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Bombesin-Like Receptor 3: Physiology of a Functional Orphan

Cuiying Xiao¹ and Marc L. Reitman^{1,*}

¹Diabetes, Endocrinology, and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA

Abstract

Bombesin-like receptor 3 (BRS-3) is an X-linked orphan Gq-coupled receptor that regulates food intake, metabolic rate, body temperature, heart rate, blood pressure, and insulin secretion. Most BRS-3 actions occur via the brain, through mechanisms including regulating sympathetic outflow. Ablation of *Brs3* causes obesity, while synthetic agonists produce weight loss.

Keywords

energy expenditure; food intake; insulin secretion; sympathetic tone; heart rate; blood pressure

Bombesin-like receptor 3 (BRS-3, also known as bombesin receptor subtype 3 and BB₃) is an orphan X-linked Gq-coupled receptor [1]. BRS-3 was named for its sequence similarity to receptors binding bombesin, a 14-amino acid frog peptide. The mammalian receptors most similar to BRS-3 bind neuromedin B or gastrin-releasing peptide. However, no endogenous ligand for BRS-3 has been identified; specifically none of neuromedin B, gastrin-releasing peptide, bombesin, or CCHamides (agonists for insect homologues) binds mammalian BRS-3 tightly. Attempts to deorphanize BRS-3 [2] have identified only low affinity agonists, including hemorphins and peptide E. Parabiotic mouse experiments did not detect a circulating ligand [3]. While it is possible that no endogenous ligand exists (e.g. the receptor's ligand-independent activity [4] contributes a basal signaling tone), we do not favor this hypothesis. Rather, evolutionary conservation of high affinity binding suggests that there is an endogenous mammalian ligand, possibly a peptide or peptide derivative.

The lack of a natural ligand has hindered study of BRS-3. For example, is the ligand a hormone, neurotransmitter, or both? Since the conditions regulating endogenous ligand production are unknown, the physiologic conditions causing BRS-3 activation are also unknown and it has been difficult to study effects of receptor activation. The welcome discovery of potent, selective synthetic ligands facilitated study of BRS-3 physiology (reviewed in [1]). However, without an endogenous ligand, one cannot know if the effects of synthetic probes faithfully mimic endogenous physiology, reflect unanticipated ligand

*Correspondence: marc.reitman@nih.gov.

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properties (e.g., biased agonism), or occur only with supra-physiologic pharmacologic doses.

The biologic effects of the BRS-3 system are detailed below and in the Table and Figure. Study of BRS-3 physiology began with the null (*Brs3*^{-/-}) mouse [5]. More recent investigations combine pharmacology with genetics, to ensure that a drug's action is 'on target' (truly due to BRS-3), as defined by the loss of the drug's effect in the *Brs3*^{-/-} mouse. This rigor is crucial because some ligands are not selective and existing BRS-3 antibodies are of uncertain specificity.

Energy homeostasis: food intake, metabolic rate, body temperature, and body weight

The best established role of BRS-3 is in the regulation of energy expenditure, food intake, and body weight. *Brs3*^{-/-} mice exhibit increased meal size, saccharine preference, and quinine aversion, reduced metabolic rate, and obesity [5–7], and pharmacological blockade of BRS-3 increases food intake and body weight. Conversely, BRS-3 agonists reduce food intake, increase metabolic rate and body temperature, and reduce body weight [8]. These actions require brain penetration by the drug. Unexpectedly, maximum agonist weight loss efficacy required continuous, full occupancy of brain receptors, suggesting that tachyphylaxis does not occur.

Some characterization of BRS-3 neurons has been reported [9], but no functional studies link specific neurons with specific physiology. Our laboratory has generated mice carrying conditional BRS-3 alleles or expressing Cre recombinase driven by the endogenous BRS-3 regulatory elements. Preliminary data indicate that activation of certain hypothalamic BRS-3 neurons reduces food intake while activation of others increases metabolic rate and body temperature.

A way to conceptualize BRS-3 physiology is that low endogenous agonist levels signal energy deficiency. It is unknown if this is more of an acute (like cholecystokinin) or chronic (like leptin) signal. Thus the *Brs3*^{-/-} mouse invokes physiological responses appropriate to the under-fed state, increasing food intake and reducing metabolic rate and body temperature, which causes weight gain. Conversely, BRS-3 agonist sends an 'energy replete' signal, reducing food intake and increasing fasting metabolic rate, leading to weight loss with presumably pharmacologic doses. Weight loss with a BRS-3 agonist is greater in obese than lean mice and reaches a plateau [8], contrasting with other mechanisms (e.g. cannabinoid-1 receptor inverse agonists) where drug treatment can produce excessive weight loss. One interpretation is that excessive weight loss triggers mechanisms that override the 'energy replete' message of BRS-3 activation. This limit on BRS-3 agonist action can be viewed either as an advantage (excessive weight loss is not a concern), or a liability (limited intrinsic efficacy of the mechanism).

Glucose-stimulated insulin secretion and insulin resistance

BRS-3 is expressed in pancreatic islets and insulinoma cell lines. BRS-3 agonists act directly on β -cells, stimulating phospholipase C, to promote glucose-stimulated insulin secretion, including on islets from a patient with type 2 diabetes. In mice, a BRS-3 agonist reduced glucose levels during an oral glucose tolerance test; this effect was lost in *Brs3*^{-/-} mice. However, *Brs3*^{-/-} mice have no islet phenotype in the absence of exogenous ligand [10]. Lack of a *Brs3*^{-/-} islet phenotype indicates the deficiency is compensated during development, or that the *in vivo* role for BRS-3 in insulin secretion is modest.

