Research Article

Improved Delivery Through Biological Membranes. XXIV. Synthesis, in Vitro Studies, and in Vivo Characterization of Brain-Specific and Sustained Progestin Delivery Systems

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Dihydropyridine ⇋ pyridinium salt-based brain-selective delivery systems were synthesized for the progestins, ethisterone, norethindrone, and norgestrel. After initial lipophilicity and in vitro studies indicated the feasibility of applying these compounds to brain-specific delivery, in vivo distribution studies were performed on one of the redox delivery systems. After systemic administration of the chemical delivery system based on norethindrone, sustained and selective delivery of the oxidized form of the drug–carrier complex was observed in the brain. In addition, a slow and sustained release of the parent steroid, norethindrone, occurred. This release produced substantially higher levels of norethindrone for more prolonged periods than the administration of norethindrone itself.

KEY WORDS: progestins; norethindrone; sustained delivery of drugs; blood–brain barrier.

INTRODUCTION

Progestins (P) are synthetic steroid derivatives that exert progesterone-like activity in the central nervous system (CNS) and in the periphery. Central actions of these agents are important in their estrogen-associated contraceptive effects (2), in their apparent ability to mitigate the severity of premenstrual tension (3), and in their ability to alter behavior (4). Unfortunately, certain progestins, especially those derived from testosterone, exert androgenic, estrogenic, and antithrombin effects as well as progestogenic actions (5). Compounds such as ethisterone (1), norethindrone (2), and norgestrel (3) elicit certain unwanted peripherally manifested effects such as alterations in metabolism, hypertension, and weight gain because of these actions (2). A method for specifically delivering progestins to the brain may be useful in mitigating these peripheral untoward actions. If this specific delivery were sustained, then dosing intervals could be decreased and the amount of progestin administered could be greatly reduced. Such a system could also be a powerful neuroendocrine probe for separating central and peripheral progestogenic actions.

The method chosen to accomplish this delivery is based on a dihydropyridine ⇋ pyridinium salt redox system (6–8). This brain-targeting chemical delivery system (CDS) was recently shown to be capable of delivering the gonadal steroids estradiol (9) and testosterone (10) to the CNS. The major aim in applying the CDS to lipophilic compounds such as steroids, which readily pass the BBB, is to produce sustained levels of these steroids in the CNS. Thus, while the administration of the parent steroid produces significant levels of that steroid in the brain, these levels rapidly fall, causing frequent dosing to maintain therapeutically significant levels. This system is theoretically described in Scheme I.

As shown, a P is derivatized to form a steroid nicotinate, followed by quaternization to give a progestin trigonellinate ion (PO⁺) and reduction to give the corresponding 1,4-dihydropyridine or progestin chemical delivery system (PCDS). Upon systemic administration of the PCDS, an extensive distribution occurs because of the relatively high lipophilicity of the dihydropyridine derivative. This metabolically labile species should then ubiquitously oxidize to form the physiologically inactive PO⁺. The inactivity of the PO⁺ is
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predicted based on reports that 17-esters of norethindrone, in themselves, lack significant progesterone receptor affinity and biological action and require hydrolysis for activity (2). The hydrophilic salt should then rapidly be eliminated from the periphery but retained in the CNS. This rapid peripheral elimination and central retention of the quaternary salts formed in vivo is well documented in other systems (7). The retention is due to the blood–brain barrier (BBB), which prevents rapid reequilibration out of the brain of polar species. With time, the “locked-in” quaternary salt hydrolyzes to produce the pharmaceutically active steroid in a sustained manner. In this system, peripheral toxicity should be reduced by preventing significant accumulation of the parent steroid. In addition, central toxicity is also attenuated since the majority of the steroid is present in the form of an inactive carrier.

In the present report, lipophilicity and in vitro studies were performed to determine the feasibility of P delivery by this method. Detailed in vivo studies were then performed to demonstrate oxidation of the CDS, rapid peripheral elimination of the pyridinium salts, and sustained central delivery of the parent steroid.

MATERIALS AND METHODS

Chemistry

Uncorrected melting points (MP) were obtained using an Electrothermal melting-point apparatus. Microcombustion analysis was performed by Atlantic Microlabs, Inc., Atlanta, Ga. Proton nuclear magnetic resonance spectra (NMR) were recorded on a Varian EM 360 or EM 390 spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to an internal standard, tetramethylsilane. Ultraviolet spectra (UV) were determined using a Hewlett Packard 8451A diode array spectrophotometer. Thin-layer chromatography (TLC) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60.

General Procedure for Synthesizing the Nicotinates

Five grams of the appropriate steroid was dissolved in 125 ml of dry, freshly distilled pyridine. To this solution were added 9 g (1.5 molar excess) of nicotinic anhydride (11,12) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). The reaction mixture was stirred at room temperature for several days protected from moisture. When TLC indicated that the reaction was completed, the solution was poured over 800 ml of ice water. The resulting solid was collected by filtration, dried over P₂O₅ in vacuo, and then recrystallized.

