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# β-Funaltrexamine inactivates ORL1 receptors in BE(2)-C human neuroblastoma cells

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**Abstract**

The potential interactions of natively expressed μ-opioid and opioid receptor-like (ORL1) receptors were studied by exposing intact BE(2)-C cells to agonists or antagonists for 1 h. Pretreatment with the μ-opioid receptor agonist, [D-Ala$^2$, *N*-Me-Phe$^4$,Gly$^5$-ol]enkephalin (DAMGO), or the ORL1 receptor agonist, orphanin FQ/nociceptin desensitized both μ-opioid and ORL1 receptor responses. β-Funaltrexamine (β-FNA) pretreatment also blocked both μ-opioid and ORL1 receptor responses, but only μ-opioid receptor binding was reduced. Moreover, β-FNA (1 μM) failed to inhibit specific ORL1 receptor binding. 

*Keywords:* μ-Opioid receptor; cAMP accumulation; Orphanin FQ/nociceptin

Orphanin FQ/nociceptin is the endogenous ligand for the opioid receptor-like (ORL1) receptor (also known by other names, including KOR-3, XOR1, LC132, ROR-C) and modulates a wide variety of behavioral responses including nociceptive sensitivity, anxiety, learning and reward (Harrison and Grandy, 2000). The ability of a μ-opioid receptor agonist to modulate orphanin FQ/nociceptin-mediated analgesia (and vice versa) varies depending on time, dose and site of administration, as does blockade of orphanin FQ/nociceptin-mediated analgesia by the irreversible μ-opioid receptor antagonist, β-funaltrexamine (β-FNA; Hao et al., 1997; Jhamandas et al., 1998). In vitro effects of μ-opioid and ORL1 receptor agonist pretreatments on μ-opioid receptor and ORL1 receptor-mediated activity are even less clear. In Chinese hamster ovary cells expressing recombinant μ and ORL1 receptors, Hawes et al. (1998) reported that orphanin FQ/nociceptin, but not [D-Ala$^2$, *N*-Me-Phe$^4$,Gly$^5$-ol]enkephalin (DAMGO), pretreatment decreased both orphanin FQ/nociceptin and DAMGO-stimulated mitogen activated protein kinase activity. In the present study, we describe a potential role for the μ-opioid receptor in ORL1 receptor activity in BE(2)-C human neuroblastoma cells, a cell line natively expressing μ-, δ-, $\kappa_3$- and ORL1 opioid receptors (Standifer et al., 1994; Mathis et al., 1999).

Liquiscint scintillation cocktail and [$^3$H]DAMGO (40.9 Ci/mmol) were purchased from National Diagnostics (Atlanta, GA) and Amersham (Arlington Heights, IL), respectively. [$^3$H]orphanin FQ/nociceptin (51 Ci/mmol), orphanin FQ/nociceptin, DAMGO and β-FNA were obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO).

BE(2)-C cells (passage 25–45; Dr. Robert Ross, Fordham University, Bronx, NY) were grown to 80–90% confluence and pretreated with vehicle (buffer or 0.1% methanol), agonist (0.1 nM orphanin FQ/nociceptin or 1 μM DAMGO) or antagonist (1 μM β-FNA dissolved in methanol) for 1 h at 37°C in culture media absent of sera. At the end of the incubation, cells were washed four to five times with ice-cold phosphate-buffered saline and assayed for receptor binding or cAMP accumulation. [$^3$H]orphanin FQ/nociceptin and [$^3$H]DAMGO binding to BE(2)-C membranes (0.6–1 mg/ml) was performed as described (Ardati et al., 1997; Standifer et al., 1994). The ability of orphanin FQ/nociceptin or DAMGO to inhibit forskolin (10 μM)-stimulated cAMP accumulation was determined in intact cells over a 10-min period (Standifer et al., 1994). Determination of Log$EC_{50}$ values was performed using nonlinear regression analysis and statistical

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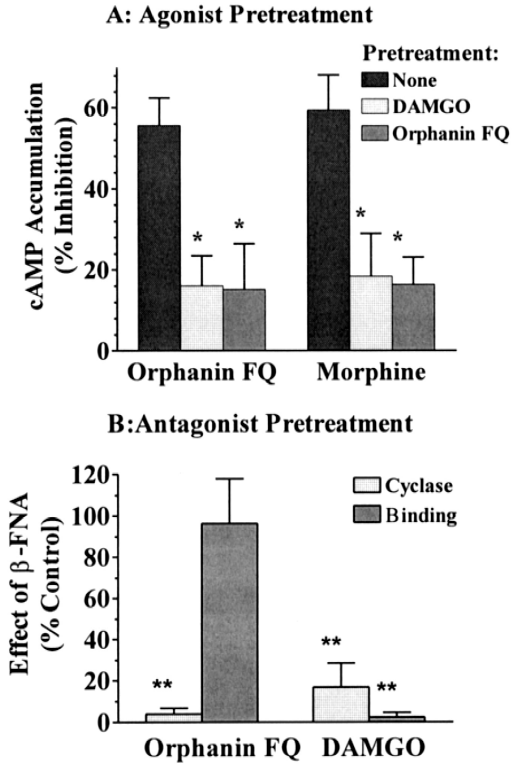


