PROBES FOR NARCOTIC RECEPTOR MEDIATED PHENOMENA 8. TRITIATION OF IRREVERSIBLE MU OR DELTA SPECIFIC OPIOID RECEPTOR AFFINITY LIGANDS TO HIGH SPECIFIC ACTIVITY[1]

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SUMMARY

Tritiation of the delta-specific opioid alkylating ligand FIT, 3 and the mu-specific opioid alkylating ligand BIT, 9 to high specific activity is described. Both compounds were derived from dibrominated amino intermediates (4 and 10, respectively) which were tritiated with tritium gas over Pd/C before conversion of the amine functions to isothiocyanates.

Key words: fentanyl, FIT, BIT, alkylator, opioid receptor, tritium.

INTRODUCTION

Since the discovery of opioid receptors in the mammalian central nervous system (1) and the subsequent demonstration of endogenous opioid ligands for these receptors (2), great effort has been devoted toward understanding the structure and function of these receptors. Early *in vivo* work by Martin (3) led him to hypothesize the existence of several opioid receptor subtypes, and subsequent data, based mainly on differential binding affinity of various ligands, has supported this hypothesis. At present, at least four receptor subtypes, designated mu, delta, kappa and sigma have been postulated and the mu and delta subtypes predominate in the rat brain (4).

[1]For paper 7 in this series, see Burke, T. R., Jr., Bajwa, B. S., Jacobson, A. E., Rice, K. C., Streaty, R. A. and Klee, W. A.-J. Med. Chem., in press, 1984.

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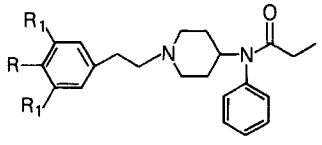
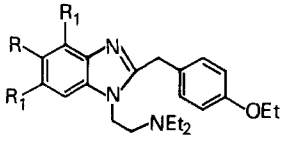
Efforts of several laboratories have also been directed toward the isolation and purification of these receptors. Radiolabeled ligands which bind with high affinity and specificity to the desired receptor subtype are valuable aids for monitoring the receptor during the purification process. Several opioid ligands have been reported which bind irreversibly to opioid receptors. In some cases, specificity for the given receptor subtype has been demonstrated (5-8).

In our recent report on a series of irreversible opioid alkylating agents (6,7) two compounds, 3 and 9 showed high specificity and affinity for the delta-receptor and the mu-receptor, respectively with 3 being the first irreversible opioid alkylating agent having delta-specificity. The delta-specific ligand 3 was derived from the 4-anilidopiperidine skeleton of the potent opioid analgesic fentanyl (1), while the mu-specific ligand 9 was derived from the benzimidazole structure of etonitazene (7). Both fentanyl and etonitazene are extremely potent opioids *in vivo*, and show high specificity for mu-receptors *in vitro* (7). In both cases the alkylating moiety is the isothiocyanate group (N=C=S) which was utilized because of its





1: R = H, R_1 = H
2: R = NH_2, R_1 = H
3: R = NCS, R_1 = H
4: R = NH_2, R_1 = Br
5: R = NH_2, R_1 = ^3H
6: R = NCS, R_1 = ^3H

7: R = NO_2, R_1 = H
8: R = NH_2, R_1 = H
9: R = NCS, R_1 = H
10: R = NH_2, R_1 = Br
11: R = NH_2, R_1 = ^3H
12: R = NCS, R_1 = ^3H

high reactivity with primary and secondary amino groups (complete reaction within minutes) compared with its reduced reactivity with hydroxyl groups (6,9).

In this report, we describe the synthesis of the delta-specific fentanyl isothiocyanate (FIT, 6) and the mu-specific benzimidazole isothiocyanate (BIT, 12) with incorporation of tritium in high specific activity. The use of the delta-specific [^{3}H]-FIT in the labeling and identification of a component of this receptor with an apparent molecular weight of 58,000 has recently been reported (10).

RESULTS AND DISCUSSION

The introduction of tritium to form both the aminofentanyl derivative 5 and the aminobenzimidazole derivative 11 relied upon tritiation of the corresponding dibromo compounds 4 and 10, respectively. The synthesis of the necessary dibromo intermediates was accomplished by reaction of the appropriate unsubstituted amines, 2 and 8 (6) with excess bromine in acetic acid.

