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Synthesis and Pharmacological Characterization of Fluorescent Opioid Receptor Probes

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Received: April 11, 1985; accepted: May 20, 1985.

Abstract: A series of five fluorescent opioid receptor probes was synthesized by coupling naloxone, oxymorphone or naltrexone with fluorescein or tetramethylrhodamine B. The series was characterized for capacity to displace ^{3}H-dihydromorphine from rat brain opioid receptors. All compounds showed receptor binding, and 1-(*N*)-fluoresceinyl naltrexone thiosemicarbazone displayed the highest mu-receptor affinity with a K_i value of 3 nM. 1-(*N*)-fluoresceinyl naloxone thiosemicarbazone was a morphine antagonist *in vivo*, approximately 6 % as potent as naloxone and naloxonazine in the mouse hot-plate test.

Opioid receptors have been studied extensively with radioactive tracer ligand displacement assays. These techniques are limited in some aspects, for instance they do not allow studies of fast kinetics of receptor-ligand interactions or direct visualization of receptor distribution in tissues. Fluorescent opioid probes might be alternative tools with particular advantages for these purposes and also for flow cytometric sorting of opioid receptor bearing membrane vesicles or cells. Fluorescent opioid peptides and a dansylated naloxone derivative have been reported previously (1, 2, 3). We have synthesized a series of morphinan-type fluorescent compounds for investigation of their properties as potential opioid receptor probes. A preliminary report on the synthesis, identification, and activities of four of these probes has appeared (4). Some probes have shown alterations in efficacy in comparison to their parent compounds (5) and prolonged duration of *in vitro* receptor blockade (6,7). The type of chemical coupling between opioid and fluorescent units was varied in order to compare chemical stability and duration of effects.

We report here the synthesis of all five probes, describe some stereochemical features as determined via high-resolution ^{1}H-NMR studies, and compare all probes for their ability to displace ^{3}H-dihydromorphine from rat brain membranes. Also, 1-(*N*)-fluoresceinyl naloxone thiosemicarbazone, the probe with the highest antagonistic activity on the guinea-pig myenteric plexus-longitudinal muscle (5) was compared to naloxone and naloxonazine for morphine antagonism in the mouse hot-plate test. Naloxonazine has been introduced as a long-acting opioid antagonist (8) and was included for comparison of duration of effects.

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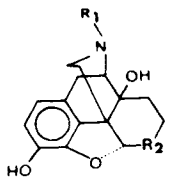
Materials and Methods

The TLC was carried out on E. Merck aluminum-supported sheets (precoated TLC sheets, silica gel 60F-254, layer thickness 0.2 mm, catalogue no. 5539). The two eluents used were: System I: $CHCl_3$: MeOH: conc. NH_4OH = 135:10:2 (v/v), and

System II: EtOH: AcOH: H_2O = 60:30:10 (v/v). The spots were observed in UV at 254 nm and at 366 nm for the fluorescent compounds. The latter were also observed in visible light. The HPLC purification and analyses were done on a Laboratory Data Control instrument. The columns used were a semi-preparative Spherisorb 10um ODS and a C18 uBondapak analytical column from Waters. ^{1}H-NMR spectra were taken on IBM-Bruker WM-400 (400 MHz) and Jeol-JNM-FX-100 (100 MHz) instruments. The mass spectra were taken on the LKB-9000 by Peter Jahnke, and MS CH-5 by Dr. Richard M. Milberg, to whom thanks are expressed.



COMPOUND	R_1	R_2
OXY	methyl	I
6-FO	"	II
NAL	allyl	I
6-FN	"	II
6-FAN	"	III
6-RN	"	IV
NALT	cyclopropylmethyl	I
6-FNX	"	II

