Activation of Bombesin receptor subtype-3 influences activity of orexin neurons by both direct and indirect pathways

著者	Furutani Naoki, Hondo Mari, Tsujino Natsuko, Sakurai Takeshi
journal or publication title	Journal of Molecular Neuroscience
volume	42
number	1
page range	106-111
year	2010-09-01
URL	http://hdl.handle.net/2297/25023

doi: 10.1007/s12031-010-9382-5

**Activation of Bombesin Receptor Subtype-3 Influences Activity of Orexin Neurons
by Both Direct and Indirect Pathways**

Naoki Furutani, Mari Hondo, Natsuko Tsujino, Takeshi Sakurai

Department of Molecular Neuroscience and Integrative Physiology, Graduate School of
Medical Science, Kanazawa University

This manuscript is related to the Yakushima 2009 symposium.

a) Running title:

BRS3 and orexin neurons

b) Takeshi Sakurai, M.D., Ph.D.

Department of Molecular Neuroscience and Integrative Physiology, Graduate School of
Medical Science, Kanazawa University, 13-1 Takaramachi, Kanazawa-shi, Ishikawa
920-8640, Japan

e-mail: tsakurai@med.kanazawa-u.ac.jp

Tel: +81-76-265-2173

Fax: +81-76-234-4224

Number of Figures: 3

Number of Pages: 20

Keywords: orexin, BRS3, patch clamp, calcium imaging, food intake, sleep

Acknowledgements

This study was supported by Grants-in-aid for Scientific Research (B), and for Young Scientists (B). Authors thank Dr. Wendy Gray for critical reading.

Abstract

The neuropeptides orexin A and orexin B (also known as hypocretin 1 and hypocretin 2), produced in lateral hypothalamic neurons, are critical regulators of feeding behavior, the reward system and sleep/wake states. Orexin-producing neurons (orexin neurons) are regulated by various factors involved in regulation of energy homeostasis and sleep/wakefulness states. Bombesin receptor subtype 3 (BRS3) is an orphan receptor that might be implicated in energy homeostasis, and is highly expressed in the hypothalamus. However, the neural pathway by which BRS3 regulates energy homeostasis is largely unknown. We examined whether BRS3 is involved in the regulation of orexin neurons. Using a calcium imaging method, we found that a selective BRS3 agonist (Ac-Phe-Trp-Ala-His-(τ Bzl)-Nip-Gly-Arg-NH₂) increased the intracellular calcium concentration of orexin neurons. However, intracellular recordings from slice preparations revealed that the BRS3 agonist hyperpolarized orexin neurons. The BRS3 agonist depolarized orexin neuron in the presence of tetrodotoxin. Moreover, in the presence of GABA receptor blockers, picrotoxin and CGP55845, the BRS3 agonist induced depolarization and increased firing frequency. Additionally, double-label in situ

1
2
3 hybridization study revealed that *Brs3* mRNA was expressed in almost all orexin neurons
4
5
6
7 and many cells around these neurons. These findings suggest that the BRS3 agonist
8
9
10 indirectly inhibited orexin neurons through GABAergic input and directly activated
11
12
13 orexin neurons. Inhibition of activity of orexin neurons through BRS3 might be an
14
15
16 important pathway for regulation of feeding and sleep/wake states. This pathway might
17
18
19
20
21 serve as a novel target for the treatment of obesity.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Introduction

The neuropeptides orexin A and orexin B were identified as endogenous ligands for two orphan G-protein-coupled receptors (GPCRs) (Sakurai, 1998). Orexin-producing neurons (orexin neurons) are localized exclusively in the lateral hypothalamic area (LHA), which is known as the feeding center. Various evidence has suggested that this neuropeptide is involved in the regulation of feeding behavior (Sakurai, 1998; Edwards, 1999; Haynes, 2000; Haynes, 2002). Subsequently, the finding that orexin deficiency causes narcolepsy in humans and animals suggested that these hypothalamic neuropeptides also play a critical role in regulating sleep and wakefulness (Chemelli, 1999; Lin, 1999; Peyron, 2000; Thannickal, 2000; Hara, 2001).

