Phenylmorphans and Analogues: Opioid Receptor Subtype Selectivity and Effect of Conformation on Activity

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The morphine-like (+)-phenylmorphan, the atypical (-)-enantiomer, and some analogues have been tested in receptor binding assays selective for opioid μ_1, μ_2, δ, κ_1, and κ_3 receptors. The affinities of all of the compounds except one, including the atypical (-)-phenylmorphan, were greatest for μ_1 and μ_2 receptors. The only exception was the (+)-9α-methyl analogue which had slightly greater affinity for the κ_1 receptor. The selective receptor binding assays provide evidence that opioids in which the phenyl ring is constrained to be equatorial on the piperidine ring can have considerable affinity for μ receptors. In addition, dose-response curves were determined for (+)- and (-)-phenylmorphan using the mouse tail-flick assay with the (+)-enantiomer found to be about 7 times more potent. Pretreatment with the selective opioid antagonists β-FNA (μ_1 and μ_2), naloxonazine (μ_1), nor-BNI (κ_1), and naltrindole (δ) suggests that the antinociceptive activity of both enantiomers is mediated through μ receptors. The pretreatment with naloxonazine, which attentuated the antinociceptive effect, shows that both (+)- and (-)-phenylmorphan are μ_1 agonists while intrathecal administration shows that both are μ_2 agonists. Conformational energy calculations on the compounds were also performed using the MM2-87 program. Consistent with previous conformational results for the phenylmorphans (*J. Med. Chem.* 1984, *27*, 1234-1237), the most potent antinociceptive compounds preferred a particular orientation of the phenyl ring.

Introduction

The enantiomers of phenylmorphan [5-(*m*-hydroxyphenyl)-2-methylmorphan] (Figures 1 and 2a) are opioid agonists with significant activity in a variety of in vivo assays of antinociception.[1-4] For example, in a tail flick assay in mice, (-)-**2a** is equipotent to morphine while (+)-**2a** is about 5 times as potent.[3] However, the enantiomers are more of interest due to their contrasting pharmacological profiles. While (+)-**2a** is morphine-like in its properties, (-)-**2a** appears to be an atypical opioid with some antagonist properties. For example, (+)-**2a** substitutes for morphine in morphine-dependent monkeys and rats whereas (-)-**2a** does not and will precipitate withdrawal symptoms.[3] Similarly, (+)-**2a** has a high capacity for inducing physical dependency whereas (-)-**2a** does not.[1-3] Finally, (+)-**2a** resembles morphine in the guinea pig ileum and mouse vas deferens assays in which (-)-**2a** has little activity.[3] This work was undertaken to clarify the role of opioid receptor subtypes on the unusual pharmacological profile of (-)-phenylmorphan. While receptor binding assays have previously been reported for the enantiomers, the radioactive ligands that were used are relatively nonspecific for opioid subtypes.[3,4] In addition to the receptor binding assays, the enantiomers were also evaluated in an in vivo mouse tail flick assay in which the mice were pretreated with selective opioid antagonists to give information regarding the opioid receptors that mediate their antinociceptive activities.

The enantiomers of phenylmorphan are also of interest with respect to a model that has been proposed for ligands that bind to opioid receptors.[5-7] The ligand model, in which the phenyl ring of all opioids binds to the same site in the receptor, is successful in rationalizing the general structure-activity relationships of opioids. Thus, a phenyl *m*-hydroxyl results in greatly increased potencies in azabicyclane opioids that are constrained to be either phenyl-axial or phenyl-equatorial.[7] Furthermore, when one superimposes the phenyl ring of phenyl-axial and phenyl-equatorial opioids, the ammonium hydrogens in both classes appear to be pointing toward the same region of the receptor space and could, therefore, interact with the same negatively charged site in the receptor. Finally, the effect of *N*-alkyl substitutions is different in phenyl-axial and phenyl-equatorial opioids.[5,7-9] For example, an *N*-allyl or related group consistently converts opioid agonists into antagonists when the phenyl ring is axial but not when it is equatorial. Similarly, an *N*-phenethyl group greatly enhances and is optimal for the potency of phenyl-axial opioids whereas phenyl-equatoral opioids respond differently to this substitution. This can be understood since N-substituents are placed in different regions of the receptor space.

