Notes

Long-Acting Opiate Agonists and Antagonists: 14-Hydroxydihydromorphinone Hydrazones

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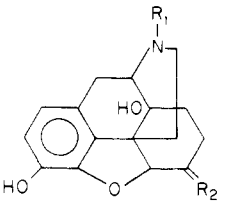
Two new long-acting hydrazone derivatives of 14-hydroxydihydromorphinones have been synthesized, oxymorphazone and naltrexazone. Both derivatives show high affinity for opiate binding sites in vitro, similar to naloxazone, the hydrazone analogue of naloxone. Sodium and manganese shifts imply that naltrexazone, like naloxazone, is a pure antagonist. By contrast, oxymorphazone inhibition of receptor binding is dramatically reduced by sodium and potentiated by manganese, suggesting it is an agonist. When given in vivo, all agents produce a significant inhibition of receptor binding for over 24 h despite extensive washing of the brain homogenates. Oxymorphone, naltrexone, and naloxone are without effect. Twenty-four hours after in vivo administration of oxymorphazone, 82% of mice are still analgetic compared to only 17% of oxymorphone-treated mice ($p < 0.005$). Twenty-four hours after naltrexazone or naloxazone treatment all mice were protected from morphine analgesia (12 mg/kg; $p < 0.005$), while naltrexone- and naloxone-treated animals did not differ significantly from saline-treated controls.

Opiates have been instrumental in our understanding of the central mechanisms of analgesia and the molecular processes involved with endogenous opioid receptor function. Despite many attempts to design irreversibly binding narcotics,[1-7] the first two agents active both in vitro and in vivo, chlornaltrexamine and chloroxymorphamine, have only recently been published.[8-10] Subsequently, a different, long-acting hydrazone derivative of naloxone, naloxazone, has been described.[11,12] We now report the synthesis and pharmacology of long-acting C_6-substituted hydrazone derivatives of oxymorphone and naltrexone and compare them to naloxazone.

The strict structural features necessary to maintain opiate activity markedly limit the location and size of potential substitutions. The C-6 carbonyl group in 14-hydroxydihydromorphinones lends itself readily to modification, and previous studies have demonstrated that changes at this position do not adversely affect the potency of the derivatives.[13,14] Furthermore, substitution of the oxygen at the C-6 position in oxymorphones by a group which has a similar spatial configuration (sp^2 or planar) to that of the carbonyl group does not modify the agonist/antagonist character of the drug.[15] We therefore elected to synthesize the hydrazone derivatives of the parent 14-hydroxydihydromorphinones which retain the sp^2 configuration at C-6 and have previously provided useful derivatives.[16]

Chemistry. The hydrazone derivatives of oxymorphone, naltrexone, and naloxone were synthesized by reacting anhydrous hydrazine with the parent compound. Thin-layer chromatograms (silica gel; $CHCl_3$–CH_3OH–NH_4OH, 132:12:0.9) of the reaction mixtures showed the formation of only one product which migrated slower than the parent ketone. Each isolated product had an R_f identical with the product in the unpurified reaction mixture, suggesting that rearrangement or further reaction did not occur during purification. Infrared spectroscopy revealed the absence of the ketone absorption normally

Chart I



	R_1	R_2
Naloxone	$-CH_2-CH=CH_2$	$=O$
Naloxazone	$-CH_2-CH=CH_2$	$=N-NH_2$
Oxymorphone	$-CH_3$	$=O$
Oxymorphazone	$-CH_3$	$=N-NH_2$
Naltrexone	$-CH_2-CH<(CH_2)_2$	$=O$
Naltrexazone	$-CH_2-CH<(CH_2)_2$	$=N-NH_2$

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Table I. Inhibition of [^{3}H]Naltrexone Binding by Oxymorphazone, Naltrexazone, and Naloxazone in Vitro

