Notes

Synthesis and Pharmacological Characterization of (±)-5,9α-Dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (Fluorophen), a Ligand Suitable for Visualization of Opiate Receptors in Vivo

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A fluorinated derivative of the benzomorphan opiate agonist phenazocine, (±)-5,9α-dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (fluorophen), was prepared by N-acylation of (±)-5,9α-dimethyl-2′-hydroxybenzomorphan with (*p*-fluorophenyl)acetyl chloride, followed by diborane reduction of the resulting amide. Fluorination produces only a twofold opiate receptor affinity loss when measured either by bioassay or receptor binding (selectivity $\mu \simeq \delta > \kappa$). Labeled with ^{18}F, fluorophen should be sufficiently potent to be useful as an in vivo probe for visualizing opiate receptors by positron emission transaxial tomography (PETT).

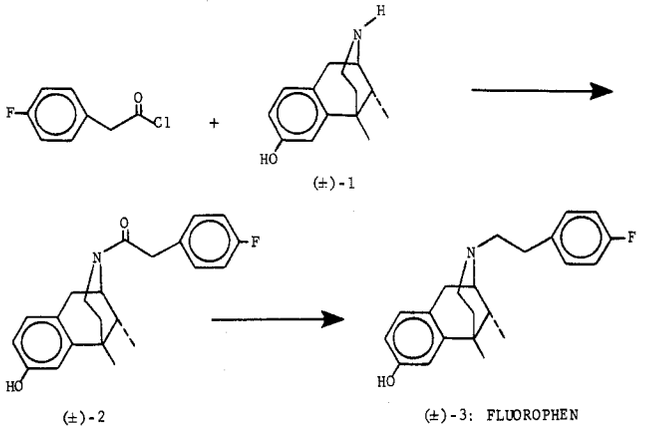
The recent demonstration of a remarkable correlation between opiate receptor distribution and terminal fields of axons marked by tract tracing suggests there are many opiatergic pathways in the brain.[1] The limbic regional distribution of these pathways, as well as the pharmacological effects of opiates, suggests that the endorphins and enkephalins play a role in the regulation of affective and drive states.[2] Although there are in vitro methods suitable for visualizing opiate receptors in human *postmortem* brains,[3,4] a method for in vivo localization of human brain opiate receptors might provide useful information about normal and pathological function.

The development of positron emission transaxial tomography (PETT) has allowed the in vivo investigation of the metabolic response of the brain to stimulation[5] and altered metabolic function of the brain in disease states.[6] To date, to the best of our knowledge, research on brain function utilizing the PETT scanner has generally involved quantitating changes in regional metabolism using 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) based on the model of Sokoloff et al.[7] The incorporation of ^{18}F into an opiate ligand would allow the in vivo localization of opiate receptors utilizing the PETT scanner. Herein we report the synthesis of a fluorine-containing opiate ligand that is six times more potent than morphine at μ opiate receptors and equipotent with [D-Ala^2,D-Leu^5]enkephalin at δ opiate receptors. This novel fluoro derivative of phenazocine (a benzomorphan with pure agonist activity[8]), fluorophen [(±)-5,9α-dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (**3**)], still retains one-half of the potency of the parent compound in in vitro bioassays and binding assays.

Results and Discussion

Racemic fluorophen was prepared as shown in Scheme I by the method May[9] described for synthesis of phenazocine. Racemic normetazocine (**1**) was converted to the corresponding amide **2**, which without purification was reduced with diborane in THF to afford fluorophen (**3**), isolated as the hydrobromide in 54% yield based on **1**. Binding assays were performed with rat brain homogenate prepared as described previously.[10] The concentrations

Scheme I



of racemic phenazocine and fluorophen required to displace one-half of the stereospecific ^3H-labeled ligand binding (IC_{50}) are shown in Table I. Binding assays were performed as previously described for [D-Ala^2,D-Leu^5]-enkephalin (DADLE) (buffer/3 mM Mn^{2+}/100 mM Na^+/2 μM GTP),[11] naloxone (buffer/100 mM Na^+),[11] and ethylketocyclazocine (EKC) (buffer/1 mM EDTA/100 mM Na^+).[12] Bioassays were performed with the guinea

† NIADDK.
‡ NIMH.

