the guinea pig LTD_4- and ovalbumin-induced bronchospasm, and the rat carregeenan paw edema models are provided in ref 4, 6, and 18-20.

Antagonism of the LTD_4-Induced Contraction of the Isolated Guinea Pig Trachea. Experimental details for the determination of the LTD_4-antagonist activity in the isolated guinea pig trachea are essentially those reported in ref 20 and 21 (see Table II).

Registry No. 1, 123723-84-2; 2, 123723-85-3; 3, 123723-86-4; 4, 123723-87-5; 5, 104325-55-5; 6, 104325-56-6; 7, 123723-88-6; 8, 123723-89-7; 9, 123723-90-0; 10, 123723-91-1; 11, 123723-92-2; 12, 121289-78-9; 13, 123723-93-3; 14, 123723-94-4; 15, 118308-98-8; 16, 118308-95-5; 17, 119603-17-7; 18, 123723-95-5; 19, 118308-97-7; 20, 118308-94-4; 21, 123723-96-6; 22, 118308-96-6; 23, 123723-97-7; 24, 123723-98-8; 25, 123723-99-9; 26, 123724-00-5; 27, 123724-01-6; 28, 123724-02-7; 29, 123724-03-8; 30, 119514-97-5; 31, 123724-04-9; 32, 123724-05-0; 33, 123724-06-1; 34, 123724-07-2; 35, 119514-96-4; 36, 123724-08-3; 37, 119514-95-3; 38, 123724-09-4; 39, 123724-10-7; 40, 123724-11-8; 41, 123724-12-9; 42, 123724-13-0; 43, 123724-14-1; 44, 123724-15-2; LTD_4, 73836-78-9; 2-(chloromethyl)quinoline, 4377-41-7; 2-(chloromethyl)naphthalene, 2506-41-4; 2-(chloromethyl)quinazoline, 6148-18-1; 2-(chloromethyl)benzothiazole, 37859-43-1; 1-methyl-2-(chloromethyl)benzimidazole, 4760-35-4; *p*-toluenesulfonamide, 70-55-3; methanesulfonamide, 3144-09-0; benzenesulfonamide, 98-10-2; 2-cyanophenol, 611-20-1; 3-cyanophenol, 873-62-1; 4-cyanophenol, 767-00-0; methyl (3-hydroxyphenyl)acetate, 42058-59-3; methyl (4-hydroxyphenyl)acetate, 14199-15-6; methyl 2-hydroxybenzoate, 119-36-8; methyl 3-hydroxybenzoate, 19438-10-9; methyl (2-hydroxyphenyl)acetate, 771-90-4; methyl α,3-dihydroxybenzeneacetic acid, 90721-46-3; methyl 3-(3-hydroxyphenyl)propanoate, 61389-68-2; 3-[(2-quinolinyl)methoxy]benzoic acid, 107813-86-5; methyl 3-[(2-quinolinyl)methoxy]benzoate, 119515-00-3; 4-[(2-quinolinyl)-methoxy]benzoic acid, 123724-16-3; 3-(3-hydroxyphenyl)propionic acid, 3480-87-3; 2-quinolinylmethyl 3-[3-(2-quinolinylmethoxy)-benzene]propanoic acid, 123724-17-4; 3-hydroxybenzyl cyanide, 25263-44-9; 2-[[3-(cyanomethyl)phenoxy]methyl]quinoline, 123724-18-5; 4-hydroxybenzyl cyanide, 14191-95-8.

Assessment of the in Vivo and in Vitro Opioid Activity of Bridged Hexahydroaporphine and Isoquinoline Molecules

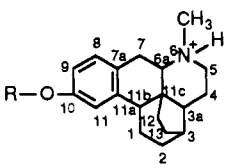
Victoria F. Roche,*,† Edward B. Roche,‡ Paul K. Lemcke,§ William S. Wire,§ and Thomas F. Burks§

Department of Pharmaceutical Sciences, School of Pharmacy and Allied Health Professions, Creighton University, Omaha, Nebraska 68178, Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68504, and Department of Pharmacology, School of Medicine, University of Arizona, Tucson, Arizona 85724. *Received June 30, 1988*

