Behavioural Brain Research 216 (2011) 519–524

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Effects of intracerebral ventricular administration of gastrin-releasing peptide and its receptor antagonist RC-3095 on learned fear responses in the rat

Zul Merali[a,b,c,e,*], Christine Mountney[a], Pam Kent[a,b], Hymie Anisman[d,e]

[a] Department of Psychology, University of Ottawa, Ottawa, ON, Canada K1N 6N5
[b] Department of Psychiatry, University of Ottawa, Ottawa, ON, Canada K1N 6N5
[c] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada K1N 6N5
[d] Institute of Neuroscience, Carleton University, Ottawa, ON, Canada K1S 5B6
[e] University of Ottawa Institute of Mental Health Research, 1145 Carling Avenue, Room 5432 Research Tower, Ottawa, ON, Canada K1Z 7K4

ARTICLE INFO

Article history:
Received 10 June 2010
Received in revised form 17 August 2010
Accepted 23 August 2010
Available online 27 August 2010

Key words:
Bombesin
Gastrin-releasing peptide
RC-3095
Conditioned emotional response
Conditioned fear
Fear-potentiated startle
Stress

ABSTRACT

Several lines of evidence have implicated bombesin and its mammalian analogue, gastrin-releasing peptide (GRP), in the mediation and/or modulation of the stress response. However, the physiological role of GRP in mediating conditioned fear responses remains to be elucidated. The objective of the present study was to characterize the role(s) of GRP and its receptor antagonist (D-Tpi6, Leu13 psi[CH2NH]-Leu14) $BB^{(6-14)}$ (RC-3095) in fear-related responses using two animal models of conditioned fear. To this end, the effects of intracerebroventricular (i.c.v.) administration of GRP (0.062, 0.30, 3.0 nmol) and RC-3095 (0.3, 3.0 and 9.0 nmol) were assessed in the conditioned emotional response (CER) and the fear-potentiated startle (FPS) paradigms. In the CER paradigm, i.c.v. administration of GRP dose-dependently (all doses) attenuated the expression of both contextual and cued fear as reflected by a reduction in freezing behavior to both the context (cage where shock was received) and cue (tone paired with shock). Conversely, pretreatment with RC-3095 (high dose), blocked the reduction of contextual and cued fear normally observed over time. Further, in the FPS paradigm, i.c.v. administration of GRP significantly attenuated the fear-potentiated startle response at medium and high doses without affecting basal startle amplitude. In contrast, pretreatment with RC-3095 at the highest dose (9.0 nmol) significantly increased the basal startle amplitude without affecting fear-potentiation, suggesting elevated fear at the onset of testing. These data provide further evidence that GRP is involved in conditioned fear responses.

1. Introduction

Bombesin (BB)-like peptides (BLPs) initially received appreciable attention owing to their potential role in the regulation of food intake and satiety [1–3]. It now appears that BLPs also serve in the mediation and/or modulation of the stress response [4]. In this regard, central BB administration can elevate plasma levels of adrenocorticotrophin-releasing hormone (ACTH) and corticosterone (CORT) and induce behaviors that are commonly associated with fear and/or stress. These behaviors include increased grooming and locomotor activity in a familiar environment, decreased food intake and locomotor activity in a novel environment, as well as disruption of fear memory consolidation in an inhibitory avoidance paradigm [5–10]. Moreover, we have shown that stressor exposure evokes the release of endogenous BLPs at several stress-relevant brain regions, including the central nucleus of the amygdala and anterior pituitary gland [11–14].

Although gastrin-releasing peptide (GRP), a mammalian counterpart of BB, appears to be involved in stress-related responses, the specific physiological role of GRP in mediating anxiety and fear responses remains unclear. Central GRP administration activates the HPA axis as reflected by an increased release of ACTH and CORT [15], and this effect can be completely blocked by pretreatment with a competitive and specific GRP (BB_2) receptor antagonist [16]. Shumyatsky et al. [17] provided evidence demonstrating that the GRP gene is highly expressed in the lateral amygdala (a region intimately involved in conditioned fear) and that the BB_2 receptors are particularly expressed on gamma-aminobutyric acid (GABA) interneurons [17]. Furthermore, they noted that BB_2-receptor-deficient mice displayed greater and more persistent long-term memory of fear, suggesting that GRP plays a role in the regulation of amygdala-dependent fear-related learning. In line with these findings, we recently demonstrated that intracerebral ventricular (i.c.v.) administration of GRP attenuated fear-potentiated startle, while its administration to either the prelimbic (PrL) or infralimbic (IL) cortex, as well as the central (CeA) or basolateral (BLA) nucleus

* Corresponding author at: University of Ottawa Institute of Mental Health Research, 1145 Carling Avenue, Room 5432, Research Tower, Ottawa, ON, Canada K1Z 7K4. Tel.: +1 613 722 6521x6551, fax: +1 613 792 3935.
E-mail address: merali@uottawa.ca (Z. Merali).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.08.027

of the amygdala, attenuated freezing in a conditioned fear paradigm [18–20].

