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Altered anxiety-related behavior in nociceptin/orphanin FQ receptor gene knockout mice

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ABSTRACT

Studies showed that nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP) agonists produce anxiolytic-like actions, while little is known about the effects of blockade of NOP receptor signaling in anxiety. To this aim, we investigated the behavioral phenotype of NOP receptor gene knockout mice ($NOP^{-/-}$) in different assays. In the elevated plus-maze and light-dark box, $NOP^{-/-}$ mice displayed increased anxiety-related behavior. In the novelty-suppressed feeding behavior and elevated T-maze, $NOP^{-/-}$ mice showed anxiolytic-like phenotype, while no differences were found in the open-field, hole-board, marble-burying, and stress-induced hyperthermia. Altogether, these findings suggest that the N/OFQ-NOP receptor system modulates anxiety-related behavior in a complex manner.

1. Introduction

In the 1990s, DNA cloning studies demonstrated that the opioid receptor family is comprised of three classical opioid receptors (recently renamed MOP, KOP and DOP receptors) and an opioid-like receptor, the NOP receptor [15]. Despite a high degree of amino acid sequence homology with the classical members of the opioid receptor family, the NOP receptor does not bind opioid ligands [50]. The endogenous ligand for the NOP receptor was independently isolated by two research groups [40,50], and named nociceptin/orphanin FQ (N/OFQ). N/OFQ is a 17-amino acid peptide that shares considerable sequence homology with dynorphin A, but does not bind to classical opioid receptors [40,50]. N/OFQ and the NOP receptor are ubiquitously expressed in the nervous system of mammals, including humans. Of particular interest is the expression of NOP receptor in brain areas involved in the processing of emotional stimuli, such as the hippocampus, septum, bed nucleus of the stria terminalis, amygdaloid complex, and the hypothalamus (for a review see [41]), which provides an anatomical substrate for the effects evoked by NOP receptor agonists in anxiety and stress-related responses (for a review see [49]).

Synthesis of novel peptide and non-peptide NOP receptor ligands, and development of knockout mice for the N/OFQ precursor (ppN/OFQ; [37]) and for the NOP receptor [44] allowed the investigation of the role played by the N/OFQ-NOP receptor system in regulating several biological functions, including anxiety- and mood-related behaviors [24]. Different laboratories have reported anxiolytic- and anti-stress-like

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actions in response to supraspinal administration of the endogenous peptide N/OFQ as well as systemic injection of the non-peptide NOP receptor agonist Ro64-6198. In 1997, Jenck et al. reported that N/OFQ reduced anxiety in rodents in different experimental models, such as elevated plus-maze, light dark-aversion, and operant conflict test [33]. Other laboratories subsequently confirmed the anxiolytic-like effects of N/OFQ in mice in the elevated plus-maze test [26], in the hole-board test [35], and in the defense test battery [29], and more recently in rats subjected to the elevated plus-maze and conditioned defensive burying test [58].

In agreement with the effects evoked by the natural peptide N/OFQ, studies have shown that peripheral administration of the NOP agonist Ro64-6198 in rats induced anxiolytic-like effects in the elevated plus-maze, fear-potentiated startle, operant conflict, and marble-burying tests [34,43]. These observations have been confirmed in rats and extended to mice and guinea pigs using unconditioned as well as conditioned tests of anxiety [57]. Additionally, pharmacological and genetic studies demonstrated that the anxiolytic-like actions evoked by N/OFQ and Ro64-6198 are mediated via activation of the NOP receptor [57,58]. However, little is known about the effects of blockade of NOP receptor signaling on anxiety. Pharmacological studies performed with non-peptide (J-113397) and peptide (UFP-101) NOP receptor antagonists demonstrated that these compounds, at doses active in blocking the anxiolytic-like effects of NOP agonists, did not modify *per se* animal behavior [57,58]. Genetic studies employing mice knockout for the N/OFQ peptide precursor (ppN/OFQ$^{-/-}$) corroborates the view that this peptidergic system plays an important role in the modulation of emotional states. In fact, ppN/OFQ$^{-/-}$ mice displayed an anxiogenic-like phenotype in the open-field, elevated plus-maze, and light-dark box tests [37].