Four novel racemic bridged hexahydroaporphine (1 and 2) and isoquinoline (3 and 4) analogues have been synthesized in an attempt to generate bicyclic derivatives of the morphinan ring system. The opioid activity of these analogues has been assessed through membrane-binding studies, in vitro studies in isolated guinea pig ileum and mouse vas deferens, and in vivo studies utilizing the mouse hot plate technique. The bridged isoquinoline precursor molecules were inactive as antinociceptives. Both the racemic phenolic hexahydroaporphine 1 and its 10-methoxy congener 2 demonstrated dose-dependent, albeit weak, antinociceptive activity when administered icv, but they induced lethal convulsions when given subcutaneously. The antinociception elicited by 1 appeared to show very weak opioid character while that caused by 2 was totally nonopioid.

Diels-Alder adducts of thebaine (oripavines) containing a bicyclic C ring are recognized as highly active opioid receptor ligands. The oripavine derivative etorphine (5) is one of the most potent of all synthetic narcotic analgetics.[1] Buprenorphine (Buprenex), an *N*-cyclopropylmethyl congener of etorphine, exhibits dose-dependent agonist and partial antagonist activities[2,3] mediated through μ receptors.[4] As an analgetic, sublingual buprenorphine is 2-3 times as potent as intramuscularly administered morphine[2,5] but has a longer duration of action[2] and a lower addiction potential.[5,6] Both etorphine and buprenorphine contain a C-ring *endo*-alkyl bridging unit connecting carbons 6 and 14. Much of the superior analgetic activity of these two bridged opioids can be attributed to high lipophilicity and effective in vivo distribution,[7,8] although enhanced receptor affinity also contributes to potency.[7]

Serious interest in bicyclic morphinans has recently been manifested. Two studies have centered on the synthesis of 6,14-exoethenomorphinans as novel analgetics.[9,10] Our laboratory has been interested in the design of isomeric bridged morphinans that contain 5,8-alkyl bridging units. In an attempt to generate the 5,8-ethano analogue (6) of the potent morphinan analgetic levorphanol, the racemic bicyclic hexahydroaporphine structures 1 and 2 have been



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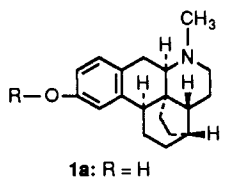
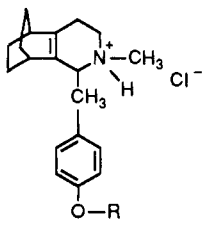
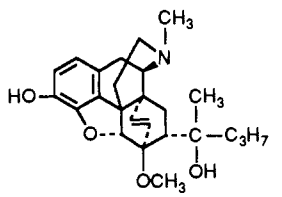
1: R = H
2: R = CH_3



1a: R = H
2a: R = CH_3



3: R = H
4: R = CH_3



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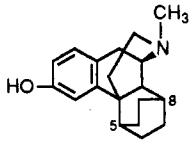

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† Creighton University.
‡ University of Nebraska Medical Center.
§ University of Arizona.

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synthesized.[11] The hexahydroaporphine ring system was generated at the expense of the morphinan system when a bridged *N*-formyloctahydroisoquinoline precursor was cyclized with anhydrous hydrofluoric acid.

The hexahydroaporphine ring system is isomeric with the morphinan ring system. Examination of Dreiding molecular models shows that the spatial relationship between functional groups deemed essential for high μ opioid receptor affinity in morphinans (phenolic hydroxy, aromatic nucleus, and tertiary amino nitrogen) are closely approximated in the isomer of **1** designated as **1a**. When molecule **1a** was aligned with a model of B/C-*trans*-morphinan (isomorphinan), the spatial similarity was particularly noteworthy as, in addition to the aforementioned groups, it also included the C-ring structural regions. Thus it was of interest to investigate the opioid activity profile of **1** and **2** in vitro and in vivo.

In view of the fact that some 1-substituted tetrahydroisoquinolines have shown opioid activity,[12,13] two bridged isoquinoline precursor molecules (**3** and **4**) were also subjected to evaluation via central membrane receptor binding, isolated tissue, and whole animal studies. It was hypothesized that the enhanced molecular flexibility of the isoquinolines might allow access to opioid receptors, even if the more conformationally restricted hexahydroaporphine derivatives proved to be of low affinity.

