Opioid Agonist and Antagonist Activities of Monofunctional Nitrogen Mustard Analogues of β-Chlornaltrexamine

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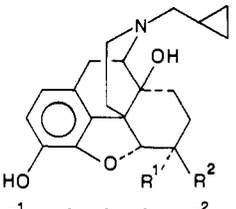
In an effort to compare the role of a monofunctional nitrogen mustard with that of its bifunctional counterpart (i.e., β-CNA, **1b**) in modulating nonequilibrium activity of opioid receptors, we have synthesized and tested *N*-(2-chloroethyl)-*N*-methylamino analogues **2a** and **2b**. Compound **2b** and β-CNA (**1b**) possessed qualitatively similar pharmacologic profiles on the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. Moreover, the corresponding epimer **2a** behaved somewhat like that reported for α-CNA (**1a**) in that it possessed irreversible agonist activity in the GPI. The similar pharmacologic profiles of the monofunctional and bifunctional nitrogen mustards suggest that possible cross-linking of receptor nucleophiles by the latter is not critical for activity. In addition, the results are consistent with the idea that the rank-order nonequilibrium activity of **2b** at different opioid receptor types is related to its relative affinity at those sites rather than to the alkylation step.

The affinity label β-chlornaltrexamine (β-CNA, **1b**)[1,2] is a nonequilibrium opioid antagonist and is employed widely as a pharmacologic tool.[3] This ligand covalently binds to at least three opioid receptor types (μ, κ, δ) in vitro and in vivo. In this regard it irreversibly blocks the effects of opioids on smooth muscle preparations and produces ultralong-lasting antagonism of opioid-induced analgesia in vivo.

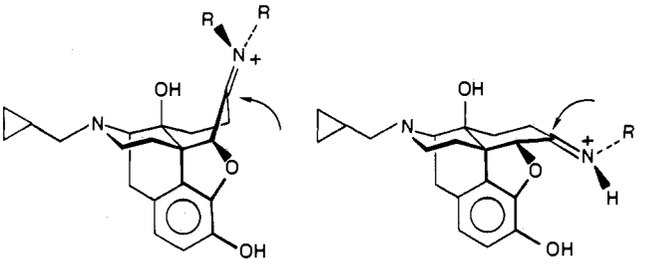
These properties have prompted us to investigate whether or not a monofunctional nitrogen mustard analogue possesses a different activity profile. This was of interest because of the potential of the bifunctional nitrogen mustard group for cross-linking of receptor nucleophiles. In this paper we describe the synthesis and biological activity of such a monofunctional analogue **2b** and its C-6 epimer **2a**.



1a, $R^1 = N(CH_2CH_2Cl)_2$, $R^2 = H$
b, $R^1 = H$, $R^2 = N(CH_2CH_2Cl)_2$
2a, $R^1 = N(CH_3)CH_2CH_2Cl$, $R^2 = H$
b, $R^1 = H$, $R^2 = N(CH_3)CH_2CH_2Cl$

Chemistry

The target compound **2b** and its epimer **2a** were synthesized from naltrexone (**3**) as outlined in Scheme I. The iminium intermediates **4** and **5** were formed from **3** and the appropriate amine by azeotropic removal of water. These intermediates were then reduced in situ to afford the amines **6** and **7b**. The stereochemistry of **7b** derived from sodium cyanoborohydride reduction of **4** is in accord with the steric course of hydride addition to other disubstituted iminium opiate intermediates.[4] Presumably, this is a consequence of pseudoallylic strain[5] forcing the C ring into a boat conformation **8** that facilitates access of hydride to the α side. Because of the absence of pseudoallylic strain in monosubstituted iminium intermediate **5**, the stereochemistry of the hydride addition product is α (**6**). In this case, the C ring has been postulated to be in a chairlike conformation **9** that is more accessible to the reducing agent on its β face. Reductive methylation of **6**



8 **9**

using formaldehyde and sodium borohydride afforded intermediate **7a**.

Replacement of the primary hydroxyl groups of **7a** and **7b** with chlorine was accomplished by the Wiley reaction[6] with either triphenylphosphine or trioctylphosphine.