Addition of 3 equivalents of bromine to a solution of 2•HCl in acetic acid gave, after work up, a 66% yield of 4•HCl. The positions of bromine substitution were determined from mass spectral fragmentation patterns [an isotopic triplet of peaks was present centered at m/e 264 ($NH_2Br_2C_6H_2CH_2$) and 278 ($NH_2Br_2C_6H_2CH_2CH_2$)] and by comparision of NMR aromatic patterns with unsubstituted 2. (The protons of the propionanalide ring system remained essentially unchanged while the two doublets at δ 6.92 and δ 6.57 of the 4-aminophenylethyl ring system in 2 collapsed to a singlet, representing two protons at δ 7.15 in the dibromo compound 4.) Tritiation of a MeOH solution of 4•HCl over 10% Pd/C under an atmosphere of tritium gas gave crude 5, which was purified by TLC.

Synthesis of benzimidazole 11 followed a similar course. Reaction of the methanesulfonate salt of 8 in acetic acid with eight equivalents of bromine gave dibromo compound 10•HCl in 26% yield. The postions of bromine

substitution were determined from the NMR aromatic pattern. The two ortho-coupled doublets of the ethoxyphenyl ring were present, while a singlet at δ 8.27 represented the proton at the 7-position of the benzimidazole ring. Tritiation with tritium gas over 10% Pd/C gave crude amine 11 which was purified by TLC.

Amines 5 and 11 were converted to the corresponding isothiocyanates 6 and 12, respectively, by reaction with thiophosgene in a biphasic chloroform-bicarbonate system. The crude reaction mixtures were purified by TLC to yield fentanyl isothiocyanate 6 with 99.9% radiochemical purity and a specific activity of 27.4 Ci/mmol, and benzimidazole isothiocyanate 12 with 95% radiochemical purity and 16.3 Ci/mmol specific activity. In the case of 12, it was essential to avoid a larger excess of thiophosgene, since in several early experiments when such an excess was employed little, if any, 12 was detected. Degradation of other nitrogenous heterocyclic ring systems is well documented (11) and it may be that similar degradation also occurs with benzimidazoles such as 11 and 12.

EXPERIMENTAL SECTION

Melting points were determined on a Fischer-Johns apparatus and are corrected. NMR spectra were recorded using a Varian 220 MHZ spectrometer with $Si(CH_3)_4$ as the internal reference. IR Spectra were recorded on a Beckman IR 4230. Electron ionization mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6E spectrometer (70eV), and accurate mass spectra were obtained using a VG Micromass 7070F spectrometer. HPLC analysis were performed using a Waters Associates Model 6000A pump equipped with a Model 660 solvent programmer, a Model 450 variable wavelength detector and a Waters μ- Porasil silica gel column (3.9 cm x 30 cm). Liquid scintillation spectrophotometry was performed using a Beckman LS-250 liquid scintillation spectrophotometer with 12 mL of either Hydrofluor or Aquasol scintillation cocktail. A counting efficiency of 45% was determined from a reference

^{3}H-standard of known activity. UV spectra were recorded on a Cary Model 15 spectrometer. Thin layer chromatography was run on EM No. 5538 aluminum backed silica gel plates (200 μ, no fluorescent indicator) except where indicated, and photoradiography was done with Kodak XAR-2 film in an X-Omatic regular cassette (Kodak) with image intensifying screen. Combustion analyses were performed by the Section on Microanalytical Services and Instrumentation, NIADDK.

N-Phenyl-N-(1-[2-(4-amino-3,5-dibromophenyl)ethyl]-4-piperidinyl)-propionamide hydrochloride (4·HCl). A solution of bromine (590 mg, 3.69 mmol) in acetic acid (2 mL) was added over two min to a stirring solution of 2·HCl (6) (500 mg, 1.23 mmol) in acetic acid (15 mL). After 30 min, the supernatant was decanted, leaving a residue of orange syrup. Trituration of the syrup with ether (50 mL) gave a yellow solid (840 mg). Crystallization from warm 2-propanol (20 mL) gave a white salt (480 mg). This was partitioned between 1 N NaOH (20 mL) and CH_2Cl_2 (2 x 50 mL), and the organic solution was dried ($MgSO_4$) and evaporated to an oil. Ethereal HCl was added to a methanolic solution of the oil followed by removal of solvent to give 4·HCl (430 mg, 66%): mp 129-133°C; EIMS: 262, 264, 266 ($NH_2Br_2C_6H_2CH_2CH_2$), 276, 278, 280 ($NH_2Br_2C_6H_2CH_2$), 507, 509, 511 (M^+); NMR ($CDCl_3$) δ 7.45-7.34 (m, 3H), 7.16 (s, 2H), 7.11-7.02 (m, 2H), 4.75-4.54 (m, 1H), 4.40 (s, 2H), 3.02-2.89 (m, 2H), 2.64-2.39 (m, 4H), 2.25-2.07 (m, 2H), 1.98-1.73 (m, 4H), 1.52-1.30 (m, 2H), 1.00 (t, 3H, J = 7 Hz).