Chemistry

The synthetic scheme developed to generate the racemic bridged hexahydroaporphine and octahydroisoquinoline ligands has been reported.[11] X-ray crystallographic analysis of the *N*-formyl precursor of **4** and of **2**·HCl indicated the presence of a single pair of enantiomers in each case. The isomer of the hexahydroaporphine structures that corresponds most closely to analgetically active levorotatory morphinans has the 3*S*,3a*R*,6a*R*,11b*S*,11c*S* configuration and is represented by structures **1a** and **2a**.

Experimental Section

Pharmacology. Opioid activity was assessed through central membrane receptor binding techniques,[14] isolated tissue studies,[15] and the mouse hot plate assay.[16]

Membrane Receptor Binding. The ability of the bridged ligands **1–4** to bind stereospecifically to the opioid receptors of rat brain was assessed via ligand-displacement experiments.[14] Male Sprague–Dawley rats (250–350 g) were sacrificed by decapitation. Whole brains minus the cerebellum were rapidly excised and homogenized in approximately 600 mL of cold 0.5 M Tris buffer (pH 7.4 at 37 °C). The homogenate was centrifuged at 22 000 rpm for 10 min at 0 °C and the resultant pellet was resuspended in 300 mL of fresh buffer. The homogenate (2.1 mL) was incubated in triplicate with 0.1 mL of varying concentrations of bridged ligand or standard. Standards utilized included (−)-morphine hydrochloride trihydrate (Merck) and levorphanol tartrate dihydrate. All bridged test ligands were analyzed as their racemic hydrochloride salts. The experiments were conducted in the presence and absence of 100 mM sodium ion. Tubes containing homogenate and 100 mM levallorphan tartrate or dextrallorphan hydrobromide were run with each experiment to allow for the determination of stereospecific binding. After the addition of [^{3}H]naloxone (New England Nuclear) to a final [^{3}H]naloxone concentration of 1.5 mM, the samples were incubated at 37 °C for 20 min. The samples were then chilled in ice for 20 min and filtered through Whatman GF/B glass-fiber filters. Each filter was rinsed twice with a 5-mL volume of ice-cold Tris buffer, transferred to a scintillation vial containing 8 mL of Aquasol scintillation cocktail (New England Nuclear), and counted at 18 °C at a counting efficiency of 41%. IC_{50} values and sodium response ratios were determined for all compounds assayed.

Isolated Tissue Studies. The bridged ligands **1–4** were analyzed for opioid receptor affinity in the mouse vas deferens (MVD) and/or the guinea pig ileum (GPI) according to the method of Shook et al.[15] Male Hartley guinea pigs (250–400 g) and male ICR mice (20–35 g) were sacrificed. Guinea pig nonterminal ileal muscle–myenteric plexus 1–2 cm in length and paired mouse vasa deferentia were harvested and suspended in 20-mL organ baths. The baths contained Krebs bicarbonate buffer (37 °C) treated continuously with a 95% O_2/5% CO_2 mixture. In the case of the MVD, $MgSO_4$ was omitted from the bathing medium. The tissue segments were attached to isometric force transducers and allowed to equilibrate for 10 min. The tissue was stretched to either 1 g (GPI) or 0.5 g (MVD) tension (previously determined to represent optimal length) and allowed to equilibrate for another 10 min. The tissue was transmurally stimulated between platinum plate electrodes at 0.1 Hz with supramaximal voltage and either 0.4 ms (GPI) or 2 ms (MVD) pulses. The electrically induced contractions were recorded on Houston or Soltec multichannel recorders. All drug substances were added to the baths in 20-μL or 60-μL increments. Each bridged analogue was tested for its ability to inhibit electrically stimulated tissue contractions after a 3-min incubation period. The opioid receptor preference of any compound exhibiting agonist action was assessed by repeating the above experiment in tissues pretreated for 2 min with an opioid antagonist. The antagonists utilized included naloxone hydrochloride (Endo Laboratories), CTP[17] (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2), a μ selective antagonist, and/or ICI 174,894 (*N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu; Cambridge Research Biochemicals), a δ-selective antagonist used only in the the MVD assay. Each bridged analogue was also tested for opioid antagonist activity by assessing its ability to reverse the action of selective agonists. PL107 ([MePhe^3,D-Pro^4]morphiceptin; Peninsula Laboratories) and tifluadom (Sandoz Pharmaceuticals), selective μ and κ agonists, respectively, were utilized in the GPI assay. PL107 and the selective δ agonist DPDPE ([D-Pen^2,D-Pen^5]enkephalin; Peninsula Laboratories) were employed in the MVD experiments.

