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Short communication

5′-Guanidinonaltrindole, a highly selective and potent κ-opioid receptor antagonist

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Abstract

5′-Guanidinonaltrindole (GNTI) possesses 5-fold greater opioid antagonist potency (K_e = 0.04 nM) and an order of magnitude greater selectivity (selectivity ratios > 500) than the prototypical κ-opioid receptor antagonist, norbinaltorphimine, in smooth muscle preparations. Binding and functional studies conducted on cloned human opioid receptors expressed in Chinese hamster ovarian (CHO) cells afforded pA_2 values that were comparable to the smooth muscle data. In view of the high selectivity and potency of GNTI, it is a potentially valuable pharmacological tool for opioid research.

Keywords: Norbinaltorphimine; Binding; κ-Opioid receptor

1. Introduction

Although opioid receptors recognize a myriad of structurally diverse ligands (Dhawan et al., 1996), very few of these compounds are highly selective antagonists for κ-opioid receptors. In this regard, norbinaltorphimine (Takemori and Portoghese, 1992) is a prototypical representative of a unique series of selective antagonists for κ-opioid receptors that is used widely as a pharmacological tool in opioid research. Here we describe a structurally novel κ-opioid antagonist, 5′-guanidinonaltrindole (GNTI) (Jones et al., 1998), whose in vitro profile is superior to that of norbinaltorphimine from the standpoint of potency and selectivity. The design of GNTI utilized the indole moiety as a scaffold to orient the guanidine group which serves as an ''address'' for recognition by the κ-opioid receptor (Olmsted et al., 1993).

2. Materials and methods

2.1. Ligands

GNTI was synthesized as described previously (Jones et al., 1998). All of the other ligands employed in this study were obtained through the National Institute on Drug Abuse (NIDA) Drug Supply Program.

2.2. Smooth muscle preparations

2.2.1. Guinea pig ileal longitudinal muscle

Ilea from randomly bred male Hartely guinea pigs (400–500 g) were taken approximately 10 cm from the ileocecal junction, and a strip of longitudinal muscle with the myenteric plexus attached was prepared by the method of Rang (1964). A 1-cm portion of this strip was mounted between two platinum electrodes, immersed in a 10.0-ml organ bath containing Krebs bicarbonate buffer (118 mM NaCl, 4.70 mM KCl, 2.52 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, 0.05 mg/ml chlortrimeton maleate, 25 mM $NaHCO_3$, and 11.48 mM glucose, pH = 7.4), held at 37°C, and continuously bubbled with 95% O_2 and 5% CO_2. Muscle strips were connected to an isometric force-displacement transducer using 4-O silk suture. Contractions of the muscle were initiated by supramaximal rectangular pulses in all preparations (stepwise increased from 10 to 80 V once per minute, 0.5 ms duration, and a frequency of 0.1 Hz) and recorded using WINDAQ (DATAQ Instruments) data acquisition and playback hardware and software on an IBM compatible computer. Muscles were allowed to equilibrate under these conditions for 90–120 min before experiments were conducted. Agonists were added cumulatively to the bath in 1–10 ml amounts

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to produce concentration–response curves of four to six points per curve. Antagonists were added to the bath at 20 or 100 nM, incubated for 15 min, and IC_{50} ratios obtained by dividing the IC_{50} of the standard agonists in the presence of the antagonist by that obtained in the absence of the antagonist in the same tissue.

2.2.2. Mouse vas deferens

Vasa deferentia from randomly bred ICR mice (30–35 g) were taken and prepared by the method of Henderson et al. (1972). Each vas deferens was mounted between two platinum ring electrodes, immersed in a 10.0-ml organ bath containing Krebs bicarbonate buffer (118 mM NaCl, 4.70 mM KCl, 2.52 mM $CaCl_2$, 1.19 mM KH_2PO_4, 25 mM HCO_3, and 11.48 mM glucose, pH = 7.4), held at 37°C, and continuously bubbled with 95% O_2 and 5% CO_2. Muscle strips were connected to an isometric force-displacement transducer using 6-O silk suture. Contractions of the muscle were initiated by supramaximal rectangular pulses in all preparations (70 V, 1.0-ms duration, and a frequency of 0.1 Hz) and recorded using WINDAQ (DATAQ Instruments) data acquisition and playback hardware and software on an IBM compatible computer. Muscles were allowed to equilibrate under these conditions for 20 min before experiments were conducted. Drugs were added cumulatively to the bath in 1–10-ml amounts to produce concentration–response curves of four to six points per curve. Antagonists were added to the bath at 100 nM, incubated for 15 min, and IC_{50} ratios obtained by dividing the IC_{50} of standard agonists in the presence of the antagonist by that obtained in the absence of the antagonist in the same tissue.