Analysis: Calcd. for $C_{22}H_{27}Br_2N_3O·HCl·H_2O$: C, 46.87; H, 5.36; N, 7.45. Found: C, 46.48; H, 5.62, N, 7.43.

N-Phenyl-N-(1-[2-(4-isothiocyanatophenyl-3,5-$^{3}H_2$)ethyl]4-piperidinyl)propanamide (6). A solution of 4·HCl (10 mg) in MeOH (500 μL) was tritiated for 2.5 h over 10% Pd/C (10 mg) under an atmosphere of 25 Ci of carrier free tritium gas. Tritiation was performed at the New England Nuclear Corp., 549 Albany St., Boston, Mass., 02118. Labile tritium was removed in vacuo, and the

residue was taken up in MeOH, filtered, reevaporated to dryness in vacuo and then dissolved in MeOH (10 mL) to yield 470 mCi of a solution of impure 5•HX(X = Cl, Br) with an activity of 47 mCi/mg of dissolved solid.

A portion of this solution (25 mCi) was evaporated to dryness under nitrogen and then partitioned between saturated aqueous $NaHCO_3$(500 μL) and $CHCl_3$ (2 x 500 μL). The $CHCl_3$ was reduced in volume under nitrogen and applied to a Whatman Silica Gel 80 plate (250 μ; 20 x 20 cm) and eluted with $CHCl_3$-MeOH (9:1). Fourteen bands (approximately 1 cm wide) were scraped off progressively from the origin to the solvent front and each band was mixed with $CHCl_3$-MeOH (9:1) (1 mL). An aliquot of each mixture was subjected to liquid scintillation spectrophotometry. Bands 9-10, representing 79% of the total eluted radioactivity were extracted with three additional aliquots (3 x 1 mL). The total extracts were combined to yield 5, (4 mCi, 16% radiochemical yield) which was used for the next step.

The solution of 5 was evaporated to dryness in vacuo, transfered to a 500 μL Wheaton reactivial using $CHCl_3$ (3 x 100 μL) and evaporated to dryness under a stream of nitrogen. The residue was dissolved in $CHCl_3$ (50 μL) and stirred with saturated aqueous $NaHCO_3$ (50 μL). Redistilled thiophosgene (2 μL) was added and the vial was then sealed and stirred at 20°C. At 60 min an additional 2 μL and at 90 min an additional 10 μL of thiophosgene were added. With TLC monitoring at approximately 30 min intervals, the reaction appeared to be complete after 100 min. The $CHCl_3$ layer was removed after 2 h, combined with $CHCl_3$ extracts of the aqueous layer (2 x 100 μL), reduced in volume under N_2, and applied to a 2 cm x 8 cm aluminum backed TLC plate. After developing ($CHCl_3$-MeOH, 9:1), the plate was cut into 8 x 1 cm bands; each band being eluted in MeOH (5 mL). A portion of each band was examined by liquid scintillation spectrophotometry. Bands 2-3, 0.5 mCi (accounting for 24% of the total eluted activity), represented a by-product of unknown composition. Bands 5-6, 0.8 mCi accounting for 42% of the total eluted radioactivity), represented

product 6. The MeOH washings from bands 5-6 were pooled to yield 6, (0.8 mCi, 20% radiochemical yield) which was stored at -20°C.

An aliquot of 6 was cochromatographed with 3 on HPLC (hexane:2-propanol, 8:2; 2 mL/min; linear gradient from 20% to 100% 2-propanol, over 10 min beginning at 16 min). Fractions were collected at 0.5 min intervals and subjected to liquid scintillation spectrophotometry. Ultraviolet absorption of eluent was recorded at 280 nm. Greater than 99.9% of eluted radioactivity chromatographed with standard 3, (fractions 9-13) indicating that 6 was greater than 99.9% radiochemically pure.

Specific activity was determined from the UV absorption of the HPLC eluate at 257 nm following injection of an aliquot of 6 (240 μCi). The UV was compared to a linear curve obtained from plotting the UV absorption of HPLC eluate for a series of known amounts of 3. This indicated that the 240 μCi represented 8.75 nmol of compound, with a specific activity of 27.4 Ci/mmol.