Mouse Hot Plate Assay. Male ICR ice (25–30 g) were used in all experiments. The number of mice per experiment ranged from 5 to 17. The mice were housed under a standard 12 hour light/dark regimen and received food and water ad libitum. Antinociception was assessed by the use of a thermostatically controlled 55 °C hot plate[16] (Technilab Instruments). Mice were placed on the hot plate to determine the control latency (in seconds) to hindpaw licking or escape jumping. Only mice exhibiting control latency values of less than 20 s were used for drug testing. All drugs were dissolved in distilled water and injected intracerebroventricularly (icv) in a dose-dependent total volume

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Table I. IC_{50}[a] Values and Sodium Response Ratios[b] Determined for Bridged Hexahydroaporphines and Isoquinolines in Displacing [^{3}H]Naloxone from Opioid Receptors

compound	$IC_{50} \pm SD$, nM		sodium response ratio
	-Na	+Na	
(−)-morphine HCl	$(2.26 \pm 0.31) \times 10^1$	$(4.87 \pm 0.48) \times 10^2$	21.55
levorphanol tartrate	(4.84 ± 0.33)	$(5.74 \pm 0.52) \times 10^1$	11.86
(1) HHA-OH·HCl	$(4.72 \pm 0.97) \times 10^2$	$(5.59 \pm 0.75) \times 10^3$	11.84
(2) HHA-MeO·HCl	inactive[c]	inactive[c]	not applicable
(3) IsoQ-OH·HCl	inactive[c]	inactive[c]	not applicable
(4) IsoQ-MeO·HCl	inactive[c]	inactive[c]	not applicable

[a] IC_{50} is the concentration of ligand that produced a 50% inhibition of stereospecific [^{3}H]naloxone binding to the opioid receptors of rat brain. All values represent the mean of three independent determinations. [b] Sodium response ratio is defined as the IC_{50} of a ligand in the presence of 100 mM sodium ion divided by the IC_{50} of the ligand in the absence of sodium ion. [c] Inactive indicates an IC_{50} greater than or equal to 10 μM.

of 3.0 or 3.3 μL according to the method of Haley and McCormick.[18] The icv route was utilized in the initial studies to determine the inherent antinociceptive activity of the bridged ligands without concern for distributive influences. In subsequent studies, the drugs were administered subcutaneously. Test latencies were determined at 5, 10, 20, 40, and, in some cases, 80 min postinjection. A maximal test latency of 60 s was used to avoid tissue damage. Antinociception was calculated as a percent of control values according to the following equation:

$$\% \text{ antinociception} = (\text{test latency} - \text{control latency})/(60 - \text{control latency}) \times 100$$

Any compound producing antinociception was reassessed in mice 5 min after pretreatment with the μ-selective antagonist naloxone and/or the κ-selective antagonist WIN 44,441-3 [(−)-1-cyclopentyl-5-(3,5,11-trimethyl-8-hydroxy-3-benzazocin-11-yl)-3-pentanone].

