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Short communication

Relative efficacies of δ-opioid receptor agonists at the cloned human δ-opioid receptor

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Abstract

The present study was conducted to determine the relative efficacies of the selective δ-opioid receptor agonists SNC80 ((+)-4-[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide), pCl-DPDPE (cyclic[D-Pen^2,4′-ClPhe^4,D-Pen^5]enkephalin) and (−)-TAN67 ((−)-2-methyl-4*a*α-(3-hydroxyphenyl)-1,2,3,4,4*a*,5,12,12*a*α-octahydro-quinolino-[2,3,3-*g*]isoquinoline). Experiments compared the abilities of the three drugs to competitively inhibit [^{3}H]naltrindole binding and also stimulate [^{35}S]GTPγS binding in membranes prepared from stably transfected Chinese hamster ovary (CHO) cells that express the cloned human δ-opioid receptor. Efficacy was determined according to the formula: efficacy = $(E_{\text{max-A}}/E_{\text{max}})(A'/A + 1) \times 0.5$. Results show that SNC80 and pCl-DPDPE had efficacy values that were about 6–7 times greater than that of (−)-TAN67.

Keywords: Efficacy; δ-Opioid receptor, human; Radioligand binding; δ-Opioid receptor agonist; [^{35}S]GTPγS binding

1. Introduction

It has long been recognized that drugs with similar receptor affinities are not necessarily equally efficacious in evoking behavioral or neurochemical effects. For example, the nonpeptidic δ-opioid receptor agonist (±)-TAN67 ((±)-2-methyl-4*a*α-(3-hydroxyphenyl)-1,2,3,4,4*a*,5,12,12*a*α-octahydro-quinolino-[2,3,3-*g*]isoquinoline) has high affinity for the δ-opioid receptors in the rat brain ($K_i = 0.7$ nM) and is also highly selective for the δ-opioid receptors (1600–2000 times greater affinity than at the μ- or κ-opioid receptors) (Suzuki et al., 1995). Yet (±)-TAN67 possesses no antinociceptive activity in the 51°C warm plate antinociceptive test (Suzuki et al., 1995).

The present study was conducted to ascertain the efficacy of the high-affinity stereoisomer (−)-TAN67 relative to two other selective δ-opioid receptor drugs, the nonpeptidic compound SNC80 ((+)-4-[(α *R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) and the peptidic agent pCl-DPDPE (cyclic[D-Pen^2,4′-ClPhe^4,D-Pen^5]enkephalin), by comparing their ability to competitively inhibit [^{3}H]naltrindole binding (Yamamura et al., 1992) and to stimulate [^{35}S]GTPγS binding (Sim et al., 1995).

Previous work in this laboratory resulted in the initial cloning of the human δ-opioid receptor (Knapp et al., 1994) and its subsequent stable transfection and expression by Chinese hamster ovary (CHO) cells (Malatynska et al., 1995). The use of a recombinant cell line expressing a homogeneous population of the human δ-opioid receptor permits the study of the receptor in isolation without the

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potentially confounding interference of other opioid receptors.

2. Materials and methods

2.1. Preparation of membranes from CHO cells

Crude membranes were prepared from CHO cells that express the human δ-opioid receptor. The recombinant CHO cells were produced by stable transfection of the human δ-opioid receptor cDNA (Malatynska et al., 1995). These cells were determined to express human δ-opioid receptor at a density of 968 ± 170 fmol/mg protein (Malatynska et al., 1995). The cell line was grown in 162 cm^2 culture flasks using Ham's F-12 with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 500 μg/ml hygromycin B. Growth medium was removed from cells grown to 80% confluency, and the monolayer was washed once with 10 ml Ca^{2+}-Mg^{2+}-deficient phosphate-buffered saline (PBS). The cells were incubated for 5 min at 37°C with 5.0 ml 1.0 mM EDTA in Ca^{2+}-Mg^{2+}-deficient PBS and detached from the flask wall by sharply striking the flask. The cells were collected by centrifugation and homogenized in 10 ml ice-cold 10 mM Tris-HCl, 1.0 mM EDTA buffer (TE buffer), pH 7.4. A crude membrane fraction was isolated by centrifugation at $40\,000 \times g$ and resuspended in 10 ml fresh TE buffer. Membranes were then incubated at 30°C for 30 min, centrifuged as described above and resuspended in assay buffer (25 mM Tris-HCl, 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) to $OD_{280} = 0.05$.