According to the ligand model, a more critical conformational factor in opioids may be the orientation of the phenyl ring relative to the piperidine ring. In opioids with symmetrically substituted piperidine rings such as mep-

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Table I. Binding Affinities (±SEM) of the Phenylmorphans and Analogues for Opioid Receptor Subtypes

compd	K_i (nM)				
	μ_1	μ_2	δ	κ_1	κ_3
(-)-2a	13 ± 4	63 ± 27	>500	200 ± 30	160 ± 40
(+)-2a	3.4 ± 0.9	19 ± 1	>500	98 ± 18	78 ± 49
(±)-2b	8.8 ± 3.0	28 ± 20	420 ± 50	110 ± 10	110 ± 50
(±)-2c	75 ± 35	>275	>500	>500	>500
(±)-2d	46 ± 10	190 ± 25	>500	70 ± 11	>300
(+)-2d	140 ± 40	>600	>500	89 ± 37	250 ± 140
(-)-2d	18 ± 14	50 ± 13	>500	49 ± 15	330 ± 63
morphine[a]	0.50 ± 0.38	2.5 ± 0.6	280 ± 50	49 ± 32	33 ± 2
U50488H[a]	370 ± 76	>500	>500	6.1 ± 1.3	>350
DPDPE[a]	82 ± 19	460 ± 150	2.9 ± 0.7	>350	>350

[a] References 26 and 27.

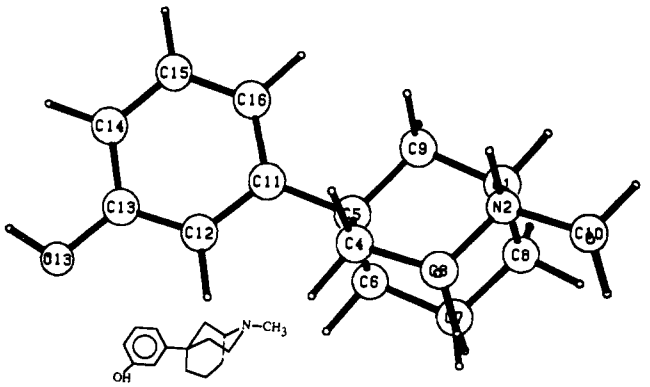

Figure 1. Global minimum for (+)-2a showing the atomic numbering.

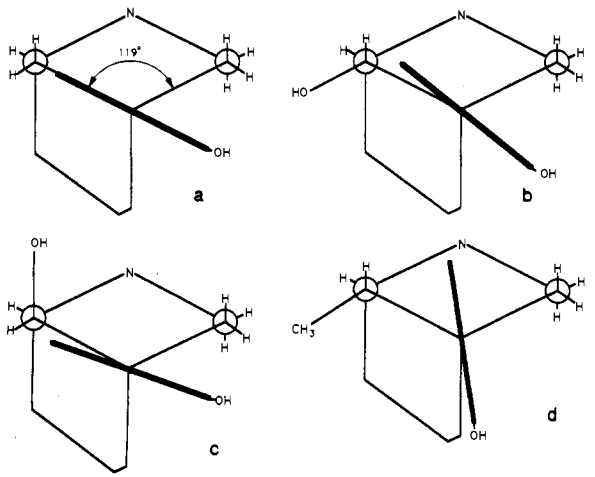

Figure 2. Global minima for (a) (+)-phenylmorphan (τ(C16–C11–C5–C4) = 119°), (b) 9α-hydroxy analogue (τ(C16–C11–C5–C4) = 104°), (c) 9β-hydroxy analogue (τ(C16–C11–C5–C4) = 135°), and (d) 9α-methyl analogue (τ(C16–C11–C5–C4) = 67°) showing the orientation of the phenyl ring. For the latter three compounds, the same enantiomer as (+)-2a is assumed.