All statistical calculations were performed as described by Tallarida and Murray.[19]

Biological Results and Discussion

Membrane Receptor Binding. The results of the brain receptor binding assay are provided in Table I. The IC_{50} values and sodium response ratios obtained for morphine and levorphanol were in agreement with values reported in the literature.[14]

All racemic bridged analogues demonstrated significantly lower affinity for opioid receptors than did the optically pure standards. The phenolic hexahydroaporphine analogue 1 showed the greatest promise as a μ receptor ligand, providing IC_{50} values approximately 1 order of magnitude higher than that of optically pure morphine in the presence and absence of sodium. It is of significance to note that meperidine has shown similar IC_{50} values in this test system.[14] Compound 1 exhibited a sodium response ratio typical of a pure μ agonist. The other bridged ligands tested were inactive in this system, providing IC_{50} values greater than 10 μM. This would argue against the concept that molecular flexibility might facilitate μ receptor binding in the isoquinoline series. Masking of the phenolic hydroxy group through methyl ether formation destroyed binding affinity in both the hexahydroaporphine and isoquinoline structures, as expected. The attenuation was most pronounced in the hexahydroaporphines (1 vs 2).

Overall, the results of the brain receptor binding studies indicated that 1 might have sufficient opioid receptor affinity to provide antinociceptive activity in vivo. The methoxylated analogue 2 might be expected to undergo metabolic conversion to 1 and thus be able to elicit an antinociceptive response in vivo.

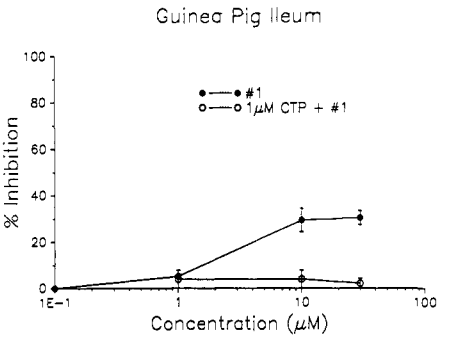

Figure 1.

Isolated Tissue Studies. None of the novel bridged analogues behaved as potent opioids in either the GPI or MVD assay. Compound 1, the most active bridged analogue in the isolated tissue preparations, required a dose of 10–30 μM to produce a maximal 30% inhibition of ileal twitch (Figure 1). That any opioid action that was involved was mediated through μ (as opposed to κ) receptors was demonstrated by the complete reversal of twitch inhibition by the μ-selective antagonist CTP (1000 nM). CTP was ineffective in reversing the agonist action of the standard κ ligand tifluadom in this assay. Naloxone (1000 nM) not only reversed the inhibition of twitch produced by 1, but increased twitch height by 27% over control values.

Compound 2 (30 μM) slowly inhibited electrically induced ileal contractions to a maximum of 27% over 10 min. Naloxone was less effective in antagonizing the effect of 2 than of 1 and CTP was totally ineffective. Compound 2 did not inhibit twitch responses in the MVD and, in fact, caused an increase in twitch height that was not blocked by the δ opioid antagonist ICI 174,864.

Compound 1 was completely inactive as an opioid agonist in the MVD assay. Compound 2 (1000 nM) produced an increase in vas twitch height that was not reversed by μ- or δ-selective antagonists. Neither bridged hexahydroaporphine analogue demonstrated opioid antagonism in either tissue. Thus, in vitro studies in the GPI, but not the MVD, demonstrated the very weak opioid action of 1 and indicated a lack of opioid agonist or antagonist activity of methoxy congener 2.

In Vivo Antinociceptive Activity. Icv Studies. In the mouse hot plate assay, both the *p*-methoxy and the phenolic isoquinoline analogues 3 and 4 were inactive as antinociceptives. Both the hexahydroaporphines 1 and 2, however, provided dose-dependent antinociception (Figure 2). A 10-μg icv dose of 1 produced an analgetic response which peaked at 79% of maximum at 10 min and had terminated by 40 min postinjection. The same dose of 2 produced antinociception that was of comparable intensity but of somewhat longer duration. The ED_{50} values for 1

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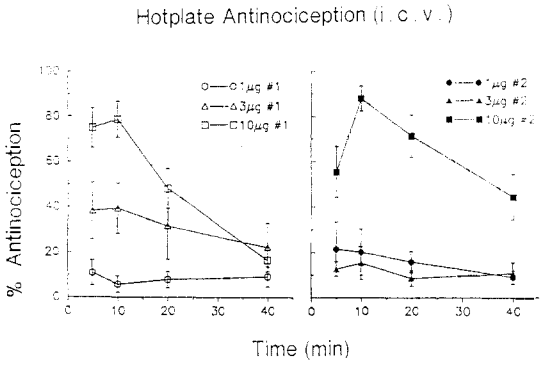

Figure 2.

