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Research article

Gastrin-releasing peptide receptor mediates the excitation of preoptic GABAergic neurons by bombesin

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HIGHLIGHTS

- GABAergic preoptic neurons express BRS3 and/or GRP receptors.
- GRP, in contrast to BRS3 agonist, mimics the excitatory effect of bombesin.
- GRP and bombesin activate a Ca^{2+}-dependent inward nonselective cationic current.
- GRP and bombesin activate Ca^{2+} release from intracellular stores.

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ABSTRACT

Bombesin, a pan agonist of the bombesin-like peptide receptor family, elicits potent hypothermia when applied centrally. The signaling mechanisms involved are not known. Here we report that GABAergic preoptic neurons express gastrin-releasing peptide (GRP) receptors and are directly excited by GRP or bombesin. This effect was abolished by a GRP receptor antagonist. A partially overlapping group of preoptic GABAergic neurons express bombesin-like receptor 3 (BRS3), however their activation results in a decrease in firing rate. The excitatory effects of bombesin or GRP were not affected by BRS3 antagonist. GRP activated a Ca^{2+}-dependent inward nonselective cationic current and Ca^{2+} release from intracellular stores. Our data indicate that GRP receptors mediate the excitatory effects of bombesin in preoptic neurons.

1. Introduction

Mammalian bombesin-like peptide receptors, a family of G protein coupled receptors that is comprised of the bombesin-like receptor-3 (BRS3), the gastrin-releasing peptide receptor (GRPR), and the neuromedin B receptor (NMBR) are widely distributed in the central nervous system and modulate metabolism and various behaviors (reviewed in Ohki-Hamazaki et al., 2005 [12]). Intracerebroventricular injection of bombesin, the pan-agonist for all three receptors, induces a fast (within minutes) and robust (3–4 °C) hypothermia, however the cellular mechanisms and receptor subtypes involved are not fully understood (Tsushima et al., 2003).

Recent studies have revealed that preoptic GABAergic neurons play an important role in the control of thermoregulatory networks that comprise also neurons of the rostral raphe pallidus and dorsomedial hypothalamus (reviewed in Morrison et al., 2012 [10]). The firing activity of preoptic GABAergic neurons provides an inhibitory tone on downstream thermoeffector neurons. A decrease in the firing rate of preoptic GABAergic neurons can account for the increased thermogenesis associated with fever (Morrison and Madden, 2014 [9]) or other hyperthermic responses (Lundius et al., 2010; Sanchez-Alavez et al., 2010 [17]. In this study we have investigated the effects of bombesin on identified preoptic GABAergic neurons and the receptor subtypes involved.

2. Materials and methods

2.1. Slice preparation

Coronal tissue slices containing the median preoptic nucleus (MnPO) were prepared from GAD65-GFP mice (28–42 d old) housed in standard conditions as previously described [7] and in accordance to procedures approved by The Scripps Research Institute Animal Welfare Committee. This transgenic mouse line expresses

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Table 1
Primers.

Primer	External sequence	Amplicon Size	Internal sequence	Amplicon Size
GRPR	F: 5′ aaccttcagcgcctaactga 3′ R: 5′ tcagtttgcagccaattctg 3′	Bp:437	F: 5′ acctgaacttggacgtggac 3′ R: 5′ ccaaagccaggctagagatg 3′	Bp:219
BRS3	F:5′ aaagcaccctgaacataccg 3′ R:5′ gtcaccaagaggaggctcag 3′	Bp:341	F: 5′ tgaatcccggaagagaattg 3′ R:5′ aatgctgctggaaggtcttg 3′	Bp:233
NMBR	F:5′ tcagaagtagcacgcattgg3′ R:5′ agcaaagggattgacacagg3′	Bp:396	F:5′ acagcatgcataccctaccc 3′ R: 5′ caatcttagccaggcgtttc 3′	Bp:242

enhanced green fluorescent protein (eGFP) under the control of the regulatory region of mouse glutamic acid decarboxylase (GAD) 65 gene. The mice were a kind gift from Dr. Gabor Szabo (Hungarian Academy of Sciences, Budapest, Hungary). The slice used in our recordings corresponded to the sections located from 0.5 mm to 0.25 mm from Bregma in the mouse brain atlas.