eridine, ketobemidone, 3-demethylprodine, and azabicyclanes, a pair of mirror image orientations of the phenyl ring are preferred.[7,10,11] In opioids with an asymmetrically substituted piperidine ring, such as the phenylmorphans and β-prodine, one or the other conformer can become preferred depending on the absolute configuration of the compound.[9,11-13] The preferred phenyl orientation of the morphine-like (+)-2a can be related to morphine using the above ligand model.[12] In contrast, the phenyl orientation of the more active enantiomer in the prodines and related compounds is the *opposite* of that observed in morphine-like opioids.[9] The prodines also appear to have anomalous structure–activity relationships compared with morphine-like opioids since a phenyl *m*-hydroxyl converts β-prodine from a potent agonist into an antagonist devoid of agonist activity.[14] The same substitution destroys the potency of the very potent α-3-allylprodine.[15] Because of these differences, it has been suggested that the prodines and related structures have a different mode of interaction with μ receptors than do phenolic opioids such as morphine.[15] The phenyl orientation in the preferred conformation of (-)-2a is essentially indistinguishable from that of the more active enantiomer of β-prodine.[12] Thus, the ligand model suggests that morphine-like compounds such as morphine itself and (+)-2a can be discriminated from atypical compounds such as (-)-2a and the more active prodine enantiomers.

A number of analogues of the phenylmorphans have also been synthesized and assayed for antinociceptive activity. These include analogues with a 9-hydroxy group in which the racemic 9α-hydroxy analogue (Figure 2b) is an agonist with potency equivalent to that of morphine while the racemic 9β-hydroxy analogue (Figure 2c) is a weak agonist at best.[16] In analogues with an α-9-methyl group (Figure 2d) the (-)-enantiomer has weak antinociceptive activity and the (+)-enantiomer is an antagonist with nalorphine-like potency.[3] These compounds were also screened on the receptor binding assays selective for opioid receptor subtypes. Finally, the conformational preferences of the analogues have been determined using MM2-87 calculations to examine if their conformational and pharmacological properties are consistent with the above ligand model.

Results

The results of the receptor binding assays are shown in Table I along with the reference compounds morphine, U50488H, and DPDPE. The affinities of the compounds

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Table II. Percent of Animals That Met Criteria of Antinociception to (+)-**2a** and (−)-**2a** after Pretreatment with the Selective Opioid Antagonists β-FNA (μ_1 and μ_2), Naloxonazine (μ_1), Nor-BNI (κ_1), and Naltrindole (δ)[a]

	control	β-FNA	naloxonazine	nor-BNI	naltrindole
(+)-**2a**					
2.5 mg/kg, sc	53	0			
5.0 mg/kg, sc	70	0[d]	20[e]	60[e]	50[e]
(−)-**2b**					
25 mg/kg, sc	50	20[b]	10[e]	50[e]	40[e]
U50,488H					
5 mg/kg, sc	60			20[e]	
DPDPE					
0.5 μg, it	60				0[d]

[a] Statistical comparisons against control values were determined using the Fisher Exact Test. [b] $p < 0.1$. [c] $p < 0.05$. [d] $p < 0.006$. [e] Not significant.

with the greatest potencies in the in vivo assays of antinociception ((+)-**2a**, (−)-**2a**, and (±)-**2b**) is greatest for the μ_1 and μ_2 receptor subtypes. None of the compounds show significant affinity for δ receptors while the affinities for the κ_1 and κ_3 receptor subtypes are intermediate. Compound (+)-**2d**, which is a pure opioid antagonist with nalorphine-like potency,[3] is noteworthy in that it retains an affinity for κ_1 sites similar to (+)-**2a** while its affinity for μ receptors has decreased 40-fold.