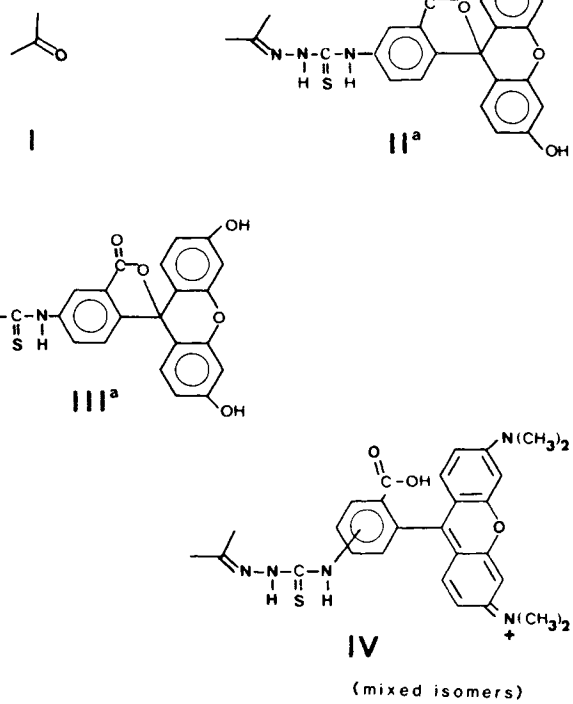


Fig. 1 Structures of oxymorphone (OXY), naloxone (NAL), naltrexone (NALT) and their derivatives.
[a]Only one of the possible pH dependent isomers of fluorescein is illustrated.

Chemistry

Synthesis of 1-(N)-fluoresceinyl naloxone thiosemicarbazone ("6-FN"): Naloxone hydrazone ["naloxazone", prepared as described (9, 10); 114 mg; 333 μmol] was added to a solution of fluorescein isothiocyanate (Sigma, isomer I; 135 mg; 347 μmol) in THF (2 ml; Fisher certified) and EtOH (4 ml; 100 %, histological quality). The reaction mixture was stirred at room temperature in the dark until it no longer contained naloxone hydrazone as shown by TLC. Thereafter, portions of EtOH and water were added to the reaction mixture. Orange crystals formed which were filtered off and dried. The structure of the crystals was determined to be that of 1-(*N*)-fluoresceinyl naloxone thiosemicarbazone (Fig. 1) based on spectroscopic evidence and elemental analysis. Major physical and spectral characteristics of 6-FN are: mp > 300°C (dec.). IR (Nujol): ν 1740 (C=C), 1635, 1590, 1500, 1325, 1268, 1208, 1182, 1110, 1028, 993, 952, 912, 852, 808, 722, 618 cm^{-1}. ^{1}H-NMR (100 MHz) (DMSO-d_6): δ 8.442 (1H, d, *J* ca. 2 Hz), 8.034 (1H, split d, *J* ca. 8 Hz, *J* ca. 2 Hz), 7.251 (1H, d, *J* ca. 8 Hz) (protons from the non-phenolic aromatic ring of the fluorescein moiety); 6.678, 6.602, 6.583 (8 H's, single peaks, aromatic protons from the phenol rings of the fluorescein and naloxone moieties); 6.11–5.10 (complex signal, vinylic H's of the allyl group); 5.029 (1H, s, bridgehead H at C-5) ppm; the upfield peaks look like those of naloxone. 400 MHz: 5.023 (H-5, major isomer, ca. 80 %, exact integration prevented by the overlap of peaks), 5.600 (H-5, minor isomer, ca. 20 %).
C, H, N. S. analysis: calculated: C, 65.74; H, 4.69; N, 7.67; S. 4.39. Found: C, 65.81; H, 4.64; N, 7.64; S, 4.34.

Synthesis of 1-(N)-fluoresceinyl oxymorphone thiosemicarbazone ("6-FO"): Oxymorphazone (0.1072 g; 0.3403 mmols) was added to a solution of fluorescein isothiocyanate (Sigma, Isomer I; 0.1419 g; 0.3644 mmol) dissolved in EtOH (4 ml; 100 %) and THF (2 ml; Fisher, certified). As soon as the oxymorphazone was added, the color of the fluoresceinyl isothiocyanate solution changed from red to orange. Reaction was carried out under agitation at room temperature for 3 hours and 45 minutes, during which time the reaction mixture was protected from light with aluminum foil.

Immediately upon mixing of the reactants, an orange precipitate began forming. After three hours of reaction a substantial amount of precipitate had formed, and EtOH (2 ml) was added to the reaction mixture. Thereafter, a sample was taken for TLC analysis, the results of which indicated that no oxymorphazone was left in the reaction system. After the reaction was terminated, the product was separated by filtration and the red solid product was washed repetitively with water and ethyl alcohol.

