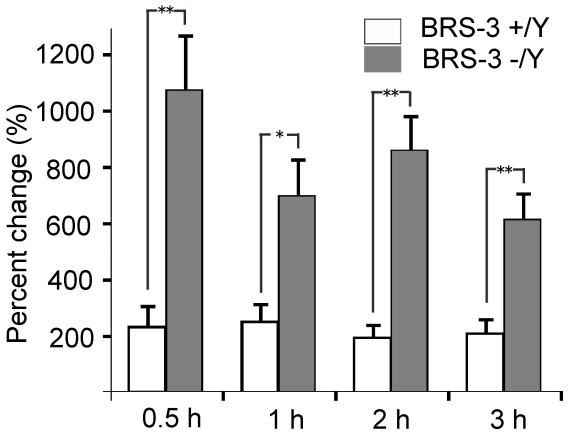

Fig. 5. Effect of intracerebroventricular MCH injection on food intake. *Relative values against the amount of food consumed with saline injection at each time point are indicated.* *, $P < 0.05$; **, $P < 0.01$.

to both peptides. This result suggests that smooth muscle contraction of fundus is mainly regulated by NMB, GRP and GRP-R. These two receptors are expressed in the preoptic area and may regulate body temperature. To examine to what extent these two receptors are concerned in thermoregulation, GRP or NMB was infused in the lateral ventricule of NMB-R-deficient mice. Although the hypothermic effect of NMB was reduced by 50% in NMB-R-deficient mice, the effect of GRP infusion was comparable to the wild-type mice. Therefore, NMB/NMB-R has an essential role in thermoregulation in parallel with GRP/GRP-R.

Despite wide distribution of *GRP-R* and *NMB-R* in embryonic brain in rat (Wada *et al.,* 1993), no developmental defect was observed in these knock-out mice brain. Since these receptors are also expressed in peripheral organs of rat embryo and in small cell lung carcinoma and colon cancer cells, the roles of these receptors in organogenesis are speculated. But it is reported that GRP/GRP-R play a only transient and non-critical role in intestinal development (Carroll *et al.,* 2002).

Concluding remarks

Studies on neuropeptides, since their discovery, extend from biochemical, pharmacological and histological to molecular and cellular biological approaches. Bombesin and its related peptides are not the exception. Molecular cloning of bombesin-like peptide receptors extensively promoted the research aiming at the cellular and molecular mechanism of their action. Powerful methods of molecular biology greatly contributed to the development of this area. But we recognize that combining methods of traditional experimental biology and others is always useful and indispensable for development of research: One of the authors learned this point in Nogent with many other things that cannot be delineated here.

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