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The nociceptin orphanin FQ peptide receptor agonist, Ro64-6198, impairs recognition memory formation through interaction with glutamatergic but not cholinergic receptor antagonists

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ABSTRACT

We previously reported that the selective nociceptin orphanin peptide (NOP) receptor agonist, Ro64-6198, impairs mnemonic function through glutamatergic-dependent mechanisms. The aim of the current study was to determine whether the amnesic effects of Ro64-6198 involve a cholinergic component. The effects of systemic administration of Ro64-6198 (0.3 and 1 mg/kg, i.p.), the cholinergic nicotinic receptor antagonist, mecamylamine (0.1 and 1 mg/kg, s.c.), the cholinergic muscarinic receptor antagonist, scopolamine (0.1 and 0.3 mg/kg, s.c.), and the glutamatergic NMDA receptor antagonist, MK-801 (0.03 and 0.1 mg/kg, s.c.), were studied in the mouse object recognition task. All compounds tested were effective in disrupting formation of long-term (24-h delay) recognition memory. Drug interaction studies were then conducted to reveal the existence of functional interactions between NOP receptors and cholinergic and/or NMDA receptors. Co-administration of silent doses of Ro64-6198 (0.3 mg/kg) and MK-801 (0.01 mg/kg) produced clear-cut memory impairment. Similar synergistic effects were observed with the combination of mecamylamine (0.03 mg/kg) and scopolamine (0.1 mg/kg). In contrast, co-administration of Ro64-6198 (0.3 mg/kg) with either mecamylamine (0.03 and 0.1 mg/kg) or scopolamine (0.1 mg/kg) was without any effect on recognition memory. These findings suggest that NOP receptor may modulate memory formation through a functional interaction with glutamatergic but not cholinergic receptors.

1. Introduction

The nociceptin/orphanin-FQ peptide (N/OFQ) represents an addition to the opioid peptide family. N/OFQ has been identified as the natural ligand of the G protein-coupled opioid receptor-like 1 receptor (ORL1) now termed NOP receptor (nociceptin orphanin FQ peptide receptor (Meunier et al., 1995; Reinscheid et al., 1995). At a cellular level, NOP receptor activation produces inhibition of the enzyme adenylyl cyclase, reduction of calcium channel conductance and activation of potassium channels (Hawes, Graziano, & Lambert, 2000; New & Wong, 2002). Through these mechanisms, N/OFQ may exert both inhibitory and disinhibitory actions depending on the regional and synaptic localisation in the central nervous system.

Studies conducted over the past decade have shown that N/OFQ and NOP receptor contribute to a host of biological functions (Calo, Guerrini, Rizzi, Salvadori, & Regoli, 2000; Chiou et al., 2007; Darland, Heinricher, & Grandy, 1998; Jenck, Ouagazzal, Pauly-Evers, & Moreau, 2000; Lambert, 2008). One of the most established findings is the inhibitory influence of this neuropeptide system on cognition. Intra-cerebral infusion of N/OFQ or systemic administration of the synthetic NOP receptor agonist, Ro64-6198, induces memory deficits in a range of cognitive tasks, including step-down passive avoidance, fear conditioning, object recognition and Morris water-maze procedures (Fornari, Soares, Ferreira, Moreira, & Oliveira, 2008; Goeldner et al., 2008; Higgins et al., 2002; Hiramatsu & Inoue, 1999; Kuzmin, Madjid, Johansson, Terenius, & Ogren, 2009; Mamiya et al., 2003; Manabe et al., 1998; Redrobe, Calo, Guerrini, Regoli, & Quirion, 2000; Sandin, Ogren, & Terenius, 2004). Conversely, antagonism of endogenous N/OFQ signalling improves learning and memory performances in rats and mice (Higgins et al., 2002; Kuzmin et al., 2009; Mamiya et al., 2003; Manabe et al., 1998). While the role of the N/OFQ system in cognition is now well

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established its mechanisms of action are still poorly understood. Electrophysiological studies reported that N/OFQ suppresses different forms of neuronal plasticity within the hippocampus including long-term potentiation (LTP) and long-term depression (LTD) by antagonizing the function of the glutamatergic NMDA receptor (Bongsebandhu-phubhakdi & Manabe, 2007; Manabe et al., 1998; Wei & Xie, 1999; Yu & Xie, 1998). Accordingly, studies conducted with knockout mice showed that ablation of NOP receptor activity results in increased intrinsic activity of hippocampal NMDA receptor, which was accompanied at the behavioral level by improved learning abilities that could be reverted by the non-competitive NMDA receptor antagonist, MK-801 (Mamiya et al., 2003). Recently, we provided evidence that the amnesic effects of the NOP receptor activation were also mediated through glutamatergic-dependent mechanisms. We found that Ro64-6198 acts synergistically with MK-801 to disrupt learning abilities in a range of cognitive tasks (Goeldner, Reiss, Wichmann, Kieffer, & Ouagazzal, 2009; Goeldner et al., 2008). More importantly, we showed that deleterious action of this agonist on recognition memory formation was linked to suppression of NMDA receptor-induced hippocampal MAPK/ERK (mitogen-activated-protein-kinases/extracellular-regulated-kinase) pathway activation, which is required for long-term information storage. In contrast, Ro64-6198 had no effect on spontaneous ERK activity during resting conditions suggesting that NOP receptor may primarily modulate changes mediated by other neurotransmitters, such as glutamate, that are triggered during learning (Goeldner et al., 2008).

