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A κ Opioid Pharmacophore Becomes a Spinally Selective κ-δ Agonist When Modified with a Basic Extender Arm

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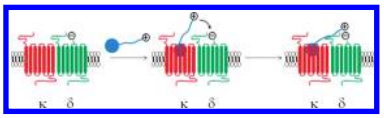
ABSTRACT We have explored the concept of a molecular extender arm attached to a κ opioid agonist pharmacophore **3** (ICI-199,441) in an effort to potentially interact with a complementary group on a neighboring opioid receptor. The molecular arm containing a terminal amine group was lengthened incrementally from 11 up to 18 atoms. Increasing the number of atoms in the arm produced virtually no change in the mouse intracerebroventricular (i.c.v.) antinociceptive potency. In contrast, the intrathecal (i.t.) potency of **6** (KDA-16) with a 16-atom arm was dramatically increased, as reflected by its antinociceptive i.c.v./i.t. ED_{50} ratio of ~130. Further lengthening led to a decreased ED_{50} ratio. In vivo selective antagonist studies of KDA-16 revealed that κ and δ opioid receptors were responsible for the greatly enhanced i.t. potency. Calcium release experiments in HEK-293 cells suggested that KDA-16 selectively activate κ-δ heteromers. These data are consistent with the reported possible presence of heteromeric κ-δ opioid receptors in mouse spinal cord but not in the brain. The use of a molecular extender arm may be useful for developing spinally selective analgesics.



KEYWORDS Opioid receptors, heteromers, molecular extender arm

Evidence for homomeric and heteromeric opioid receptors[1−4] has led to the design of bivalent ligands that have the potential for bridging opioid receptors with two pharmacophores linked to one another through a spacer.[5,6] Such ligands whose spacer is in the range of 18−21 atoms have pharmacological properties that are consistent with the activation or antagonism of heteromeric opioid receptors in vivo and in vitro.[7] Thus, bivalent ligands have been reported for targeting of heteromeric μ-κ, κ-δ, and μ-δ opioid receptors. Significantly, the spacer length for the presumed bridging of receptors is in the range of 16−21 atoms for these bivalent ligands, which is of sufficient length for cross-linking the receptor protomers in an oligomeric array.[5−10]

Different pharmacological selectivity profiles upon intrathecal (i.t.) versus intracerebroventricular (i.c.v.) administration have been observed during the study with the bivalent ligand KDN-21 (**1**),[6] which has κ and δ opioid receptor antagonist pharmacophores tethered with a spacer. KDN-21 was characterized by substantially higher i.t. receptor selectivity and potency with little or no i.c.v. selectivity. Similarly, a single pharmacophore κ-δ opioid receptor agonist 6′-GNTI (**2**)[11] was reported to be active i.t. but only weakly active i.c.v. (Figure 1). Studies of standard κ and δ agonists and antagonists that exhibit i.c.v./i.t. selectivity ratios, which are dependent on the route of administration, also suggest different phenotypic receptors.[12] When taken together, these studies have suggested that δ-κ opioid receptor heteromers are possibly localized in the spinal cord but not in the brain.

As opioid agonists that selectively activate spinal cord opioid receptors could be a viable approach to reducing side effects elicited by analgesics at supraspinal sites, we have considered the possibility that an opioid pharmacophore with a molecular arm of specific length could potentially interact with a residue on a neighboring receptor, thereby modulating opioid selectivity. Considering the existence of some acidic amino acid residues on the extracellular loops of opioid receptors, we envisaged that a protonated amine group on a molecular arm might associate with a carboxylate group on a neighboring receptor to modify opioid activity. Such ligands might be capable of bridging protomers in homomers or heteromers.