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Table I. Relative Potencies of (±)-Phenazocine and (±)-Fluorophen in Decreasing ^{3}H-Labeled Ligand Specific Binding in Rat Brain Homogenate

^{3}H-labeled ligand (concn, nM)		IC_{50},[a] nM	rel potency
[D-Ala^2,D-Leu^5]enkephalin (2.5)	[Leu^5]enkephalin	11 ± 5	1.0
	(±)-phenazocine	26 ± 8	0.42
	(±)-fluorophen	50 ± 11	0.22
(−)-naloxone (1.0)	(−)-morphine	200 ± 30	1.0
	(±)-phenazocine	82 ± 25	2.4
	(±)-fluorophen	180 ± 60	1.1
(±)ethylketocyclazocine (2.8)	(±)-EKC	8.4 ± 1.7	1.1
	(±)-phenazocine	65 ± 52	0.13
	(±)-fluorophen	125 ± 39	0.07

[a] IC_{50} = concentration displacing 50% of specifically bound ^{3}H-labeled ligand. Values represent mean plus or minus SEM of two or more experiments, each in triplicate.

Table II. Relative Potency of Various Opiates in Several Bioassays

	guinea pig ileum		mouse vas deferens		rabbit vas deferens	
opiate	IC_{50},[a] nM	rel potency	IC_{50},[a] nM	rel potency	IC_{50}, nM	rel potency
(−)-morphine	77.0 ± 16.0	1.0				
[Leu^5]enkephalin			9.4 ± 1.6	1.0		
(±)-ethylketocyclazocine					47.1 ± 9.0	1.0
(±)-phenazocine	9.8 ± 1.9	7.9	8.0 ± 2.4	1.2	121 ± 26	0.4
(±)-fluorophen	12.1 ± 2.4	6.4	8.6 ± 1.9	1.1	157 ± 33	0.3

[a] IC_{50} = concentration inducing 50% of the maximal effect. Values represent mean plus or minus SEM; $n = 6$.

pig ileum myenteric plexus–longitudinal muscle preparation (which sensitively detects μ pharmacological activity),[13] the mouse vas deferens preparation (which very sensitively detects δ opiate pharmacological activity),[14] and the rabbit vas deferens preparation (which is sensitive for detecting κ pharmacological activity).[15] The various opiate receptor subtypes are classified according to Martin et al.[16] and Lord et al.[17]

Phenazocine is a pure opiate agonist with greater clinical pain-relieving potency than morphine.[18] Fluorine substitution in the para position of the *N*-phenethyl moiety produces only a small loss in opiate receptor affinity, not an unexpected result given the well-studied structure–activity relationships of the morphinans and 6,7-benzomorphans.[13,19] The binding assays detect an approximately twofold receptor loss for assays selective for μ, δ, and κ opiate agonist activity (Table I). In vitro smooth muscle bioassays showed a slight trend toward receptor affinity loss, which was not statistically significant (Table II). In close agreement with the binding assay data, fluorophen was about half as potent as phenazocine as an analgesic in mice injected subcutaneously and tested for analgesia by the hot plate [0.30 mg/kg (0.23–0.40)] and Nilsen [0.64 mg/kg (0.45–0.93)] methods, employing the usual protocol.[19] Fluorophen has the subtype selectivity pattern of the parent compound phenazocine. Both bioassays and receptor binding assays suggest that fluorophen has a potent but nonselective preference for μ and δ opiate receptors, relative to κ opiate receptors. Still, it should be possible to achieve selective μ and δ opiate receptor visualization by coadministering appropriate doses of nonradiolabeled selective ligands. In this way, with ^{18}F-labeled fluorophen, μ receptors could be visualized after administration of a δ ligand to block ^{18}F-fluorophen's δ binding component and, in other experiments, vice versa. In any case, fluorophen has sufficiently high affinity for opiate receptors (in the nanomolar range), as measured by both bioassay and receptor assay, that reasonable in vivo receptor-binding signal-to-noise ratios should be achieved after low-dose administration.[20] A striking opiate receptor distribution gradient across primate cortex has been described,[21] which should make visualization with existing PETT technology feasible.