Recent studies of the efferent and afferent systems of orexin-producing neurons, as well as phenotypic characterization of mice with genetic alterations in the orexin system, have suggested further roles of orexin in the coordination of emotion, energy homeostasis, reward, drug addiction, and arousal. Orexin neurons receive abundant input from the limbic system, preoptic sleep-promoting neurons, and hypothalamic neurons implicated in energy homeostasis. Orexin neurons are also regulated by peripheral

1
2
3 metabolic cues, including ghrelin, leptin and glucose, suggesting that orexin neurons
4
5
6
7 might provide a link between energy homeostasis and sleep/wake states (Yamanaka,
8
9
10 2003b).

11
12
13
14 Bombesin receptor subtype 3 (BRS3) is a member of the bombesin receptor
15
16
17 subfamily of GPCRs, which also includes the neuromedin B- and gastrin-releasing
18
19
20 peptide receptors (NMBR and GRPR) (Gorbulev, 1992). BRS3 couples to endogenous
21
22
23 Gq family proteins and increases intracellular calcium concentration. BRS3 has a unique
24
25
26 pharmacological profile and does not bind with high affinity with any known
27
28
29 bombesin-related peptides. Although a number of molecules that can activate human
30
31
32 BRS3 have been developed (Mantey, 1997; Liu, 2002; Weber, 2003; Boyle, 2005;
33
34
35 Gonzalez, 2008; Zhang, 2009), its natural ligands are still unknown (Jensen, 2008).

36
37
38
39 BRS3 is primarily expressed in the CNS, and is present in the highest amounts
40
41
42
43 in the hypothalamic area where orexin neurons are localized (Ohki-Hamazaki, 1997a;
44
45
46 Yamada, 1999; Liu, 2002; Jennings, 2003; Sano, 2004). Although the normal
47
48
49 physiological function of BRS3 is largely unknown, mice lacking the BRS3 gene develop
50
51
52
53
54
55
56 late onset obesity, accompanied by a reduced metabolic rate and increased food intake
57
58
59
60
61
62
63
64
65

(Ohki-Hamazaki, 1997b). In good agreement with the *Brs3*^{-/-} mouse study, a BRS3 agonist, Bag-1, reduced food intake and increased metabolic rate (Guan, 2010). These studies suggested that BRS3 might be an important regulator of feeding behavior and energy homeostasis. A recent study showed that the anorectic effects of the BRS3 agonist remained intact in *neuropeptide Y*^{-/-}, *agouti-related peptide*^{-/-}, *melanocortin 4 receptor*^{-/-}, *cannabinoid receptor 1*^{-/-} and *leptin receptor*^{db/db} mice (Guan, 2010). These results suggest that the effect of the BRS3 agonist might be mediated through other feeding mechanisms. However, the neural pathways of feeding behavior mediated by BRS3 are largely undefined. The orexin pathway is one of the possible pathways by which the BRS3 agonist evokes its effects, because orexin neurons are regulated by several factors involved in energy metabolism, and expression of BRS3 were found in the LHA. To investigate this possibility, we evaluated the effect of a BRS3 agonist on orexin neurons. In this study, we found that a BRS3 agonist activated orexin neurons directly, while it also inhibited these neurons through activation of local GABAergic interneurons.

Materials and Methods

Drugs

The BRS3 agonist, neuromedin B (Peptide Institute, Osaka, Japan), tetrodotoxin (TTX) (Wako, Osaka, Japan), picrotoxin (Sigma, St. Louis, MO), and CGP55845 (Wako) were dissolved in extracellular solution. We also used a synthesized a selective BRS3 agonist (Ac-Phe-Trp-Ala-His (τ Bzl)-Nip-Gly-Arg-NH₂) (Boyle, 2005).

Animals

All experimental procedures involving animals were approved by the Kanazawa University Animal Care and Use Committee and were in accordance with NIH guidelines. All efforts were made to minimize animal suffering and discomfort and to reduce the number of animals used. Genetically-modified mice used in this study were *Orexin/YC2.1* mice (Tsujino, 2005) and *Orexin/EGFP* mice (Yamanaka, 2003a).