The neurotransmitter acetylcholine has long been implicated in learning and memory processes (Pepeu & Giovannini, 2010; Winters, Saksida, & Bussey, 2008) and prior neurochemical studies have also suggested the existence of negative functional interaction between N/OFQ and central cholinergic systems. I.e. N/OFQ reduces cholinergic transmission in many brain structures, including the hippocampus (Cavallini, Marino, Beani, Bianchi, & Siniscalchi, 2003; Itoh, Konya, Takai, Masuda, & Nagai, 1999), while deletion of NOP receptor gene enhances basal hippocampal acetylcholine levels (Uezu et al., 2005). In the present study, we used the novel object exploration task to explore for the first time the contribution of cholinergic component to the inhibitory influence of NOP receptors on recognition memory. Recognition memory is generally distinguished into spatial and visual object recognition memories that have been suggested to depend on different but overlapping neuronal substrates (Steckler, Drinkenburg, Sahgal, & Aggleton, 1998b; Winters et al., 2008). For instance, the perirhinal cortex is essential for processing of the object features, and hence for formation of the visual objects memory, while the hippocampus is critical for spatial information processing and for formation of associations involving the object and the location (or context) in which it was encountered. Many variants of novel object exploration task have been developed to study the different facets of recognition memory (Steckler, Drinkenburg, Sahgal, & Aggleton, 1998a). Here, we used a procedure that involves both visual and spatial components to examine the potential contribution of cholinergic mechanisms to the amnesic effects of Ro64-6198. The procedure consists of an acquisition session during which mice are presented with an object in a familiar open field arena followed by delayed test trial, in which the previously experienced sample object (familiar object) is presented together with a novel object introduced into an empty spatial location. We first characterized the effect of Ro64-6198, MK-801, the cholinergic nicotinic receptor antagonist, mecamylamine, and the cholinergic muscarinic receptor antagonist, scopolamine, on formation of long-term (24 h delay) recognition memory. Drug interaction studies were then conducted to reveal the existence of a functional interaction between NOP receptors and cholinergic and/or NMDA receptors.

2. Materials and methods

2.1. Animals

Eight week old C57BL/6N (BL6N) male mice were purchased from Charles River Laboratory (France) and housed 4 per cage with free access to food and water. Mice were maintained on a 12:12 h light/dark cycle (lights on from 7 am to 7 pm) at 22 °C, and allowed to acclimate to housing conditions until testing, at age 12–15 weeks. All experimental procedures were carried out according to the guidelines of the European Union and were approved by the local ethic committee (CREMEAS).

2.2. Drugs

Ro64-6198 (F. Hoffman-La Roche, Basel, Switzerland), MK-801 (Sigma, France), scopolamine hydrobromide (Sigma, France) and mecamylamine (Sigma, France) were dissolved in physiological saline (0.9% NaCl). Drugs were injected at a volume of 10 ml/kg either intra-peritoneally (Ro64-6198) or sub-cutaneously (MK-801, scopolmaine and mecamylmine), 30 min before testing. The doses of the various compounds used were selected based on previous studies (Dodart, Mathis, & Ungerer, 1997; Feiro & Gould, 2005; Goeldner et al., 2008).

2.3. Object recognition task and experimental design

2.3.1. Object recognition procedure

Testing was carried out in dimly lit (50-Lux) open-field arenas ($44 \times 44 \times 17$ cm^3, Panlab, Barcelona, Spain) fitted with infra-red beam frames which provide automated measures of locomotor activity (Actitrack, Barcelona, Spain). The general procedure consisted of three different phases carried out over three consecutive days. On the first day, mice were habituated to the open-field arena for 30 min. The following day, they were submitted to a 10 min acquisition trial in the presence of an object A (dice or marble) placed in the northeast (NE) or northwest (NW) corner of the open-field arena. The presentation of the object (dice or marble) as well as its position (NE or NW) was counterbalanced between mice for each treatment. The time spent exploring the object (T_1A), defined as head oriented towards the object within a distance of about 2 cm, was hand-scored with a stopwatch. Minimum exploration time was set to 3 s, and mice that did not reach this criterion were excluded from the study. On the third day, mice were submitted to a 10-min test trial in the presence of the familiar object (A) placed in the same corner, and a novel object (B) placed in the opposite corner. The time spent exploring the two objects (T_2A and T_2B, respectively) was manually scored. After each run, arenas and objects were cleaned. The experimenter scoring mouse behavior was blind to the treatment. The recognition index (RI) was defined as $(T_2B/(T_2A + T_2B) \times 100)$. A RI of 50% corresponds to chance level whereas a higher RI reflects active recognition.

2.3.2. Experimental design

2.3.2.1. Experiment I. Effect of Ro64-6198 and MK-801 administered alone or conjointly. One group of naïve mice received injections of Ro64-6198 (0, 0.3 and 1 mg/kg, $n = 6$ per dose) or MK-801 (0, 0.03 and 0.1 mg/kg, $n = 6$–8 per dose) before the acquisition and were tested the following day drug free. A second group of mice received injections of Ro64-6198 (0.03 mg/kg, $n = 10$) and MK-801 (0.01 mg/kg, $n = 9$) either separately or concomitantly ($n = 10$) before acquisition and were tested the following day. The control group ($n = 10$) received conjoint injections of the corresponding vehicles.