Dose–response curves for antinociception were generated for (+)-**2a** and (−)-**2a** administered both subcutaneously and intrathecally. The latter experiment was performed to determine if both enantiomers are μ_2 agonists, since it has been shown that the μ receptors that mediate spinal antinociception are μ_2 receptors.[17] Pretreatment with the μ_1-selective antagonist naloxonazine (see next paragraph) suggests that both are also μ_1 agonists. Administered subcutaneously, the ED_{50} of (+)-**2a** (with 95% confidence limits) was 3.2 (1.9, 6.7) mg/kg while that of (−)-**2b** was 22. (12., 39.) mg/kg. Administered intrathecally, (+)-**2a** was again more potent with an ED_{50} (with 95% confidence limits) of 4.0 (1.8, 15.) μg compared to 16. (5.6, 38.) μg for (−)-**2a**. The ratio of the binding affinities of (+)-**2a** and (−)-**2a** for μ_1, μ_2, κ_1, and κ_3 receptors tends to parallel their potencies in the in vivo assays of antinociception. That is, the receptor affinities of (+)-**2a** are consistently 2 to 4-fold higher than those of (−)-**2a** for each of these receptor subtypes which is roughly consistent with their antinociceptive potencies.

The effects of pretreatment with selective opioid antagonists on the antinociception of (+)-**2a** and (−)-**2a** are displayed in Table II. These results indicate that the antinociceptive activity of both enantiomers is mediated through μ receptors, since the μ_1- and μ_2-selective antagonist β-FNA and the μ_1-selective naloxonazine attenuated the antinociceptive activity whereas there was little, if any, effect by the κ_1-selective nor-BNI and δ-selective naltrindole at doses which reverse the activity of the κ-selective agonist U50488H and the δ-selective agonist DPDPE, respectively.

The global minima for (+)-**2a**, **2b**, **2c**, and **2d** are shown in Figure 2. The various energy minima present are summarized in Table III, and the barrier for rotation of the phenyl ring is shown in Figure 3. As was found previously for (+)-**2a**,[12] there are three pairs of energy minima for rotation of the phenyl ring with conformers differing by 180° having essentially the same energy. The pair of lowest

Table III. Locations[a] and Steric Energies (kcal/mol) of Potential Energy Minima for Rotation of the Phenyl Ring in (+)-**2a** and the Corresponding Enantiomer in the Analogues[b]

compd	60°	energy	120°	energy	180°	energy
(+)-**2a**	66°	22.5	119°	21.3	167°	22.3
2b	69°	21.2	104°	20.8	185°	22.9
2c	50°	19.8	135°	17.4	*c*	
2d	67°	25.4	*c*		191°	28.6

[a] Torsion angle is C16–C11–C5–C4 (see Figure 1). [b] Only minima in the vicinity of 60°, 120°, and 180° are included since there is little energy difference caused by a 180° rotation of the phenyl ring. [c] No stable minimum.

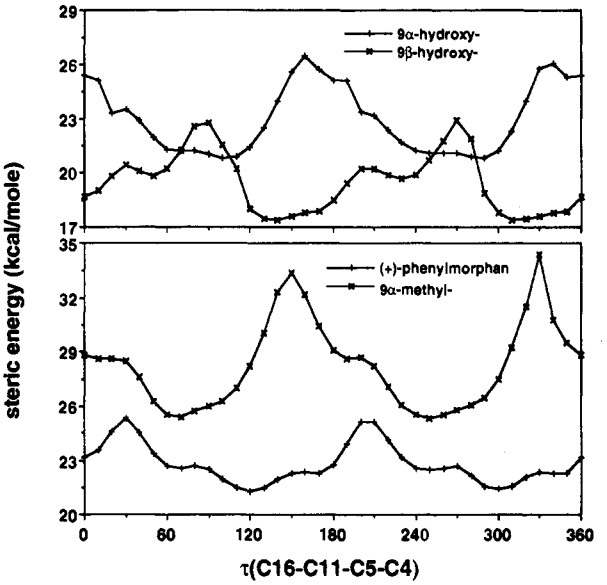

Figure 3. Effect of phenyl rotation on the conformational energy of (+)-**2a** and analogues. The same absolute configuration as (+)-**2a** has been assumed for the analogues.

