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Bombesin-induced enhancement of memory consolidation in male and female rat pups: Role of glutamatergic and dopaminergic systems

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ABSTRACT

Previous studies have shown that the neuropeptide bombesin (BBS) enhances consolidation of specifically for inhibitory avoidance memory in adult rats. However, its effect on memory consolidation during premature period is not clear as well. Thus, this study evaluated the effect of BBS and its interaction with glutamatergic and dopaminergic systems on memory consolidation in rat pups. Male and female rat pups (30 days old) were trained in an inhibitory avoidance (IA) task (0.5 mA, 3 s footshock). Memory retention was tested 24 h later during which the latency to re-enter to the shock compartment was recorded. First, the effects of different doses (0.001, 0.0025, 0.005, 0.01 and 0.02 mg/kg) of BBS injected immediately following training were tested. Then, the effect of the most effective dose of BBS obtained in the previous experiment was examined in the presence of the glutamate NMDA receptor antagonist MK-801 (0.05 mg/kg), the dopamine D1 receptor antagonist SCH-23390 (0.05 mg/kg) and the dopamine D2 receptor antagonist sulpiride (20 mg/kg). Findings indicate that BBS significantly enhances memory consolidation at all tested doses in male pups and at a dose of 0.01 mg/kg in female pups. MK-801, SCH-23390 and sulpiride administration before BBS injection in individual groups significantly blocked BBS-induced memory enhancement. Our findings indicate that similar to adult rats, BBS enhances memory consolidation in developing rat. This enhancing effect is mediated, at least in part, via an interaction with glutamatergic and dopaminergic systems.

1. Introduction

Memory consolidation is regulated by a variety of hormones, and neuropeptides such as bombesin (BBS) – like peptides and neurotransmitters (McGaugh, 2000; Silva, 2003; Tonegawa et al., 2003). BBS is a 14 amino acid peptide first isolated from the skin of the European amphibian, and subsequent studies have shown the presence of BBS-like peptides, e.g. gastrin releasing peptide (GRP), neuromedin B and C in the peripheral and central nervous system of the mammalian (Walsh et al., 1979). Their receptors and the corresponding mRNA are present and differentially distribute within the CNS (Moody and Merali, 2004a). Developmentally, binding sites for radiolabeled BBS are noticeable in the embryonic rat CNS and reach adult-like levels in post-natal day 10, while BBS-like immumoreactivity reaches mature levels around the third post-natal week (Gillati and Moody, 1984). In both adult and developing rats, the concentrations of BBS-like peptides are higher in the hypothalamus than the midbrain or hindbrain. The density of BBS binding sites is significantly greater in the hippocampus and olfactory tubercle than the midbrain (Gillati and Moody, 1984).

BBS-like peptides affect a wide range of physiological and pathological processes such as neuroendocrine functions, growth of tumor cells, feeding and different behaviors (McCoy and Avery, 1990; Moody and Merali, 2004a; Ohki-Hamazaki et al., 2005; Patel et al., 2006; Roesler et al., 2006a). Peripheral post-training administrations of both BBS or GRP enhance retention test performance in T-maze foot-shock avoidance (Flood and Morley, 1988) and one trial inhibitory avoidance task (Flood and Morley, 1988; Rashidy-Pour and Razvani, 1998) in adult rats and mice, while the blockade of BBS or GRP receptors impairs memory retention (Martins et al., 2005; Presti-Torres et al., 2007). GRP receptor antagonist or PKA, MAPK, PKC signaling inhibitors prevent BBS-induced memory retention (Roesler et al., 2006b).

Several studies indicated that central but not systemic injection of BBS leads to severe grooming behavior in adult mice and rats (Brown et al., 1977; Cowan et al., 1985; Katz, 1980; Ladenheim and Ritter, 1988). On the other hand, in immature 20 days old and even younger rats grooming behavior occurs through systemic injection of BBS (Jackson and Kitchen, 1989). These findings indicate that systemic injection of BBS could not alter grooming behavior in adult rats, while it

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could affect grooming in pup rats, suggesting that in ontogeny, blood brain barrier matures along with the development of the animal.

To date most researchers have investigated the effects of BBS on memory processing in adult rats. The ontogeny of cognitive responses to BBS has been largely neglected. As mentioned above, binding sites for BBS are present in the CNS of developing infant rats and the levels of BBS-like immumoreactivity reaches mature levels around the third post-natal week (Gillati and Moody, 1984). Previous studies have shown the involvement of NMDA or dopamine receptors in the effects of BBS on some behaviors in adult rats (Koga et al., 2011; Merali and Piggins, 1990b), but their possible interaction in developing infant rats is not known. A previous study has shown that the adult pattern of NMDA receptors emerged postnatal day 14 with a little change across development in rat forebrain (Insel et al., 1990). Ontogeny studies of both dopamine D 1 and D 2 receptors in rodent brains demonstrate that the density of receptors is relatively low at birth and increases at a steady rate during the first 3–4 weeks postnatal period (Rao et al., 1991; Schambra et al., 1994; Srivastava et al., 1992).

The present study contains two parts and was undertaken to investigate the effects of BBS, and its possible interaction with NMDA or dopamine receptors on memory processing in male and female rat pups. The first part was designed to determine the dose-response effects of BBS on memory consolidation of one trial training in both genders. The second part investigated the possible role of NMDA or dopamine D1 or D2 receptors on BBS effects on memory processing in developing rats.