	[^{3}H]naltrexone binding assay[a]							
	standard		NaCl		$MnCl_2$		NaCl + $MnCl_2$	
addition	cpm	decrease, %	cpm	decrease, %	cpm	decrease, %	cpm	decrease, %
none	4930 ± 143		6070 ± 75		4080 ± 138		5840 ± 72	
oxymorphazone								
4 nM	4860 ± 32	2	5800 ± 106	5	3180 ± 164	22	5240 ± 41	10
10 nM	3750 ± 98	24	5520 ± 85	9	2550 ± 61	37	4560 ± 80	22
naltrexazone								
4 nM	2980 ± 97	39	3730 ± 144	39	2570 ± 82	38	3480 ± 39	40
10 nM	1910 ± 78	61	2200 ± 17	64	1550 ± 55	62	2240 ± 129	62
naloxazone								
10 nM	4400 ± 42	11	4644 ± 64	24	3135 ± 62	23	4470 ± 24	23

[a] Binding assays were performed with [^{3}H]naltrexone (1.3 nM) and rat brain homogenates under the following conditions: (1) standard, no additions; (2) addition of NaCl, 100 mM; (3) addition of $MnCl_2$, 1 mM; (4) addition of both NaCl, 100 mM, and $MnCl_2$, 1 mM. Results represent specific binding and are the means of triplicate samples ± SEM. The experiment has been replicated three times.

seen in each of these dihydromorphinone derivatives. Reaction at the C_6 carbon was also indicated by a downfield shift in the C_5 hydrogen absorption in the NMR spectrum of all three derivatives. Mass spectroscopy using chemical ionization showed intense M + 1 ions at 316, 356, and 342 for oxymorphazone, naltrexazone, and naloxazone, respectively. The presence of a free NH_2 group was indicated by a positive TNBS test.[17,18] All three derivatives slowly produced a reddish-brown color, in contrast to hydrazine which rapidly formed a deep red color. Quantitative titration of the compounds with TNBS also indicated that the product is the monosubstituted hydrazone. These data confirmed the proposed structures as presented in Chart I. We have named these derivatives oxymorphazone, naltrexazone, and naloxazone, respectively.

Pharmacology. Binding assays were used to evaluate the affinity of these compounds for opiate binding sites in vitro (Table I). Binding reversibly under these assay conditions, the new agents displaced [^{3}H]naltrexone binding with potencies only slightly less than their ketone precursors. Under similar assay conditions, neither the monosubstituted hydrazine phenelzine (0.1 mM) nor the hydrazide isoniazid (0.1 mM) inhibitied either [^{3}H]naloxone or [^{3}H]dihydromorphine binding by more than 10%.[12] Sodium ions, which selectively lower agonist binding to opiate receptors,[19] markedly reduced oxymorphazone's inhibition of [^{3}H]naltrexone binding with no reduction in the inhibition of either naltrexazone or naloxazone. Manganese ions, which enhance opiate agonist binding,[20] increased oxymorphone's potency with little effect on naltrexazone or naloxazone. Prolonged in vitro inhibition of [^{3}H]opiate binding by these compounds was obtained by incubation of brain homogenates with the three hydrazone derivatives (10 μM) for 30 min at 25 °C, followed by two standard wash procedures. The ^{3}H-labeled [D-Ala^2,Met^5]enkephalinamide binding in homogenates treated with the hydrazone derivatives (oxymorphazone, 467 ± 42 cpm; naltrexazone, 314 ± 33 cpm; naloxazone, 308 ± 45 cpm) was significantly reduced for all three drugs ($p < 0.001$) compared to control tissue incubated with naltrexone (1184 ± 78 cpm), oxymorphone (1247 ± 45 cpm), and naloxone (987 ± 86 cpm) at identical concentrations. The nature and mechanism of the prolonged in vitro blockade of opiate binding sites by these hydrazone derivatives remain unknown.

These derivatives were then tested for in vivo effects on both [^{3}H]opiate binding and analgesia. As seen in Table

Table II. Effects of in Vivo Oxymorphazone, Naltrexazone, and Naloxazone Treatment on [^{3}H]Opiate Binding and Analgesia[a]

treatment	analgesia, % (no. analgesic/ no. tested)	^{3}H-labeled [D-Ala^2,Met^5]-enkephalinamide binding, cpm
(1) oxymorphazone	82 (9/11)[b]	1350 ± 94 ($n = 7$)[c]
oxymorphone	17 (2/12)	1830 ± 157 ($n = 8$)
(2) naltrexazone	0 (0/6)[b]	1971 ± 171 ($n = 3$)[b]
naltrexone	67 (4/6)	3720 ± 386 ($n = 3$)
saline	100 (6/6)	
(3) naloxazone	0 (0/13)[b]	1688 ± 185 ($n = 4$)[b]
naloxone	54 (7/13)	2625 ± 145 ($n = 4$)
saline	63 (5/8)	