Pharmacological Results

The activities of the reference compound, β-chlornaltrexamine, and the monofunctional nitrogen mustards **2a** and **2b** are summarized in Tables I and II. These compounds were evaluated for agonist and antagonist activities on the electrically stimulated guinea pig ileum longitudinal muscle[7] (GPI) and the mouse vas deferens[8] (MVD) preparations. All compounds were tested for their agonist and irreversible antagonist activities. In regard to the latter, this was accomplished by first determining the concentration–response curves for standard agonists, morphine, ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin (DADLE). The preparations were incubated for 30 min with the nitrogen mustard (20 nM). This was followed by thorough washing (20 times) and redetermining the activity of the standard agonists.

On the GPI **2b** behaved initially as a full agonist. Over the 30-min incubation period, however, the agonist response disappeared and the electrically stimulated muscle twitch returned to its control level. The activity profile of β-CNA was somewhat similar in that it also displayed initial agonism followed by sustained antagonism. How-

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Scheme I

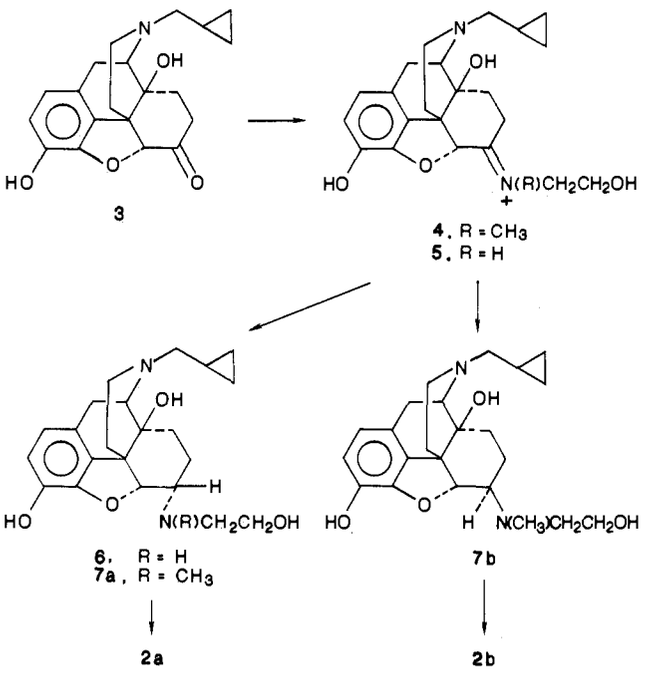

Table I. Opioid Agonist Activities of β-Chloroethylamines on the GPI and MVD

compd	potency ratio (GPI)[a]	IC_{20} (MVD),[b] nM
1b	*c*	*c*
2b	5.3 ± 3.2	10
2a	159 ± 64	3

[a] Relative to morphine = 1.0 (morphine IC_{50} = 0.29 ± 0.07 μM). [b] Partial agonists with maximum responses of 30–40% and the IC_{20} values are presented. [c] Transient agonism (<5 min) observed at 20 nM in the GPI and 200 nM in the MVD.

Table II. Irreversible Antagonist Activities of β-Chloroethylamines on the GPI and MVD

	IC_{50} ratio[a]		
	GPI		
compd	morphine	EK	MVD, DADLE
1b (β-CNA)	11.1 ± 1.1[b]	12.7 ± 1.5	10[c]
2b	48.8 ± 10.6[b]	17.3 ± 2.4	2.9 ± 0.7
2a	*d*	*d*	5.0 ± 2.2

[a] IC_{50} ratio = IC_{50} after antagonist treatment/control IC_{50}. All values are means of at least three determinations. [b] IC_{20} ratio reported due to partial agonist activity of morphine after exposure to antagonist. [c] IC_{50} ratio for leucine-enkephalin rather than DADLE (ref 11). [d] Irreversible agonist activity precluded assessment of antagonist activity.

ever, its agonism was of a transient nature by comparison, with a duration of a 3–5-min period. In contrast to **2b**, its epimer **2a** showed irreversible agonist activity that could not be reversed by naloxone after incubation. The initial agonist activity was 30 times greater than that of **2b**.

It was found that 30-min exposure of the GPI to **2b** irreversibly inhibited the agonist effect of morphine. This was characterized by a shifting of the concentration–response curve to higher concentration and a lowering of the maximum response. Under identical conditions the EK concentration–response curve was shifted in a parallel fashion to the right by a factor of 17. By comparison, β-CNA appeared to be somewhat less potent in its ability to antagonize the effect of morphine and more potent with respect to the blockage of EK; the antagonism of both agonists by β-CNA was irreversible.