Given the colocalization of δ and κ receptors in spinal cord axons of rodents[13] and reports on the possible association of these receptors as heteromers,[12] we have explored such an approach through use of a molecular arm attached to a κ opioid receptor agonist pharmacophore **3** (ICI-199,441),[14] in an effort to design spinally selective ligands (Table 1). The constitution of the molecular arm attached to the pharmacophore was designed to maintain a hydrophobic−hydrophilic balance and allowed us to readily adjust the arm length. It is composed of a number of glycine units (n = 0−3) and a diglycolic alkyldiamine moiety in which the methylene unit

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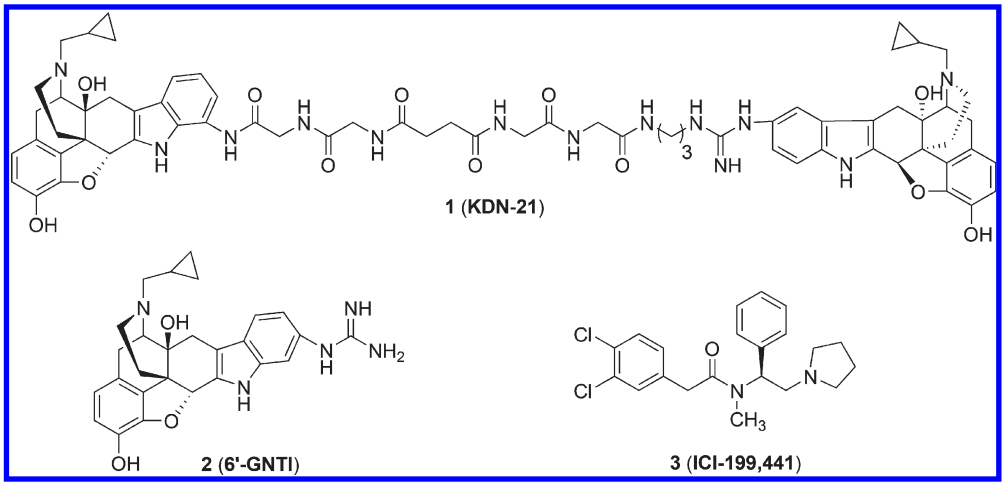
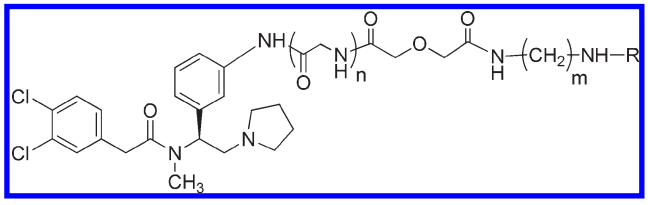


Figure 1. Structures of KDN-21, 6′-GNTI, and ICI-199,441.

Table 1. Opioid Agonist Selectivity of Ligands (**4**−**8**) in the Mouse Tail-Flick Assay[a] upon Intracerebroventricular or Intrathecal Administration



					ED_{50} (nmol/mouse)		
compd	compd name[b]	n	m	R	i.c.v.	i.t.	i.c.v./i.t. ratio
3	ICI-199,441	N/A	N/A	N/A	0.58 (0.41−0.83)	0.55 (0.47−0.64)	1.05
4	KDA-11	0	4	CH_3	0.52 (0.37−0.73)	0.15 (0.11−0.21)	3.45
5	KDA-14	1	4	CH_3	0.48 (0.34−0.66)	0.17 (0.12−0.24)	2.81
6	KDA-16	2	3	CH_3	0.58 (0.24−1.43)	0.0045 (0.003−0.08)	130.91
7	KDA-18	3	2	CH_3	0.40 (0.28−0.59)	0.035 (0.022−0.042)	11.43
8	N/A	2	3	CHO	0.47 (0.32−0.69)	0.020 (0.011−0.038)	23.44

[a] Ref 12. [b] Ref 15.

is varied (m = 2−4). The molecular arm was extended from the meta-amino position of pharmacophore **3**, since this attaching point has been proved not to radically change the selectivity or potency of the pharmacophore.[5] The series consisted of four ligands **4**−**7** (KDA-11 to KDA-18)[15] having an arm length from 11 to 18 atoms between the pharmacophore and the terminal secondary methylamino group.