2.2. Determination of agonist stimulation of [^{35}S]GTPγS binding

Membranes were incubated with appropriate concentrations of agonist drugs in the presence of 0.1 nM [^{35}S]GTPγS (1250 Ci/mmol, New England Nuclear, Boston, MA, USA) in a total assay buffer of 1.0 ml. After 90 min incubation at 30°C, the reaction was terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters, followed by four washes with 4 ml ice-cold 25 mM Tris/120 mM NaCl, pH 7.4. All samples were run in duplicate. Bound radioactivity was measured by liquid scintillation spectrophotometry after an overnight extraction with EcoLite scintillation cocktail (ICN Biomedicals, Costa Mesa, CA, USA).

2.3. Determination of agonist inhibition of [^{3}H]naltrindole binding

Membranes were incubated with appropriate concentrations of agonist drugs in the presence of 0.5 nM

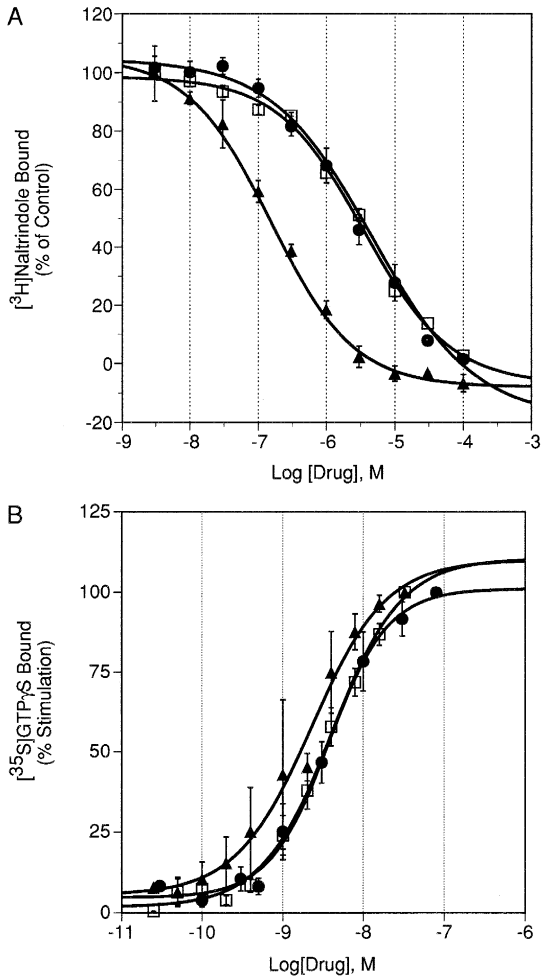

Fig. 1. (A) Effect of various δ-opioid receptor agonists on [^{3}H]naltrindole binding to membranes prepared from CHO cells stably expressing the human δ-opioid receptor. Membranes were incubated with [^{3}H]naltrindole (500 pM) in the presence of: SNC80 (open squares, $n = 4$); pCl-DPDPE (solid circles, $n = 6$) and (−)-TAN67 (solid triangles, $n = 4$). The symbols and vertical bars represent the mean inhibition ± S.E.M. expressed as percent control of specific [^{3}H]naltrindole bound. Control [^{3}H]naltrindole binding in the absence of δ-opioid receptor agonist was 5376 ± 1673 dpm for SNC80, 4195 ± 262 dpm for pCl-DPDPE, and 2275 ± 913 dpm for (−)-TAN67; there were no statistically significant differences between any of these groups (one-way ANOVA). The calculated mean IC_{50} values were 3875 nM for SNC80, 3520 nM for pCl-DPDPE, and 197 nM for (−)-TAN67. The calculated K_d value for [^{3}H]naltrindole was 9.3 pM. (B) Stimulation by various opioid agonists of [^{35}S]GTPγS binding to membranes prepared from CHO cells stably expressing the human δ-opioid receptor. Membranes were incubated with [^{35}S]GTPγS (100 pM) in the presence of: SNC80 (open squares, $n = 6$); pCl-DPDPE (solid circles, $n = 5$) and (−)-TAN67 (solid triangles, $n = 3$). The symbols and vertical bars represent the mean response ± S.E.M. expressed as percent stimulation of [^{35}S]GTPγS binding of each sample with basal [^{35}S]GTPγS bound set as 0% and maximal agonist-stimulated [^{35}S]GTPγS bound set as 100%. Basal [^{35}S]GTPγS binding in the presence of GDP (50 μM) was 46.4 ± 7.6 fmol/mg protein for SNC80, 46.8 ± 12.1 fmol/mg protein for pCl-DPDPE and 28.2 ± 6.6 fmol/mg protein for (−)-TAN67; there were no statistically significant differences between any of these groups (one-way ANOVA).