On the MVD, both epimers displayed partial agonist activity, with **2b** somewhat less potent than **2a**. β-CNA produced a transient agonist effect of short duration (less than 2 min). Both of the nitrogen mustard compounds (**2a**, **2b**) shifted the concentration–response curves of DADLE in a parallel fashion to higher concentrations by factors of 3–5.

Discussion

The monofunctional nitrogen mustard **2b** displayed an antagonist profile that was qualitatively similar to that of its bifunctional analogue, β-CNA, in that it irreverisbly blocked the agonist effect of morphine, EK, and DADLE on the smooth muscle preparations. Since these ligands posses μ, κ, and δ selectivity, respectively, the data suggests that **2b** may covalently bind to these opioid receptor types. In this regard the rank-order blockage of these receptor types is identical with that of β-CNA.[3]

The fact that all three opioid receptor types are irreversibly blocked is a consequence of the high reactivity of the aziridinium intermediate derived from the nitrogen mustard compounds. However, rank-order irreversible activity is probably related to the affinity of the nitrogen mustard for the different receptor types.[9] This is in contrast to affinity labels with less reactive electrophiles where covalent selectivity is determined by the position of a neighboring nucleophile on the receptor (secondary recognition).[10]

A notable difference between **2b** and β-CNA was the duration of initial agonism upon incubation with the GPI. While this was transient with β-CNA, this agonism took 30 min to disappear in the case of **2b**. The exact nature of the agonism is not certain, but it is possible that it may arise from reversible complexation of opioid receptors, whereupon subsequent alkylation affords antagonism. The shorter duration of agonism of β-CNA may be related to its apparently greater ability to irreversibly block the effect of EK at κ receptors.

Of interest is the observation that **2a** behaves quite differently from its epimer **2b** in the GPI. In this regard **2a** exhibited highly potent agonism that was not reversed through washing or attempted naloxone displacement. A similar profile was reported for the β-CNA epimer, **1a**, where κ receptors were implicated in this effect.[11]

The similar pharmacologic profiles of **2b** and β-CNA indicate that the bifunctional nature of β-CNA is not of critical importance in the reversible blockage of opioid receptors. Moreover the data support the idea that primary recognition (rather than a second recognition step)[10] is the determining factor in the alkylation of opioid receptor types with reactive electrophiles such as aziridinium.

Experimental Section

General Procedures. Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and were within ±0.4% of the theoretical values. IR spectra were obtained on a Perkin-Elmer 281 instrument. NMR spectra were recorded at ambient temperature on Varian A-60, T-60, FT-80 or JEOL FX 90Q instruments using tetramethylsilane as an internal standard. Optical rotations were determined with a 1-dm cell on a Perkin-Elmer 141 polarimeter. Mass spectra were determined on AE 1 MS-30 or Finnegan 4000 instruments. Electron-ionization mass spectra

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were conducted at 20 or 70 eV, and chemical-ionization mass spectra were conducted with either ammonia or methane as reagent gas. All TLC data were determined with Eastman "Chromagram" 13181 plastic-backed sheets (silica gel) with fluorescent indicator. Unless otherwise stated, all reagents and solvents were reagent grade and were used without prior purification. All chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI. Gifts of drugs that were gratefully received were naltrexone hydrochloride, from the National Institute on Drug Abuse, Rockville, MD. Hydrochloride salts were precipitated from solutions of free bases in nonpolar solvents by the addition of ethanolic HCl, followed by filtering and drying.