In situ hybridization

Double in situ hybridization was performed according to procedures previously described (Mieda, 2006). Digoxigenin (DIG)-labeled riboprobes for *mBrs3* were synthesized from a 1196 bp fragment of murine *Brs3* cDNA (nucleotides -172 - +1367 from the initiation codon) containing 1196 bp of the whole coding region cloned into the pCRII vector

(Invitrogen). Fluorescein (FITC)-labeled riboprobes for *prepro-Orexin* were synthesized by in vitro transcription. pBluescript II SK (+) containing a 0.5 kb fragment encoding Gln33-Val130 of *prepro-Orexin* was used as a template for in vitro transcription (Sakurai, 1998). The DIG and FITC-labeled probes were detected by means of anti-DIG (1/2000) and anti-FITC (1/1000) antibodies conjugated with alkaline phosphatase (Roche Diagnostics, Basel, Switzerland). Alkaline phosphatase activity was detected with NBT/BCIP and INT/BCIP (Roche Diagnostics).

Brain slice preparation

Orexin/YC2.1 mice (3-8 weeks old) and *Orexin/EGFP* mice (3-6 weeks old) were anesthetized with forane (Abbott, Osaka, Japan). The mice were decapitated under deep anesthesia. Brains were isolated in ice-cold cutting solution consisting of (mM): 280 sucrose, 2 KCl, 10 HEPES, 0.5 CaCl₂, 10 MgCl₂, 10 glucose, pH 7.4, bubbled with 100% O₂. Brains were cut coronally into 300-μm slices with a vibratome (VTA-1000S, Leica, Germany). Slices containing the LHA were transferred for 1 hr to an incubation chamber at room temperature filled with physiological solution containing (mM): 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 CaCl₂, 1.0 MgSO₄, 26 NaHCO₃, 11 glucose, pH 7.4, bubbled

with 95% O₂/5% CO₂.

Calcium imaging of orexin neurons

Optical recordings were performed on a fluorescence microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a cooled charge-coupled device (CCD) camera (CoolSNAP HQ2, Roper Scientific, Tucson, AZ) controlled by MetaFluor 5.0.7 software (Universal Imaging, West Chester, PA). YC2.1 was excited through a 440DF20 filter, and its fluorescent image was subjected to dual emission ratio imaging through two emission filters (480DF30 for ECFP, 535DF26 for EYFP) controlled by a filter changer (ProscanII, Prior Scientific Instruments, Cambridge, UK).

Electrophysiological recording

Orexin/EGFP mice were used for whole cell recordings. The slices were transferred to a recording chamber (RC-27L, Warner Instrument Corp., CT, USA) at room temperature on a fluorescence microscope stage (BX51WI, Olympus, Tokyo, Japan). Neurons that showed GFP fluorescence were used for patch-clamp recordings. The fluorescence microscope was equipped with an infrared camera (C-3077, Hamamatsu Photonics, Hamamatsu, Japan) for infrared differential interference contrast (IR-DIC) imaging and a

CCD camera (JK-TU53H, Olympus) for fluorescent imaging. Each image was displayed separately on a monitor. Recordings were carried out with an Axopatch 700B amplifier (Axon Instruments, Foster City, CA) using a borosilicate pipette (GC150-10, Harvard Apparatus, Holliston, MA) prepared by a micropipette puller (P-97, Sutter Instruments, Pangbourne, UK) and filled with intracellular solution (4-10 M Ω), consisting of (mM): 125 K-gluconate, 5 KCl, 1 MgCl₂, 10 HEPES, 1.1 EGTA-Na₃, 5 MgATP, 0.5 Na₂GTP, pH7.3 with KOH. Osmolarity of the solution was checked by a vapor pressure osmometer (model 5520, Wescor, Logan, UT). The osmolarity of the internal and external solutions was 280-290 and 320-330 mOsm/l, respectively. The liquid junction potential of the patch pipette and perfused extracellular solution was estimated to be -16.2 mV and was applied to the data. The recording pipette was under positive pressure while it was advanced toward an individual cell in the slice. A tight seal of 0.5-1.0 G Ω was made by applying negative pressure. The membrane patch was then ruptured by suction. The series resistance during recording was 10-25 M Ω and was compensated. The reference electrode was an Ag-AgCl pellet immersed in the bath solution. During recordings, cells were superfused with extracellular solution at a rate of 1.0-2.0 ml/min using a peristaltic

1
2
3 pump (K.T. Lab, Japan) at RT.
4
5
6
7
8
9

10 **RESULTS**

11 ***Selective BRS3 agonist influences activity of orexin neurons by direct and indirect*** 12 13 **pathways**

