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Communications to the Editor

Mutational Evidence for a Common κ Antagonist Binding Pocket in the Wild-Type κ and Mutant μ[K303E] Opioid Receptors†

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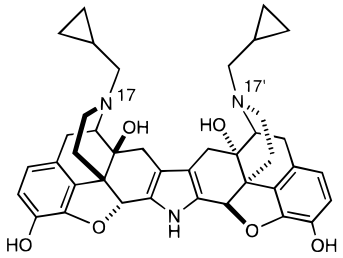
The selectivity of the prototypical κ opioid receptor (OP_2)[1] antagonist norbinaltorphimine (*nor*-BNI, **1**) has been attributed[2] to the presence of an "address"[3] moiety which interacts with a key residue that is unique to this G protein-coupled receptor. In this regard, it has been established that the N-17′ basic group of **1** contributes to the κ selectivity, and it has been suggested that the protonated N-17′ basic nitrogen atom might ion-pair with an acidic residue on the receptor. Subsequent studies with mutant κ receptors revealed that Glu297,[4] located at the top of transmembrane helix 6 (TM6), could provide such an acidic residue. Modeling of *nor*-BNI docked to the κ receptor has suggested that the protonated N-17 and N-17′ groups interact with two acidic residues: a conserved aspartate (Asp138) residue within the cavity created by the 7TM bundle and the nonconserved Glu297.[5]

We now wish to report that the use of the indole moiety of the δ opioid antagonist naltrindole[6] (NTI, **3**) as a scaffold[7] to hold a C5′-guanidinyl "address" has afforded a derivative, GNTI (**2**), with enhanced binding and selectivity at the cloned κ receptor. Additionally,



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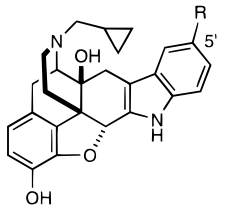

2, R=NHC(=NH)NH_2
3, R=H

we have found that the mutant opioid receptor, μ-[K303E], that contains a glutamate residue in a position equivalent to that in the wild-type (wt) κ receptor binds **1** and **2** with high affinity and selectivity. These results are consistent with the importance of a properly oriented basic group[7] that interacts with Glu297 in conferring κ anatgonist selectivity and support the idea that this residue is the primary recognition element of the κ "address" subsite for **1** and **2**. Moreover, it suggests that the wt κ receptor and mutant μ receptor (K303E) each possess a similar recognition site for accommodating the antagonist pharmacophore that contains the N-17 basic group.

The design rationale for GNTI (**2**) involved attachment of the guanidinyl group to the C5′ position of **3** in order to approximate the distance between N-17 of the antagonist pharmacophore and N-17′ of the "address" in **1**. Alignment of the antagonist pharmacophore and the basic groups of **1** and **2** illustrates (Figure 1) that the indolic moiety of **2** functions as a rigid molecular scaffold to fix the C5′-guanidinyl pendant in an orientation that is similar to that of N-17′ in *nor*-BNI (**1**). The importance of an ionic group as a contributor to the potency and selectivity of **2** was evaluated with CNGNTI (**4**) ($pK_a = 2.76$),[8] as its electron-withdrawing cyano functionality greatly reduces the basicity of the guanidine.

Compounds **2** and **4** were both derived from a common amine precursor as outlined in Scheme 1. Raney nickel-catalyzed reduction of 5′-nitronaltrindole (**5**)[6] afforded the 5′-amino derivative **6**. GNTI (**2**) was obtained from **6** using a guanidylation protocol.[9] Trifluoroacetic acid

† This communication is dedicated to the memory of Professor A. E. Takemori.

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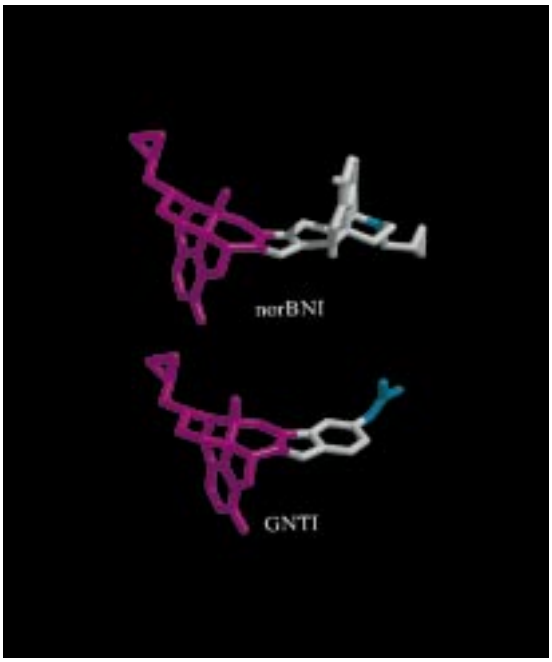