5-Amino-4,6-dibromo-2-(4-ethoxybenzyl)-1-diethylaminoethylbenzimidazole hydrochloride (10·HCl). A solution of 8·CH_3SO_3H (6) (366 mg, 1.0 mmol) in acetic acid (20 mL) was stirred at 20°C while bromine (160 mg, 1.0 mmol) was added affording a colorless solution. An additional 1.13 (7 mmol) of bromine was slowly added. The mixture was stirred for 2 h during which time a yellow solid separated. The mixture was filtered and the filter cake washed first with 2-propanol then ether, yielding a yellow solid (640 mg). The solid was heated with 20% aqueous acetone (50 mL) and the red supernatant decanted leaving a small amount of insoluble residue, which was discarded. The mixture was rendered alkaline by addition of aqueous NaOH, and the mixture was extracted with $CHCl_3$ (2 x 50 mL), dried ($MgSO_4$) and evaporated to give a red oil (308 mg). The oil was dissolved in MeOH, ethereal HCl was added, and the solution decolorized with activated charcoal. Filtration and concentration to 2 mL followed by addition of ether (6 mL) gave white 10·HCl. Recrystallization from MeOH-ether gave 10·HCl (147 mg, 26%): mp 156-159°C; NMR (DMSO-d_6): δ

8.27 (s, 1H), 7.39 (d, 2H, J = 9 Hz), 6.89 (d, 2H, J = 9 Hz), 4.54 (s, 2H), 3.82-3.70 (m, 8H), 3.27-2.98 (m, 6H), 1.27 (t, 3H, J = 7 Hz), 1.18 (t, 6H, J = 7 Hz).

High resolution mass spectrum ($C_{22}H_{28}N_4OBr_2$): 522.0629; Found: 552.0641.

2-(4-Ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanobenzimidazole-4,6-$^3H_2$ (12). A sample of 10·HCl (10 mg) was tritiated with tritium gas as indicated for the dibromo 4 yielding 173 mCi of crude 11 · HX(X=Cl,Br) (17.3 mCi/mg). Crude 11 was dissolved in MeOH and an aliquot (8 mCi) was removed, concentrated under nitrogen, applied to an aluminum backed silica gel TLC plate (8 cm x 12 cm) and developed ($CHCl_3$-MeOH-NH_4OH, 9:1:0.1). A photoradiogram (exposure time of 1 h) indicated one main band and several minor bands. The main band (R_f = 0.6) was cut from the plate and eluted with MeOH (6 mL) to yield 11 (2.8 mCi 35% radiochemical yield). The solvent was removed by rotary evaporation in vacuo and the residue dissolved in $CHCl_3$ (3 x 100 µL), and transfered to a 300 µL reactivial. The solvent was evaporated under nitrogen and the residue redissolved in $CHCl_3$ (20 µL). The $CHCl_3$ solution was stirred with saturated aqueous $NaHCO_3$ (20 µL) in the presence of redistilled thiophosgene (660 nmol; 4 µL of a solution of 10 µL of thiophosgene in 780 µL of $CHCl_3$).

The reaction was discontinued at 35 min and the $CHCl_3$ layer was removed and combined with $CHCl_3$ extracts of the aqueous phase (3 x 50 µL). The solvent was evaporated under nitrogen to a small volume, applied to an aluminum backed TLC plate (1 cm x 10 cm) and eluted ($CHCl_3$:MeOH, 9:1). Photoradiographic visualization of the resulting plate showed 12 as the main band (R_f = 0.85). This was cut from the plate and eluted with MeOH (2 mL) to yield 12 (2.2 mCi, 79% radiochemical yield) which was stored as a solution in MeOH at -20°C.

Radiochemical purity of 12 was determined both by TLC and HPLC. An aliquot of 12 was mixed with a small amount of 9 and developed on TLC

($CHCl_3$-MeOH, 9:1). The plate was then cut into bands, each band was eluted and examined by liquid scintillation spectrophotometry. The position of 9 (R_f = 0.84) was determined by brief exposure to I_2 vapor prior to cutting. A total of 95% of radioactivity coeluted with 9. A slower moving impurity accounting for 3% of the total radioactivity was also present.

A sample of 12 was also cochromatographed with 9 on HPLC (hexane:2-propanol, 3:1; 2 mL/min). Fractions were collected at 0.5 min intervals and optical absorbance was recorded at 280 nm. Subjecting each fraction to liquid scintillation spectrophotometry showed that 98% of total eluted radioactivity cochromatographed with 9 (fractions 4-12). However, the impurity seen on TLC was not resolved under these conditions. It can be concluded that 12 is at least 95% radiochemically pure.

Specific activity was determined by comparing the UV spectrum of 12 (0.34 mCi/mL) with that of 1.0×10^{-5}M solution of 9. The optical density of 12 was 1.57 x the optical density of 9 at 280 mm, indicating that 12 was 1.57×10^{-5}M representing a specific activity of 16.3 Ci/mmol.

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