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Nociceptin/orphanin FQ and NOP receptor gene regulation after acute or repeated social defeat stress

Megan K. Green, Darragh P. Devine *

University of Florida, Department of Psychology, Behavioral Neuroscience Program, P.O. Box 112250, Gainesville, FL 32611-2250, USA

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ABSTRACT

Antagonists of the NOP receptor have antidepressant effects in rodent models, suggesting that the N/OFQ–NOP system may play an important role in affective disorders. Furthermore, multiple lines of experimental evidence link N/OFQ neurotransmission with physiological and behavioral responses to stress. One possibility is that disregulated expression of the N/OFQ peptide neurotransmitter and/or the NOP receptor may participate in the etiology of stress-induced psychopathology. In the present set of experiments, we compared gene expression for prepro-N/OFQ and NOP receptor in groups of rats that were exposed to differing regimens of social defeat stress. Male Long-Evans rats were exposed to no social defeat, a single, acute social defeat or to repeated social defeats with or without an acute defeat on the final day. *In situ* hybridization was conducted with ^{35}S-labelled riboprobes aimed at prepro-N/OFQ mRNA or NOP receptor mRNA. Expression was analyzed by quantification of optical density in limbic and extra-limbic forebrain regions. There were no statistically significant changes in prepro-N/OFQ mRNA expression after stress exposure in any of the brain regions analyzed. However, the rats that were exposed to acute social defeat displayed elevations in NOP receptor mRNA expression in the central and basomedial nuclei of the amygdala and in the paraventricular nucleus of the hypothalamus. Additionally, the rats that were acutely stressed after a history of repeated social defeat also displayed elevated levels of NOP receptor mRNA expression in the paraventricular nucleus of the hypothalamus. These results suggest that the N/OFQ–NOP receptor system is affected by acute stress exposure, particularly in limbic regions. This stress-induced upregulation of NOP receptor gene expression further supports the possibility that disregulation of the N/OFQ–NOP system may contribute to behavioral and hormonal disregulation following stress.

1. Introduction

There is extensive evidence that the peptide neurotransmitter, nociceptin/orphanin FQ (N/OFQ) and its cognate receptor, NOP, play important roles in expression of emotionally-relevant behaviors and in activation of the hypothalamic–pituitary–adrenal (HPA) axis. Many studies report that intracerebroventricular (icv) injections of N/OFQ (Jenck et al., 1997; Gavioli et al., 2002; Vitale et al., 2006) or systemic injections of the synthetic agonist Ro 64–6198 (Jenck et al., 2000; Dautzenberg et al., 2001; Varty et al., 2005) produce decreases in expression of anxiety-related behaviors of rodents. However, we found increases in anxiety-related behaviors after icv or intra-limbic injections of N/OFQ in rats (Fernandez et al., 2004; Green et al., 2007), and Kamei and colleagues (2004) reported both anxiolytic and anxiogenic-like effects after icv injections in mice. Griebel and colleagues (1999) found that N/OFQ decreases anxiety-related behaviors only in conditions where the mice are exposed to unavoidable severe stress (forced contact with a threatening stimulus). Furthermore, Gavioli and colleagues (2007) found both heightened and suppressed expression of anxiety-related behaviors in NOP receptor knockout mice, depending upon the type of test used. Thus, it appears that N/OFQ neurotransmission is implicated in regulation of anxiety states, but the specific effect may depend upon currently unidentified characteristics of the test.

More consistent results have been obtained in tests of the antidepressant-like effects of NOP receptor antagonists. Although N/OFQ did not alter immobility measures in forced swim and tail suspension tests after icv administration, the NOP receptor antagonists [Nphe^1]-nociceptin (1–13)-NH_2 (Redrobe et al., 2002), UFP-101 (Gavioli et al., 2003, 2004), J-113397 (Redrobe et al., 2002), and SB-612111 (Rizzi et al., 2007) each exerted antidepressant-like effects in these tests.

Studies of the effects of N/OFQ on activity of the HPA axis have uniformly revealed that N/OFQ elevates circulating adrenocorticotropic hormone (ACTH) and corticosterone (CORT) concentrations after injection into the lateral ventricles (Devine et al., 2001;

* Corresponding author. Tel.: +1 352 273 2174; fax: +1 352 392 7985.
E-mail address: dpdevine@ufl.edu (D.P. Devine).

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Nicholson et al., 2002; Fernandez et al., 2004; Leggett et al., 2006) and limbic structures (Green et al., 2007) in unstressed and mildly stressed rats. Furthermore, the N/OFQ-induced activation of the HPA axis is accompanied by increased expression of corticotrophin releasing hormone (CRH) mRNA in the paraventricular hypothalamus (PVN) and by increased proopiomelanocortin (POMC) mRNA expression in the pituitary (Leggett et al., 2006). These effects of N/OFQ administration resemble the effects of acute stress exposure (see Harbuz and Lightman, 1989) and are blocked by concurrent icv administration of the NOP receptor antagonist UFP-101 (Leggett et al., 2006).

Interestingly, N/OFQ content of the basal forebrain is reduced by acute restraint stress, and these neuronal stores are replenished within 24 h (Devine et al., 2003). This suggests that stress causes release of endogenous N/OFQ, and that biosynthetic activity returns N/OFQ stores to normal levels after release.