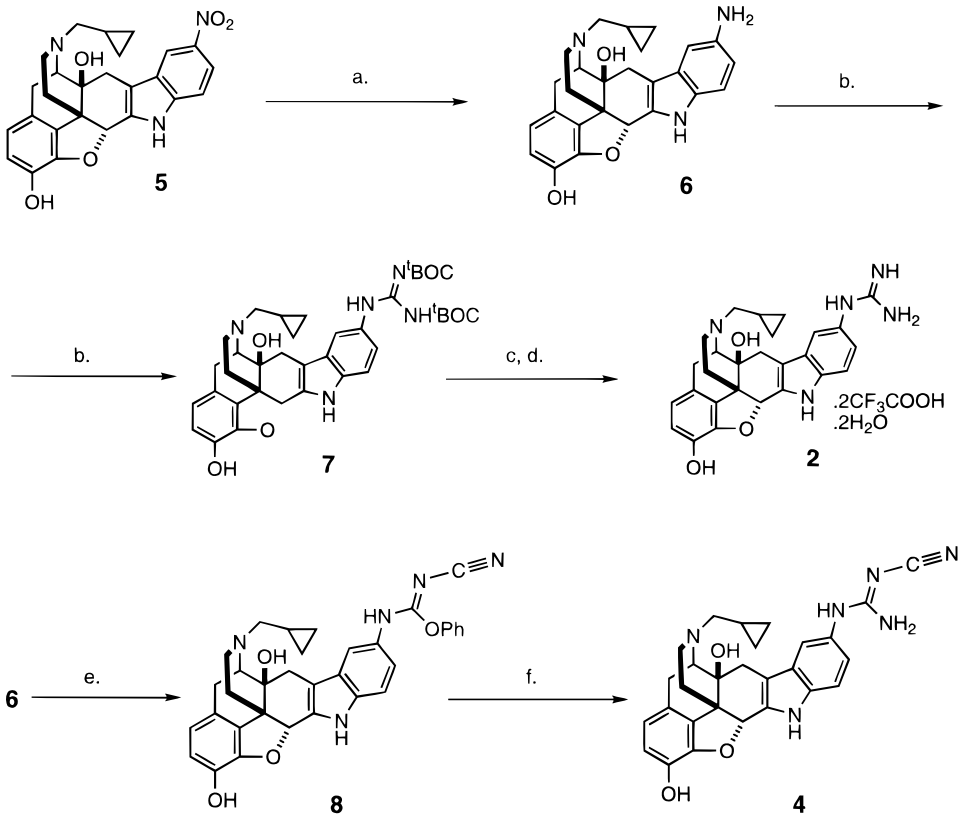
Figure 1. 3D-Alignment of *nor*-BNI (**1**) (above) and GNTI (**2**) (below). The antagonist pharmacophore is indicated in magenta and the κ "address element" in blue. The indolic and pyrrole "spacers" are gray. Images were created using MIDAS 2.0 on a Silicon Graphics Workstation.

deprotection of intermediate **7** afforded **2** as its bistrifluoroacetate salt in excellent yield after reverse-phase HPLC.[10] CNGNTI (**4**) was obtained by sequential nucleophilic displacement of phenoxide from the diphenyl-*N*-cyanocarbonimidate synthon,[11] initially using **6** to afford isourea **8** followed by aqueous ammonolysis.

The in vitro pharmacological data for GNTI (**2**) and its cyano analogue CNGNTI (**4**) are listed in Table 1. Compounds **2** and **4** as well as the established prototypical opioid antagonist **1** were tested on the electrically stimulated guinea-pig ileal longitudinal muscle[12] (GPI) and the mouse vas-deferens[13] (MVD) preparations as previously described.[14] The compounds were incubated with the preparations 15 min prior to testing with either morphine (M), (−)-ethylketazocine (EK), or [D-Ala^2,D-Leu^5]enkephalin (DADLE). The data illustrate that GNTI (**2**) is an extremely potent, highly selective κ opioid receptor antagonist and was approximately 4-fold more potent than *nor*-BNI (**1**), with selectivity ratios that were 1 order of magnitude greater.

Table 2 lists the binding of **1**, **2**, and **4** for the wt and mutant κ[E297K] κ receptors as well as for the wt and mutant μ[K303E] μ receptors. The notion that Glu297 at the top of TM6 may be important for antagonist selectivity at κ opioid receptors has been reported in connection with the binding of *nor*-BNI.[4] Both ligands **1** and **2** display large affinity changes on mutation of the glutamate residue to lysine (E297K) in the κ receptor and from lysine to glutamate (K303E) in the μ receptor. These point mutations affected the affinity by almost 2 orders of magnitude irrespective of the receptor type. The discovery that a single-point mutation in the "address" recognition locus of the μ receptor allows a highly selective κ antagonist to recognize the μ[K303E] mutant as a "κ" receptor highlights the crucial

Scheme 1[a]



[a] Reagents: (a) Ra-Ni, CH_3CH_2OH, $N_2H_4 \cdot H_2O$, N_2(g), rt, 2 h, 70%; (b) (^{t}BOCNH)$_2$CS, Hg(II)Cl_2, DMF, 0 °C rt, $(CH_3CH_2)_3N$, 2 h, 76%; (c) TFA, N_2(g), CH_2Cl_2, 0 °C rt; (d) RP-HPLC, 83%; (e) $(C_6H_5O)_2CNCN$, CH_3CH_2OH, N_2(g), 24 h, 60 °C, 82%; (f) NH_4OH, CH_3CH_2OH, 70 °C, 48 h, 77%.