14 Recent studies have suggested that the activity of orexin neurons is influenced by various
15
16 factors involved in sleep/wake states or feeding behavior. It is also possible that BRS3
17
18 may regulate the activity of orexin neurons, because *Brs3*^{-/-} mice developed obesity and
19
20 impairment of glucose metabolism (Ohki-Hamazaki, 1997b). To examine the possibility
21
22 that BRS3 is involved in regulation of the activity of orexin neurons, we applied a
23
24 selective BRS3 agonist (Ac-Phe-Trp-Ala-His (τBzl)-Nip-Gly-Arg-NH₂) to orexin
25
26 neurons using brain slice preparations of *Orexin/YC2.1* transgenic mice, in which orexin
27
28 neurons specifically express the calcium-indicator protein, yellow cameleon (YC) 2.1
29
30 (Tsuji, 2005), and observed the change in intracellular calcium concentration ([Ca²⁺]_i)
31
32 of orexin neurons. In the presence of tetrodotoxin (TTX), the BRS3 agonist increased the
33
34 YFP/CFP ratio, showing that [Ca²⁺]_i was increased (Fig. 1A). The BRS3 agonist-induced
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 increase in $[Ca^{2+}]_i$ was concentration-dependent (Fig. 1B) (n=3-6). Of YFP-positive
4
5
6
7 neurons tested, 91.8% were activated by the BRS3 agonist (n=49), but they showed no
8
9
10 response to neuromedin B, an NMBR agonist (n=11).
11
12

13
14 We also studied the effect of the BRS3 agonist on orexin neurons in detail by
15
16
17 slice patch clamp recording. Unexpectedly, BRS3 agonist (1 μ M) bath application
18
19
20
21 potently hyperpolarized and decreased the firing frequency of orexin neurons under the
22
23
24 whole cell current clamp mode (n=7, Fig. 2A). On the other hand, the BRS3 agonist
25
26
27 (1 μ M) induced depolarization of orexin neurons in the presence of TTX (Fig. 2B).
28
29
30
31 Furthermore, the BRS3 agonist (1 μ M) induced depolarization and increased the firing
32
33
34 frequency of orexin neurons in the presence of both picrotoxin and CGP55845 (GABA_A
35
36
37 and GABA_B receptor blockers, respectively) (Fig. 2C). These observations suggest that
38
39
40
41 the BRS3 agonist induced depolarization of orexin neurons directly, but more potently
42
43
44 hyperpolarized these neurons through activation of GABAergic interneurons.
45
46
47

48 ***Brs3* and *Orexin* are colocalized in lateral hypothalamic neurons**

49
50
51

52
53 To examine the tissue localization of BRS3, we performed double-label in situ
54
55
56 hybridization. We found that BRS3-expressing neurons were widely distributed in the
57
58
59
60
61
62
63
64
65

1
2
3 CNS. Relatively large numbers of cells were found in the cortex, hippocampus, amygdala
4
5
6
7 and LHA (Fig. 3A and B). We found that almost all neurons expressing *Orexin* mRNA
8
9
10 expressed *Brs3* mRNA (Fig. 3C and D).
11
12

13 14 **Discussion** 15

16
17 Although BRS3 still remains an orphan receptor, it is thought to be a possible drug target
18
19
20
21 for treating obesity and the metabolic syndrome, because BRS3-deficient mice show late
22
23
24 onset obesity and impaired glucose metabolism.
25
26

27
28 In the present study, we found that a selective BRS3 agonist hyperpolarized all
29
30
31 orexin neurons under current clamp recording (Fig. 2A) but depolarized them in the
32
33
34 presence of GABA receptor antagonists, picrotoxin and CGP55845 (Fig. 2C). This result
35
36
37 suggests that the inhibitory effect on orexin neurons was mediated by activation of
38
39
40 GABAergic input. Consistently, under inhibition of neural input by TTX, the effect of the
41
42
43 agonist on orexin neurons was excitatory, and intracellular Ca^{2+} of orexin neurons was
44
45
46 increased presumably through activation of the Gq-subfamily of G- proteins (Fig. 2B). In
47
48
49
50
51
52
53 situ hybridization study revealed that *Brs3* mRNA was expressed widely in hypothalamic
54
55
56 areas (Fig. 3A and B), in agreement with previous studies (Ohki-Hamazaki, 1997a;
57
58
59
60
61
62
63
64
65

