Probes for Narcotic Receptor Mediated Phenomena 22. (1) Synthesis and Characterization of Optically Pure [^{3}H](+)-4-[(α*R*)-α-((2*S*,5*R*)-4-Propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide, [^{3}H]SNC 121, a Novel High Affinity and Selective Ligand for Delta Opioid Receptors

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SUMMARY

The synthesis of unlabelled and labelled SNC 121, a selective nonpeptide ligand for the delta opioid receptor is reported. [^{3}H]SNC 121 of specific activity of 26.8 Ci/mmol, was synthesized by catalytic tritiation of the optically pure precursor SNC 80.

KEY WORDS: [^{3}H](+)-4-[(α*R*)-α-((2*S*,5*R*)-4-Propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide ([^{3}H] SNC 121); Delta Opioid Receptors; SNC 80; [^{3}H]SNC 121.

INTRODUCTION

It is well established that endogenous peptides (endorphins) interact with multiple populations of opioid receptors which have been classified into three major types, known as mu (μ), delta (δ) and kappa (κ) (2). The opioid receptors are members of the seven-transmembrane spanning domain receptor family and are coupled to adenyl cyclase mediating agonist inhibition of cyclic AMP (cAMP) formation (3). The recent cloning of these receptors and the development of highly selective radiolabelled ligands will facilitate studies at the molecular level and lead to a better understanding of their functional properties.

The discovery of opioid analgesics lacking the undesirable side effects associated with typical μ receptor agonists such as morphine has been a goal of opioid research for many years. It is known that agonists acting at the δ opioid receptor produce analgesia in animal models of pain and they appear to show beneficial modulatory effects including enhancement of potency and efficacy of morphine (4, 5) and limited ability to produce respiratory depression (6) and adverse gastrointestinal effects (7). Most of the ligands used for the characterization of the δ opioid receptor have been peptidic in nature. The efficacy of these compounds is limited due to the inability of these peptides to cross the blood brain barrier and their susceptibility to metabolism

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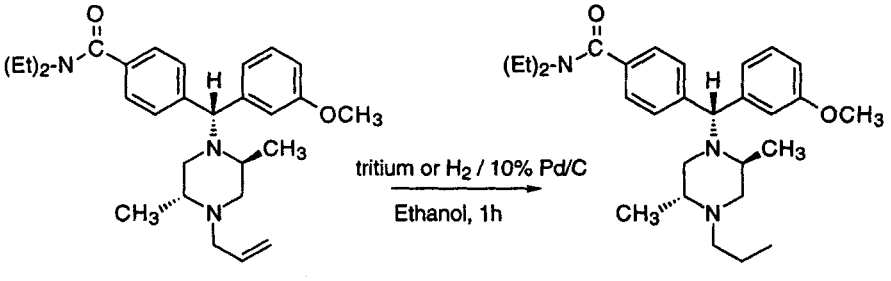
(8). Thus, the development of a nonpeptide, highly selective radiolabelled δ opioid receptor ligand became one of our research goals.

A major lead in this area was provided by the discovery in the Burroughs-Wellcome laboratories of BW373U86, a racemic, nonpeptide agonist with δ opioid selectivity (9). Recently, we reported the synthesis and absolute configuration of the optically pure enantiomers of BW373U86, its benzylic epimers, and their methyl ethers from enantiomeric piperazines (10). The same chiral piperazine we employed for the synthesis of the (+)-enantiomer of BW373U86 was later utilized in a highly diastereoselective synthesis of this material (11). From this first series of compounds, we found that SNC 80 [(+)-1] exhibited a remarkable 2000 fold μ/δ selectivity in both receptor binding and bioassays (10). Based on this lead compound, we synthesized and analyzed the effect of the replacement of the *N*-allyl substitution present in our optically pure precursor with a propyl side chain, which would allowed us to introduce the tritium label in the last step of our synthetic scheme. Since it is very well established that drug enantiomers can show different and in some cases opposite pharmacological effects, optically pure ligands for drug-receptor studies are required (12). The enantiomerically pure tritiated ligand [(+)-2, [^{3}H]SNC 121] has proved to be a valuable tool for study of the structure, function and pharmacological role of the δ opioid receptor (13, 14). Here we describe the synthesis of unlabeled and [^{3}H]labelled SNC 121.

RESULTS AND DISCUSSION

The synthetic route for preparing SNC 121 [(+)-2], is outlined in Scheme 1. The optically pure precursor SNC 80 [(+)-1] was synthesized according to the method previously described by Calderon *et al.* (10). Catalytic hydrogenation of the allylic double bond of SNC 80 over 10% Pd/C for 1 hour afforded the unlabelled SNC 121 in high yield. Longer reaction times resulted in significant amounts of side products corresponding to the hydrogenolysis of the *trans*-2,5-dimethylpiperazine. Thus, catalytic tritiation of (+)-1 over 10% Pd/C with carrier free tritium gas in ethanol, during 1 hour followed by TLC purification afforded [^{3}H]SNC 121 (52.5% radiochemical yield, 26.8 Ci/mmol).