Ligands **4**−**7** were first evaluated for agonist activity in the mouse tail flick assay (Table 1) by either intracerebroventricular (i.c.v.) or intrathecal (i.t.) administration. All ligands (**4**−**7**) containing a basic group at the terminus of the extender arm exhibited antinociceptive activity characteristic of full agonists. When given i.c.v., these ligands displayed potencies similar to the parent ligand **3** (ICI-199,441). In contrast, when administered intrathecally, a potency increase that ranged from 3 to 122 times higher than the parent compound **3** was observed. It is noteworthy that the highest i.t. potency was achieved at a chain length of 16 atoms (**6**, KDA-16), and further extension of the arm led to reduce the activity. In this regard, **6** was exceptionally potent by the i.t. route (ED_{50} = 4.5 pmol) relative to i.c.v. administration (ED_{50} = 580 pmol). Ligand **6** afforded the highest i.c.v./i.t. ED_{50} ratio of ∼130, as compared to the ratio of 1.05 for the parent compound **3** (Table 1). Depending on the arm length, other ligands in this series also showed 3−11-fold increase of their i.c.v./i.t. ratio. Thus, the in vivo data were consistent with our original design concept that attachment of a molecular extender arm to the pharmacophore might increase efficacy.

To evaluate the importance of a basic group on the terminus of the molecular arm, analogue **8** with a formamide moiety to reduce the basicity of the amino group was synthesized as a control ligand for KDA-16 (**6**). In vivo evaluation showed that **8** possessed an i.c.v./i.t. ED_{50} ratio of ∼23, exhibiting an i.c.v. potency equivalent to that of the ligand **6** but with 4.4-fold less i.t. potency relative to **6**. The reduced i.c.v./i.t. potency ratio suggested that the terminal-protonated amino group in KDA-16 might be involved in association with a counterion on a neighboring receptor or the same receptor.

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Table 2. Antagonism Effects on Ligand **6** with Selective Antagonists upon Intrathecal Administration

antagonist	norBNI (κ)	NTI (δ)	CTOP (μ)
ED_{80} ratio[a]	28.50[b]	9.97[b]	no change[c]

[a] ED_{80} ratio = ED_{80} of ligand in the presence of antagonist divided by the ED_{80} of the ligand alone. [b] This is a one point experiment. Dose of norBNI, 2.5 nmol/mouse; 20 min peak time; NTI, 5.0 nmol/mouse; 20 min peak time; and CTOP, 6.0 pmol/mouse; 20 min peak time. [c] No change means that with the antagonist, the ligand ED_{80} was not significantly different from the control value and no shift was expected.

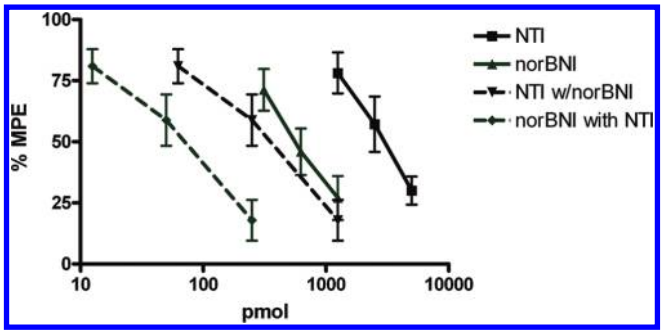

Figure 2. Synergism of norBNI and NTI against ligand **6** (20 pmol, i.t.) in HSD:ICR(CD1) mice. norBNI in the presence of NTI (♦) was 9.20 times more potent than norBNI alone (▲). NTI in the presence of norBNI (▼) was 9.28 more potent than NTI alone (■). Antinociception is quantified as the percent maximal possible effect (%MPE), which is calculated as %MPE = (test − control/10 − control) × 100.

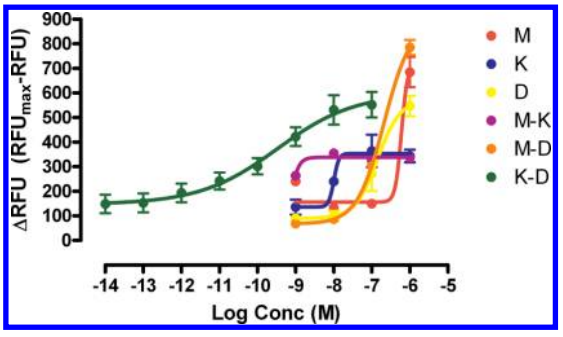

Figure 3. Intracellular calcium release assay mediated by increasing concentrations of the ligand **6** was conducted in HEK-293 cells stably expressing opioid receptors. The response was measured as relative fluorescence units (RFU) in the *Y*-axis (no. of replications ≥ 3). ΔRFU is the change in RFU, which is calculated by subtracting the lowest baseline value after time = 32 s from the maximal RFU measured during that interval.