In summary, fluorphen is a potent μ and δ opiate receptor agonist in a variety of in vitro assays. Since it should be possible to synthesize this drug with the positron-emmitting fluoride isotope ^{18}F, it is potentially useful as a means of visualizing the distribution of opiate receptors with the PETT scanner. However, before fluorophen can be used in this application, further experiments to define its in vivo opiate receptor distribution pattern, pharmacokinetics, and toxicity will be required.

Experimental Section

A Fisher-Jones apparatus was used for melting point determination (corrected). Microanalysis was performed by the Laboratory of Chemistry's Section on Microanalytical Services and Instrumentation. IR (Beckman 4230), NMR (Varian HR-220), and mass spectra (Hitachi Perkin-Elmer RMU-6E) were consistent with the assigned structure.

(±)-5,9α-Dimethyl-2-[2-(4-fluorophenyl)ethyl]-2′-hydroxy-6,7-benzomorphan (Fluorophen, 3) Hydrobromide. Similar to the procedure of May and Eddy[9] for the synthesis of phenazocine, a suspension of 2.17 g (10 mmol) of normetazocine[22]

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in 30 mL of MeOH was stirred overnight with 4.2 g (30 mmol) of powdered anhydrous K_2CO_3 and 2.95 mL of (4-fluorophenyl)acetyl chloride [prepared by reaction of (4-fluorophenyl)acetic acid with $SOCl_2$]. The mixture was diluted with 5 mL of H_2O and evaporated to a white slurry. An additional 20 mL of H_2O was added; the mixture was extracted (2 × 20 mL of $CHCl_3$) and the combined extract was dried (Na_2SO_4) and evaporated, yielding 3.82 g of syrup. Crystallization from ligroin/ether gave 3.06 g of white solid, which was reduced directly as follows.

A 1.00 g (2.8 mmol) portion of the solid was dissolved in 10 mL of dry THF and refluxed with 11.0 mL of 1.0 M B_2H_6 in THF. After 2 h, 5 mL of MeOH was cautiously added, and the mixture was evaporated to a syrup and then taken up in 20 mL of MeOH and stirred with 5 mL of 37% HCl at 90 °C (bath temperature) for 1 h. Evaporation of the MeOH left a residue, which was treated with 20 mL of H_2O, neutralized (NH_4OH, pH 7), and extracted (3 × 50 mL of $CHCl_3$). The $CHCl_3$ was dried (Na_2SO_4) and evaporated to give 980 mg of foam, which was dissolved in 5 mL of 2-propanol, acidified with a few drops of 48% HBr, and then diluted with 3 mL of ether to yield 847 mg of **3**·HBr as white prisms: mp 150–153 °C; CIMS (CH_4), QM 340; NMR ($CDCl_3$, free amine) δ 0.86 (d, 3 H, J = 7 Hz), 1.32 (s, 3 H), 1.43–1.25 (m, 1 H), 2.36–1.89 (m, 3 H), 3.00–2.68 (m, 6 H), 3.20 (br s, 1 H), 6.68 (dd, 1 H, J = 8 and 2 Hz), 6.76 (d, 1 H, J = 2 Hz), 7.00–6.86 (m, 3 H), 7.16–7.07 (m, 3 H); IR ($CHCl_3$, free amine) 3300, 2960–2841, 1610, 1586, 1494 cm^{-1}; NMR of the HBr salt confirmed the presence of 1 mol of 2-propanol of solvation. Anal. Calcd for $C_{22}H_{28}FNO·HBr·C_3H_8O·^1/_2H_2O$: C, 61.35; H, 7.41; N, 2.86. Found: C, 60.99; H, 7.32; N, 2.82.