17-(Cyclopropylmethyl)-4,5α-epoxy-6β-[*N*-(2-hydroxyethyl)-*N*-methylamino]morphinan-3,14-diol (7b). To a solution of naltrexone (**3**; 4.49 g, 13.18 mmol) and 2-(methylamino)ethanol (2.97 g, 19.5 mmol, 3.17 mL) in benzene (100 mL) was added benzoic acid (6.44 g, 52.72 mmol). The solution was refluxed 24 h with a Dean–Stark trap for azeotropic removal of water to form the iminium benzoate **4**. The benzene was removed under vacuum and the residue was taken up in toluene (100 mL). The toluene solution was added to a stirred solution of $NaCNBH_3$ (0.91 g, 14.5 mmol) in methanol (100 mL) over 15 g of molecular sieves (3 Å). The reaction was allowed to proceed for 15 h without stirring and was filtered, quenched with water (20 mL), and evaporated to dryness. The residue was dissolved in water (100 mL), basified with NH_4OH, and extracted with $CHCl_3$ (3 × 150 mL). The $CHCl_3$ extracts were pooled, dried (Na_2SO_4), and filtered. Removal of solvent in vacuo left 5.22 g of crude product, which was crystallized from ethyl acetate to yield 1.63 g (31%) of **7b**: mp 221–223 °C dec; $[\alpha]^{25}_D$ −192.2° (*c* 1.0, MeOH); TLC, R_f 0.38 (EtOAc/MeOH/NH_4OH, 90:10:3); NMR ($CDCl_3$) δ 6.19–6.50 (2 d, 1 H each, Ar *H*), 4.48 (d, *J* = 8.35 Hz, C-5 *H*), 2.4 (s, 3 H, NCH_3); EIMS, *m/e* 400 (M^+). Anal. ($C_{23}H_{32}O_4N_2$) C, H, N.

6β-[*N*-(2-Chloroethyl)-*N*-methylamino]-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol Dihydrochloride (2b·2HCl). To a stirred slurry of **7b**·2HCl (1.82 g, 3.85 mmol) in acetonitrile (50 mL) over 3-Å molecular sieves (5 g) were added triphenylphosphine (5.04 g, 19.25 mmol) and carbon tetrachloride (56 mL). The reaction mixture was stirred at 25 °C under nitrogen for 12 h. The mixture was decanted from the molecular sieves, and the solvent was removed in vacuo. The solid residue was digested by stirring in benzene, dissolved in water (50 mL), and washed with $CHCl_3$ (3 × 50 mL) to remove triphenylphosphine oxide. The aqueous solution was evaporated in vacuo to leave crude **2b**·2HCl (2.35 g) contaminated with triphenylphosphine oxide. The crude product was purified with 87 g of dry column grade silica gel and 17 mL of ethyl ether/ammonium hydroxide (99:1) which was equilibrated for 1.5 h before packing. Crude **2b**·2HCl (290 mg) was deposited onto a minimum amount of cellulose and loaded onto the top of the column. The column was eluted, and fractions containing pure **2b** were pooled. A volume of toluene 50% that of the eluate was added, followed by removal of NH_4OH and most of the solvent in vacuo. Bubbling HCl gas into the remaining solution resulted in the precipitation of **2b**·2HCl. Removal of the remaining toluene in vacuo afforded 23 mg (10%) of **2b**·2HCl: mp 260–270 °C; $[\alpha]^{23}_D$ −67° (*c* 0.5, MeOH); TLC, R_f 0.72 (EtOAc/MeOH/NH_4OH, 90:10:3); NMR ($CDCl_3$, free base) δ 6.75–6.47 (2 d, 1 H each, Ar *H*), 4.54 (d, *J* = 7.91 Hz, C-5 *H*), 2.41 (s, 3 H, NCH_3); EIMS, *m/e* 418 (M^+). Anal. ($C_{23}H_{31}O_3N_2Cl$·2HCl) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-6α-[*N*-(2-hydroxyethyl)amino]morphinan-3,14-diol (6). A solution of naltrexone (**3**) (4.5 g, 13.2 mmol), ethanolamine (1.26 mL, 1.21 g, 19.8 mmol), and a catalytic amount of toluenesulfonic acid, in toluene (150 mL), was refluxed 20 h in a flask connected to a Dean–Stark trap for azeotropic removal of water. The resulting solution of imine **5** was concentrated to a volume of 15 mL in vacuo. To this solution was added absolute ethanol (50 mL) and sodium borohydride (0.25 g, 6.9 mmol). The mixture was stirred for 7 h at 25 °C, after which TLC indicated that the reaction had ceased at about 75% completion. The mixture was acidified to pH 5 with ethanolic HCl, and additional sodium borohydride (0.17 g, 4.6 mmol) was added. After 12 h of reaction, the reaction was quenched by the addition of water (50 mL), and the ethanol was evaporated in vacuo. The remaining aqueous solution was basified with NH_4OH and extracted with CH_2Cl_2 (3 × 75 mL). The organic extracts were pooled, dried (Na_2SO_4), filtered, and evaporated to leave a residue, which was taken up into absolute ethanol and crystallized to yield 3 g (59%) of **6**. Recrystallization from ethyl acetate and methanol afforded pure **6**: mp 213–215 °C; $[\alpha]^{22}_D$ −230° (*c* 1.0, MeOH); TLC, R_f 0.38 (EtOAc/MeOH/NH_4OH, 80:20:5); NMR (Me_2SO-d_6) δ 6.55–6.36 (2 d, 1 H each, Ar *H*), 4.46 (d, *J* = 3.6 Hz, C-5 *H*); EIMS, *m/e* 386 (M^+). Anal. ($C_{22}H_{30}O_4N_2$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-6α-[*N*-(2-hydroxyethyl)-*N*-methylamino]morphinan-3,14-diol (7a). To a stirred solution of **6**·2HCl (0.461 g, 1.01 mmol) in absolute ethanol (5.2 mL) were added 2.6 mL of aqueous 37% formaldehyde (32 mmol) and sodium borohydride (0.36 g, 9.5 mmol) at 0 °C. When addition was complete, the temperature was raised to 25 °C and the mixture was allowed to stand for 12 h. Ethanolic HCl was added dropwise to bring the reaction mixture to pH 7. Additional formaldehyde (1.3 mL, 16 mmol) and sodium borohydride (0.18 g, 4.75 mmol) were added to the mixture, and the reaction was continued for 2 h at 25 °C and then for 1 h at 40 °C. Water was added and the ethanol then was removed in vacuo. The remaining aqueous solution was extracted with ethyl acetate (4×). The organic extracts were pooled and washed with brine. Removal of solvent in vacuo left 0.38 g (91%) of crude **7a**. Crystallization from ethyl acetate/methanol followed by recrystallization from diethyl ether gave pure **7a** (251 mg, 62%): mp 143–145 °C; $[\alpha]^{22}_D$ −229° (*c* 1.0, MeOH); TLC, R_f 0.32 (EtOAc/MeOH/NH_4OH, 90:10:3); NMR (Me_2SO-d_6) δ 6.62–6.35 (2 d, 1 H each, Ar *H*), 4.65 (d, *J* = 3.2 Hz, C-5 *H*); EIMS, *m/e* 400 (M^+). Anal. ($C_{23}H_{32}N_2O_4$) C, H, N.

