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Effect of novel nociceptin/orphanin FQ–NOP receptor ligands on ethanol drinking in alcohol-preferring msP rats

D. Economidou [a,*], A. Fedeli [a], R. Martin Fardon [b], F. Weiss [b], M. Massi [a], R. Ciccocioppo [a,b]

[a] Department of Experimental Medicine and Public Health, University of Camerino, Via Scalzino 3, 62032 Camerino, MC, Italy
[b] The Scripps Research Institute, Molecular and Integrative Neurosciences Department, La Jolla, CA, USA

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ABSTRACT

Activation of the NOP receptor by the endogenous ligand nociceptin/orphanin FQ (N/OFQ) reduces alcohol consumption in genetically selected alcohol-preferring Marchigian Sardinian (msP) rats. The present study evaluated the effect of three newly synthesized peptidergic and one brain-penetrating heterocyclic NOP receptor agonists on alcohol drinking in the two bottle choice paradigm. MsP rats were intracerebroventricularly (ICV) injected with the NOP receptor agonists OS-462 (0.5 and 1.0 μg), UFP-102 (0.25 and 1.0 μg) or UFP-112 (0.01 and 0.05 μg), or with Ro 64-6198 (0.3 and 1.0 mg/kg) given intraperitoneally (i.p.) and tested for 10% alcohol consumption. Results showed decreased alcohol consumption after treatment with all three peptidergic NOP receptor agonists (OS-462, UFP-102 and UFP-112). OS-462 (at the 1.0 μg dose) and UFP-102 (at the 0.25 μg dose) induced a significant increase in food intake as well. Surprisingly, Ro 64-6198 was ineffective at the 0.3 mg/kg dose, whereas it increased ethanol and food consumption at the 1.0 mg/kg dose. Pre-treatment with the selective μ-receptor antagonist naloxone (0.5 mg/kg, i.p.) reduced these effects of 1.0 mg/kg of Ro 64-6198. These findings confirm that activation of brain NOP receptors reduces alcohol drinking in msP rats and demonstrate that OS-462, UFP-102 and UFP-112 act as potent NOP receptor agonists. On the other hand, Ro 64-6198 increased alcohol drinking, an effect probably induced by a residual agonist activity of this compound at μ-opioid receptors. Overall, the results indicate that OS-462, UFP-102 and UFP-112 may represent valuable pharmacological tools to investigate the functional role of the brain N/OFQ system.

1. Introduction

Nociceptin/orphanin FQ (N/OFQ), the endogenous ligand for the NOP opioid receptor, previously referred to as ORL-1 or OP_4 receptor [31,44], is known to be structurally related to the opioid peptide dynorphin A [32,33,45,46]. However, N/OFQ does not bind to MOP, DOP or KOP opioid receptors, nor do opioid peptides activate the NOP receptor [45,46]. In contrast, N/OFQ activates with high selectivity the NOP receptor [45], eliciting intracellular responses with the same intracellular mechanisms as classic opioid receptors [45]. Interestingly, however, N/OFQ has been found to act in the brain as a functional antiopioid peptide by blocking opioid-induced supraspinal analgesia or morphine-induced conditioned place preference [6,34,35,38,39].

Brain mapping studies have revealed a neuroanatomical distribution of N/OFQ and the NOP receptor different from that of other opioid peptides and receptors [1,8,16,21,37,40,47]. A wide distribution of this peptidergic system has been found in various corticomesolimbic structures, including the amygdala,

* *Corresponding author*. Tel.: +39 0737 403321; fax: +39 0737 630618.
E-mail address: daina.economidou@unicam.it (D. Economidou).
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the bed nucleus of the stria terminalis and various frontocortical areas. Interestingly, such brain areas are known to be involved in the regulation of the motivational properties of drugs of abuse [14,28,50] and an important involvement of the N/OFQ–NOP receptor system in the control of reward and drug abuse processes has been now well established [5]. For instance, pre-treatment with N/OFQ blocks ethanol-, morphine- and cocaine-induced conditioned place preference [4,6,29,39] and reduces ethanol intake in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats both in the two bottle free-choice and operant self-administration paradigms [4,7]. Finally, microdialysis studies demonstrated that central administration of this peptide significantly attenuates the increase of extracellular dopamine (DA) induced in the nucleus accumbens (Nacc) after administration of cocaine or morphine [12,30].

Because of the well documented role of the N/OFQ–NOP receptor system in drug and alcohol reward as well as the ability of this opioid peptide to exert its effects without activating classic opioid receptors (MOP, DOP and KOP), great interest is presently devoted to the development of novel NOP receptor agonists with favorable pharmacodynamic and pharmacokinetic properties such as brain permeability following peripheral administration. Recently, several peptidergic NOP receptor agonists as well as one non-peptidergic brain-penetrating NOP receptor agonist, Ro 64-6198, have been synthesized [49].