[^{3}H]naltrindole (32 mmol/Ci, New England Nuclear) under identical conditions of assay buffer, tissue concentration, incubation time, incubation temperature, and post-incubation wash buffer that were employed in the [^{35}S]GTPγS experiments. Nonspecific binding was determined using 10 μM naltrexone. Maximum [^{3}H]naltrindole binding was $< 10\%$ of added ligand in all samples. All samples were run in duplicate.

2.4. Drugs

Agonist drugs tested included SNC80 (Calderon et al., 1994), pCl-DPDPE (Toth et al., 1990) and (−)-TAN67 (Nagase et al., 1994). All drugs were prepared in aqueous solution.

2.5. Data analysis

Data from the [^{3}H]naltrindole and [^{35}S]GTPγS binding assays were analyzed using Graph Pad Prism (San Diego, CA, USA). The efficacy of the δ-opioid receptor agonists was determined by the method of Ehlert (1985), in which efficacy $= (E_{max\text{-}A}/E_{max})(A'/A + 1) \times 0.5$, where $E_{max\text{-}A}$ is the maximum functional response evoked by the test drug, E_{max} is the maximum possible agonist-induced functional response, A' is the K_i for inhibition of [^{3}H]naltrindole binding by the test drug, and A is the EC_{50} value for stimulation of [^{35}S]GTPγS binding by the test drug. Differences in control [^{3}H]naltrindole binding levels and basal [^{35}S]GTPγS binding levels among groups were evaluated using one-way analysis of variance (ANOVA). Differences in K_i and EC_{50} values for δ-opioid receptor agonists were evaluated using Student's *t*-test.

3. Results

SNC80, pCl-DPDPE and (−)-TAN67 all competitively inhibited [^{3}H]naltrindole binding and stimulated [^{35}S]GTPγS binding in membranes prepared from CHO cells that stably express the human δ-opioid receptor. In the [^{3}H]naltrindole binding study, results demonstrate that the affinity of (−)-TAN67 for the human δ-opioid receptor is approximately 20–25-times greater than those of SNC80 and pCl-DPDPE which are approximately equal (Fig. 1A). In the [^{35}S]GTPγS binding study (Fig. 1B), findings show that the potency of (−)-TAN67 to stimulate [^{35}S]GTPγS binding is about 2-times greater than those of SNC80 and pCl-DPDPE which are approximately equal (Table 1).

