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Impairments of social behavior and memory after neonatal gastrin-releasing peptide receptor blockade in rats: Implications for an animal model of neurodevelopmental disorders

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Abstract

The gastrin-releasing peptide receptor (GRPR) has been implicated in central nervous system (CNS) diseases, including neurodevelopmental disorders associated with autism. In the present study we examined the effects of GRPR blockade during the neonatal period on behavioral measures relevant to animal models of neurodevelopmental disorders. Male Wistar rats were given an intraperitoneal (i.p.) injection of either saline (SAL) or the GRPR antagonist [D-Tpi^{6}, Leu^{13} psi(CH_2NH)-Leu^{14}] bombesin (6–14) (RC-3095; 1 or 10 mg/kg) twice daily for 10 days from postnatal days (PN) 1 to 10. Animals treated with RC-3095 showed pronounced deficits in social interaction when tested at PN 30–35 and impaired 24-h retention of memory for both novel object recognition (NOR) and inhibitory avoidance (IA) tasks tested at PN 60–71. Neither short-term memory tested 1.5 h posttraining nor open field behavior were affected by neonatal GRPR blockade. The implications of the findings for animal models of neurodevelopmental disorders are discussed.

Keywords: Bombesin-like peptides; Gastrin-releasing peptide receptor; RC-3095; Social behavior; Memory; Neurodevelopmental disorders

1. Introduction

The gastrin-releasing peptide (GRP)-preferring type of bombesin (BB) receptor (GRPR, also known as BB2 receptor) has been increasingly implicated in regulating normal brain function as well as in the pathogenesis of neurological and psychiatric disorders (for recent reviews, see Moody and Merali, 2004; Roesler et al., 2006a). The GRPR is a G-protein coupled receptor expressed in the cell membranes of several tissues, including neuronal dendrites and cell bodies (Wolf and Moody, 1985; Zarbin et al., 1985; Battey and Wada, 1991; Kamichi et al., 2005). GRPR activation by the amphibian peptide BB or its mammalian counterpart, GRP, affects a range of cellular and neuroendocrine functions (Moody and Merali, 2004; Ohki-Hamazaki et al., 2005; Roesler et al., 2006a).

Recent studies have indicated that GRP and the GRPR are implicated in regulating the formation and extinction of emotional memory in brain areas including the dorsal hippocampus and basolateral amygdala (Flood and Morley, 1988; Santo-Yamada et al., 2001; Shumyatsky et al., 2002; Roesler et al., 2003; Santo-Yamada et al., 2003; Roesler et al., 2004b,c;

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Martins et al., 2005; Venturella et al., 2005; Dantas et al., 2006; Luft et al., 2006; Roesler et al., 2006b). In addition, increasing evidence suggests that the GRPR is a molecular target for the development of novel therapeutics for the treatment of central nervous system (CNS) disorders including memory dysfunction, Alzheimer's disease (AD), schizophrenia, anxiety, and brain cancer (Ito et al., 1994; Kiaris et al., 1999; Santo-Yamada et al., 2001; Shumyatsky et al., 2002; Meller et al., 2004; Moody and Merali, 2004; Roesler et al., 2004a,b,c; Martins et al., 2005; Luft et al., 2006; Roesler et al., 2006a,b).

Central nervous system diseases possibly involving the GRPR also include neurodevelopmental disorders associated with autism (Ishikawa-Brush et al., 1997; Shumyatsky et al., 2002; Marui et al., 2004; Roesler et al., 2006a). An X;8 translocation occurring in the first intron of the GRPR gene has been identified in a female patient with multiple exostoses and autism accompanied by mental retardation and epilepsy, indicating that the GRPR is a candidate gene in autism, and changes in the function of brain GRPRs during development might play a role in producing behavioral features associated with neurodevelopmental disorders (Ishikawa-Brush et al., 1997). Behavioral alterations in rodents relevant to autism include social interaction deficits, stereotyped behavior, and impaired cognitive function (for recent reviews, see DiCicco-Bloom et al., 2006; Moldin et al., 2006; Moy et al., 2006). The effects of pharmacological manipulation of the GRPR during the neonatal period on some aspects of adult behavior relevant for stress and anxiety have been described (Piggins and Merali, 1992a,b; Piggins et al., 1993). However, previous studies have not verified the effects of GRPR blockade during development on other behaviors relevant for models of CNS disorders, such as social behavior and cognitive function.