The discovery of benzodiazepines in the early sixties and their remarkable success in the treatment of anxiety has strongly influenced the development of animal models of anxiety, including the elevated plus-maze and the light-dark box tests. The predictive validity of these models is mainly based on their ability to detect the pharmacological actions of benzodiazepines [2]. However, in the last 20 years, the introduction of selective serotonin reuptake inhibitors (SSRI) into clinical practice has revolutionized the treatment of affective disorders. Chronic administration of SSRI has been shown to be effective not only in the treatment of depression, but also in virtually all kinds of anxiety disorders, i.e. panic disorder, social phobic, post-traumatic stress disorder, obsessive-compulsive disorder and general anxiety disorder [27]. This clinical evidence has challenged the predictive validity of animal models of anxiety. Importantly, the most commonly used animal models of anxiety, i.e. elevated plus-maze, light-dark transition and social interaction tests, have minimal sensitivity to the anxiolytic-like effects of antidepressants [8]. By contrast, repeated administration of SSRI and tricyclic antidepressants seems to exert anxiolytic-like effects in rodents subjected to models of anxiety such as novelty-suppressed feeding behavior, elevated T-maze test, mouse defense test battery, while the acute treatment with the same drugs is able to induce anxiolytic-like effects in the mouse marble-burying and in the isolation-induced ultrasonic vocalizations in guinea-pig pup assays [8]. Such differences in predictive validity of animal models of anxiety support the view that these assays could reflect different aspects involved in anxiety and fear process, which are mediated through distinct neuroanatomical, and neurochemical circuits [8].

Considering that little information is available regarding the effects of blockade of NOP receptor signaling on anxiety, we decided to characterize the phenotype of NOP receptor gene knockout mice in several models of anxiety. This was performed in NOP$^{-/-}$ mice employing a battery of tests including; elevated plus-maze, light-dark box, hole-board, open-field, marble-burying, novelty-suppressed feeding behavior, elevated T-maze, and stress-induced hyperthermia tests.

2. Methods

All experiments and experimental procedures were conducted in accordance with the standards of the European Communities Council directives (86/609/EEC), national regulations (D.L. 116/92) and international standards of animal welfare recommended by the Society for Neuroscience.

2.1. Animals

Experiments were conducted using adult male NOP$^{+/+}$ and NOP$^{-/-}$ mice 2–3 months old (weighing 28–35 g). These animals were obtained by backcrossing hybrid C57BL/6J × 129 animals [44] with CD-1 mice for nine generations in order to produce a line based prevalently on CD-1 genetic background. Animals were all genotyped by polymerase chain reaction. Details of the generation and breeding of mutant mice have been reported previously [44,4]. Mice were housed in groups of 4–5 per cage (20 cm × 27 cm × 14 cm high), maintained on a 12 h light/dark cycle (lights off at 19:00 h) and provided with laboratory food (Extruded Global Rodent Diet, Harlan Teklad, Oxon, UK) and water *ad libitum*. Experiments were performed between 10.00 and 13.00 h in a quiet and darkened room. Mice were acclimated to the room at least 2 h before the test. Between each trial, the apparatus employed was cleaned and dried before the test.

Little is known about the potential carry over effect of experience of one behavioral test to another [59]. However, mice repeatedly exposed to the same test display altered responses when reassessed in that given animal model of anxiety [31]. Taking into account these considerations, our experiments were performed by subjecting different groups of NOP$^{+/+}$ and NOP$^{-/-}$ mice to the procedure described in Table 1.

2.2. General health and gross neurological examination

General health was evaluated by measuring body weight of both NOP$^{+/+}$ and NOP$^{-/-}$ mice at different ages (15, 30, 45, 60, 90 and 120 days after birth). Gross neurological function was assessed by evaluating normal reflexes such as eye blink, ear twitch, and whisker-orienting reflex of a freely moving animal as described by Crawley and Paylor [17]. These reflexes were measured by simply touching the eye, the tip of the ear, and the whiskers with a cotton-tipped swab. The presence or the absence of responses was recorded. Visual reflexes were