Water was added to the mother liquor and an orange precipitate formed which was separated by filtration and also washed repetitively with water and ethyl alcohol.

The first crop of crystals was a red solid (0.0851 g), and the second crop an orange solid (0.0353 g). After the mother liquor from the second crystallization was allowed to stand, further quantities of red precipitate formed, and this precipitate was separated by filtration (0.0409 g). Thus, the overall yield was 67.3 %. Analytical data: IR(Nujol): ν 1748, 1652, 1602, 1498, 1208, 1173, 1102, 1028, 993, 928, 850, 802, 722, 662 cm^{-1}. ^{1}H-NMR (DMSO-d_6) (400 MHz): δ 2.32 (N-CH_3), 5.02 (H-5, major isomer, 81.4 %), 5.59 (H-5, minor isomer, 18.6 %), the aromatic region splitting like in 6-FN; ^{13}C-NMR δ for some characteristic peaks of the opiate moiety: 168.38 (C-

6), 143.64 (C-4), 140.47 (C-3), 131.66 (C-12), 123.74 (C-11), 119.05 (C-1), 117.17 (C-2), 88.30 (C-5), 69.55 (C-14) ppm.

Synthesis of 1-(N)-fluoresceinyl naltrexone thiosemicarbazone ("6-FNX"): Naltrexazone (0.0573 grams; 1.61 x 10^{-4} mol) was dissolved in a minimum amount of THF and the resulting solution added (together with the EtOH rinsings from the container in which the dissolution was carried out) to a stirred solution of fluoresceinyl isothiocyanate (Sigma, Isomer I; 0.0641 g; 1.65 x 10^{-4}mol) in THF (1 ml; Fisher, certified) and EtOH (2 ml). As soon as the natrexazone was added, the color of the fluoresceinyl isothiocyanate solution began changing from red to orange. Reaction was carried out under agitation at room temperature while the reaction solution was protected from light with aluminum foil. After approximately one hour and 5 minutes of reaction, a small quantity of precipitate was formed and a sample taken for TLC analysis. Thereafter, additional EtOH was added until further precipitation was obtained. After one hour and 35 minutes the reaction was stopped by filtering out the solid precipitate. The solid product was washed twice with EtOH, yielding a crystallizate of 1-(*N*)-fluoresceinyl naltrexone thiosemicarbazone ("6-FNX") (0.0223 g), determined by TLC analysis to be substantially pure.

Further crystallization from the mother liquor yielded an additional quantity of 6-FNX (0.0182 g). By adding water to the remaining mother liquor, a third crop of crystalline 6-FNX was obtained (0.0410 g). The third crop was washed with ethyl alcohol and water. Overall yield at this point was 67.9 %. Further crystallization from the mother liquor provided a fourth crop of 6-FNX crystals (0.0138 g) bringing the total yield to 79.4 %.

IR(Nujol): ν 1740, 1640, 1592, 1500, 1323, 1280, 1210, 1180, 1111, 1032, 998, 918, 855, 808, 622 cm^{-1}. ^{1}H-NMR (DMSO-d_6) (400 MHz): δ 0.49, 0.12 (split signal, cyclopropyl CH_2's), 5.028 (H-5, major isomer, ca. 80 %, exact integration prevented by peak overlaps), 5.600 (H-5, minor isomer, ca. 20 %), the aromatic splitting like that in 6-FN.

