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Inverse agonism by Dmt–Tic analogues and HS 378, a naltrindole analogue

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Abstract

The potent δ-opioid receptor antagonist H-2′,6-L-tyrosine(Dmt)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic-OH) exhibited partial inverse agonism (EC_{50} = 6.35 nM, E_{max} = −18.87%) for [^{35}S]GTPγS binding and H-Dmt–Tic-NH_2 was a neutral antagonist (no effect up to 30 μM). In contrast *N*,*N*(CH_3)$_2$-Dmt–Tic-NH_2 was a full inverse agonist (EC_{50} = 2.66 nM, E_{max} = −35.95%) similar to ICI 174864 ([*N*,*N*-diallyl-Tyr^1,Aib^{2,3},Leu^5]enkephaline) but with a 3.5-fold higher EC_{50}. In comparison, naltrindole was a neutral antagonist while its analogue HS 378 was a partial inverse agonist (E_{max} = −12.99%).

Keywords: Dmt–Tic; Inverse agonism; Antagonism

Dimethyl-L-tyrosine (Dmt) and the Dmt-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) pharmacophore represent important elements for high δ affinity and selectivity δ-opioid receptor antagonism; being orders of magnitude greater than Tyr cognates (Lazarus et al., 1998; Salvadori et al., 1995). While increased activity by Dmt might stabilize the rotational motion of the aromatic ring by dimethylation to reinforce the tyramine hydroxyl group to interact with the receptor (Bryant et al., 1998), peptide stability could also prolong the biological half-life (Sasaki et al., 1999). A requirement for multiple aromatic centers was based on conformational analysis of H-Tyr–Tic-OH/-NH_2, H-Dmt–Tic-OH (Bryant et al., 1998) and *cyclo*(Dmt–Tic) (Bryant et al., 1998), in which a low energy conformation of *cyclo*(Dmt–Tic) superimposed with a rms of 0.3 Å (Bryant et al., 1997) on the X-ray diffraction structure of *N*,*N*(CH_3)$_2$-Dmt–Tic-OH (Flippen-Anderson, personal communication). Augmentation of the hydrophobicity of Dmt–Tic through amidation (Salvadori et al., 1995) and N-alkylation produced analogues with high δ affinity, selectivity and enhanced δ antagonism (Bryant et al., 1998; Lazarus et al., 1998). Furthermore, since amidation elevated μ-receptor affinity (Lazarus et al., 1998; Salvadori et al., 1995), the development of C-terminal hydrophobic Dmt–Tic analogues greatly enhanced μ-opioid receptor properties (Salvadori et al., 1999). The change in receptor selectivity and pharmacological activity provided the impetus to investigate other biochemical events effects since these potent pseudopeptides have the potential to be exquisite tools for defining the action of δ receptors. Therefore, the functional assay using the binding of [^{35}S]GTPγS was employed to explore the interaction of opioid receptor ligands with their receptors and G proteins.

HEK(human embryonic kidney)-2938 cells expressing human δ, μ- and κ-opioid receptors, produced in AstraZenca (Valiquette et al., 1996), were grown in suspen-

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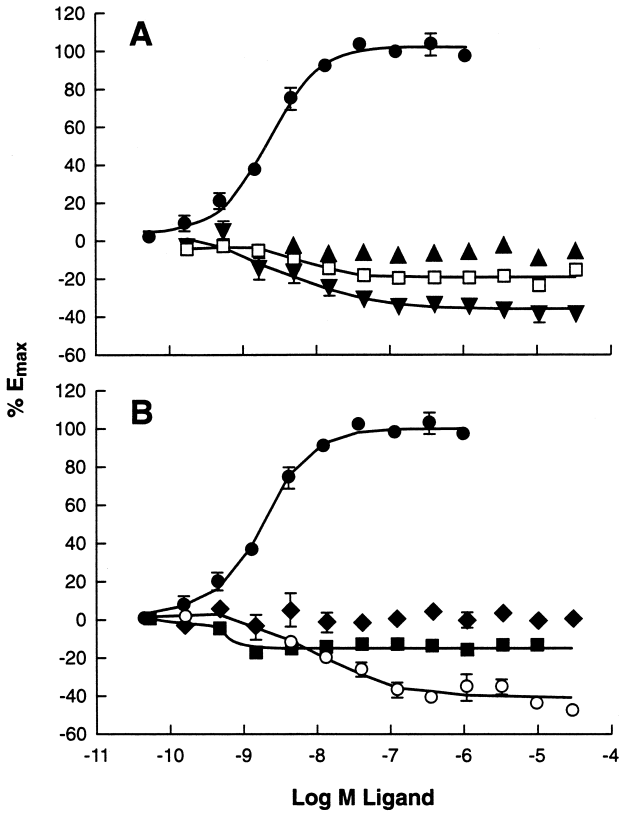

Fig. 1. Competitive binding curves on the stimulation of the incorporation of [^{35}S]GTPγS into the cloned human δ-opioid receptor by the δ agonist SNC-80 (●). (A) The binding curves demonstrate that H-Dmt–Tic-NH_2 (▲) was a neutral agonist, whereas H-Dmt–Tic-OH (□) was a partial inverse agonist, while *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2 (▼) exhibited a full inverse agonist properties. (B) Controls with the same data for SNC-80 (in panel A) using naltrindole (♦) as a neutral antagonist and ICI-174,864 (□) as a recognized full inverse agonist, to demonstrate that HS 378 (■) is a partial inverse agonist.

sion. A membrane fraction (P_2), prepared by homogenization and differential centrifugation, was combined with the test compounds in 0.2 nM [^{35}S]GTPγS in 50 mM HEPES, pH 7.4, 20 mM NaOH, 5 $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 0.1% BSA (bovine serum albumin) and 15 μM GDP (guanosine-5′-diphosphate). After 60 min at 22 C, membrane bound radioactivity was determined by rapid filtration. Control and stimulated (E_{max}) [^{35}S]GTPγS binding were determined in the absence and presence of reference agonists for each of the opioid receptors: 3 μM SNC-80 (μ receptor agonist) for δ, 30 μM DAMGO ([D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin for μ, or 1 μM U69593, a κ receptor agonist). The effects were fit to a four-parameter logistic model (GraphPad Prism™) and expressed as EC_{50} and $\%E_{max}$.