SCHEME 1



(+)-1, SNC 80 **(+)-2**, SNC 121

The lack of nonpeptide ligands with high affinity and selectivity for the δ opioid receptor has hampered an understanding of the pharmacologic and physiologic functions of this receptor. We

wished to synthesize a selective radioligand to address this problem. Experiments with unlabelled SNC 121 indicated that this ligand had high affinity and selectivity (>1000-fold vs. μ receptors) for rat brain δ receptors (13). Tritiated SNC 121 and its nonradioactive analogues have proved to be useful probes at the molecular level in studies using the cloned human δ opioid receptor (14).

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. Combustion analyses were determined at Atlantic Microlabs, Atlanta, GA. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 4600 mass spectrometer. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were performed using a Varian Gemini-300 spectrometer. Chemical shifts are expressed in parts per million on the δ scale relative to TMS internal standard. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), br (broad). Ultraviolet (UV) spectra were recorded using a Hewlett-Packard 8450 and using a Beckman DU-65 UV/VIS spectrophotometer. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 250 microns. Radioactivity determinations were carried out with a Beckman LS-6000 IC liquid scintillation analyzer using hydrofluor scintillation cocktail.

(+)-4-[(α *R*)-α-((2*S*,5*R*)-4-Propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide [(+)-2]. A solution of **(+)-1** (10) (10 mg, 0.022 mmol) in ethanol (1 mL) containing 10% Pd/C (5 mg) was stirred for 1 h at room temperature under an atmosphere of H_2. The solution was filtered and the volatiles were evaporated under reduced pressure to give 9 mg (89%) of **(+)-2** as a white crystalline residue, homogeneous by TLC (solvent: ethyl acetate). This compound was recrystallized from acetonitrile-water, mp 137-138 °C ; ^{1}H-NMR (*CDCl*$_3$) δ *0.85* (t, *J* = *7.3 Hz, 3H*), *0.95* (d, *J* = *6.4 Hz, 3H*), 1.11-1.25 (m, 9H), 1.38-1.51 (m, 2H), 1.84-1.90 (m, 1H), 2.10-2.20 (m, 2H), 2.42-2.48 (m, 1H), 2.55-2.62 (m, 3H), 2.79-2.84 (m, 1H), 3.28 (br s, 2H), 3.53 (br s, 2H), 3.77 (s, 3H). 5.20 (s, 1H), 6.72-6.82 (m, 3H), 7.20-7.30 (m, 3H), 7.47 (d, *J* = 8.1 Hz, 2H); CIMS *m/z* 452 (M+1); [a]$^{22}_D$ +14.92 (*c* 0.68, methanol). Anal. calc for ($C_{28}H_{41}N_3O_2$): C 74.46, H 9.15, N 9.30%; Found: C 74.38, H 9.10, N 9.32%.

[^{3}H]-(+)-4-[(α *R*)-α-((2*S*,5*R*)-4-Propyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide [^{3}H]SNC 121. A solution of **(+)-1**, (12 mg, 0.022 mmol) in ethanol (1 mL) containing 10% Pd/C (5 mg) was stirred for 1 h at room temperature under an atmosphere of carrier free tritium gas at New England Nuclear, Boston, MA. The solution was filtered and the volatiles were evaporated and diluted with ethanol for storage (1535 mCi). A 153.5 mCi (crude product) portion of this in 1 mL of ethanol was used for purification in our laboratory. The pure [^{3}H]SNC 121 was obtained by TLC purification on one 20 cm x 20 cm x 0.5 *mm* plate eluting with ethyl acetate followed by extraction of the band co-migrating with unlabelled **(+)-2** with 20 ml of ethyl acetate. After evaporating the solvent, the residue was reconstituted with 100 ml of absolute ethanol. Liquid scintillation counting indicated a yield of 80.6 mCi of [^{3}H]SNC 121 from 153.5 mCi crude product which corresponds to a 52.5% radiochemical yield, specific activity = 26.8 Ci/mmol (from UV analysis of the solution at λ = 274

nM; ε_{274} = 3172 liter. cm^{-1}. mol^{-1}) and in greater than 95% radiochemical purity as determined by HPLC analysis on a Zorbax TMS column. Since the radiochemical yield is based on the amount of crude material returned from the tritiation, it represents a recovery yield. The mobile phase was a 45 min linear gradient at 1 ml/min from 25% to 100% B where A was 0.1% aqueous trifluoroacetic acid (TFA) and B was 75:0.1:24.9 acetonitrile /TFA/water. Under these conditions [^{3}H]SNC 121 eluted in a single sharp peak at 18 min (13). The compound showed no significant deterioration after several months of storage at -80°C in ethanol at a concentration of 1 mCi/ml.

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