2.3. Preparation of membranes

Receptor binding studies were conducted on human opioid receptors transfected into Chinese hamster ovary (CHO) cells. The μ cell line was maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum and 400 μg/ml GENETICIN (G418 sulfate). The δ-opioid receptor cell line is maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum and 500 μg/ml hygromycin B. The κ-opioid receptor cell line was maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum, 400 μg/ml geneticin (G418 sulfate) and 0.1% penicillin/streptomycin. All cell lines were grown to full confluency, then harvested for membrane preparation. The membrane used for functional assays was prepared in buffer A (20 mM Hepes, 10 mM $MgCl_2$, and 100 mM NaCl at pH 7.4) and the membrane for binding assays was prepared in 50 mM Tris buffer, pH 7.7. Cells were harvested by scraping the plates with a rubber policeman and then centrifuging at $500 \times g$ for 10 min. The cell pellet was suspended in buffer A or Tris buffer, homogenized in a Polytron Homogenizer, and centrifuged at $20{,}000 \times g$ for 20 min. The cell pellet was washed in buffer A or Tris, centrifuged at $20{,}000 \times g$ for another 20 min and finally suspended in a small amount of buffer to determine protein content. Membrane was aliquoted in small vials at a concentration of 6 mg/ml per vial and stored at −70°C and used as needed.

2.4. Opioid receptor binding assays

Binding assays were conducted using tritiated [D-Ala^2, *N*-Me-Phe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO), tritiated [D-Ala^2,D-Leu^5]enkephalin ([^{3}H]Cl-DPDPE), and (+)-(5α, 7α, 8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide [^{3}H]U69,593 for μ-, δ- and κ-opioid receptors, respectively. For μ- and δ-opioid receptor binding, cell membranes were incubated with the appropriate radioligand and unlabeled drug in a total volume of 200 μl in 96-well plates, usually for 1 h at 25°C. For κ-opioid receptor binding cell membranes were incubated in a total volume of 2 ml in tubes rather than plates, as the number of opiate receptors or receptor occupancy in κ-opioid receptor cell line has not been as high as in other cell lines. For routine experiments, membranes were incubated with the test compounds at concentrations ranging from 10^{-5} to 10^{-10} M. After the incubation, samples were filtered through glass fiber filters by using a Tomtec cell harvester. Filters were dried overnight before radioactivity levels were determined. Nonspecific binding was determined by using 1 μM of the unlabeled counterpart of each radioligand. K_i values were calculated using the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + L/K_d)$ (Cheng and Prusoff, 1973) where L is radioligand concentration and K_d is the binding affinity of the radioligand, as determined previously by saturation analysis.

2.5. [^{35}S]GTPγS binding for functional determinations

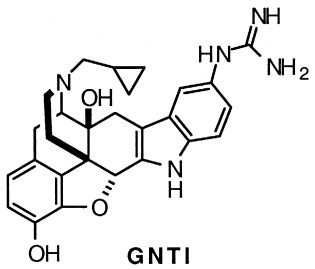
Membranes prepared as described above were incubated with [^{35}S]GTPγS (50 pM), GDP (usually 10 μM), and the desired compound, in a total volume of 200 μl, for 60 min at 25°C. Samples were filtered over glass fiber filters and counted as described for the binding assays. A dose–response curve for each of the standard full agonists (DAMGO, DPDPE, and U69,593, for μ-, δ-, and κ-opioid receptors, respectively) constructed in the presence and absence of GNTI. For each compound a full Schild analysis was conducted, utilizing a full agonist dose–response curve in the presence of at least three concentrations of the antagonist.

3. Results

3.1. Studies with smooth muscle preparations

GNTI potently antagonized the agonist effect of the selective κ-opioid receptor agonists, with K_e values differ-

Table 1
Antagonism of opioid agonists by GNTI in smooth muscle preparations



GNTI

Agonist[a] (receptor type)	[GNTI], nM[b]	IC_{50} ratio[c] (n)	K_e, nM[d] GNTI	K_e, nM nor-BNI	GNTI, % relative potency[e]
U-50488H (κ)	20	488 ± 34 (3)	0.041	0.20	100
(−)-EK (κ)	20	132 ± 33 (11)	0.153	0.56	26.8
Dynorphin-A-(1–11) (κ)	20	29.1 ± 6.2 (5)	0.712	ND[f]	5.76
DADLE (δ)	100	1.87 ± 0.85 (3)	NC[g]	10.64	NC[g]
DPDPE (δ)	100	4.72 ± 1.71 (5)	27	ND[f]	0.15
DAMGO (μ)	100	4.94 ± 1.63 (5)	25	21.7	0.16
Morphine (μ)	100	4.35 ± 1.63 (5)	30	13.7	0.14
$OFQ/N_{(1-17)}$ (ORL-1)	100	0.99 (2)	NC[g]	> 500	NC[g]