2.2. Whole-cell patch-clamp recording

The aCSF contained (in mM) the following: 130 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2 $CaCl_2$, 1 $MgSO_4$, and 10 glucose, osmolarity of 300–305 mOsm, equilibrated with 95% O_2 and 5% CO_2, pH 7.4. Other salts and agents were added to this medium. A set of experiments was carried out in Ca^{2+}-free aCSF which contained (in mM) 130 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 0.5 EGTA, 3 $MgSO_4$, and 10 glucose. In a set of experiments the extracellular solution was exchanged with a Na^+-free buffer ("NMDG external solution") containing 155 *N*-methyl-D-glucamine, 3.5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose, and 10 HEPES (pH 7.4, adjusted with HCl). For cell-attached recordings the patch pipette was filled with aCSF. Whole-cell recordings were carried out using either a K^+ or a Cs^+ pipette solution. The K^+ pipette solution contained (in mM) 130 K-gluconate, 5 KCl, 10 HEPES, 2 $MgCl_2$, 0.5 EGTA, 2 ATP and 1 GTP (pH 7.3) was used in all experiments. The Cs^+ pipette solution contained (in mM): 130 Cs-methanesulphonate, 10 CsCl, 10 HEPES, 2 $MgCl_2$, 0.5 EGTA, 2 ATP, 1 GTP (pH 7.4). The electrode resistance after back-filling was 2–5 MΩ. All voltages were corrected for the liquid junction potential (−13 mV and −7 mV, respectively). Data were acquired with a MultiClamp 700 B amplifier (Molecular Devices, Sunnyvale, CA, USA) digitized using a Digidata 1320A interface and the Pclamp9.2 software package. The sampling rate for the continuous recordings of spontaneous activity was 50 kHz. The recording chamber was constantly perfused with extracellular solution (2–3 ml min^{-1}). The agonists were applied locally using a perfusion pencil system (tip diameter 100 μm, Automate Scientific) driven by gravity, while the antagonists were bath-applied. The perfusion was kept on for 90 s in most experiments, except those measuring effects on synaptic activity for which the incubation time was 3–4 min. The temperature of the external solution was controlled with a TC–344 B temperature controller and an inline heater (Warner Instruments, Hamden, CT, USA) and was maintained at 36–37 °C.

2.3. Ca^{2+} imaging

Fura-2 fluorescence signals were acquired with a CCD camera (Hamamatsu ORCA-ER) connected to its frame grabber driven by Slidebook software (Intelligent Imaging Innovations, Denver, CO, USA). An ultra-high-speed wavelength switcher Lambda DG-4 (Sutter Instruments, Novato, CA, USA) provided alternating excitation for ratiometric Fura-2 measurements. The filters were 340HT15 and 380HT15. The illumination source was a standard xenon lamp. The sampling frequency of 0.2 Hz was sufficiently fast to capture responses to bombesin and GRP. At this excitation frequency, photobleaching and phototoxicity were minimal. Fura-2AM loading and data acquisition were carried out as described in our previous studies.

2.4. Data analysis and curve fitting

All data represent mean ± SD. Data analysis and curve fitting was carried out using the SigmaPlot software package (Systat Software, Inc., San Jose, CA, USA). One-way Anova with Tukey's post hoc test ($P < 0.05$) was used for comparison of multiple groups. The concentration–response data of agonist actions were fitted to the Hill equation: $E = E_{max}/\{1 + (EC_{50}/[Agonist])^n\}$, where [Agonist] represents the agonist concentration, n is the Hill coefficient and E_{max} is the maximum effect as a percentage of the maximum histamine response in the same cell. Synaptic activity was quantified and analyzed statistically as described previously [21,20]. Briefly, synaptic events were detected and analyzed (amplitude, kinetics, frequency) off-line using a peak detection program (Mini Analysis program, Synaptosoft, Decatur, NJ, USA). Events were detected from randomly selected recording stretches of 2 min before and during bombesin or GRP treatment. Statistical significance of the cumulative distributions of the measured parameters (inter-event interval, amplitude, rise time, time constant of decay) were assessed with the Kolmogorov-Smirnov two-sample test (K-S test, $P < 0.05$) using the Mini Analysis program. The averages for the measured parameters (frequency, amplitude, rise time, time constant of decay) for each experiment were obtained using the Mini Analysis program. Event frequency was calculated by dividing the number of events by the duration (in seconds) of the analyzed recording stretch.

2.5. Chemicals

Bag-1 and bantag-1, BRS3 agonist and antagonist, respectively, were a kind gift from Dr Marc Reitman (Merck, Rahway, NJ USA). Bombesin, GRP, BIM 23042 were purchased from Tocris (Ellisville, MO, USA). RC-3095, TTX, CNQX, AP-5 and gabazine, BAPTA-AM were purchased from Sigma (St Louis, MO, USA).

2.6. Cell harvesting, reverse transcription and PCR

MnPO neurons in slices were patch-clamped and then harvested into the patch pipette by applying negative pressure. The content of the pipette was expelled in a PCR tube. dNTPs (0.5 mM), 50 ng random primers (Invitrogen) and H_2O were added to each cell to a volume of 16 μl. The samples were incubated at 65 °C for 5 min and then put on ice for 3 min. First strand buffer (Invitrogen), DTT (5 mM, Invitrogen), RNaseOUT (40 U, Invitrogen) and SuperScriptIII (200 U, Invitrogen) were added to each sample to a volume of 20 μl followed by incubation at room temperature for 5 min, at 50 °C for 50 min and then at 75 °C for 15 min. After reverse transcription samples were immediately put on ice. 1 μl of RNAse H was added to samples and kept at 37 °C for 20 min. PCR assays were carried out using pairs of nested primers (Table 1) as described previously [7,19].