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European Journal of Pharmacology 579 (2008) 110–115
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# Quantitative study of [(pF)Phe$^4$,Arg$^{14}$,Lys$^{15}$]nociceptin/orphanin FQ-NH$_2$ (UFP-102) at NOP receptors in rat periaqueductal gray slices

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Received 20 May 2007; received in revised form 2 October 2007; accepted 4 October 2007
Available online 11 October 2007

**Abstract**

The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is a novel member of the opioid receptor family with little affinity for traditional opioids. This receptor and its endogenous ligand, N/OFQ, are widely distributed in the brain and are implicated in many physiological functions including pain regulation. [(pF)Phe$^4$,Arg$^{14}$,Lys$^{15}$]N/OFQ-NH$_2$ (UFP-102) is a newly developed peptide agonist of NOP receptors. In this study, we quantitatively investigated the effect of UFP-102 at native NOP receptors of the ventrolateral periaqueductal gray (PAG), a crucial midbrain area involved in pain regulation and enriched with NOP receptors, using blind patch-clamp whole-cell recording technique in rat brain slices. UFP-102, like N/OFQ, induced an outward current in ventrolateral PAG neurons and increased the membrane current elicited by a hyperpolarization ramp from −60 to −140 mV. The current induced by UFP-102 was characterized with inward rectification and had a reversal potential near the equilibrium potential of $K^+$ ions, indicating that UFP-102 activates G-protein coupled inwardly rectifying $K^+$ channels. The effect of UFP-102 was concentration-dependent with the maximal effect similar to that of N/OFQ. The $EC_{50}$ value was 11±2 nM, which is 5 fold lower than that of N/OFQ. The effect of UFP-102 was not affected by naloxone while competitively antagonized by UFP-101 ([Nphe$^1$,Arg$^{14}$,Lys$^{15}$]N/OFQ-NH$_2$), a potent NOP receptor antagonist, with a p$A_2$ value of 6.7. These results suggest that UFP-102 is a full agonist at the postsynaptic NOP receptors of the midbrain of rats and is 5 fold more potent than N/OFQ.


*Keywords:* Nociceptin/orphanin FQ; UFP-102; UFP-101; NOP receptors; Potassium channels; Periaqueductal gray

## 1. Introduction

A new member of the opioid receptor family was identified in 1994 when opioid receptors were cloned. It was named initially as ORL1, opioid-like orphan receptor, because it is highly homologous to classical opioid receptors but has little affinity for traditional opioids (Mollereau et al., 1994). Its endogenous agonist was simultaneously identified by two groups and was termed nociceptin (Meunier et al., 1995) or orphanin FQ (Reinscheid et al., 1995). This receptor was, thereafter, renamed after its endogenous ligand as nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor and was classified as a non-opioid branch of the opioid receptor family (NC-IUPHAR, 2004). N/OFQ has been implicated in many physiological or pathological functions, including pain regulation, stress response, feeding, locomotor activity, learning and memory, pituitary functions, and immune and cardiovascular controls (Darland et al., 1998; Peluso et al., 1998; Calo' et al., 2000; Mogil and Pasternak, 2001; Chiou et al., 2007). Heterogeneity of NOP receptors has been suggested from the findings that there are splicing variants of NOP receptor transcripts and more than one specific binding site of N/OFQ in the rodent brain (Mathis et al., 1997). Furthermore, functional heterogeneity of NOP receptors has been revealed by a receptor ligand, Ro 64-6198 (Chiou et al., 2004; Kuzmin et al., 2004). Hence,

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doi:10.1016/j.ejphar.2007.10.006

the development and characterization of NOP receptor ligands would be of help in revealing the physio/pathological roles of N/OFQ and clarifying the possible diversity of NOP receptors.

[(pF)Phe$^4$,Arg$^{14}$,Lys$^{15}$]N/OFQ-NH$_2$ (UFP-102) is a novel peptide agonist of NOP receptors developed by substituting the 4th, 14th and 15th amino acid of N/OFQ with (pF)Phe, Arg and Lys, respectively (Guerrini et al., 2005). These modifications make UFP-102 more potent than N/OFQ in several assays performed *in vitro* on expressed recombinant NOP receptors ($CHO_{hNOP}$) and native receptors of several peripheral preparations and cortex synaptosomes (Carra et al., 2005). It also displayed longer duration of action than N/OFQ *in vivo* (Carra et al., 2005; Economidou et al., 2006). In this study, we have quantitatively investigated the pharmacological characteristics of UFP-102 at the postsynaptic NOP receptors of rat brain slices containing the midbrain periaqueductal gray (PAG), which is enriched with dense NOP receptors (Anton et al., 1996).