energy minima for (+)-**2a** occur at τ(C16–C11–C5–C4) = 119° and −61° with the largest barrier to rotation being 4.0 kcal/mol. The conformational behavior of the potent agonist **2b** (for the equivalent enantiomer) is similar to that of (+)-**2a** in that the global minima occur at 104° and −72° with a maximum barrier of 5.7 kcal/mol. For the very weak agonist **2c** (for the equivalent enantiomer), the global minima have shifted to 135° and −48° with a maximum barrier of 5.5 kcal/mol. While the region around 120° is only 0.6 kcal/mol higher than the global minima, the region around 100°, where the minimum occurs for **2b**, is quite unfavorable with an energy that is 4.2 kcal/mol higher. The compound **2d** (assuming the same absolute configuration as (+)-**2a**) has a similar energy curve as **2b** but the global minima have shifted to 67° and −110° with a maximum barrier of 9.1 kcal/mol. Significantly, the region around 120° is now quite unfavorable with an energy 2.8 kcal/mol higher than the global minima. The region around 100° is 0.9 kcal/mol less favorable than the global minima.

Discussion

The receptor binding data (Table I) and the in vivo mouse tail-flick data after pretreatment with selective opioid antagonists (Table II) provide evidence that compounds that are constrained to be phenyl-equatorial can also have considerable affinity for and act through μ receptors. Pretreatment with the μ_1-selective antagonist naloxonazine attenuated the antinociceptive activity of (+)-**2a** and (−)-**2a** showing that both are μ_1 agonists. Administered intrathecally, both enantiomers once again have antinociceptive activity showing that both are also μ_2

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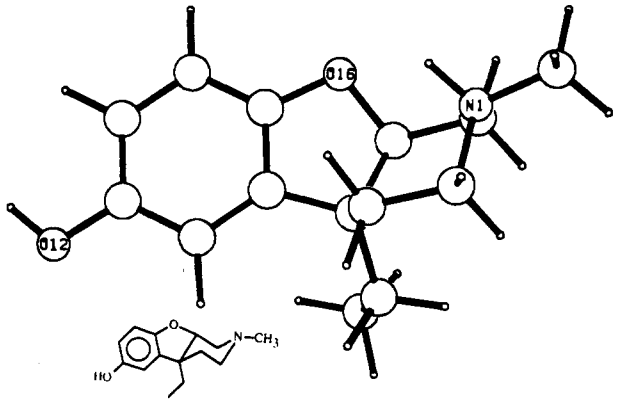

Figure 4. Benzofuro[2,3-*c*]pyridin-6-ol which is active as μ agonist.[18] The same enantiomer as (+)-**2a** is assumed.

agonists.[17] This provides additional evidence as to the validity of the ligand model discussed in the Introduction in which the phenyl ring of all opioids binds to the same site in the receptor, and the direction of the ammonium hydrogen is more important than the actual position of the ammonium nitrogen.

For the phenylmorphans, in which the major conformational degree of freedom is rotation about the phenyl–piperidine bond, it was previously proposed that the region in the vicinity of one of the pair of global minima (τ(C16-C11-C5-C4) = 119°) is likely to be responsible for antinociceptive activity.[12] The conformational energy results for the various analogues studied here (Figure 3) are consistent with a region in the vicinity of ~100° being responsible for antinociception. For **2b**, in which the racemate has about the same antinociceptive potency as the phenylmorphans, the minimum occurs at 104°. Significantly, of the three minima for (+)-**2a** between 0° and 180° (~60°, ~120°, and ~180°), the region in the vicinity of 180° has become quite unfavorable for **2b**. For **2c**, which has little antinociceptive activity, the global minimum occurs at 135° and the region from 60° to 110° is quite unfavorable. For **2d**, the global minimum occurs at 67° (assuming the same absolute configuration as (+)-**2a**) and the compound appears to either be a weak agonist or a pure antagonist depending on the absolute configuration. The region in the vicinity of ~100° is easily accessible for (+)-**2a**, which has a low barrier to rotation but appears to be less accessible to **2c** and **2d**.