Biochemical Methods. For the binding assays, male Sprague–Dawley rats were killed by decapitation, the brains were rapidly removed, and the cerebellum was dissected away. The brains were homogenized in 50 vol of 0.05 M Tris–HCl buffer, pH 7.4, at 4 °C in a Brinkman Polytron (setting 6). The resulting homogenate was centrifuged at 18000 rpm for 15 min. After centrifugation, the supernatant was decanted and the pellets of the crude membrane preparation were resuspended in 2.5 vol of 0.05 M Tris-HCl, pH 7.4, and frozen. In the standard binding assay, 1-mL aliquots of the thawed crude membrane preparation were incubated in triplicate. For [^{3}H]DADLE, the incubation medium consisted of 0.05 M Tris-HCl at pH 7.4 and 1% bovine serum albumin. For naloxone, the medium consisted of 0.05 M Tris-HCl, 100 mM NaCl, 2 μM GTP, bacitracin at 0.5 mg mL^{-1}, and aprotinin at 100 kallikrein inhibitory units/mL. For EKC, the medium consisted of 0.05 M K_2HPO_4·HCl buffer, 1 mM EDTA, 100 mM NaCl, pH 7.4. To each was added the appropriate ^{3}H-labeled ligand at about 50000 cpm with either unlabeled ligand, unlabeled phenazocine, or unlabeled fluorophen to give a final dilution of 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} M. The resulting mixtures were incubated at 25 ([^{3}H]DADLE) or 4 °C ([^{3}H]naloxone and [^{3}H]-EKC) for 30 (if 25 °C) or 60 min (if 4 °C), and incubation was terminated by rapid filtration through glass-fiber circles (Whatman GF/B) and rinsed twice with cold buffer. Binding of ^{3}H-labeled ligand was quantitated by measuring the radioactivity of the membrane-laden filter in 10 mL of scintillation cocktail after agitation of the counting vials for 30 min.

Bioassays were prepared as follows. For guinea pig ileum, male guinea pigs weighing 400 to 450 g were sacrificed by decapitation, and the terminal ileum was exposed and dissected free. The distal 10 cm of ileum adjacent to the ileocecal junction was discarded. A portion of ileum approximately 10 cm in length was slid onto a glass rod, and the longitudinal muscle was mobilized from the circular muscle along the mesenteric border by careful stroking with a cotton pledget soaked in Krebs solution. The entire longitudinal muscle with the adherent myenteric plexus was then separated from the circular muscle by gentle blunt dissection. For mouse vas deferens, male mice weighing 20 to 25 g were killed by decapitation, and the vasa deferentia were dissected out en bloc with the seminal vesicle. Adherent blood vessels and connective tissue were removed by careful blunt dissection, the vasa were separated from the seminal vesicle, and the contents were expelled. For rabbit vas deferens, male rabbits weighing between 2500 and 3000 g were killed and decapitated, and the vasa deferentia were prepared as above. After dissection, all tissues were placed in organ baths in warm Krebs solution (NaCl, 118 mM; KCl, 4.75 mM; $CaCl_2$, 2.54 mM; K_2HPO_4, 1.19 mM; $NaHCO_3$, 25 mM; and glucose, 11 mM, at 37 °C), gassed with 95% O_2/5% CO_2, and placed under resting tension (1000 mg for guinea pig ileum, 100 mg for mouse vas deferens, and 500 mg for rabbit vas deferens). Platinum electrodes were used to deliver stimuli (supramaximal rectilinear pulses of 0.1-ms duration at 0.1 Hz) to the myenteric plexus or the intramural nerves. Contractions were recorded isometrically (Grass transducers, FT 03) and displayed on a Grass polygraph.

Registry No. (±)-**1**, 52079-30-8; (±)-**2**, 86436-93-3; (±)-**3**, 86495-14-9; (±)-**3**·HBr, 86436-94-4; (*p*-fluorophenyl)acetyl chloride, 459-04-1.

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Some Biological Properties of the Impure Dichloride Salt of Tetrakis[*p*-(dimethylamino)phenyl]ethylene and a Pinacolone

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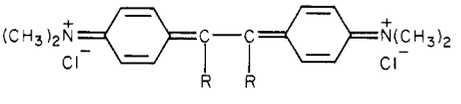
The impure dichloride salt of tetrakis[*p*-(dimethylamino)phenyl]ethylene and a pinacolone that is a substituted acetophenone show several biological properties, one of which is activity against lymphosarcoma in mice. The involvement, if any, of free radicals in the biological properties of these substances is discussed.

Work[1] carried out in our laboratories has shown that the unpurified salt (1)[2] of tetrakis[*p*-(dimethylamino)-phenyl]ethylene has some biological activity. Subcutaneous injection of impure 1 into rats depressed the leucocyte count and prevented tumor formation in rats fed



1, R = $(CH_3)_2NC_6H_4^-$

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