[a] Groups of mice were treated with either oxymorphone or oxymorphazone; naltrexone, saline, or naltrexazone; or naloxone, saline or naloxazone at 200 mg/kg and assayed 18–20 h later for analgesia as described in the Experimental Section. Oxymorphone and oxymorphazone analgesia was determined directly by tail-flick latencies before and 18–20 h after injection. Naltrexazone, naltrexone, naloxazone, and naloxone effects were determined 18–20 h later by measuring the analgesia produced by morphine (12 mg/kg) ip, as described in the Experimental Section. Additional groups of mice were similarly treated with either oxymorphone or oxymorphazone; naltrexone or naltrexazone; or naloxone or naloxazone, and binding was determined 18–20 h later. Since saline-treated animals give results identical with either oxymorphone, naltrexone, or naloxone under these conditions (Pasternak, unpublished observations), saline animals were not included in these assays. Note that binding in treatment groups 1, 2, and 3 were performed in different experiments and, therefore, binding in oxymorphone, naltrexone, and naloxone animals is not the same. [b] $p < 0.005$. [c] $p < 0.02$.

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II, oxymorphazone, naltrexazone, and naloxazone significantly inhibited the binding of ^{3}H-labeled [D-Ala^2,Met^5]-enkephalinamide 18–24 h after in vivo administration. Oxymorphone, naltrexone, and naloxone were without effect. As with the in vitro inhibition of binding described above, this decreased binding was not reversed by extensive washing procedures. This blockade of binding sites appears to be specific. Although all three hydrazone compounds significantly depressed [^{3}H]opiate binding (Table II), phenelzine at an equimolar dose in vivo had no effect 18 h later on [^{3}H]naloxone binding.[12] In addition, in vivo administration of naloxazone (200 mg/kg) has no significant effects 18 h later on α- or β-adrenergic, benzodiazepine, or muscarinic acetylcholine receptors.[12] Analgesia was tested in mice treated with these compounds (Table II). Oxymorphone administration produced analgesia in only 17% (2/12) of treated mice after 24 h. Oxymorphazone produced analgesia in 82% of mice after 24 h, with 50% (4/8) of mice similarly treated in additional experiments still analgesic after 48 h.

Since naltrexazone and naloxazone produced no analgesia alone, their ability to block morphine analgesia was investigated. Morphine analgesia in naloxone- or naltrexone-treated mice was not significantly different from saline-treated animals. Naloxazone and naltrexazone, which decrease [^{3}H]opiate binding for over 24 h, totally prevented morphine analgesia (12 mg/kg) in all mice examined. Direct injection of naloxazone intracerebroventricularly (10 g/brain) also blocked morphine analgesia 24 h later.[21]

This study describes two new long-acting opiates, oxymorphazone and naltrexazone. Unlike the alkylating agents chlornaltrexamine and oxymorphamine, the mechanism through which these hydrazone derivatives produce their long-acting effects both in vivo and in vitro remain unknown. Although the prolonged receptor blockade both in vivo and in vitro was not reversed by extensive wash procedures and appeared to correlate with long-lasting pharmacological effects, there is no conclusive evidence of covalent linkages between the hydrazone drugs and opiate binding sites. The high doses of drugs used in these studies to maximize the long-lasting in vivo effects on analgesia probably result from a variety of factors, which includes the lower entry of naloxazone into the brain compared to naloxone.[12] Further studies are in progress to elucidate the mechanism of action of these drugs.

Experimental Section

Chemistry. All melting points were taken on a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 237. NMR spectra were recorded on a Varian A-60A spectrophotometer with Me_4Si as the internal standard. Mass spectra were obtained on a DuPont-CEC 21-492 instrument at the Rockefeller University Mass Spectrometry Laboratory. Microanalysis was performed at Rockefeller University's Microanalytical Laboratory. Naloxone, naltrexone and oxymorphone were a generous gift from Endo Laboratories. ^{3}H-Labeled [D-Ala^2,Met^5]enkephalinamide and Formula 963 Scintillator fluor were obtained from New England Nuclear and Sprague–Dawley rats and ICR/CD1 mice from Charles River.