The purpose of this study was to evaluate the effect of three newly synthesized peptidergic NOP receptor agonists (OS-462, UFP-102 and UFP-112) and of the brain penetrating agent Ro 64-6198 on home-cage voluntary ethanol drinking in msP rats.

2. Methods

2.1. Animals

Male genetically selected alcohol-preferring rats were used. These rats were bred in the Department of Experimental Medicine and Public Health of the University of Camerino (Marche, Italy) for 50 generations from Sardinian alcohol-preferring (sP) rats of the 13th generation, provided by the Department of Neurosciences of the University of Cagliari [15,17]. These animals are referred to as Marchigian Sardinian P (msP) rats. At the time of the experiments, their body weight ranged from 400 to 450 g. The rats were housed in a temperature (20–22 °C) and humidity (45–55%) controlled vivarium on a reverse 12-h light:12-h dark cycle (lights off at 9:00 a.m.). Rats were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). All procedures were conducted in adherence with the European Community Council Directive for Care and Use of Laboratory Animals.

2.2. Surgical procedures

For intracranial surgery, rats were anesthetized by intramuscular injection of 100–150 μl of a solution containing tiletamine hydrochloride (58.17 mg/ml) and zolazepam hydrochloride (57.5 mg/ml). A guide cannula for injections into the lateral ventricle was stereotaxically implanted and cemented to the skull. The following coordinates, taken from the atlas of Paxinos and Watson [43], were used: antero-posterior = −1.0 mm with respect to bregma, lateral = 1.8 mm from the sagittal suture, ventral = 2 mm from skull surface.

For ICV administration, compounds were injected through a stainless-steel injector protruding 2.5 mm beyond the cannula tip. Experiments began one week after surgery. Cannula placement was verified before the experiment by ICV injection of 100 ng of angiotensin II. Only animals that showed a clear dipsogenic response (at least 6 ml of water in 5 min) were used for further testing.

2.3. Drugs

The NOP receptor agonist OS-462 (*N*-a-6-guanidinohexyl-3,5-dimethyl-ltyrosyl-L-tyrosyl-*N*-[(R)-1-(2-naphthyl)ethyl]-L-argininamide, MW = 823) corresponds to Example 25 of the European Patent [22,25,42] and was provided by Nippon Shinyaku, Co. Ltd., Kyoto, Japan. The NOP receptor agonists UFP-102 ([(pF)Phe4,Arg14,Lys15]N/OFQ-NH2, MW = 2725) and UFP-112 ([(pF)Phe4Aib7Arg14Lys15]N/OFQ-NH2, MW = 2738) were generously provided by Dr. Remo Guerrini of the Department of Pharmaceutical Sciences, University of Ferrara, Italy. Ro 64-6198 was kindly provided by Hoffmann-La Roche (Basel, Switzerland). Naloxone was purchased from Tokris (Cookson Ltd., UK).

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, MW = 438.017) was dissolved in a solution containing 10% DMSO, 10% Tween-80, 80% distilled water and administered intraperitoneally (i.p.) at a volume of 1 ml/kg. Naloxone was dissolved in distilled water and was given i.p. at a volume of 1 ml/kg. All other NOP receptor agonists were dissolved in sterile isotonic saline and injected ICV in a volume of 1 μl.

2.4. Experimental procedure

At 3 months of age, msP rats were selected for ethanol preference by offering them free choice between water and 10% ethanol (w/w) 24 h a day for 15 days. Water and ethanol were available in graduated drinking tubes equipped with metallic drinking spouts and fluid was measured by reading the volume consumed from the graduated burettes. Food intake was measured by weighing the food containers and taking into account spillage. Ethanol, water and food intakes are expressed as g/kg to reduce the influence of differences in body weight.

The rats used in the present experiments all showed 24 h ethanol intake of 6–7 g/kg with ethanol preference [ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h × 100] greater than 90%. Rats were given free access to ethanol, water and food for 24 h/day until a stable baseline alcohol intake was established. Subsequently, ethanol availability was restricted to 30 min/day for animals treated with OS-462, UFP-102 or UFP-112 and to 60 min/day for animals treated with Ro 64-6198. Ethanol was made available at the beginning of the rats' dark phase (9:00 a.m.). All animals were habituated to the experimental procedure by receiving 3–4 i.p. or ICV injections before initiation of the experiments (pre-treatment period). All experiments were carried out according to a between-subject design, in which each group of rats received a single dose of a single compound.