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A potent and highly selective nonpeptidyl nociceptin/orphanin FQ receptor (ORL1) antagonist: J-113397

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Abstract

We discovered a potent nociceptin/orphanin FQ receptor (ORL1) receptor antagonist, J-113397 (1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one). J-113397 inhibited [^{125}I][Tyr^{14}]nociceptin binding to Chinese hamster ovary (CHO) cells expressing ORL1 receptor in a dose-dependent manner (IC_{50}; 2.3 nM), but showed 600-fold or less affinity for μ-, δ- and κ-opioid receptors. Nociceptin/orphanin FQ-induced suppression of cyclic AMP accumulation elicited by forskolin was completely inhibited by J-113397 with an IC_{50} value of 26 nM. These results indicate that J-113397 is a potent and selective nonpeptidyl antagonist of the ORL1 receptor.

Keywords: Nociceptin/orphanin FQ; ORL1 receptor; Opioid

Nociceptin, also termed orphanin FQ (Meunier et al., 1995; Reinscheid et al., 1995), is a peptide consisting of 17 amino acids that was recently identified as an endogenous ligand for the nociceptin/orphanin FQ receptor (ORL1) (Mollereau et al., 1994), and has been thought to exert various biological effects on nociception, locomotion, and autonomic regulation in vivo (Meunier, 1998). Although studies with mice lacking ORL1 receptors revealed involvement of this peptide in cognitive function (Manabe et al., 1998), lack of a potent and selective antagonist of ORL1 receptor has prevented definitive clarification of the physiological roles of the nociceptin/orphanin FQ–ORL1 receptor system. We screened ORL1 receptor antagonists using our chemical library, and here in report J-113397, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one, as the first potent and selective antagonist of the ORL1 receptors (Fig. 1A).

Binding studies were performed using membrane fractions isolated from Chinese hamster ovary (CHO) cells expressing either human ORL1 receptors (CHO-ORL1), human μ-, δ-, or κ-opioid receptors. [^{125}I][Tyr^{14}]nociceptin binding assays were conducted according to the method of Reinscheid et al. (1995). The binding affinities for μ-, δ-, and κ-opioid receptors were determined by competition binding study with [^{3}H]diprenorphine, [^{3}H]DADLE, and [^{3}H]U-69593, respectively. Each membrane was incubated in 50 mM Tris–buffer (pH 7.4) with radioligand at 25°C for 2 h. The antagonistic activity of J-113397 was determined by cyclic AMP assay. CHO-ORL1 cells were incubated with nociceptin/orphanin FQ and/or J-113397 in Locke's buffer, pH 7.4 (154 mM NaCl, 5.6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5.6 mM glucose, 10 mM HEPES, 0.3 mM 3-isobutyl-1-methylxanthine), containing 10 μM forskolin at 25°C for 10 min. Cyclic AMP levels were determined using a BIOTRAK cyclic AMP direct assay kit (Amershampharmacia Biotech).

J-113397 inhibited [^{125}I][Tyr^{14}]nociceptin binding to CHO-ORL1 with an IC_{50} value of 2.3 ± 0.26 nM (Fig. 1B), but only inhibited [^{3}H]diprenorphine, [^{3}H]DADLE and [^{3}H]U-69593 binding to human μ-, δ- and κ-opioid receptor, respectively, at concentrations of more than 100 nM. The IC_{50} values for J-113397 were only 2200 ± 350 nM for μ-opioid receptors, $> 10{,}000$ nM for δ-opioid receptors, and 1400 ± 180 nM for κ-opioid receptors (Fig. 1B). In order to assess whether J-113397 is an agonist or

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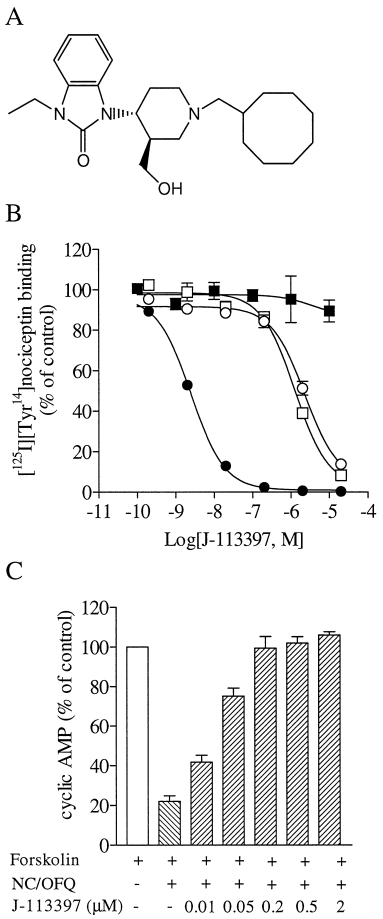

Fig. 1. (A) Structure of J-113397. (B) Inhibitory activities of J-113397 in radioligand bindings to (●) ORL1, (○) μ-, (■) δ-, and (□) κ-opioid receptors. Membranes from CHO cells expressing ORL1, μ-, δ-, and κ-opioid receptors were incubated with [^{125}I][Tyr^{14}]nociceptin, [^{3}H]diprenorphine (μ), [^{3}H][D-Ala^{2}, D-Leu^{5}]enkephalin ([^{3}H]DADLE) (δ), and [^{3}H](+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzene acetamide ([^{3}H]U-69593) (κ), respectively, in the presence of various concentrations of J-113397. (C) Effects of J-113397 on 1 nM nociceptin/orphanin FQ (NC/OFQ)-produced suppression of cyclic AMP accumulation elicited by forskolin (10 μM) in CHO-ORL1. Data are the mean ± S.E.M. of three experiments.

antagonist of ORL1 receptor, we examined the effects of this compound on cyclic AMP formation in CHO-ORL1 cells. Nociceptin/orphanin FQ dose-dependently suppressed the accumulation of cyclic AMP elicited by 10 μM forskolin with an EC_{50} value of 0.22 ± 0.011 nM (data not shown). J-113397 (0.01–2 μM) inhibited the effects of nociceptin/orphanin FQ on cyclic AMP-formation in a dose-dependent manner with an IC_{50} value of 26 ± 3.1 nM (Fig. 1C). These results demonstrate that J-113397 is a potent antagonist of ORL1 receptor with high selectivity over other classical opioid receptors.

The nociceptin/orphanin FQ derivative, [Phe^{1}Ψ(CH_2-NH)Gly^{2}]nociceptin-(1–13)-NH_2, was reported to be a peptidyl antagonist of the ORL1 receptors (Guerrini et al., 1998). However, this peptide was shown to possess agonistic activity in a cyclic AMP assay with CHO cells transfected with human ORL1 receptor (Butour et al., 1998). Therefore, it is still debatable whether [Phe^{1}Ψ(CH_2-NH)Gly^{2}]nociceptin-(1–13)-NH_2 acts as an antagonist or agonist of the ORL1 receptor. In contrast, J-113397 behaved as an antagonist of ORL1 receptor in the cyclic AMP assay with CHO-ORL1 although [Phe^{1}Ψ(CH_2-NH)Gly^{2}]nociceptin-(1–13)-NH_2 behaved as an agonist of the ORL1 receptor in the same condition (data not shown). Thus, our nonpeptidyl antagonist J-113397 may be a valuable tool to study the physiological roles of nociceptin/orphanin FQ.

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