2. Materials and methods

2.1. Animals

Wistar male (n = 97) and female (n = 128) pup rats (30 days old) weighing 60–70 g were used. Animals were kept in a controlled temperature (22 ± 2^{oC}) room and a 12-h regular light/dark cycle (light on from 07:00 to 19:00), housed 5 to 6 per cage with free access to food and water. All experiments were conducted between 14:00 and 17:00 h. The experimental protocol was approved by the Ethical Review Board of Semnan University of Medical Sciences (93/584217). All of the experimental trials were conducted in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

Bombesin (BBS, 0.001, 0.0025, 0.005, 0.01 and 0.02 mg/kg), the NMDA receptor antagonist MK-801 (0.05, 0.075 and 0.1 mg/kg), the dopamine D1 receptor antagonist SCH-23390 (SCH, 0.05 mg/kg), and the dopamine D2 receptor antagonist sulpiride (SUL, 20 mg/kg) were purchased from Sigma (England). All drugs were prepared in physiological saline and injected intraperitoneally (i.p) in volume of 1 ml/kg. BBS doses or vehicle were injected immediately following training. The antagonists (either NMDA or D1 or D2) or vehicle were given immediately before BBS injection.

2.3. Inhibitory avoidance training

The apparatus used for Inhibitory avoidance (IA) training consisted of a two – compartment box. The lit chamber (22 × 21 × 23 (h) cm) made from transparent plastic was connected by a guillotine door to the dark compartment of the same dimensions with black opaque walls and ceiling. Stainless steel bares characterized 3 mm in diameter and 1 cm apart consisted in the floor of dark chamber through which a current pulse could be delivered from a source of constant current to plantar surface of paw. All experimental animals were habituated to the equipment at first. Animal was placed in the lit chamber and 5 s later, the guillotine door was opened. After entering the rat into the dark chamber, the door was closed and then animal was returned into the home cage and 30 min later, habituation trial was repeated and followed 30 min later again by the acquisition trial during which after closing the guillotine door a 50-Hz, 0.5-mA constant current shock within 3 s was applied immediately after the rat had entered into the dark chamber. In the end of acquisition trial, rats were injected with either antagonist or vehicle and then immediately followed by either BBS or vehicle injection. Then, rats were returned to home cage. 24 h after training, the rat was placed in the lit chamber and after 5 s, the guillotine door was opened and the latency (*sec*) of entering into the dark chamber (step-through latency, STL) was recorded. This latency time was considered as the measure of memory retention. Ten minutes was considered as cut off point to entering into the dark chamber. Animals were used only once in the test.

2.4. Hot plate test

Pain behavioral response was detected using a hot-plate device (Harvard Apparatus Limited, Fircroft way, Edenbridge, Kent) at 52 °C and licking of paw was considered as pain response. After habituating to the plate, rats were placed on the hot plate and delay for licking the paw (*sec*) was recorded as nociceptive threshold. The mean value for licking was used in the statistical analysis. A cut off time of 60 s was selected in order to *avoid tissue damage.*

2.5. Statistical analysis

Data are expressed as mean ± SEM. All data were analyzed by one-way analysis of variance followed by Tukey's multiple comparison post hoc tests. Student *t*-test was used to compare two independent groups. $P < 0.05$ was considered significant.

3. Experiments and results

3.1. Experiment 1

The first experiment examined the dose-response effects of post-training peripheral injection of different doses of BBS on memory consolidation. Fifty-seven male (n = 8–10 in each group) and sixty female (n = 10 in each group) animals were randomly divided into six groups to receive saline (SAL, 1 ml/kg), or BBS (0.001, 0.0025, 0.005, 0.01 and 0.02 mg/kg) immediately after the learning trial. The animals received two injections: the first injection was SAL which immediately followed by the second injection (SAL or BBS)·The retention test was done 24 h later. In line with efforts made to reduce the total number of animals used, the data from control animals were used in the subsequent experiments examining the interaction between BBS and NMDA or dopamine receptors antagonists. Thus, the same as of the animals in experiments 2 and 3, the rats of experiment 1 received 2 consecutive injections.

Post-training BBS administration showed a dose-dependent effect on memoryretention test which was performed 24 h after IA training in male (Fig. 1A), and female pups (Fig. 1B). One-way ANOVA showed a significant difference among the male ($F_{5, 51} = 78.1$, $P < 0.0001$), and female groups ($F_{5, 54} = 6.88$, $P < 0.0001$). Post-hoc comparisons indicated that BBS at a dose of 0.01 mg/kg ($P < 0.0001$) in female pups and all tested doses of ($P < 0.05$ to $P < 0.0001$) in male pups significantly enhanced memory consolidation compared to the control group.

3.2. Experiment 2

This experiment examined the effect of BBS (0.01 mg/kg) on memory consolidation in the presence or absence of the NMDA receptor antagonist MK-801. Since the blockade of the NMDA receptor itself impairs memory consolidation, we first examined the dose-response effects of MK-801 (0.05, 0.075 and 0.1 mg/kg) on memory consolidation to obtain the ineffective dose of MK-801. The female rat pups were