Additional evidence for the biologically active form of the phenylmorphans is the recently reported μ-receptor activity of racemic benzofuro[2,3-*c*]pyridine-6-ols[18] (Figure 4), which are good analogues to the preferred conformers of the phenylmorphans. In these phenyl-equatorial compounds, the phenyl ring cannot rotate and the equivalent dihedral angle is 92° for the energy-minimized structure in the corresponding enantiomer. In morphine, which is constrained to be phenyl-axial, the equivalent dihedral angle is 84° based on the morphine hydrochloride trihydrate crystal structure.[19]

On the basis of the pharmacological results presented here, the cause of the atypical (mixed agonist–antagonist) properties of (−)-**2a** remains unclear. There is no apparent difference between the enantiomers in the receptor binding assays aside from a relatively consistent 2–4-fold affinity difference in favor of (+)-**2a**. Of course, receptor binding assays do not indicate agonist or antagonist properties. The affinity of (−)-**2a** for opioid receptors is relatively unaffected by Na^+ ions unlike that of (+)-**2a**, which suggests that (−)-**2a** is only a partial agonist.[4] In the in vivo assays of antinociception, however, both enantiomers appear to be acting through μ receptors and both appear to be capable of eliciting a full response. On the basis of the relationship between the relative receptor affinities of the two enantiomers and their in vivo antinociceptive potencies, it appears that both may be full agonists with respect to that property. It has been known for a long time that opioid mixed agonist–antagonists can have analgesic properties.[20] This suggests that there may be a receptor reserve ("spare receptors") for antinociception in which only a fraction of the available receptors need to be occupied to produce the full agonist response. Under these conditions, it is well known that a partial agonist with low efficacy may be capable of producing the same maximum effect as a full agonist and will, therefore, appear to be a full agonist.[21] Thus, it is possible for a partial agonist like (−)-**2a** to appear to be a full agonist for properties for which there is a receptor reserve and a mixed agonist–antagonist for properties in which all receptors must be occupied to elicit the maximum response.

After the completion of this work and the initial reviews of this manuscript, the absolute configuration of (+)-**2d** was determined to be (1*R*,5*S*,9*R*) by X-ray crystallography.[22] Thus, the pure antagonist (+)-(1*R*,5*S*,9*R*)-**2d** corresponds to the atypical (−)-(1*R*,6*S*)-**2a**, and the weak agonist (−)-(1*S*,5*R*,9*S*)-**2d** corresponds to the morphine-like (+)-(1*S*,5*R*)-**2a**.[13]

Experimental Section

Computational Methods. Energy minimization of the compounds in this study were performed with respect to all internal coordinates using the MM2-87 program and parameter set developed by Allinger and Yuh.[23,24] There were no missing parameters. All calculations were performed for the protonated compound. The dielectric constant was set to 80 in all calculations to approximate a water solution and to prevent electrostatic forces from dominating the calculations. For **2b** and **2c**, the hydroxyl group was set at its optimum value. To ensure complete convergence, the criterion was set to $^1/_8$ of its default value except for the energy barrier calculations for the rotation of the phenyl ring which used the default value for the convergence criterion.

Materials. The phenylmorphan enantiomers and analogues were obtained from Everette May except for (−)-**2d**, which was obtained from Arthur Jacobson through the Drug Testing Program of the Committee for Problems of Drug Dependence. Radioligands (^{3}H-[D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO), ^{3}H-[D-Ala^2,D-Leu^5]enkephalin (DADL), ^{3}H-[D-Pen^2,D-Pen^5]-enkephalin (DPDPE), and [^{3}H]U69593) and Formula 963 scintillation fluor were purchased from New England Nuclear Corp. (Boston, MA), halothane from Halocarbon Laboratories, Inc. (Hackensack, NJ), cold DPDPE from Peninsula Laboratories

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(Belmont, CA), and naltrindole hydrochloride and norbinaltorphimine hydrochloride (nor-BNI) from Research Biochemicals, Inc. (Natick, MA). β-Funaltrexamine (β-FNA) was obtained from the Research Technology Branch of the National Institute on Drug Abuse. U50,488H was a generous gift from Upjohn Pharmaceutics (Kalamazoo, MI). [^{3}H]Naloxone benzoylhydrazone (NalBzoH) was synthesized as described previously.[25] Naltrindole and nor-BNI were used as salts and NalBzoH and U50,488 as free bases. Fresh calf brains were obtained locally, dissected into the appropriate brain region, and prepared as previously reported[26] and frozen. Tissue prepared in this manner and maintained at −70 °C retained its binding for at least 3–4 weeks. Frozen guinea pig brains were obtained from Charles River (Wilmington, MA). Each brain was thawed, and the cerebellar tissue was prepared and frozen as described previously.[27]

