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(2*S*,3*R*)TMT-L-Tic-OH is a potent inverse agonist at the human δ-opioid receptor

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Abstract

We examined the pharmacologic effect of β-methyl-2′,6′-dimethyltyrosine-L-tetrahydroisoquinoline-3-carboxylic acid ((2*S*,3*R*)TMT-L-Tic-OH) on G protein activation in membranes prepared from Chinese Hamster Ovary cells transfected with cDNA of the human δ-opioid receptor. (2*S*,3*R*)TMT-L-Tic-OH inhibited G protein activation to 58% of basal with an EC_{50} of 0.72 nM as determined by [^{35}S]GTPγS binding. These findings suggest that (2*S*,3*R*)TMT-L-Tic-OH is a highly potent inverse agonist at the human δ-opioid receptor.

Keywords: δ-Opioid receptor; Inverse agonist; G protein

Investigators have identified potent and highly selective antagonists that act at the μ-, κ- and δ-opioid receptors. In the case of the δ-opioid receptor, analogues of [Leu]-enkephalin have been synthesized that have agonist, antagonist and inverse agonist properties (Mullaney et al., 1996; Schiller et al., 1999). It is now known that when 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) is substituted in the L conformation at the second position of the enkephalin peptide, the affinity and selectivity of the peptide for δ-opioid receptors is improved (Schiller et al., 1992). Some Tic containing compounds have previously been shown to act as δ-opioid receptor-selective antagonists (Schiller et al., 1992). Recently, we synthesized a new analogue of this class of δ-opioid receptor-selective ligand, β-methyl-2′,6′-dimethyltyrosine-L-Tic-OH ((2*S*,3*R*)-TMT-L-Tic-OH) (Liao et al., 1997). We report here that this dipeptide is a highly potent inverse agonist at the human δ-opioid receptor.

We examined the modulation of G proteins in membranes prepared from a Chinese hamster ovary (CHO) Cell line that had previously been transfected with cDNA of the human δ-opioid receptor (hDOR/CHO) (Malatynska et al., 1995). Membranes were prepared and G protein activity was determined as [^{35}S]GTPγS binding according to our previous protocol (Hosohata et al., 1998). Briefly, hDOR/CHO cells were grown in HAMS-F12 with 10% fetal bovine serum, G418 (500 μg/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml). Adherent cells were removed from tissue culture plates with phosphate-buffered saline (PBS) containing 0.02% EDTA. Cells were sedimented, washed with PBS and homogenized with a dounce homogenizer into Tris (10 mM)/EDTA (1 mM) pH = 7.4. Cells were again sedimented and homogenized as above into assay buffer (25 mM Tris, 150 mM NaCl, 2.5 mM $MgCl_2$, 1.0 mM EDTA, 50 μM GDP, 30 μM bestatin, 10 μM captopril and 0.1 mM phenylmethylsulfonyl fluoride, pH = 7.4). Membranes were incubated with 0.1 nM [^{35}S]GTPγS (1250 Ci/mmol, New England Nuclear, Boston, MA) in a total volume of 1 ml assay buffer with increasing concentrations of opioid drugs in duplicate. Incubation continued for 90 min at 30°C. Membranes were resuspended to a final density of OD_{280} = 0.05. Bound [^{35}S]GTPγS was separated from free by vacuum filtration through Whatman GF/B glass filters followed by four washes with ice cold 25 mM Tris/120 mM NaCl, pH = 7.4. Filters were counted in EcoLite (ICN Biochemicals, Costa Mesa, CA) and individual experiments analyzed as a

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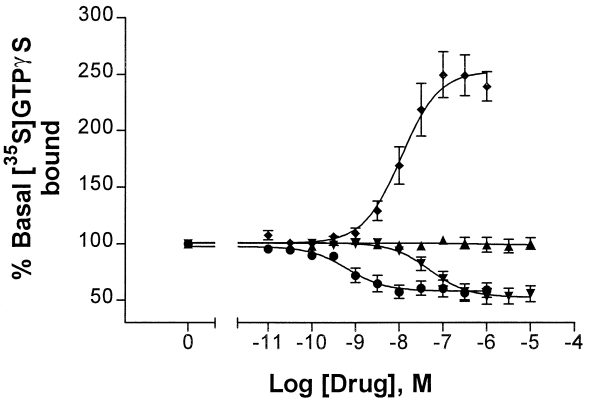

Fig. 1. Effect of a δ-opioid agonist, antagonist and inverse agonists on basal [^{35}S]GTPγS binding to hDOR/CHO membranes. The graph represents the mean ± standard error of the mean for each drug. Cell membranes were incubated with increasing concentrations of DPDPE (♦), naltrexone (▲), ICI 174,864 (▼) or (2*S*,3*R*)TMT-L-Tic-OH (●) for 90 min at 30°C. DPDPE stimulated 254 ± 18% basal G protein activation with a potency of 12.5 ± 2.0 nM, whereas naltrexone was a neutral antagonist. ICI 174,864 and (2*S*,3*R*)TMT-L-Tic-OH were inverse agonists that mediated a reduction in [^{35}S]GTPγS binding to 52 ± 7 and 58 ± 7% of basal, respectively. Differences in the intrinsic activities of ICI 174,864 and (2*S*,3*R*)TMT-L-Tic-OH did not reach statistical significance using a *t*-test. In contrast, the potencies of these drugs; 56 ± 17 and 0.72 ± 0.15 nM, respectively; were significantly different by *t*-test ($P < 0.05$).

sigmoidal dose response curve (Hill slope = 1) using Prism ver. 2 (GraphPad, San Diego, CA). Data are expressed below as the mean ± standard error of the mean. $N \geq 3$ for all conditions.

[D-Phe^2, D-phe^5]enkephalin (DPDPE) stimulated [^{35}S]GTPγS binding to 254 ± 18% basal binding with an EC_{50} of 12.5 ± 2.0 nM (Fig. 1). In contrast, the nonselective opioid drug naltrexone was a neutral antagonist in this system over the entire concentration range of drug tested (10 pM–10 μM). When we tested (2*S*,3*R*)TMT-L-Tic-OH we found it to function as an inverse agonist in this system. [^{35}S]GTPγS binding was reduced to a minimum of 58 ± 7% of basal with a potency of 0.72 ± 0.15 nM. We also examined the effect of ICI 174,864 on G protein activation. Consistent with a previous report (Mullaney et al., 1996), this drug also was an inverse agonist at the cloned hDOR. ICI 174,864 reduced [^{35}S]GTPγS binding to 52 ± 7% of basal with a potency of 56 ± 17 nM.

These data indicate that (2*S*,3*R*)TMT-L-Tic-OH is an inverse agonist at the cloned δ-opioid receptor of > 50-fold potency as compared to ICI 174,864. Both drugs have similar intrinsic activities. In hDOR/CHO membranes, DPDPE acted as an agonist, naltrexone as a neutral antagonist and both (2*S*,3*R*)TMT-L-Tic-OH and ICI 174,864 as inverse agonists in the [^{35}S]GTPγS binding assay of G protein activation. Our findings suggest that (2*S*,3*R*)TMT-L-Tic-OH is a highly potent inverse agonist that stabilizes a conformation of the δ-opioid receptor that inhibits receptor coupling to G proteins under experimental conditions where both agonist and neutral antagonist activity can be determined. Thus, (2*S*,3*R*)TMT-L-Tic-OH may be a useful pharmacological tool to determine structural features of the δ-opioid receptor involved in G protein activation.

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