Synthesis of 1-(N)-tetramethylrhodaminyl-B-naloxone thiosemicarbazone ("6RN"): Tetramethylrhodaminyl-B-isothiocyanate (Sigma, 10 mg; 23 μmol) was almost completely dissolved in a mixture of THF and EtOH (approximately 3 ml), producing a wine-red solution. To this solution was added naloxazone, i.e., naloxone hydrazone, (8.5 mg; 25 μmol). After the naloxazone was added, the solution changed from wine-red to a brownish color and became cloudy. After 33 minutes of reaction, a sample of the reaction solution was removed and subjected to TLC analysis. The results of this analysis did not indicate the presence of any remaining naloxazone. Approximately one hour after the addition of naloxazone, the reaction mixture was quenched by addition of water. Upon quenching, the solution warmed slightly, but no precipitate formed. Thereafter, the solution was concentrated to approximately its initial volume by removal of solvent on a rotary evaporator under mild heating. Significant foaming was observed during the concentration of the solution, and a precipitate formed which was separated by filtration. Thereafter, the mother liquor was subjected to further evaporation to one quarter of the original volume, upon which additional precipitation occurred, producing a second crop of crystals which was also removed by filtration. Both crops of crystals were dried in a vacuum oven. The product was 1-(*N*)-tetramethylrhodaminyl-B-naloxone thiosemicarbazone. Analytical data: IR (Nujol): ν 1628, 1595, 1560, 1522, 1508, 1368, 1343, 1302, 1253, 1231, 1190, 1116, 1030, 930, 890, 803, 725, 620, 600 cm^{-1}.

Synthesis of N-(1-N-allyl-14-hydroxynordihydro-6-morphinanyl)-N'-fluoresceinyl-thiourea ("6-FAN"), fluorescein-labeled 6-alpha-naloxamine: 6-alpha-naloxamine·2 HCl, was prepared as described (13). The configurational purity of 6-alpha naloxamine was unambigously established via a ^{13}C-NMR analysis.

The ^{13}C-NMR spectrum of a single isomer shows only one set of characteristic peaks, and only one peak per carbon. The mixture of two isomers shows two sets of peaks, one set per each isomer. The presence of both isomers does not influence the chemical shifts of the individual isomers present in the mixture. Since the carbon chemical shifts of 6-alpha- and 6-beta-naloxamine have not been previously reported, they are given in Table I. The ring C of the 6-alpha compound is expected to adopt a twist boat conformation and ring C of the 6-beta epimer a chair conformation (14). 22.0 mg (55 μmol) was dissolved in 200 μl EtOH and 20 μl Et_3N. Fluorescein isothiocyanate ("FITC"; Sigma; isomer I; 24.4 mg; 63 μmol) was dissolved in 300 μl Et_3N. The two solutions were thereafter mixed and maintained at room temperature in the dark (wrapped in aluminum foil) for 2 h to allow the condensation reaction to occur between the amine and the isothiocyanate. The progress of the reaction was followed by TLC. After 2 h some water was added to the reaction mixture and the mixture was concentrated by evaporation on a rotary evaporator. A portion of EtOH was added, and orange crystals were formed. Additional crystals were obtained by cooling the mother liquor. As indicated by the ^{1}H-NMR, TLC, and HPLC analyses, the product was substantially pure *N*-(1-*N*-allyl-14-hydroxynordihydro-6-alpha-morphinanyl)-*N*'-fluoresceinyl-thiourea. TLC data are given in Table II. The ^{1}H-NMR spectrum of 6-FAN is very similar to that of 6-FN with the exception of the expected differences for the H-5, H-6 regions.

Table I. ^{13}C-NMR (Bruker 400) Chemical Shifts in D_2O

Carbon	6-Alpha-Naloxamine 2 HCl*	6-Beta-Naloxamine 2 HCl*
1	121.76	121.77
2	119.64	119.47
3	138.59	141.05
4	145.91	142.35
5	86.34	89.45
6	70.57	71.23
7	30.65	29.66
8	29.57	27.79
9	56.85	56.54
10	18.70	21.90
11	124.18	122.82
12	129.04	129.62
13	46.82	47.18
14	62.71	63.44
15	24.15	23.60
16	46.01	46.90
17	48.16	53.51
18	126.64	126.67
19	127.24	127.01

*Prepared and purified by crystallization as described in ref. 13.