1
2
3 Yamada, 1999; Guan, 2010; Jennings, 2003; Sano, 2004). Double-label in situ
4
5
6
7 hybridization study showed that almost all orexin neurons also expressed *Brs3* mRNA
8
9
10 (Fig. 3C and D). Both electrophysiological and histochemical analyses suggested that the
11
12
13 BRS3 agonist activated orexin neurons directly through activation of BRS3 on orexin
14
15
16
17 neurons. However, the direct depolarizing effect of the BRS3 agonist appeared only when
18
19
20
21 neural input was suppressed by TTX or a GABA blocker. This suggests that the indirect
22
23
24
25 inhibitory effect predominated over the direct excitatory effect. Consistently, we
26
27
28
29 observed many BRS3-positive neurons in the LHA by in situ hybridization, which could
30
31
32
33 possibly be GABAergic interneurons, although further studies are required to reveal the
34
35
36
37 origin of GABAergic input to orexin neurons.

38
39 From a physiological viewpoint, unknown natural ligands of BRS3 also might
40
41
42
43 inhibit the activity of orexin neurons and suppress feeding behavior. Although a recent
44
45
46
47 report suggested that a BRS3 agonist increased inhibitory synaptic transmission of the
48
49
50
51 arcuate nucleus (Guan, 2010), other pathways regulating feeding behavior have not yet
52
53
54
55 been clarified. This study revealed a new pathway of a BRS3 agonist. Our results also
56
57
58
59
60
61
62
63 suggest the new possibility that BRS3 might be an important factor in sleep/wake
64
65

1
2
3 regulation, because the orexin system is considered to be a critical regulator of
4
5
6
7 sleep/wake regulation.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure legends

Fig. 1

FRET calcium imaging study showing that the BRS3 agonist influenced orexin neuron activity, using a hypothalamic slice from *Orexin/YC2.1* mice. A, In the presence of tetrodotoxin (TTX), bath application of the BRS3 agonist (1 μ M) increased the YFP/CFP ratio, suggesting that the BRS3 agonist (1 μ M) increased [Ca²⁺]_i in orexin neurons. B, The BRS3 agonist induced a concentration-dependent increase in [Ca²⁺]_i (EC₅₀: 45.7 $\pm$ 2.4 nM).

Fig. 2

A, Under whole cell current clamp mode, the BRS3 agonist (1 μ M) induced hyperpolarization of orexin neurons and decreased firing frequency. B, In the presence of TTX, orexin neurons were depolarized by the BRS3 agonist (1 μ M). C, In the presence of both picrotoxin (GABA_A blocker) and CGP55845 (GABA_B blocker), the BRS3 agonist (1 μ M) depolarized orexin neurons and increased their firing frequency.

Fig. 3

Double-label in situ hybridization histochemistry showing distributions of *Orexin* (brown) and *Brs3* (blue) mRNA. A, *Brs3* mRNA was distributed widely in the CNS, especially in the cortex, hippocampus, amygdala and LHA. B, Schematic representation of distribution of *Brs3* and *Orexin* mRNA (bregma -1.34 mm). C. High power view of lateral hypothalamic area. D, High power view of region in rectangle in C. *Orexin* mRNA colocalized with *Brs3* mRNA. Yellow arrowheads show colocalization.

References

- Boyle RG, Humphries J, Mitchell T et al. (2005) The design of a new potent and selective ligand for the orphan bombesin receptor subtype 3 (BRS3). *J Pept Sci.* 11, 136-141.
- Chemelli RM, Willie JT, Sinton CM et al. (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell.* 98, 437-451.
- Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR (1999) The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol.* 160, R7-12.
- Gonzalez N, Hocart SJ, Portal-Nunez S et al. (2008) Molecular basis for agonist selectivity and activation of the orphan bombesin receptor subtype 3 receptor. *J Pharmacol Exp Ther.* 324, 463-474.
- Gorbulev V, Akhundova A, Buchner H, Fahrenholz F (1992) Molecular cloning of a new bombesin receptor subtype expressed in uterus during pregnancy. *Eur J Biochem.* 208, 405-410.
- Guan XM, Chen H, Dobbelaar PH et al. (2010) Regulation of energy homeostasis by bombesin receptor subtype-3: selective receptor agonists for the treatment of obesity. *Cell Metab.* 11, 101-112.
- Hara J, Beuckmann CT, Nambu T et al. (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron.* 30, 345-354.
- Haynes AC, Jackson B, Chapman H et al. (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept.* 96, 45-51.
- Haynes AC, Chapman H, Taylor C et al. (2002) Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept.* 104, 153-159.
- Jennings CA, Harrison DC, Maycox PR, Crook B, Smart D, Hervieu GJ (2003) The distribution of the orphan bombesin receptor subtype-3 in the rat CNS. *Neuroscience.* 120, 309-324.
- Jensen RT, Battey JF, Spindel ER, Benya RV (2008) International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. *Pharmacol Rev.* 60, 1-42.