The pharmacological selectivity of **6** was characterized using the selective antagonists, norBNI[16] (κ), NTI[17] (δ), and CTOP[18] (μ) (Table 2). When administered i.t., compound **6** was potently antagonized at κ and δ receptors. Using the same antagonist dose i.c.v. did not cause a significant shift, possibly due to differential sensitivity of different phenotypic receptors to these antagonists in the brain and cord. Given the evidence for the coexistence of κ and δ receptors in spinal neuronons[6,7,13] and reported studies with ligands selective for heteromeric δ-κ receptors, these data suggest that KDA-16 (**6**) was able to activate both κ and δ opioid receptors in the mouse spinal cord but not in the brain. In this regard, the finding that norBNI and NTI synergistically antagonized **6** upon i.t. administration is of possible significance (Figure 2), given its high i.c.v./i.t. ED_{50} selectivity ratio and κ/δ selectivity. One possibility is that this arises as a consequence of allosterism between heteromeric κ-δ opioid receptors.[6,7,12]

To investigate this further, ligand **6** was tested using the calcium release assay[19] on HEK-293 cells stably expressing μ, κ, δ, κ-δ, κ-μ, and μ-δ opioid receptors. These six cell lines also contained a transiently expressed chimeric $G_i$iq protein, which stimulates the release of calcium upon activation. This study revealed that **6** exhibited potent agonist activity in the subnanomolar range on cells coexpressing κ/δ opioid receptors. In contrast, other coexpressed and singly expressed receptors were activated 66−335 times less potently (Figure 3). Given that κ and δ opioid receptors are known to organize as heteromers in cultured cells,[1,11] the results of the cell-based study are consistent with our in vivo observations and suggest that KDA-16 (**6**) might selectively activate κ-δ receptor heteromers rather than κ or δ homomers.

Considering the flexibility of the 16-atom molecular arm, we attribute the substantially greater i.t. potency of KDA-16 (**6**) to the possible association of its protonated amine with acidic amino residues (Glu or Asp) on an extracelluar loop of its neighboring δ opioid receptor. The κ opioid pharmacophore might first associate with a κ opioid receptor, followed by bridging of the arm to a carboxylate residue on a neighboring δ receptor (Figure 4). It may be more than a coincidence that the extender molecular arm of **6** is close to the range of spacers in bivalent ligands that target heteromeric receptors, given its potent activity and selectivity in the spinal cord. The molecular extender arm approach could be applied to design spinally selective ligands as potential analgesics that have minimal supraspinal side effects.

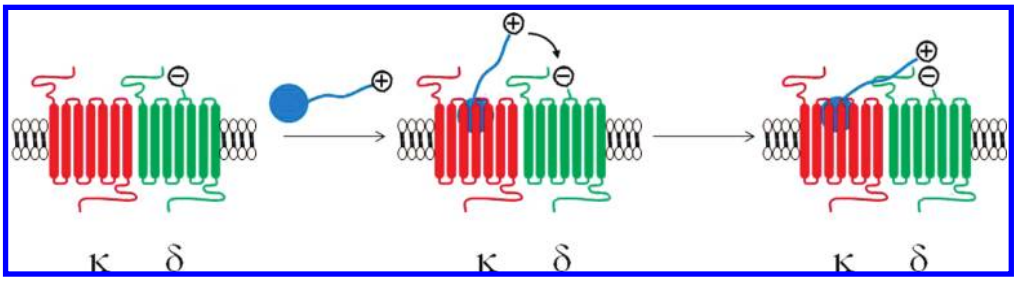

Figure 4. Conceptual model of the interaction of **6** (KDA-16) with heteromeric κ-δ opioid receptors. The κ pharmacophore (blue) binds to the κ receptor, placing its cationic protonated amine moiety in proximity to an anionic moiety on an outer loop of the associated δ receptor, resulting in bridging to both receptors. Arm lengths shorter or longer than that in ligand **6** are not optimal for association of counterions.

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SUPPORTING INFORMATION AVAILABLE Experimental procedures and analytical data for compounds **4**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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