Table II. R_f Values

Compound	System I R_f	System II R_f	Comment
6-alpha naloxamine	0.045	0.31	spot observed in UV (254 nm)
FITC+Et_3N+HCl	0	0.81	orange spot
FAN	0	0.58	orange spot

Binding Assay

All compounds were tested for displacement of ^{3}H-dihydromorphine (Amersham Inc., U. K., specific activity 79.2 Ci/mmole) from opioid receptors in rat brain membrane preparations. The osmotically shocked membrane homogenate used in the opioid receptor binding assay was a single batch prepared from whole rat brain minus cerebellum. All compounds were freshly dissolved (1 mM in H_2O, fluorescein and rhodamine compounds with equimolar NaOH added) and diluted for use. Stability of solutions was checked by reverse phase HPLC (Waters C18 uBondapak, 20 minute gradient of 5 to 35 % isopropanol, 0.1 % ammonium acetate pH 5.9) just before assay. Triplicate samples of the compounds were incubated in physiological HEPES buffer pH 7.4 with tracer ligand and membranes for 30 minutes at 37°C, the minimum time to reach equilibrium for some compounds. The incubation was stopped by rapid cooling on ice and centrifugation in a Beckman microfuge for 5 minutes. The supernatant was removed in a sling and the radioactivity in the membrane pellet counted (15, 16). The data were analyzed using the program LIGAND of Munson and Rodbard (17). A full standard curve for dihydromorphine was run first and used in all subsequent fits.

Mouse Hot-Plate Test

The antinociceptive effect of morphine and antagonism of this effect by naloxone and derivatives was determined using the Up-and-down method of Dixon (18) as described in detail elsewhere (19). Briefly, mice weighing 25 g (NMRI strain, Anticimex, Solna, Sweden) were tested on a 54°C hot-plate. The latency for fore- or hindpaw licking or jumping was determined for all mice in a pre-test. The mean latency and standard deviation for each group (10 mice) were calculated. In the screening following administration of compounds, antinociception was defined as latency prolonged more than 2 standard deviations from the pre-test mean. Three series of experiments were performed:

1) The ED50 of morphine 30 min after *s.c.* administration was determined, as well as the *s.c.* doses of the various antagonists sufficient to reverse latency to the pre-test level when injected 15 min prior to 25 mg/kg morphine.

2) The antagonists were administered *s.c.* at the equi-antagonistic dose blocking the acute effect of 25 mg/kg of morphine as determined in experiment 1, and the ED50 of morphine was determined 6 h after antagonist administration.

3) Massive doses (40 mg/kg) of the antagonists naloxone and naloxonazine were given *s.c.* and the ED50 of morphine was determined 24 h later.

Results

Chemistry

The structures of the parent compounds and products are shown in Fig. 1. The absence of contamination of the various hydrazones used in the syntheses by parent compounds was confirmed rigorously. Mass spectral analysis gave the corresponding correct parent ions (naloxazone M^+ *m/e* 341 (100 %), no naloxone peak of *m/e* 327). TLC and IR also indicated pure products. Both electron impact and chemical ionization mass spectral modes were attempted for identification/purity check of 6-FN, as a representative compound. No parent peak was observed in either modes. (Thanks are expressed to Dr. Lennart Meurling for these mass spectral studies).

In analyzing 6-FN with HPLC, we observed the existence of one major and one minor peak. Since the elemental analysis for 6-FN was satisfactory, we proposed that the peaks corresponded to isomers of the parent opioid. The compound in the major peak was separated on HPLC and its opioid receptor binding was tested against the original mixture containing two peaks. No significant difference was observed between the mixture and the pure major compound. In repeated HPLC experiments with the purified major peak of 6-FN we observed again two peaks, which were even more noticeable when an analytical column was used, indicating a possible equilibrium between to isomers. This prompted us to perform a high resolution (400 MHz) ^{1}H-NMR study of 6-FN and its analogues in an attempt to confirm the structures of the isomers. The NMR spectra of 6-FN, 6-FNX, and 6-FO (400 MHz; in DMSO-d_6) were all similar in that they showed two peaks for H-5 of the opioid unit. These were ascribed to the *anti*-thiosemicarbazone (major peak at 5.02–5.03 ppm; ca. 80 %) and *syn*-thiosemicarbazone (minor peak at 5.59–5.60; ca. 20 %), respectively. The *syn-anti* stereochemistry was assigned as described (10–12).

The fluorescent properties of a representative probe were as follows.

