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Research Report

The nociceptin system and hippocampal cognition in mice A pharmacological and genetic analysis

Alexander Kuzmin[a], *Nather Madjid*[a], *Björn Johansson*[a,b], *Lars Terenius*[b], *Sven Ove Ögren*[a,*]

[a]*Department of Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden*
[b]*Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, SE-17177, Sweden*

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ABSTRACT

This study examines the effects of NOP agonists nociceptin/orphanin FQ (N/OFQ) and Ro 64-6198, NOP antagonists [Nphe[1]]N/OFQ(1-13)-NH_2 Nphe[1] and naloxone benzoylhydrazone (NalBzoH) on spatial memory in NMRI mice and pronociceptin (proNC) knockout (KO) mice using the water maze task. N/OFQ, administered i.c.v. (1, 5 and 10 nmol/mouse) and into hippocampal CA3 (1 nmol/mouse, bilaterally), impaired acquisition and retention in the maze. Impairments were blocked by pre-treatment with Nphe[1] (10 nmol, i.c.v.). Ro 64-6198 (0.1–0.3–1 mg/kg i.p.) also dose-dependently impaired learning. However, pre-treatment with NalBzoH (1 mg/kg, s.c.) failed to modify the effects of Ro 64-6198. Nphe[1] (10 nmol/mouse i.c.v.) and NalBzoH (1 mg/kg, s.c.) by themselves failed to affect maze performance, despite a tendency for enhanced performance. Prepro N/OFQ knockout (ppN/OFQ -/-) showed evidence of improved learning, evident at retention trials and in reversal training. ppN/OFQ -/- mice were not impaired by N/OFQ (10 nmol i.c.v.) in the task, suggesting that changes in postsynaptic NOP receptors may occur in such KO mice. It is concluded that N/OFQ and NOP receptors have an important role in hippocampus-dependent spatial learning and memory, probably by modulation of glutamatergic functions.

1. Introduction

Nociceptin/orphanin FQ (N/OFQ; Cox et al., 2000) is a natural ligand for the G protein-coupled N/OFQ peptide receptor (NOP), previously termed the opioid receptor-like 1 receptor (ORL1) (Meunier et al., 1995; Reinscheid et al., 1995), which has been shown to have multiple physiological roles in the CNS (Calo et al., 2000). Despite a structural similarity to opioids, N/OFQ has negligible affinity for opioid receptors and its pharmacological effects are not sensitive to naloxone (Meunier, 1997). The enrichment of NOP and its mRNA in cortical and limbic regions of the brain (Henderson and McKnight, 1997; Sim-Selley et al., 2003) and the immunohistochemical localization of NOP receptors in the hippocampus, brainstem and cortex (Darland et al., 1998; Meunier 1997; Neal et al., 1999a,b; Henderson and McKnight, 1997) have led to the suggestion that N/OFQ and its receptor may be important in cognitive, emotional and attentional processes.

The neuropeptide N/OFQ has been implicated in cognitive processes, based also on pharmacological evidences (Higgins

* *Corresponding author*. Fax: +46 8 302875.
E-mail address: sven.ove.ogren@ki.se (S.O. Ögren).

Abbreviations: N/OFQ, nociceptin/orphanin FQ; Nphe[1], [Nphe[1]]N/OFQ(1-13)-NH_2; NOP, N/OFQ receptor; Ro 64-6198, ((1S,3aS)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride)

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et al., 2002; Kuzmin et al., 2003; Mamiya et al., 2003; Manabe et al., 1998; Nagai et al., 2007; Noda et al., 2000; Sandin et al., 1997, 2004, Walker et al., 2002). Several studies have indicated a possible role of N/OFQ in hippocampal plasticity and hippocampally dependent learning and memory. For instance, N/OFQ has been shown to inhibit the induction of long-term potentiation (LTP) in the dentate gyrus and CA1 region of rat hippocampal slices (Yu et al., 1997; Yu and Xie, 1998). Moreover, intrahippocampal infusions of N/OFQ produced biphasic effects on spatial learning in rats with memory facilitation at low doses and memory impairment at higher doses (Sandin et al., 1997, 2004). Intracerebroventricular (i.c.v.) injections of N/OFQ in mice were found to impair step-down passive avoidance learning (Nabeshima et al., 1999) and spatial learning in rats (Redrobe et al. 2000). Moreover, activation of the hippocampal NOP receptor inhibited formation of long-term recognition memory, related to upregulation of phosphorylation of extracellular signal-regulated kinase (ERK) (Goeldner et al., 2008). It was suggested that the inhibitory effect of the NOP receptor on long-term memory was partly due to reduction of glutamatergic function at NMDA receptors in the hippocampus (Goeldner et al., 2008).