Naloxazone (Ia). Naloxone (1.0 g) was slowly added to anhydrous hydrazine (4.4 mL, Eastman) dissolved in absolute ethanol (6 mL). The solution was stirred at room temperature for 90 min, after which time it was added carefully to a 5% sodium borate solution. The aqueous solution was extracted three times with chloroform and the combined organic extracts were then back-washed with 5% sodium borate solution, dried over anhydrous sodium sulfate, and concentrated by evaporation in vacuo. Petroleum ether (30–60 °C) was added to the residue and the solution was allowed to stand while the product crystallized: yield 50%; mp 161–163 °C; IR ($CHCl_3$) ν_{max} 3300 (br) 1650, 1620, 1460 cm^{-1}; NMR ($CDCl_3$) δ 6.4–6.8 (2 H), 4.9–5.3 (m, 3 H), 4.80 (s, H_5); MS (chemical ionization) intense M + 1 = 342. Anal. Calcd for $C_{19}H_{23}O_3N_3$: C, 66.84; H, 6.79; O, 14.06; N, 12.31. Found: C, 66.61; H, 6.90; O, 14.36; N, 12.13.

Naltrexazone (Ib). The procedure used in the synthesis of Ia was followed using naltrexone (1.0 g): yield of product 55%; mp 157–160 °C; IR ($CHCl_3$) no carbonyl absorption; NMR ($CDCl_3$) δ 6.4–6.8 (2 H), 4.81 (s, H_5), 0.5–0.9 (5 H, cyclopropyl); MS M + 1 = 356. Anal. Calcd for $C_{20}H_{25}O_3N_3$: C, 67.58; H, 7.09; N, 11.82; O, 13.51. Found: C, 67.85; H, 7.17; N, 11.53; O, 13.45.

Oxymorphazone (Ic). A procedure similar to the one employed in the synthesis of Ia using oxymorphone (250 mg) gave a 40% yield of Ic: mp 135–138 °C; IR (Nujol) no carbonyl absorption; NMR (methanol-d_4) δ 6.6 (s, 2 H), 4.79 (s, H_5), 2.35 (s, 3 H); MS M + 1 = 316. Anal. Calcd for $C_{17}H_{21}O_3N_3$: C, 64.74; H, 6.71; N, 13.33; O, 15.22. Found: C, 64.56; H, 6.67; N, 13.25; O, 15.52.

Pharmacology. All drugs given in vivo were dissolved in water (10 mg/mL) with glacial acetic acid and injected subcutaneously on the back of the neck 15–20 min later. Lethality from the hydrazones (200 mg/kg) after 24 h was less than 15%.

Binding Assays. Brain homogenates were prepared and receptor binding was performed with [^{3}H]naltrexone or ^{3}H-labeled [D-Ala^2,Met^5]enkephalinamide as previously described.[22] In brief, brains were homogenized in 50 mM Tris buffer (pH 7.7 at 25 °C) and extensively washed to remove any endogenous or exogenous opioids. The standard wash procedure included: (1) centrifugation, 40000g × 15 min; (2) resuspension, 50 vol (w/v); (3) incubation at 37 °C for 30 min; (4) centrifugation as before; (5) resuspension and assay. Assays were performed in triplicate on 2-mL aliquots in the presence and absence of levallorphan (1 μM) at 25 °C for 30 min. All values represent specific binding (binding in the absence minus binding in the presence of levallorphan) and are reported as the mean ± SEM. All experiments have been replicated three times. Student's t test was used to examine statistical significance.

Tail-Flick Assay. Analgesia was evaluated with the tail-flick assay as described previously.[23] Base-line latencies were determined for each mouse and compared to the latency for the same mouse after administration of opiate. Analgesia is defined as a latency at least twice the base-line latency. Oxymorphazone and oxymorphone were tested by comparing the latencies 18–20 h after administration to base-line levels prior to the drug. Naltrexazone and naloxazone effects on morphine analgesia were tested by establishing a base-line latency 18–20 h after naloxazone, naloxone, naltrexazone, naltrexone, or saline treatment, injecting morphine sulfate (12 mg/kg ip), and comparing the latency 20 min later to the base-line values. Analgesia was defined as an increase in latency equal to the base line. Fisher's exact test was used to examine the statistical significance between treatments.

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