The development of several series of GRPR antagonists has allowed the investigation of the effects of pharmacological blockade of the GRPR on behavior (Roesler et al., 2003; Santo-Yamada et al., 2003; Roesler et al., 2004b,c; Martins et al., 2005; Venturella et al., 2005; Dantas et al., 2006; Luft et al., 2006; Roesler et al., 2006b). The GRPR antagonist [D-Tpi^{6}, Leu^{13} psi(CH_2NH)-Leu^{14}] bombesin (6–14) (RC-3095) was developed by Schally and colleagues as an experimental anticancer drug (Radulovic et al., 1991; Yano et al., 1992; Halmos and Schally, 1997; Szepeshazi et al., 1997; Schwartsmann et al., 2005, 2006). Biochemical studies have provided evidence that RC-3095 acts as a selective GRPR antagonist (Radulovic et al., 1991; Yano et al., 1992; Pinski et al., 1992a,b,c; Halmos and Schally, 1997; Szepeshazi et al., 1997). The pharmacokinetics of RC-3095 when administered systemically in rats has been described. Thus, RC-3095 levels in blood reached a peak at 15 min after a single s.c. injection of a dose of 100 μg, and virtually disappeared from circulation within 5 h after injection. In spite of its short half-life, RC-3095 could still induce prolonged biological effects (e.g., tumor suppression and decrease in levels and mRNA expression of the epidermal growth factor receptor (EGFR)) (Szepeshazi et al., 1997). In the brain, i.c.v. administration of RC-3095 blocks GRP-induced neuroendocrine effects (Pinski et al., 1992a,c), and infusion of RC-3095 into the dorsal hippocampus blocks BB-induced modulation of memory consolidation (Roesler et al., 2006b). Although to date there is no direct evidence that RC-3095 administered systemically can enter the adult brain, we have previously shown that systemic injections of RC-3095 induce pronounced effects on brain function in adult rats and mice (Meller et al., 2004; Roesler et al., 2004b,c).

The aim of this study was to examine the effects of neonatal GRPR blockade on behavioral measures relevant for animal models of neurodevelopmental and psychiatric disorders. We investigated social behavior, open field behavior, aversive memory, and recognition memory, in rats given systemic injections of the GRPR antagonist RC-3095 during the neonatal period.

2. Methods

2.1. Animals

Pregnant Wistar rats were obtained from the State Health Science Research Foundation (FEPPS-RS, Porto Alegre, Brazil). After birth each litter was adjusted within 48 h to eight rat pups, and to contain offspring of both genders in about equal proportions. Each pup was kept together with its mother in a plastic cage with sawdust bedding in a room temperature of 21 ± 1 °C and a 12/12 h light/dark cycle. At the age of 4 weeks, pups were weaned and the males were selected and raised in groups of three to five rats. For postnatal treatments, animals were given standardized pellet food and tap water *ad libitum*. Weight gain was measured at postnatal days (PN) 1–10, 15, 30, 60, and 90. All behavioral experiments were performed at light phase between 09:00 h and 16:30 h. The same animals were used in different behavioral experiments, but not all animals were used in all experiments: a subset of the animals tested for open field behavior was used for the social behavior test and memory tasks. All experimental procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23 revised 1996). The protocol for this research was approved by the Institutional Ethics Committee of the Pontifical Catholic University. All efforts were made to minimize the number of animals and their suffering.

2.2. Drugs and pharmacological procedures

Male pups were given two daily intraperitoneal (i.p.) 1 ml/kg injections of saline (SAL; NaCl 0.9%), or RC-3095 (1 or 10 mg/kg) dissolved in SAL; Zentaris GmbH, Frankfurt, Germany), at PN 1–10. RC-3095 has been consistently used in previous studies as a tool to investigate the role of GRPR in brain function (Roesler et al., 2003, 2004b,c; Martins et al., 2005; Venturella et al., 2005; Dantas et al., 2006; Luft et al., 2006; Roesler et al., 2006b). Drug solutions were prepared immediately prior to administration. Drug doses were chosen on the basis of previous studies (Roesler et al., 2004b,c). The treatment regimen (i.e., two daily injections of SAL or RC-3095 from PN 1 to 10) was chosen on the basis of previous studies examining the effects of pharmacological manipulation of the GRPR with BB or a GRPR antagonist on brain function in rats (Piggins and Merali, 1992b; Piggins et al., 1993). Because the blood–brain barrier is not yet complete at the neonatal period, peptides injected systemically in rats during the neonatal period are likely to enter the brain and produce direct effects on neural function (Kastin et al., 1980; Wagner et al., 1999; Reglodi et al., 2003).

2.3. Social interaction test

Impaired social behavior is a key behavioral feature of rodent models of autism spectrum disorders and schizophrenia (Mohn et al., 1999; Schneider and Przewlocki, 2005; DiCicco-Bloom et al., 2006; Moldin et al., 2006; Moy et al., 2006). Social interaction was tested at PN 30–35. This age was chosen on the basis of previous studies on rat models of autism (Schneider and Przewlocki, 2005). Animals were tested under dim/light and unfamiliar conditions, in