Reduction of NOP receptor function through NOP gene deletion in mice facilitated cognitive function, indicated by improved water maze acquisition, passive avoidance retention and facilitated LTP induction (Manabe et al., 1998; Noda et al., 1998). Although those effects cannot directly be linked to hippocampal signaling, the cognitive impairments appeared not to involve changes in nociceptive thresholds or anxiety-related behavior (Nishi et al., 1997; Mamiya et al., 1998). Mice lacking the N/OFQ peptide ppN/OFQ(-/-) mice (Köster et al., 1999) represent an alternative to the N/OFQ KO mouse to study the role of endogenous N/OFQ in cognition. In one study, ppN/OFQ(-/-) mice showed slightly improved spatial learning in comparison with wild type littermates, assessed in the water maze task and improved memory performance in the probe trial (Higgins et al., 2002). In another study, ppN/OFQ(-/-) failed to alter spatial acquisition and memory in the water maze (Köster et al., 1999). Notably, these mice displayed signs of elevated anxiety-like behavior when compared to their wild type and heterozygous littermates (Köster et al., 1999; Reinscheid and Civelli, 2002). The observed differences in cognitive performance between NOP(-/-) and ppN/OFQ(-/-) mice have presently no adequate explanation.

Studies on the functional role of the N/OFQ receptor with pharmacological tools have been hampered by the lack of potent and selective synthetic ligands. However, a non-peptidergic ligand ((1S,3aS)-8-(2,3,3a,4,5,6-Hexahydro-1H-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one hydrochloride (Ro 64-6198), which has been shown to be a full NOP receptor agonist with an affinity close to that of N/OFQ itself, can be administered by the systemic route (Jenck et al., 2000; Shoblock, 2007; Wichmann et al., 2000). The pharmacological specificity of Ro 64-6198 was confirmed by the absence of behavioral effects in NOP receptor KO mice (Higgins et al. 2001). Two peptidergic ligands, selective for the NOP receptor have also recently been characterized as antagonists: [Nphe^1]N/OFQ(1-13)NH_2 (Calo et al., 2000; Guerrini et al., 2000), abbreviated NPhe^1, and [NPhe^1, Arg^{14}, Lys^{15}]nociceptin(1-13)NH_2 (UFP-101, Calo et al., 2002). The antagonistic properties and high selectivity and affinity of both peptidergic ligands have been confirmed in several *in vivo* assays (Calo et al., 2002). However, these peptidergic antagonists can only be used after intracerebral injection (Redrobe et al., 2000). Naloxone benzoylhydrazone (NalBzoH) is a combined opioid receptor and N/OFQ receptor antagonist which can be given systemically (Bigoni et al., 2002). Behavioral studies have shown that NalBzoH is an antagonist of the *in vivo* effects of N/OFQ. For instance, systemic administration of NalBzoH completely inhibited N/OFQ-induced hyperalgesia and hypolocomotion (Noda et al., 1998) and it also blocked the impairing effect of N/OFQ on passive avoidance retention (Mamiya et al., 1999). Importantly, NalBzoH loses its analgesic properties in NOP receptor KO mice (Noda et al., 1998).

Although the available data implicate N/OFQ and NOP in cognition, their specific roles are still not well defined. A controversy remains whether N/OFQ can both impair or improve memory. For instance, intrahippocampal injection of N/OFQ produced a biphasic effect on spatial learning in the rat, which both were related to activation of NOP (Sandin et al., 2004). Also in the passive avoidance test and in the Y-maze spontaneous alternation tests, N/OFQ impaired learning performance at nanomolar doses in normal mice, but normalized the performance in scopolamine-impaired mice at very low doses (doses down to femtomols) (Hiramatsu and Inoue, 2000). These results indicate that N/OFQ has bidirectional modulatory effects on learning and memory, but the mechanisms underlying the impairments at high doses and the improvement at low doses are still unknown. Furthermore, the basal activity of N/OFQ is probably low, since none of the tested NOP antagonists have shown any significant effect on hippocampally dependent memory performance, in contrast to the receptor KO mice (Redrobe et al., 2000; Sandin et al., 2004). It is therefore likely that N/OFQ has differential roles in learning and memory in normal rodents compared to KO mice, either by acting on different subtypes or binding sites of the NOP receptors located in the hippocampal neurocircuitry involved in cognitive processes or acting on additional receptors related to nociceptin. Such differences may underlie the variable effects on cognitive performance observed after genetic loss of the nociceptin peptide or receptor (Mamiya et al., 1998; Manabe et al., 1998; Köster et al., 1999; Higgins et al., 2002).

In view of the conflicting results on spatial learning obtained in NOP receptor and pro-NC KO mice, the main aim of the present study was to further examine the role of the endogenous NOP receptor system in learning and memory using mice. A major aim was to characterize and analyze the effects on spatial learning and memory by the use of NOP receptor ligands and mice with deletion of the N/OFQ peptide. Since N/OFQ is believed to modulate memory via hippocampal mechanisms, the effects of intraventricular and intrahippocampal drug administration were compared. Cognitive functions were assessed in the Morris water maze, a spatial memory task involving hippocampal functions (Morris et al., 1982). The first set of studies investigated the effects of NOP ligands (the natural peptide and synthetic compounds) either alone or in combination using systemic, i.c.v. or intrahippocampal administration. It was of special interest to analyze the effects (including sensitivity to antagonists) of N/OFQ after i.c.v. and intrahippocampal administration with those obtained by Ro 64-6198 given systemically. The studies also