In contrast to these findings, Roesler et al. [21] reported that microinjection of a selective BB_2-receptor antagonist directly into the BLA impaired memory retention on an inhibitory avoidance task, suggesting that blockade of BB_2 receptors impairs aversive memory, while we reported that blockade of BB_2 receptors in the IL, CeA or BLA results in sometimes contradictory, dose-dependent results on conditioned freezing. Thus, while there are data supporting a role for BLPs in fear and stress-related responses, current data are sparse and not fully congruent.

The aim of this set of experiments was to further characterize the role of GRP and its receptor in fear-related responses using animal models of conditioned fear. To this end, the effects of i.c.v. administration of GRP, and a BB_2-receptor antagonist, RC-3095, were assessed in rodent paradigms thought to reflect anxiety and/or fear; namely the conditioned emotional response (CER) and the fear-potentiated startle (FPS) paradigms.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Charles River Laboratories, St-Constant, Quebec) weighing between 250 and 275 g at time of surgeries were used. Animals were housed individually in a temperature and humidity controlled environment on a 12-h light/dark cycle (lights on at 07:00 h) and were permitted 1 week to acclimatize to the vivarium prior to being used. Throughout the study, all animals had free access to food (Purina Rat Chow) and tap water. All experiments were conducted in accordance with the Canadian Council of Animal Care, and were approved by the animal care committee of the University of Ottawa.

2.2. Surgery

Animals were anesthetized with the inhalant halothane at 2.5% and were stereotaxically implanted with a stainless steel guide cannula (22 gauge, 5.5 mm length; Plastics One, Roanoke, VA), aimed at the third ventricle (A/P: −4.4 mm; D/V: −4.4 mm; L: 0 mm; obtained from Paxinos and Watson [22]. The cannulae were anchored to the skull with 4 stainless steel screws and dental acrylic. Removable stylets (Plastics One, Roanoke, VA) were inserted into the guide cannulae until the experimental day. Animals were allowed 1 week recovery before testing. During the recovery period animals were acclimated to handling as well as mock central injection procedures.

2.3. Drugs and injections

All drugs were delivered into the 3rd ventricle in a 3 μL volume infused over 60-s via an injection cannula (0.5 mm longer than the guide cannula). The injector was connected to a 10 μL Hamilton Syringe with polyethylene tubing, which delivered the drug at a flow rate of 0.5 μL/min over a 1-min interval (pump: Harvard Apparatus, MA). Following drug infusion, the injector was left in place for an additional 60 s to ensure diffusion of the drug away from the cannula tip.

GRP (Phoenix Pharmaceuticals, Inc.) and the BB_2-receptor antagonist RC-3095 (D-Tpi6, Leu13 psi[CH2NH]-Leu14) BB (6–14); Sigma–Aldrich) were each dissolved in Krebs ringer buffered saline solution (KRB) consisting of (in nmol; 2.7 K^+, 145 Na^+, 1.35 Ca^{2+}, 1.0 Mg^{2+}, 150 Cl^- ascorbate, pH 7.4). The control (vehicle) animals received an equivalent volume of KRB alone. In the GRP study, animals were randomly assigned to 1 of 4 groups (n = 8–10 rats/group): vehicle (KRB); 0.062 nmol GRP (Low); 0.3 nmol GRP (Med); 3.0 nmol GRP (Hi). In the RC-3095 study, animals were randomly assigned to 1 of 4 drug conditions (n = 8–10/group): vehicle (KRB); 0.3 nmol RC-3095 (Low); 3.0 nmol RC-3095 (Med); 9.0 nmol RC-3095 (Hi). Doses were chosen based on previous findings from our laboratory using these compounds and a review of published literature [15,18,23–29].

2.4. Conditioned emotional response

2.4.1.1. Apparatus

The conditioning chamber (Coulbourn Instruments) measured 31 cm × 25 cm × 30 cm. The front and back walls were made of clear Plexiglas and two side walls made of stainless steel panels. The floor was composed of 16 stainless steel rods (4 mm diameter, 1.4 cm apart), which were connected to a Coulbourn Instruments shock generator (model H13–16) that delivered constant current. A Sonalert tone generator (75 kHz, low setting—Coulbourn Instruments) was situated in the top rear panel and provided the conditioning auditory cue.