Intriguingly, a non-selective BRS-3 agonist increased glucose uptake in skeletal muscle cells from obese humans [11]. It is not known if similar physiology exists in mice, which are not reported to express BRS-3 in muscle. Older, obese (but not younger, lean) *Brs3*^{-/-} mice show increased insulin levels, indicating insulin resistance [5]. Pending further data, a working hypothesis is that the insulin resistance is secondary to the obesity.

Heart rate and blood pressure

Brs3^{-/-} mice have a reduced resting heart rate, but with physical activity the heart rate increases disproportionately, reaching a similar maximum as active controls. Resting blood pressure was similar in *Brs3*^{-/-} and control mice and increased more with activity in the *Brs3*^{-/-} mice. Treatment with a BRS-3 agonist increased blood pressure and heart rate in wild type, but not in *Brs3*^{-/-} mice. The reduced heart rate in *Brs3*^{-/-} mice is likely explained by reduced resting sympathetic tone, with a compensatory increase in blood pressure to the normal range [12]. BRS-3 is not required for activity-induced increases in heart rate and blood pressure.

Other possible physiological roles

BRS-3 expression has been noted in multiple tumor types (lung carcinoma, carcinoid, renal cell, Ewing sarcoma, pancreatic, pituitary, ovarian, prostate) [1]. Since the tissues from which the cancers originate typically express little BRS-3, some tumor cells may upregulate BRS-3 expression. This has generated interest in using BRS-3 as a biomarker to improve tumor classification and in the development of BRS-3-binding reagents for tumor imaging and for targeting delivery of anti-tumor drugs to tumor tissues.

In rat, BRS-3 immunoreactivity was reported in the myenteric and submucosal ganglia, a localization consistent with a role in gastrointestinal motility [1].

Brs3^{-/-} mice do not have a strong behavioral phenotype, but have been reported to show less 'anxiety' by some measures in an elevated plus maze and a decreased response to social isolation and a novel environment [6]. However, BRS-3 agonist did not affect rat behavior in an elevated plus maze [8].

BRS-3 mRNA is present in testis of some species [1]. BRS-3 mRNA is also expressed at higher levels in female than male mice in the medial amygdala, which is implicated in sex behavior, and in the bed nucleus of the stria terminalis [13]. Male *Brs3*^{-/-} mice have

apparently normal fertility. Since all published reports use male mice, there is no information on possible sex-dimorphic BRS-3 physiology.

BRS-3 agonists stimulate sympathetic tone to brown fat and the heart. Clinically, an agonist caused subjects to feel jittery and have erections [14], consistent with increased sympathetic tone. It is possible that BRS-3 stimulates multiple aspects of the sympathetic nervous system.

Therapeutic implications

How do the physiologic actions of BRS-3 inform prospects for clinical use of selective BRS-3 agonists or antagonists? BRS-3 agonists have weight loss efficacy in multiple preclinical species, and likely could be used in drug combinations for the treatment of obesity [8]. A major effect of BRS-3 agonists in mice is BAT activation with a persistent increase in metabolic rate; it will be important to see if this action also occurs in humans and if the magnitude is clinically meaningful. Glucose lowering efficacy is a bonus in the obese population, but the glucose reduction in the mouse studies indicates that the effect is likely too small for a BRS-3 agonist to be developed primarily for diabetes. To date, one BRS-3 agonist has been administered clinically and it transiently increased blood pressure [14]. An increase in blood pressure by BRS-3 agonists, even transiently, is undesirable in an obesity treatment since this is a cardiovascular risk factor, and a main goal in treating obesity is the reduction of cardiovascular risk. Thus the therapeutic potential of BRS-3 ligands is unclear, pending better understanding of BRS-3 physiology.

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Figure 1.

Model of the BRS-3 system.

A postulated energy sufficiency signal ('energy replete') is generated in the periphery and/or brain and is proposed to cause release of an endogenous agonist ligand, which functions as a hormone and/or neurotransmitter. The endogenous ligand activates BRS-3 neurons in the hypothalamus and other brain nuclei. Brain BRS-3 activation inhibits food intake and increases sympathetic nervous tone to brown adipose tissue (BAT), increasing energy expenditure and body temperature, to heart, increasing heart rate, and to arteries, increasing blood pressure. The effects shown are those elicited with a synthetic BRS-3 agonist and thus may include effects of supra-physiologic stimulation. BRS-3 agonist also acts directly on pancreatic β -cells, increasing glucose-stimulated insulin secretion. Pathways upstream of BRS-3 are shown in blue and those downstream are in red.

TablePhenotype of *Brs3*^{-/-} mice and *in vivo* actions of BRS-3 agonists.

Physiologic process	Tissue where ligand acts	<i>Brs3</i> ^{-/-} phenotype	Agonist effect	Ref
Food intake	Brain	Increased (slight)	Reduce	[5–8]
Metabolic rate, body temperature	Brain	Reduced (slight)	Increase	[5, 8]
Body weight and adiposity	Brain	Increased	Reduce	[5, 7, 8]
Glucose-stimulated insulin secretion	Pancreatic β -cells	Similar to wild type	Increase	[10]
Heart rate	Brain	Reduced (resting only)	Increase	[5, 12]
Blood pressure	Brain	Increased (active only)	Increase	[5, 12, 14]
Erections	Unknown	Unknown	Erections	[14]
Behavior (elevated plus maze)	Brain	Reduced ‘anxiety’	No effect	[6, 8]
Glucose uptake	Muscle	Unknown	Increase	[11]
Unknown	Testis	Unknown	Unknown	see [1]
Unknown	Intestinal neurons	Unknown	Unknown	see [1]
Unknown	Tumors	Unknown	Unknown	see [1]