Fig. 1. Orphanin FQ/nociceptin-mediated inhibition of forskolin-stimulated cAMP accumulation in BE(2)-C cells is sensitive to μ-opioid agonist (A) and antagonist (B) treatments. (A) Intact cells were assayed for the ability of orphanin FQ/nociceptin (0.1 nM) or morphine (1 μM) to inhibit forskolin (10 μM)-stimulated cAMP accumulation after a 1 h pretreatment with DAMGO (1 μM) or orphanin FQ/nociceptin (0.1 nM). $^{*}P < 0.01$ by ANOVA compared to controls; $n = 3$–4. (B) Intact cells were treated with β-FNA (1 μM) for 1 h prior to assay of orphanin FQ/nociceptin-(0.1 nM) and DAMGO-(10 μM) mediated inhibition of forskolin-stimulated cAMP accumulation and [$^{3}$H]orphanin FQ/nociceptin (1 nM) and [$^{3}$H]DAMGO (1 nM) binding. $^{**}P < 0.05$ by *t*-test compared to controls, $n = 3$.

comparisons were made using an unpaired *t*-test or analysis of variance (ANOVA) as indicated (GraphPad Prism Version 3.00 for Windows 95/98, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Orphanin FQ/nociceptin and DAMGO inhibited cAMP accumulation ($-\text{LogEC}_{50}$ values: $12.43 \pm 0.45$ and $6.65 \pm 0.33$, respectively), consistent with previous reports (Mathis et al., 1999; Standifer et al., 1994). Pretreatment of BE(2)-C cells for 1 h with an ORL1 (orphanin FQ/nociceptin) or μ-(DAMGO) opioid receptor agonist desensitized the response of both receptors (Fig. 1A, $^{*}P <$ 0.01 by ANOVA compared to control; $n = 3$–4). The maximal ORL1 receptor response to orphanin FQ/nociceptin (0.1 nM) was reduced over 70% by both treatments; concentration–response curves were flattened to such an extent that $\text{LogEC}_{50}$ values could not be accurately estimated after treatment. The μ-opioid receptor response to a single concentration of morphine (1 μM) was reduced by both treatments, as well. To rule out any contribution of the μ-opioid receptor to the orphanin FQ/nociceptin response, cells were pretreated with 1 μM β-FNA, a concentration sufficient to reduce μ-opioid receptor binding and inhibition of DAMGO-mediated cAMP accumulation by 95% and 87%, respectively (Fig. 1B; $^{**}P < 0.05$ by *t*-test, compared to controls: $4.8 \pm 1.6$ fmol/mg protein and $75.4 \pm 1\%$, respectively). Surprisingly, β-FNA treatment also completely blocked the ability of orphanin FQ/nociceptin to inhibit cAMP accumulation compared to vehicle-treated controls ($65.6 \pm 16.7\%$), but had no effect on basal or forskolin-stimulated levels of cAMP ($54.6 \pm 11.9$ and $130 \pm 28$ pmol/mg, respectively). Again, $\text{LogEC}_{50}$ values could not be accurately estimated after pretreatment. This blockade did not appear to be mediated through the ORL1 receptor because the same β-FNA pretreatment failed to reduce specific [$^{3}$H]orphanin FQ/nociceptin binding (Fig. 1B; vehicle-treated control $35.8 \pm 6.48$ fmol/mg protein; $n = 3$). In a separate study, 1 and 10 μM β-FNA failed to inhibit specific [$^{3}$H]orphanin FQ/nociceptin (1 nM) binding ($84.7 \pm 17.7\%$ and $74 \pm 21.6\%$ control, respectively; $n = 3$); a $K_i$ value for β-FNA at that site could not be determined.

β-FNA covalently binds to Lys$^{233}$ of the μ-opioid receptor (Chen et al., 1996). This residue is conserved among all opioid receptors, except ORL1; the selective irreversible nature of β-FNA appears to result from tertiary structures specific to the μ-opioid receptor (Chen et al., 1996). Clearly, the actions of β-FNA on orphanin FQ/nociceptin receptor activity are not mediated through the orphanin FQ/nociceptin binding site on ORL1. These findings suggest that cross talk between μ-opioid and ORL1 receptors, distinct from the binding site, may contribute to ORL1 function.

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