Experiments were conducted in the ventrolateral region of the PAG, which is a crucial site for morphine-induced supraspinal analgesia (Yaksh et al., 1976) and also the action site that N/OFQ reverses the antinociceptive effect of morphine (Morgan et al., 1997). Previous studies have shown that N/OFQ activates inwardly rectifying $K^+$ channels, which are coupled by G-protein (Ikeda et al., 1997), in most of the tested neurons in the ventrolateral PAG (Vaughan and Christie, 1997; Chiou, 1999, 2001; Chiou and Fan, 2002; Chiou et al., 2002, 2004, 2005). The effect of UFP-102 at the NOP receptors of ventrolateral PAG neurons was, therefore, quantified by the increment of this G-protein coupled inwardly rectifying $K^+$ (GIRK) current. The potency and efficacy of UFP-102 were compared with those of N/OFQ.

## 2. Materials and methods

All animal experiments were approved by the Institutional Animal Care and Use Committee of College of Medicine, National Taiwan University. All efforts were made to minimize the number of animals used. The dissection of PAG slices, electrophysiological recordings, and data analysis were similar to that described in the previous report (Chiou et al., 2005).

Coronal midbrain slices (400 μm) containing the PAG were dissected from 9–17 day-old rats (Wistar strain) and equilibrated in the artificial cerebral spinal fluid (aCSF) at room temperature for at least 1 h before recording. The aCSF consisted of (mM): NaCl 117, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaH_2PO_4$ 1.2, $NaHCO_3$ 25 and dextrose 11.4, and was oxygenated with 95% $O_2$/5% $CO_2$, (pH = 7.4). Slices were mounted on a submerged recording chamber and perfused with the oxygenated aCSF at a rate of 2–4 ml/min. Blind patch-clamp whole-cell recording was conducted at 30 °C. The internal solution contained (mM): $K^+$ gluconate 125, KCl 5, $CaCl_2$ 0.5, BAPTA 5, Hepes 10, MgATP 5, and GTPtris 0.33 (pH = 7.3). The electrode resistance was 4–8 MΩ.

To study the activation of GIRK channels by UFP-102, a hyperpolarization ramp protocol was applied. The cell was held at −70 mV, stepped to −60 mV for 100 ms, ramped from −60 mV to −140 mV for 400 ms, and then stepped back to −70 mV (Fig. 1, inset). After whole-cell configuration was formed, hyperpolarization ramps were given every 30 s. Membrane currents elicited by hyperpolarization ramps were recorded through an amplifier (Axopatch 200B, Axon Instruments Inc., Union City, C.A.) with a PC computer running pClamp 7 (Axon Instruments Inc.) and simultaneously recorded

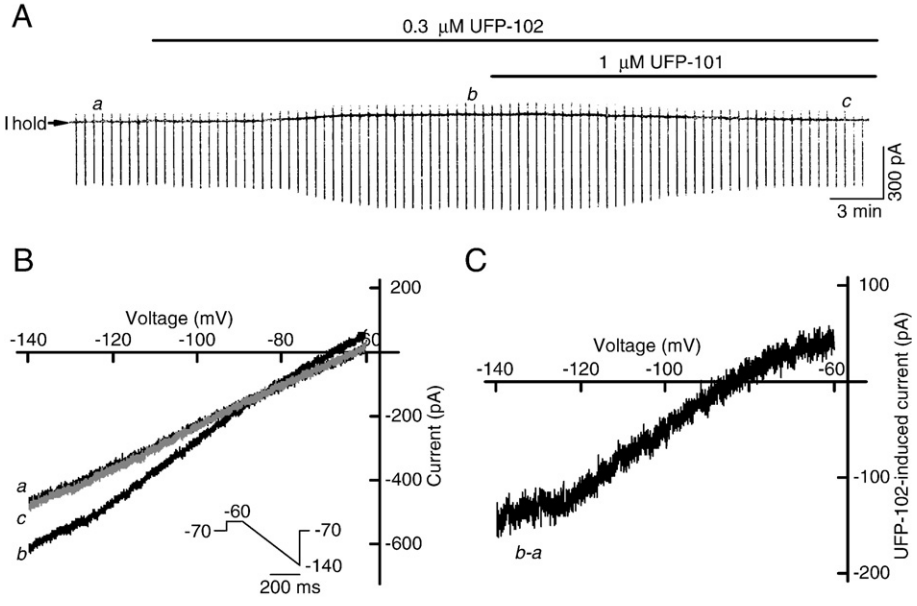


Fig. 1. UFP-102 activated GIRK current in ventrolateral PAG neurons in a manner blocked by UFP-101. Membrane currents were evoked by hyperpolarization ramps from −60 to −140 mV at 0.2 mV/ms every 30 s from the holding potential of −70 mV (inset). A: The chart recording of the membrane current of a neuron treated with 0.3 μM UFP-102, followed by 1 μM UFP-101. The baseline of the traces resembles the holding current ($I_{hold}$) of the recorded neuron. B: The current–voltage (*I–V*) curve of the membrane current in the control (*a*) or the presence of UFP-102 (*b*) or UFP-102 plus UFP-101 (*c*). C: The *I–V* curve of UFP-102-induced current, which was obtained by subtracting the current in the control from that during exposure to UFP-102 (*b–a*).