- 1
2
3 Lin L, Faraco J, Li R et al. (1999) The sleep disorder canine narcolepsy is caused by a
4 mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 98, 365-376.
5
6 Liu J, Lao ZJ, Zhang J et al. (2002) Molecular basis of the pharmacological difference
7 between rat and human bombesin receptor subtype-3 (BRS-3). *Biochemistry*. 41,
8 8954-8960.
9
10 Mantey SA, Weber HC, Sainz E et al. (1997) Discovery of a high affinity radioligand for
11 the human orphan receptor, bombesin receptor subtype 3, which demonstrates
12 that it has a unique pharmacology compared with other mammalian bombesin
13 receptors. *J Biol Chem*. 272, 26062-26071.
14
15 Mieda M, Williams SC, Richardson JA, Tanaka K, Yanagisawa M (2006) The
16 dorsomedial hypothalamic nucleus as a putative food-entrainable circadian
17 pacemaker. *Proc Natl Acad Sci U S A*. 103, 12150-12155.
18
19 Ohki-Hamazaki H, Wada E, Matsui K, Wada K (1997a) Cloning and expression of the
20 neuromedin B receptor and the third subtype of bombesin receptor genes in the
21 mouse. *Brain Res*. 762, 165-172.
22
23 Ohki-Hamazaki H, Watase K, Yamamoto K et al. (1997b) Mice lacking bombesin
24 receptor subtype-3 develop metabolic defects and obesity. *Nature*. 390, 165-169.
25
26 Peyron C, Faraco J, Rogers W et al. (2000) A mutation in a case of early onset narcolepsy
27 and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat*
28 *Med*. 6, 991-997.
29
30 Sakurai T, Amemiya A, Ishii M et al. (1998) Orexins and orexin receptors: a family of
31 hypothalamic neuropeptides and G protein-coupled receptors that regulate
32 feeding behavior. *Cell*. 92, 573-585.
33
34 Sano H, Feighner SD, Hreniuk DL et al. (2004) Characterization of the bombesin-like
35 peptide receptor family in primates. *Genomics*. 84, 139-146.
36
37 Thannickal TC, Moore RY, Nienhuis R et al. (2000) Reduced number of hypocretin
38 neurons in human narcolepsy. *Neuron*. 27, 469-474.
39
40 Tsujino N, Yamanaka A, Ichiki K et al. (2005) Cholecystokinin activates
41 orexin/hypocretin neurons through the cholecystokinin A receptor. *J Neurosci*. 25,
42 7459-7469.
43
44 Weber D, Berger C, Eickelmann P, Antel J, Kessler H (2003) Design of selective
45 peptidomimetic agonists for the human orphan receptor BRS-3. *J Med Chem*. 46,
46 1918-1930.
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 Yamada K, Wada E, Imaki J, Ohki-Hamazaki H, Wada K (1999) Hyperresponsiveness to
4 palatable and aversive taste stimuli in genetically obese (bombesin receptor
5 subtype-3-deficient) mice. *Physiol Behav.* 66, 863-867.
6
7
8 Yamanaka A, Muraki Y, Tsujino N, Goto K, Sakurai T (2003a) Regulation of orexin
9 neurons by the monoaminergic and cholinergic systems. *Biochem Biophys Res*
10 *Commun.* 303, 120-129.
11
12
13 Yamanaka A, Beuckmann CT, Willie JT et al. (2003b) Hypothalamic orexin neurons
14 regulate arousal according to energy balance in mice. *Neuron.* 38, 701-713.
15
16
17 Zhang L, Nothacker HP, Wang Z, Bohn LM, Civelli O (2009) Pharmacological
18 characterization of a selective agonist for bombesin receptor subtype-3. *Biochem*
19 *Biophys Res Commun.* 387, 283-288.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure1
[Click here to download high resolution image](#)

Furutani et al. Figure 1

Furutani et al. Figure 2

Furutani et al. Figure 3