Table 1 compares the efficacies of SNC80, pCl-DPDPE and (−)-TAN67. Since all three δ-opioid receptor agonists stimulated [^{35}S]GTPγS binding to the same E_{max} level (about 2-fold over basal [^{35}S]GTPγS binding), $E_{max\text{-}A}/E_{max}$ reduces to 1 and the equation is simplified to the following: efficacy $= (A'/A + 1) \times 0.5$, or more simply, $(K_i/EC_{50} + 1) \times 0.5$. Results show that SNC80 and pCl-DPDPE had efficacy values that are about 6–7-times greater than that of (−)-TAN67.

4. Discussion

The concept of efficacy was introduced by Stephenson (1956) in an effort to explain how drugs might evoke effects of equal magnitude while occupying different proportions of target receptors. Efforts have been made to determine the efficacy of various agonist drugs in vivo and in vitro using a variety of paradigms. For example, many of these approaches are based on the assumptions that opioid receptors in the mouse vas deferens or guinea pig ileum are identical with comparable opioid receptors in the brain. It is further assumed that opioid receptors in the mouse and human brains are similar. Experimentally, recombinant CHO cell lines that stably express the human δ-opioid receptor allows the study of that receptor with intact signal transduction and second messenger systems without the confounding contributions of closely related receptors that are co-expressed in vivo. Studies of drug efficacy in such a homogenous population of the cloned human δ-opioid receptor can yield valuable information about the efficacies of drugs.

The results of the [^{3}H]naltrindole competitive inhibition study indicate that of the three δ-opioid receptor agonists, (−)-TAN67 possesses the greatest affinity for the human δ-opioid receptor, approximately 20–25-times greater than

Table 1
Efficacies of δ-opioid receptor agonists at the cloned human δ-opioid receptor

Agonist	K_i (nM)	*n*	EC_{50} (nM)	*n*	Efficacy	Relative efficacy
SNC80	65.6 (45.4–85.8)	4	5.0 (1.9–8.1) [a]	6	7.06	1.00
pCl-DPDPE	79.7 (8.8–150)	6	6.9 (1.1–12.7) [a]	5	6.28	0.89
(−)-TAN67	3.1 (2.4–3.8)	4	2.9 (1.8–4.1)	3	1.03	0.15

The K_i value was determined from [^{3}H]naltrindole competitive inhibition experiments using the Cheng-Prusoff equation (Cheng and Prusoff, 1973), and the EC_{50} value was determined from the [^{35}S]GTPγS stimulation experiments. Values in parentheses indicate the 95% confidence intervals and *n* = number of independent experiments. Efficacy was determined according to the equation described in Section 2.5 of the text. Relative efficacy was determined by dividing the efficacy of each agonist by the efficacy value for the most efficacious agonist (SNC80). Significance of difference: [a] $P < 0.05$ between K_i and EC_{50} for the same drug (Student's *t*-test).

SNC80 and pCl-DPDPE. The results of the [^{35}S]GTPγS stimulation study reveal all three δ-opioid receptor agonists to have EC_{50} values in the nanomolar range. However, when efficacies were determined, a quite different picture emerges. Since all three δ-opioid receptor drugs are full agonists, their efficacies were determined solely by their K_i and EC_{50} values, and the ratios of K_i to EC_{50} confer greater efficacies to SNC80 and pCl-DPDPE, about 6–7-times greater than that of (−)-TAN67. This is consistent with the demonstrated experimental antinociceptive activity of SNC80 and pCl-DPDPE in vivo (Bilsky et al., 1995; Ayres et al., 1989) and the modest antinociceptive activity of (±)-TAN67 in vivo (Suzuki et al., 1995).

In summary, CHO cells stably transfected with human δ-opioid receptor can be used for the investigation of δ-opioid receptor agonist affinity and potency. The present study demonstrates that such a system can be used to establish the efficacy for δ-opioid receptor agonists. The results show that in this system, δ-opioid receptor agonists exhibited a rank order of efficacy of SNC80 = pCl-DPDPE > (−)-TAN67.

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