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Nociceptin and the NOP receptor in aversive learning in mice

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Abstract
The endogenous neuropeptide nociceptin (N/OFQ), which mediates its actions via the nociceptin receptor (NOP), is implicated in multiple behavioural and physiological functions. This study examined the effects of the NOP agonists N/OFQ and the synthetic agonist Ro 64-6198, the antagonists NNN and NalBzoH, as well as deletion of the Pronociceptin gene on emotional memory in mice. The animals were tested in the passive avoidance (PA) task, dependent on hippocampal and amygdala functions. N/OFQ injected intraventricularly (i.c.v.) prior to training produced a biphasic effect on PA retention; facilitation at a low dose and impairment at higher doses. Ro 64-6198 also displayed a biphasic effect with memory facilitation at lower doses and impairment at a high dose. None of the agonists influenced PA training latencies. NNN did not significantly modulate retention in the PA task but antagonized the inhibitory effects of N/OFQ. NalBzoH facilitated memory retention in a dose-dependent manner and blocked the impairing effects of N/OFQ. However, neither NNN nor NalBzoH blocked the memory-impairing effects of Ro 64-6198. Finally, the Pnoc knockout mice exhibited enhanced PA retention latencies compared to the wild type mice. The biphasic effect of the natural ligand and Ro 64-6198 and the failure of the antagonists to block the action of Ro 64-6198 indicate complexity in ligand-receptor interaction. These results indicate that brain

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nociceptin and its NOP has a subtle role in regulation of mechanisms of relevance for treatment of disorders with processing disturbances of aversive events e.g. Alzheimer's disease, anxiety, depression and PTSD.

1. Introduction

The nociceptin receptor (NOP receptor, previously the Orl1 receptor) (Mollereau et al., 1994; Reinscheid et al. 1995) is a G protein-coupled receptor, encoded by the opioid receptor-like1 gene (*Oprl1*) and belonging to the opioid family (Calo et al., 2000; Civelli, 2008; Toll et al., 2016). The search for its endogenous agonist resulted in the discovery of the neuropeptide Nociceptin/Orphanin FQ (N/OFQ) shown to be a heptadecapeptide (FGGFTGARKSARKLANQ) and an agonist for the NOP receptor (Civelli, 2008; Meunier, 1997; Meunier et al., 1995; Reinscheid et al. 1995). Similar to other opioid peptides, N/OFQ is synthesized from a precursor protein, Prepro N/OFQ, encoded by the Prepronociceptin gene, Pnoc (Mollereau et al., 1994; Saito et al., 1995). Although N/OFQ shares a 17-amino-acid sequence homologous with the opioid peptide dynorphin A (Guerrini et al., 2000), it has negligible affinity for opioid receptors (Meunier et al., 1995) and differs pharmacologically from the opioid family (Calo et al., 2000; Jenck et al., 2000; Meis, 2003). However, some results suggest that the N/OFQ and opiate systems can interact in different brain functions (Mogil et al., 1996; Walker et al., 2002).

The N/OFQ system has been associated with a variety of physiological and biological functions in the PNS and CNS (Calo et al., 2000; Civelli, 2008; Lambert, 2008; Toll et al., 2016). The localization and expression of N/OFQ and NOP in the brain and pharmacological evidence (Darland et al., 1998; Henderson and McKnight, 1997; Meunier, 1997; Neal et al., 1999; Sim-Selley et al., 2003) have implicated the N/OFQ system in nociceptive activity, motor function, stress, depression, addiction and anxiety (Gavioli and Calo' 2013; Jenck et al., 2000; Kuzmin et al., 2003; Mallimo and Kusnecov, 2013; Toll et al., 2016) as well as learning and memory (Manabe et al., 1998; Nagai et al., 2007; Noda et al., 2000; Reinscheid et al., 1995; Sandin et al., 1997, 2004).

Studies in mice and rats have shown that intraventricular (i.c.v.) or intrahippocampal (i.h.) administration of N/OFQ can modify learning and memory in tasks such as contextual fear, step down/through passive avoidance (PA), and hippocampal-dependent spatial learning (Fornari et al., 2008; Goeldner et al., 2009; Hiramatsu and Inoue, 1999; Kuzmin et al., 2004; Mamiya et al., 1999; Roozendaal et al., 2007; Sandin et al., 1997, 2004). N/OFQ administered i.c.v. impaired step-down PA in mice (Mamiya et al., 1999; Nabeshima et al., 1999) and spatial learning in rats (Redrobe et al., 2000). In mice, N/OFQ administered i.c.v. (1, 5 and 10 nmol/mouse) or i.h. (1 nmol/mouse, bilaterally) into the CA3 region dose-dependently impaired acquisition and retention in the water maze task (Kuzmin et al., 2009). The potential role of N/OFQ in hippocampal plasticity is further supported by the observation that N/OFQ inhibits long-term potentiation (LTP) in slices of the dentate gyrus and CA1 region of the rat hippocampus (Yu et al., 1997; Yu and Xie, 1998).

Previous results in transgenic mice have shown that a genetic loss of the NOP receptor can result in facilitatory effects on both spatial and aversive learning and memory, as well as enhanced hippocampal LTP in the CA1 region (Manabe et al., 1998; Noda et al., 2000). However, studies with constitutive Pnoc knockout mice have reported discrepant results. Thus, Pnoc knockout mice were reported to improve hippocampal-dependent memory (Higgins et al., 2002; Kuzmin et al., 2009), without modulating LTP in the CA1 area (Higgins et al., 2002), while another study failed to find evidence for improved spatial learning in Pnoc knockout mice (Köster et al., 1999).

The nonpeptide NOP receptor ligand Ro 64-6198, which readily enters the brain after systemic administration (Shoblock, 2007), has become an important tool in studies of the functional role of brain N/OFQ (Higgins et al., 2001; Jenck et al., 2000). It behaves as a full NOP receptor agonist with a subnanomolar affinity for the NOP receptor (Jenck et al., 2000; Wichmann et al., 2000). The receptor specificity of Ro 64-6198 has been confirmed by the absence of its behavioural effects in NOP receptor knockout mice (Higgins et al., 2001).

Ro 64-6198 mimics some, but not all, of the motor and cognitive effects reported after i.c.v. administration of N/OFQ (Higgins et al., 2002; Jenck et al., 2000; Kuzmin et al., 2004). Like N/OFQ, Ro 64-6198 has been shown to dose-dependently impair memory retention in mice in contextual fear and PA paradigms (Goeldner et al., 2009; Higgins et al., 2002; Hiramatsu et al., 1999; Roozendaal et al., 2007) as well as spatial learning in the water maze (Higgins et al., 2002; Kuzmin et al., 2009).

Based on results of N/OFQ administered i.h. N/OFQ is suggested to have a bidirectional role in spatial learning (Redrobe et al., 2000; Sandin et al., 1997, 2004). Low doses of N/OFQ facilitate and high doses impair spatial memory (Sandin et al., 2004). Notably, both effects are blocked by putative NOP antagonists (Redrobe et al., 2000; Sandin et al., 2004). However, in view of the inconsistent results with NOP antagonists, these results have remained controversial (Andero, 2015).

The aims of this study are three-fold; first to demonstrate if brain N/OFQ has a bidirectional role in PA memory, N/OFQ given i.c.v. was compared with systemically administered Ro 64-6198, using a wide range of doses. To be able to detect both impairing and facilitating effects on PA memory, the strength of the unconditioned stimulus (UCS) was adjusted (see Madjid et al., 2006). Secondly, to investigate if the effects on PA memory by N/OFQ and Ro 64-6198 are blocked