Results with the pseudopeptides H-Dmt–Tic-OH, H-Dmt–Tic-NH_2 and *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2, the control standards, naltrindole and its analogue HS 378 (Schmidhammer et al., 1998) on the binding of [^{35}S]GTPγS by the δ agonist SNC-80 are detailed in Fig. 1. The data are summarized as follows: H-Dmt–Tic-OH was a partial inverse agonist with an E_{max} of −18.87% (Fig. 1A) in spite of its high δ affinity, selectivity and antagonism (Lazarus et al., 1998) and similar to HS 378 (E_{max} = −12.90%) (Fig. 1B). However, H-Dmt–Tic-NH_2, whose receptor binding properties and bioactivity are less than H-Dmt–Tic-OH (Lazarus et al., 1998), was a neutral antagonist without effect up to 30 μM (Fig. 1A), comparable to that of naltrindole (Fig. 1B). Although *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2 exhibits nearly 50-fold greater δ antagonism than H-Dmt–Tic-OH (Lazarus et al., 1998), it exhibited full inverse agonism with an E_{max} of −35.95% which was essentially the same as ICI 174864 (*N*,*N*-diallyl-Tyr,Aib,Phe,Leu-OH) (−39.58%). Interestingly, *N*,*N*-$(CH_3)_2$-Dmt–Tic-NH_2 exhibits weak μ antagonist activity (Lazarus et al., 1998)and weak μ antagonism in the [^{35}S]GTPγS binding assay without activity toward κ receptors (data not shown). Inhibition of the basal binding of [^{35}S]GTPγS (EC_{50}) was 6.35, 2.66 and 9.25 nM for H-Dmt–Tic-OH, *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2 and ICI-174,864, respectively. Since the E_{max} for *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2 and ICI-174,864 were nearly equivalent, *N*,*N*$(CH_3)_2$-Dmt–Tic-NH_2 is a more potent inverse agonist by 3.5-fold. HS 378 was a partial inverse agonist.

These data are consistent with the concept that the non-peptide δ-opiate antagonist naltrindole and Dmt–Tic pharmacophore pseudopeptides interact differently with respect to the δ-opioid receptor. These results on the inhibition of the basal activity of G proteins confirm that the Dmt–Tic pharmacophore represents a potent class of δ-opioid receptor antagonists (Lazarus et al., 1998; Salvadori et al., 1999) and gives rise to the prospect that they might have potential clinical and therapeutic applications.

References

Bryant, S.D., Balboni, G., Guerrini, R., Salvadori, S., Tomatis, R., Lazarus, L.H., 1997. Opioid diketopiperazines: refinement of the δ opioid antagonist pharmacophore. Biol. Chem. 378, 107–114.

Bryant, S.D., Salvadori, S., Cooper, P.S., Lazarus, L.H., 1998. New δ opioid antagonists as pharmacological probes. Trends Pharmacol. Sci. 19, 42–46.

Lazarus, L.H., Bryant, S.D., Cooper, P.S., Guerrini, R., Balboni, G., Salvadori, S., 1998. Design of δ-opioid peptide antagonists for emerging drug applications. Drug Dev. Today 3, 284–294.

Salvadori, S., Attila, M., Balboni, G., Bianchi, C., Bryant, S.D., Crescenzi, O., Guerrini, R., Picone, D., Tancredi, T., Temussi, P.A., Lazarus, L.H., 1995. δ Opioidmimetic antagonists: prototypes for designing a new generation of ultraselective opioid peptides. Mol. Med. 1, 678–689.

Salvadori, S., Guerrini, R., Balboni, G., Bianchi, C., Bryant, S.D., Cooper, P.S., Lazarus, L.H., 1999. Further studies on the Dmt–Tic pharmacophore: hydrophobic substituents at the C-terminus endows δ antagonists to manifest μ agonism or μ antagonism. J. Med. Chem. 42, 5010–5019.

Sasaki, Y., Suto, T., Ambo, A., Ouchi, H., Yamamoto, Y., 1999. Biological properties of opioid peptides replacing Tyr at position 1 by 2,6-dimethyl-Tyr. Chem. Pharm. Bull. 47, 1506–1507.

Schmidhammer, H., Krassnig, R., Greiner, E., Schultz, J., White, A., Berzetei-Gurske, I.P., 1998. Synthesis and biological evaluation of 14-alkoxymorphinans: Part 15. Novel delta opioid receptor antagonists with high affinity and selectivity in the 14-alkoxy-substituted indolomorphinan series. Helv. Chim. Acta 81, 1064–1069.

Valiquette, M., Vu, H.K., Yue, S.Y., Wahlestedt, C., Walker, P., 1996. Involvement of Trp-284, Val-296, and Val-297 of the human δ-opioid receptor in binding of δ-selective ligands. J. Biol. Chem. 271, 18789–18796.