[a]The guinea pig ileum was employed for κ- and μ-opioid receptor agonists and the mouse vas deferens (MVD) was used for δ-opioid receptor agonists and nociceptin. Abbreviations: (−)-EK, (−)-ethylketazocine; U50488H, trans-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; DADLE, [D-Ala^2,D-Leu^5]enkephalin; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; OFQ/N-(1–17), orphanin FQ/nociceptin, Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln.
[b]Employed as the bis-trifluoroacetate salt.
[c]IC_{50} of the agonist in the presence of GNTI (20 or 100 nM) divided by the control IC_{50} in the same preparation.
[d]$K_e = [GNTI]/(IC_{50}\ ratio - 1)$.
[e](K_e values of GNTI vs. U50488H divided by the K_e values of GNTI vs. μ-, κ- or δ-opioid receptor agonists) × 100.
[f]Not determined (ND) experimentally.
[g]Not calculated (NC)since the IC_{50} ratio is not significantly different from unity.

ing over a 17-fold range (Table 1). The κ-opioid antagonist potency of GNTI was approximately five times greater than that of norbinaltorphimine. The μ- and δ-selective were antagonized very weakly, and orphanin-FQ/nociceptin, Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln [OFQ/N-(1–17)] was not antagonized at all.

3.2. Binding studies on human opioid receptors

Competition of GNTI with (+)-(5α, 7α, 8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide [^{3}H]U69,593 (κ), [^{3}H]DAMGO (μ), and [^{3}H]Cl-DPDPE (δ) revealed it to possess relatively high affinity ($K_i = 0.18$ nM) for κ-opioid receptors and substantially lower affinity for μ and δ sites (Table 2). The opioid receptor selectivity ratios were 206 (μ/κ) and 389 (δ/κ).

Table 2
Binding and function of GNTI using human cloned opioid receptors expressed in CHO cells

Opioid receptor type	Binding[a]	Function[b]	
	K_i (nM)	pA_2	Slope
κ	0.18 ± 0.10	10.40 ± 0.10	−1.04 ± 0.09
μ	36.9 ± 2.3	8.49 ± 0.09	−1.00 ± 0.05
δ	70.0 ± 0.3	7.81 ± 0.06	−1.00 ± 0.05

[a][^{3}H]U69,593, [^{3}H]DAMGO, and [^{3}H]Cl-DPDPE were employed as radioligands for κ-, μ-, and δ-opioid receptors.
[b]Agonists employed were (+)-(5α, 7α, 8β)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide (U69,593), DAMGO, and DPDPE for κ-, μ-, and δ-opioid receptors.

3.3. Functional studies on human opioid receptors

Antagonism of U-69593, DAMGO, and DPDPE by GNTI was determined by measuring the change in binding of [^{35}S]GTPγS upon incubation with GNTI (Befort et al., 1996). The ability of GNTI to compete with the above agonists were expressed as pA_2 values. GNTI was most effective at κ-opioid receptors ($pA_2 = 10.4$), with substantially lower antagonist potency at μ-opioid and δ-opioid receptors (81- and 389-fold lower potency, respectively) (Table 2).

4. Discussion

This study has shown GNTI to be an exceptionally potent antagonist of κ-selective opioid agonists in both the

guinea pig ileum preparation and in human cloned κ-opioid receptors. It is fivefold more potent and an order of magnitude more selective than the prototypical κ-opioid antagonist, norbinaltorphimine (Takemori and Portoghese, 1992). The higher selectivity for GNTI relative to nor-BNI is due both to its higher potency at κ-opioid receptors and relatively lower potency at μ- and δ-opioid receptors.

Given that GNTI is an analogue of the δ-opioid antagonist, naltrindole (Takemori and Portoghese, 1992), it is noteworthy that GNTI possesses relatively weak δ-opioid receptor antagonist activity. This is most likely a consequence of unfavorable interaction of the guanidine group with the ''address'' locus of the δ-opioid receptor, as 5′-substitution on naltrindole has been reported (Portoghese et al., 1990) to reduce δ-opioid antagonist potency.

The high κ-opioid antagonist potency of GNTI is a likely consequence of ionic interaction of its guanidinium group with the non-conserved Glu^{297} residue at the top of transmembrane helix 7 of the κ-opioid receptor, as demonstrated through site-directed mutagenesis studies (Hjorth et al., 1995; Jones et al., 1998).

Finally, the substantial difference in K_e values (~17-fold) for the GNTI-induced antagonism of *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U50488H) and dynorphin-A-(1–11) is noteworthy, given that the ligands have approximately the same agonist potency in the guinea pig ileum. The different K_e values may reflect differences in the bonding modes of U50488H and dynorphin-A-(1–11) (Paterlini et al. 1997; Subramanian et al., 1998) to κ-opioid receptors or the involvement of different subpopulations of κ-opioid receptors.

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