Receptor Binding. The μ_1 and μ_2 binding was performed using calf thalamic membranes.[26] The μ_1 assay used [^{3}H]DADL (0.7 nM) with DPDPE (10 nM) to block δ receptors while μ_2 binding was performed with [^{3}H]DAMGO (0.7 nM) with DSLET (5 nM) to block μ_1 sites. The δ binding was determined with [^{3}H]DPDPE (1 nM) in calf frontal cortex. The κ_1 binding was determined in 2-mL aliquots of guinea pig cerebellar membranes (10 mg/mL wet weight tissue) incubated with [^{3}H]U69593 (1.4 nM) in 50 mM Tris buffer (pH 7.6) at 25 °C for 60 min.[27] For κ_3 binding, calf striatal membranes were incubated with [^{3}H]NalBzoH (1 nM) in potassium phosphate buffer (50 mM; pH 7.2) in the presence of K_2-EDTA.[27] After incubation, samples were filtered over glass fiber filters. All determinations were performed in triplicate, and each experiment was replicated three times. Nonspecific binding was determined in the presence of levallorphan (1 mM). Only specific binding is reported. All values were presented as means ±SEM. K_i values of unlabeled compounds were calculated: $K_i = (IC_{50})/(1 + S)$ where S = (concentration of radioligand)/(K_D of radioligand).[28,29]

Animals. Male CD-1 mice (25–35 g; Charles River Breeding Laboratories, Wilmington, MA) were maintained on a 12-h light/dark cycle with food and water available ad libitum. Mice were housed in groups of five until testing.

Antinociceptive Assay. Antinociception was determined quantally using the radiant heat tail-flick technique[30] in which the latency to withdraw the tail from a focussed light stimulus was measured electronically using a photocell. Base-line latencies (2.0–3.0 s) were determined before experimental treatments for all animals as the mean of two trials. Antinociception was defined as a doubling or greater of the base-line latency. A maximum latency of 10 s was used to minimize tissue damage.

Dose–response curves for (+)-**2a** and (−)-**2a** in the tail-flick assay were determined with groups of mice (n = 10). With subcutaneous administration, the ED_{50}s with 95% confidence limits are 3.2 (1.9, 6.7) and 22. (12., 39.) mg/kg for (+)-**2a** and (−)-**2a**, respectively. Animals treated with β-FNA (40 mg/kg, sc) and naloxonazine (35 mg/kg, sc) received the antagonist 24 h prior to testing with the agonist, as described previously.[17,31] Naltrindole (20 mg/kg, sc) and nor-BNI (10 mg/kg, sc) were administered immediately prior to testing with the agonist. To ensure that the κ_1 antagonist nor-BNI and the δ antagonist naltrindole were active, groups of mice (n = 5) were administered either the κ-selective agonist U50,488H or the δ-selective agonist DPDPE alone or with their selective antagonist.

Dose–response curves for (+)-**2a** and (−)-**2a** were also determined using intrathecal administration (n = 10) since it has been shown that the μ receptors in spinal antinociception are μ_2 receptors since they are antagonized by the selective μ antagonist β-FNA but unaffected by the μ_1-selective naloxonazine.[17] In contrast, the μ receptors in supraspinal antinociception appear to be μ_1 receptors since they are antagonized by both β-FNA and naloxonazine.[17] The ED_{50}s with 95% confidence limits are 4.0 (1.8, 15.) and 16. (5.6, 38.) μg for (+)-**2a** and (−)-**2a**, respectively. Intrathecal injections were made by lumbar puncture[32] under light halothane anesthesia using a Hamilton 10-μL syringe fitted to a 30-gauge needle with V1 tubing.

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