Table 1. Opioid Antagonist Potencies in Smooth Muscle Preparations

		K_e (nM)[a]			selectivity ratio	
compd	compd	EK (κ)[b]	M (μ)[b]	DADLE (δ)[c]	κ/μ	κ/δ
1	*nor*-BNI	0.56	13.7	10.6	25	19
2	GNTI	0.14	30	115	208	799
3	NTI	45	29.4	0.13	(δ/μ = 226)	(δ/κ = 346)
4	CNGNTI	3.13	16.13	9.08	5	3

[a] K_e = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of the antagonist (100 nM) divided by the IC_{50} of the agonist alone in the same preparation. K_e values were calculated from an average of at least three IC_{50} ratio values. [b] Determined using the guinea-pig ileum preparation. [c] MVD determined using mouse vas-deferens preparation. The agonists employed were ethylketazocine (EKC), morphine (M), and [D-Ala^2,D-Leu^5]enkephalin (DADLE).

Table 2. Effect on Binding Affinity of Mutational Exchange of Glu297 in the κ Receptor and Lys303 in the μ Receptor

	$K_i \pm$ SEM (n), nM[a]			
compd	wt κ F_{ana}[b]	κ[E297K] F_{mut}[c]	wt μ F_{mut}[c]	μ[K303E] F_{mut}[c]
1	0.12 ± 0.04 (8)	12.5 ± 0.92 (5)	101.9 ± 10.2 (3)	0.77 ± 0.13 (5)
	1.0	123	800	9.3
2	0.09 ± 0.01 (3)	12.9 ± 0.48 (2)	9.23 ± 1.39 (3)	0.06 ± 0.02 (3)
	0.75	143	103	0.67
4	0.27 ± 0.01 (3)	2.48 ± 0.82 (3)	4.04 ± 1.23 (3)	0.29 ± 0.05 (3)
	2.25	9.2	15	1.1

[a] The K_i values were determined in competition binding using [^{3}H]diprenorphine in transiently expressed rat COS-7 cells and analyzed by whole cell binding. The number of individual determinations is indicated in parentheses (n). [b] F_{ana} = K_i(analogue)/K_i(*nor*-BNI on the wt κ receptor). [c] F_{mut} = mutational factor, K_i(mutant receptor)/K_i(wt κ receptor).

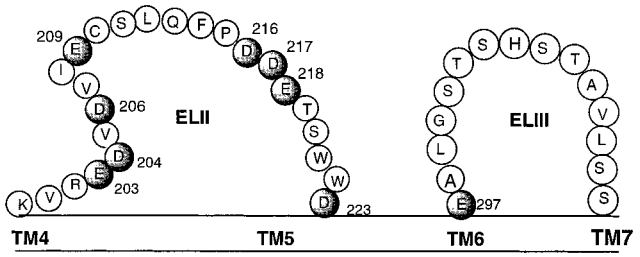


Figure 2. Extracellular loop regions (ELII and ELIII) of the κ opioid receptor (acidic residues are shaded).

role of an acidic residue in this position for conferring κ selectivity. In this regard it is noteworthy that GNTI has extremely high affinity for both wt κ and μ[K303E] receptors. Interestingly, the finding that **4** has one-third to one-fifth the affinity of **2** for the wt κ and mutant μ receptors suggests that a H-bonding component also is involved in the interaction of the glutamate residue with the guanidinyl group.

Significantly, the κ "address" recognition locus for *nor*-BNI (**1**) and GNTI (**2**) differs from that of the endogenous peptide agonist dynorphin-A, which requires the highly acidic extracellular loop II for high potency and selectivity (Figure 2).[15−17] Thus, it appears that the binding of peptidic κ agonists and nonpeptide κ antagonists may involve a common "message"[3] recognition cavity but different "address" recognition loci.[18]

In conclusion, this study suggests that the κ "address" recognition locus for κ antagonists is determined primarily by the glutamate residue. Placement of this acidic wt κ residue into an equivalent position in the μ receptor facilitates recognition, as reflected by the binding of the indolomorphinan GNTI (**2**) and the bivalent ligand *nor*-BNI (**1**) to wt κ and mutant μ-[K303E] receptors. These data provide persuasive evidence that the recognition loci for *nor*-BNI and GNTI are very similar in the κ receptor and that the μ[K303E] receptor also contains an analogous recognition cavity.

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