Since N/OFQ and the NOP receptor are implicated in regulation of affect and in HPA axis responses, we investigated the possibility that exposure to emotional stress may alter gene expression in this peptide neurotransmitter system. In these experiments, we used the social defeat model of emotional stress. In this widely used model, a young naïve male rat (the "intruder") is placed into the cage of an experienced and larger male rat (the "resident"). The resident male rat characteristically exerts dominance by pinning the intruder, and the intruder characteristically submits, by displaying a supine posture in response (see Miczek et al., 2004). Accordingly, the social defeat procedure emulates natural social hierarchies and takes advantage of social stressors that rats may experience in their natural habitats.

Social defeat exposure activates cortical and limbic circuits (Matsuda et al., 1996; Martinez et al., 1998; Chung et al., 1999; Nikulina et al., 2004) that are thought to be important in processing of emotional stressors (Herman and Cullinan, 1997). Furthermore, regional forebrain expression of *c-fos* is modified by repeated exposure to social defeat. Some limbic areas (e.g. bed nucleus of stria terminalis, medial amygdala) continue to have elevated *c-fos* expression, whereas other areas (e.g. septum, central amygdala) no longer express elevated *c-fos* after repeated defeat (Martinez et al., 1998). In addition, repeated defeat produces substantial increases in the duration of limbic *c-fos* activation (Matsuda et al., 1996). Thus, alterations in responding during repeated exposure to social defeat may reveal interesting stress-induced plasticity in neural circuits that process emotional regulation (Koolhaas et al., 1997; Miczek et al., 2004; Buwalda et al., 2005). Accordingly, we examined prepro-N/OFQ and NOP receptor mRNA after acute and repeated social defeat using *in situ* hybridization histochemistry.

2. Methods

All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were pre-approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida.

2.1. Animals

Thirty male and 14 female Long-Evans rats (Harlan, Indianapolis) were housed in 43 × 21.5 × 25.5 cm polycarbonate cages on a 12–12 h light–dark cycle (lights on at 7:00 am) in a climate-controlled vivarium (temperature 21–23 °C, humidity 55–60%). Standard laboratory chow and tap water were available *ad libitum*.

Fourteen of the male rats (weighing 600–700 g at the time of the experiment) were vasectomized and pair-housed with the 14 female rats (200–225 g at arrival). These male rats were trained to exhibit dominant behaviors in the home cage (by repeated exposure to naïve smaller "intruder" males), and were used as "residents" in the social defeat procedure (see below). The remaining 16 males, weighing approximately 300 g each, were used as experimental intruder rats. The intruder rats were pair-housed throughout the course of the experiment.

2.2. Social defeat procedure

The experimental rats were randomly assigned to 4 treatment groups. The rats of Group 1 were not exposed to social defeat (no stress controls). Group 2 rats were exposed to a social defeat session on the 6th day of the experiment only (no repeated stress, acute stress). Group 3 was exposed to a social defeat session on each of the first 5 days, but did not experience a defeat session on the 6th day (repeated stress, no acute stress), and Group 4 rats were defeated on each of the 6 experimental days (repeated stress, acute stress). This experimental design allowed us to examine the effects of acute defeat on the N/OFQ–NOP system (in comparison with unstressed controls), to evaluate whether the tone of the system changed over repeated exposures (i.e. habituation or sensitization), and to assess whether the response to an acute defeat changed with repeated stress experience. The group assignments and stress exposures of the rats are summarized in Table 1. In all cases of repeated social defeat sessions, the intruder rat was exposed to a new resident for each encounter, in order to prevent habituation in the interactions between the resident and intruder rats.

The social defeat procedure was conducted in 2 stages. During the first stage, the resident female was removed from the home cage, and 10 min later, the intruder was placed directly into the cage with the male resident. The encounter continued for 5 min or until the intruder was defeated 3 times (whichever occurred first). Each defeat was counted when the intruder displayed submissive behavior (supine posture for at least 2 s) with the resident standing over the intruder, maintaining physical contact. This first stage was also terminated if the intruder exhibited freezing behavior for 90 s. Immediately after stage 1, each intruder rat was removed from the resident's cage and placed individually into a 10 × 10 × 15 cm (inner dimensions) double-walled wire mesh cage. The intruder within the wire cage was then placed back into the resident's cage until 10 min had passed from the start of stage 1. This allowed for equalization of the duration of stress exposure of the intruder rats, and limited the number of defeats per session to a maximum of three. Following the 10-min session, the intruder rat was returned to its home cage and the female was returned to

Table 1
Summary of rat group assignments: The rats were assigned to groups that experienced differing regimens of repeated and acute social defeat exposure, and all the brains were harvested at equivalent times on day 6.

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1 (No stress)	No stress	No stress	No stress	No stress	No stress	No stress
2 (Acute stress)	No stress	No stress	No stress	No stress	No stress	Social defeat
3 (Repeated stress)	Social defeat	Social defeat	Social defeat	Social defeat	Social defeat	No stress
4 (Repeated + acute stress)	Social defeat	Social defeat	Social defeat	Social defeat	Social defeat	Social defeat