2.4.1.2. Procedure

All subjects completed 1 day of training followed by a day of testing 24-h later. Training for contextual fear occurred 1 week after surgery, while cued fear training followed 2 weeks from surgery. During the contextual training phase, subjects were placed in the conditioning chamber where they received 6 footshocks (1.0 mA; 1-s in duration) with an average intertrial interval (ITI) of 1-min. Cued fear training included 6 pairings of a 20-s tone with a 1.0 mA (1-s) continuous footshock. The shock was delivered during the final second of the 20-s tone. Again, each trial was delivered at an average ITI of 1 min.

On the test days, rats were infused with the drug 15-min before testing. Contextual fear was assessed (over a 4-min period for the GRP study and over a 15-min period for the RC-3095 study) by placing them in the conditioning chamber where they had previously been shocked. The difference in timing between the agonist and antagonist condition was based on previous findings that 4 min was sufficient for the agonist to demonstrate effects [19], whereas pilot work using RC-3095 injected i.c.v. indicated that the antagonist would require a longer test time to demonstrate its effects. Freezing behavior (the absence of movement excluding involuntary respiratory movements) was assessed using a time-sampling method. The absence or presence of complete immobility during every 5-s sampling epoch was recorded over the course of the test period. Evaluations of freezing were conducted by trained experimenters blind to the drug condition.

To test for CER in the cued condition, rats were placed in a novel environment similar to the training chamber; however, these chambers were modified in that the walls were covered in black laminate, while the floor was smooth and covered with bedding chips. Animals were allowed a 1-min exploration period and were subsequently presented with the conditioned cue (tone—that had previously been paired with footshock). A total of 15 tones (each 20-s in duration) were presented at 1-min intervals. Freezing was scored as described in the contextual test. Between each training and testing session, cages were cleaned with 70% ethanol.

2.5. Fear-potentiated startle

2.5.1.1. Apparatus

The startle apparatus (Coulbourn Instruments) consisted of a sound attenuated chamber containing 2 calibrated platforms (18 cm × 10 cm) designed to measure the animal's startle response [30]. Animals were placed in a Teflon cage (18.5 cm × 11 cm), positioned atop the platforms. The cage floor consisted of metal rods (4 mm diameter spaced 1.8 cm apart) that were connected to shock generators. A high frequency speaker mounted (24 cm) above the platforms generated white noise, while tones were generated by a sonalert model tone generator (75 kHz—Coulbourn Instruments).

2.5.1.2. Procedure

The fear-potentiated startle training and testing spanned 4 days. On Day 1, rats received 30 random bursts of white noise (95, 110, and 115 db) over 16 min to establish baseline startle amplitudes and acclimatize animals to the startle chamber. On Day 2, animals received their first conditioning session (CS-US pairing); rats received 7 trials (randomized 1 min intertrial interval—ITI) pairing a 4 s tone (75 kHz) with a 0.6 mA, 0.5 s footshock administered during the last 500 ms of the tone. Forty-eight hours later (Day 4), animals were tested for fear-potentiation. Briefly, drugs were injected 15 min prior to testing, at which point animals received 20 trials consisting of 110 db white noise bursts (random 1 min ITI), followed by 5 tone-paired noise trials, and finally, 5 noise-alone trials. Between tests, cages were cleaned with 70% ethanol.

2.6. Histologies

Following completion of the experiment, rats received an overdose of pentobarbital and 1 μL of India ink (25%) was delivered through the injection cannula. Animals were then sacrificed and their brains were removed and frozen. Locations of the cannulae were verified histologically upon thionin staining of the sections.

2.7. Statistics

For CER, data were analyzed separately for the agonist and antagonist conditions, as were the data from the contextual and cued freezing conditions. For the contextual test in the agonist condition, the raw freezing scores were transformed into a percentage of sampling bins during which freezing occurred over the four 1-min intervals. These percentages were then analyzed using a mixed-measures analysis of variance (ANOVA) with Treatment condition as the between-group measure and Time (minutes 1–4) as the within-group measure. For the antagonist contextual condition and the cued tests, percentage of freezing scores were calculated for the 15 1-min bins during the test, and subsequently collapsed over three 5-min time blocks. These values were then analyzed using a mixed-measures ANOVA with Treatment condition as the between-group measure and Time (three 5-min blocks)