17β-[3-Pyridinyl(carboxy)oxy]-pregn-4-en-20-yn-3-one (4). Ethisterone (1) (5 g, 0.016 mol) was reacted with nicotinic anhydride in pyridine for 7 days. The solid obtained was recrystallized from aqueous methanol. The yield was 88%. MP 203–204.5°C. TLC 60:40 hexane:ethyl acetate Rf = 0.11 (H₂SO₄/char). UV (MeOH) nm 230, 242. NMR (CDCl₃) δ 1.03 (s, 3H, angular CH₃); 1.20 (s, 3H, angular CH₃); 1.36–2.63 (m, 20H, skeletal protons); 2.70 (s, 1H, alkynyl proton); 5.73 (s, 1H, α,β-unsat. proton); 7.43 (m, 1H, pyridine C-5 proton); 8.30 (m, 1H, pyridine C-4 proton); 8.80 (m, 1H, pyridine C-6 proton); 9.20 (m, 1H, pyridine C-2 proton). Analysis calculated for C₂₇H₃₂NO₅: C, 77.40; H, 7.43; N, 3.36. Found: C, 77.57; H, 7.52; N, 3.35.

17β-[3-Pyridinyl(carboxy)oxy]-19-norpregn-4-en-20-yn-3-one (5). Norethindrone (2) (5 g, 0.017 mol) was stirred for 7 days in a pyridine solution of nicotinic anhydride and DMAP. After initial workup, 5 was recrystallized from aqueous methanol. The yield was 84%. MP 199–201°C. TLC 60:40 hexane:ethyl acetate Rf = 0.09 (H₂SO₄/char). UV (MeOH) nm 242. NMR (CDCl₃) δ 1.07 (s, 3H, angular CH₃); 1.0–3.33 (m, 20H, skeletal protons); 2.70 (s, 1H, alkynyl proton); 5.85 (s, 1H, α,β-unsat. proton); 7.29–7.53 (m, 1H, pyridine C-5 proton); 8.17–8.4 (m, 1H, pyridine C-4 proton); 8.70–8.93 (m, 1H, pyridine C-6 proton); 9.23 (s, 1H, pyridine C–2 proton). Analysis calculated for C₂₇H₃₄NO₅: C, 77.42; H, 7.20; N, 3.47. Found: C, 77.32; H, 7.22; N, 3.44.

13-Ethyl-17β-[3-pyridinyl(carboxy)oxy]-18,19-dinorpregn-4-en-20-yn-3-one (6). Norgestrel (3) (2 g, 0.006 mol) was stirred in a solution of nicotinic anhydride and DMAP for 20 days. Compound 6 was recrystallized from aqueous ethanol. The yield was 70%. MP 202–206°C. UV (MeOH) nm 230, 242. NMR δ (CDCl₃) 0.73–3.32 (m, 2H, skeletal protons + 13-ethyl group); 2.80 (s, 1H, alkyl proton); 5.80 (s, 1H, α,β-unsat. proton); 7.23–7.57 (m, 1H, pyridine C-5 proton); 8.13–8.40 (m, 1H, pyridine C-4 proton); 8.67–8.87 (m, 1H, pyridine C-6 proton); 9.17 (s, 1H, pyridine C-2 proton). Analysis calculated for C₂₇H₃₂NO₅: C, 77.70; H, 7.43; N, 3.36. Found: C, 77.72; H, 7.49; N, 3.30.

General Procedure for Quaternization

The steroid-17-trigonelline iodides were obtained by dissolving 2 g of the appropriate steroid nicotinate in acetonitrile. Two milliliters of methyl iodide was added to the solution, which was then heated at reflux for 12 hr. The resulting solid was collected by filtration and dried.

1-Methyl-3-[[pregn-4-en-20-yn-3-one-17β-yloxy]carbonyl]pyridinium Iodide (7). Compound 4 (2 g) was derivatized as described above. The yield was 98% (2.62 g). MP 226–227°C. UV (MeOH) nm 224. NMR (δ-d5-DMSO) δ 1.05 (s, 3H, angular CH₃); 1.20 (s, 3H, angular CH₃); 0.70–2.97 (m, 20H, skeletal protons + 1H alkyl proton); 4.50 (s, 3H, N⁺-CH₃); 5.63 (s, 1H, α,β-unsat. proton); 8.13–8.5 (m, 1H, pyridinium C-5 proton); 8.83–9.13 (m, 1H, pyridinium C-4 proton); 9.15–9.40 (m, 1H, pyridinium C-6 proton); 9.46 (bs, 1H, pyridinium C-2 proton). Analysis calculated for C₂₇H₃₄NOI: C, 60.11; H, 6.08; N, 2.50; I, 22.72. Found: C, 60.30; H, 6.14; N, 2.45; I, 22.55.

1-Methyl-3-[[19-norpregn-4-en-20-yn-3-one-17β-yloxy] carbonyl] pyridinium Iodide (8). Compound 5 (2 g) was derivatized as described above. The yield was 95%. MP 207–210°C. UV (MeOH) nm 224. NMR (δ-d5-DMSO) δ 1.03 (s, 3H, angular CH₃); 0.66–2.70 (m, 20H, skeletal protons); 3.73 (s, 1H, alkyl proton); 4.48 (s, 3H, N⁺-CH₃); 8.10–8.46 (m, 1H, pyridinium C-5 proton); 8.90–9.13 (m, 1H, pyridinium C-4 proton); 9.15–9.37 (m, 1H, pyridinium C-6 proton); 9.47 (bs, 1H, pyridinium C-2 proton). Analysis calculated for C₂₇H₃₂NOI·½H₂O: C, 58.48; H, 5.78; N, 2.53; I, 22.94. Found: C, 58.19; H, 5.99, N, 2.49; I, 22.64.

1-Methyl-3-[[13-ethyl-18,19-dinorpregn-4-en-20-yn-3-one-17β-yloxy]carbonyl]pyridinium Iodide (9). Compound