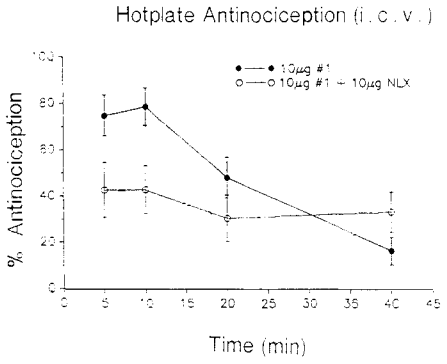

Figure 3.

and 2 were calculated at 4.07 and 5.42 μg, respectively. By comparison, morphine sulfate gave an ED_{50} of 79.4 ng. Naloxone (1 μg icv) had little effect on the antinociception produced by 1, although 1 μg of naloxone antagonized responses to icv morphine (3 μg). The initial antinociceptive phase of 1 was attenuated, but not totally blocked, by 10 μg of naloxone (Figure 3). The response at 20 and 40 min postinjection was not significantly different from that observed in animals not receiving naloxone. WIN 44,441-3, at 1- or 10-μg doses, provided an attenuation of 1-induced antinociception that was indistinguishable from that produced by 10 μg of naloxone in the first 20 min.

Methoxy hexahydroaporphine congener 2 displayed a nonopioid antinociceptive activity (Figure 4). This activity was unaffected by 10-μg icv doses of either naloxone or WIN 44,441-3.

Subcutaneous Studies. No antinociception was elicited by either 1 or 2 after subcutaneous administration of 3 mg/kg doses. At doses of 10 mg/kg or higher, all animals tested convulsed and died within 25 min of drug administration.

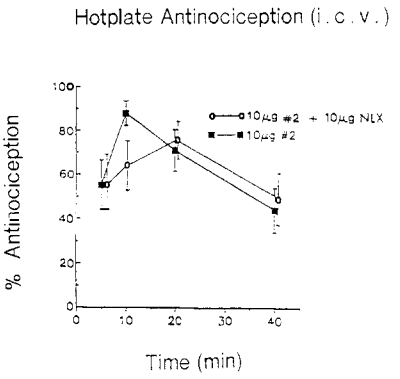

Figure 4.

Conclusions

Both bridged hexahydroaporphines 1 and 2 provide dose-dependent antinociceptive activity when administered icv. Compound 1 appears to exhibit very weak μ opioid agonist action. The phenolic nature of 1 presumably allows very limited access to μ receptors, and a portion of the antinociception produced by this compound is proposed to come from μ-receptor stimulation. Masking the phenol though methyl ether formation destroys opioid receptor affinity altogether and antinociceptive action proceeds through an alternate, as yet undefined, pathway. That compounds 1 and 2 share a common nonopioid antinociceptive mechanism is suspected. When administered peripherally in doses greater than or equal to 10 mg/kg, both agents induce lethal convulsions. Whether these toxic manifestations are elicited by one or both isomers is unknown. The nature of the transforamtion from therapeutic to toxic activity is a focus of future studies.

Acknowledgment. Victoria F. Roche gratefully acknowledges the American Foundation for Pharmaceutical Education (W. Paul Briggs memorial fellowship), the Biomedical Research Support Grant program of the NIH (No. 3-270-0774-100), and the Health Future Foundation at Creighton University (James E. Keck faculty development award) for their financial support of various portions of this work. We gratefully acknowledge the generous donation of CTP by Dr. V. J. Hruby (University of Arizona); of tifluadom by Sandoz Ltd. (Basel, Switzerland); of dextrallorphan hydrobromide, levallorphan tartrate dihydrate, and levorphanol tartrate by Hoffman-La Roche (Nutley, NJ); and of WIN 44,441-3 by Sterling Winthrop (Rensselaer, NY).

Registry No. (±)-1a, 91817-15-1; (±)-2a, 91877-79-1; (±)-3, 91817-29-7; (±)-4, 91817-14-0.