6α-[*N*-(2-Chloroethyl)-*N*-methylamino]-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3,14-diol Dihydrochloride (2a·2HCl). To **7a**·2HCl (0.118 g, 0.25 mmol) in dry acetonitrile (10 mL) were added carbon tetrachloride (0.38 g, 2.5 mmol) and trioctylphosphine (0.37 g, 1 mmol) at 23 °C. The mixture was heated to 41 °C for 10 h, after which additional carbon tetrachloride (0.5 mL, 5.2 mmol) and trioctylphosphine (0.5 g, 1.7 mmol) were added. The reaction was continued for 6 h at 41 °C, after which the solvents were evaporated to leave a viscous oil. Carbon tetrachloride (1 mL, 10.4 mmol) was added and the solution was stirred for 3 h at 23 °C. The mixture was added dropwise to ethyl ether (30 mL) and the resulting precipitate was separated by centrifugation. Dissolution in ethanolic HCl (1 mL) and reprecipitation from ether (3×) afforded 67 mg (55%) of **2a**·2HCl·H_2O as an amorphous solid: mp 210 °C (dec without melting); $[\alpha]^{26}_D$ −179.6° (*c* 1.0, MeOH); TLC, R_f 0.88 (ethyl ether/MeOH/NH_4OH, 90:10:2); NMR (free base, $CDCl_3$) δ 6.75–6.45 (2 d, 1 H each, Ar *H*), 4.82 (d, *J* = 3.6 Hz, C-5 *H*), 2.52 (s, 3 H, NCH_3); EIMS, *m/e* 418 (M^+). Anal. ($C_{23}H_{31}O_3N_2Cl$·2HCl·H_2O) C, H, N, Cl.

Acknowledgment. This work was supported by the National Institute on Drug Abuse. We thank Victoria Darrow Elliott for the capable technical assistance.