6-FN: a) A 10^{-6}M solution in pH 7 phosphate buffer showed an excitation maximum at 493 nm and an emission maximum at 513 nm, as compared to 493 nm and 510-511 nm, respectively, for free fluorescein. b) The UV absorption maximum of a 10^{-5}M solution (phosphate buffer) was at 493 nm (A = 0.410). c) Fluorescence quantum yield of 6 FN compared to free fluorescein was approximately 67 % at 10^{-9} M. d) Fluorescence polarization was 0.038 for 6-FN and 2.022 for free fluorescein (these fluorescence measurements were done by Dr. Walter B. Dandliker to whom thanks are expressed).

Opioid Receptor Binding

The results obtained from statistical analysis of ^{3}H-DHM displacement studies are given in Table III. A better fit for dihydromorphine binding was obtained with a 2-site rather than a 1-site model (F = 6; D.F. = 11), but some compounds gave ambiguous Ki values with this model. Since dihydromorphine predominantly occupies mu-sites, receptor-type selectivity of the test compounds cannot be accurately determined from these data, and Ki values are shown as obtained for a 1-site model. However, altered type selectivity in comparison to its parent naloxone has previously been observed for 6-FN which showed about equal affinity for mu and delta receptors (4).

In vivo Studies

The equi-antagonistic doses of subcutaneously administered naloxone, naloxonazine which is a dimer of two opioid units, and 6-FN in the mouse hot-plate test were determined as the doses required to block the analgesic effects of 25 mg/kg

Table III. Ki Values as Obtained by Fitting Data to a 1-Site Mathematical Model.

Compound	Ki + SE (nM)
Dihydromorphine	3.1 ± 0.34
Naloxone	1.6 ± 0.26
6-RN	5.3 ± 0.78
6-FN	11.5 ± 1.3
6-FAN	18.9 ± 3.1
Oxymorphone	1.1 ± 0.20
6-FO	12.8 ± 2.1
Naltrexone	0.43 ± 0.065
6-FNX	2.6 ± 0.43

morphine (*s.c.*). This dose of morphine was about 17 times higher than its ED50, which was determined to be 1.5 (95% confidence limits 0.65–3.43) mg/kg. The values obtained were 2.4 (1.0–6.0) μmol/kg for naloxone, 2.1 (0.9–4.9) μmol/kg for naloxonazine and 38 (16–96) μmol/kg for 6-FN.

In a second experiment, the persistence of the effects of these equi-antagonistic doses was tested 6 h after *s.c.* administration. No significant shift of the morphine ED50 was observed for any compound.

In order to compare these experiments with published data on naloxonazine (20), a third experiment was performed with higher doses of naloxone (40 mg or 110 μmol/kg) and naloxonazine (40 mg or 62 μmol/kg). The morphine ED50 was 5-fold higher than that of the saline treated control group 24 h after naloxonazine. 6-FN could not be tested at such high doses because of its lower solubility.

Discussion

Modification of morphinan-type opioids at the C-6 carbon has previously been shown to be compatible with opioid receptor activity (21, 22). The bonds connecting the C-6 carbon of the opioid to the fluorescent moieties are of interest in terms of chemical stability and duration of effects. The existence of isomers of several of the probes might also need further analysis, but if rapid isomerization is taking place as indicated with 6-FN, isolation of the minor isomers would be difficult. 6-FN, 6-FNX and 6-RN appear to dissociate slowly from receptors, since inhibition of tracer binding is resistant to several washes (6, 7). They also show somewhat prolonged effects on the guinea-pig ileum myenteric plexus-longitudinal muscle (5). Several long-acting opioid analogues have been described by Sayre et al. (23), in which the chemical substitution in the vicinity of the C-6 carbon has been related to long-acting effects. The mechanism of the interaction with the receptor is not known in detail. Some compounds have highly reactive side groups and are presumably reacting covalently with a nucleophile near the binding site. Similar interactions have been proposed for opioid azines (8). If any such chemical transformation is taking place between the fluorescent probes and the receptor, it would be an important factor for their possible applications. If the probes were to bind covalently to the receptor, this would interfere with their use in conventional studies of fast kinetics of receptor-ligand interactions, although they might give other information on the receptor. Such reactions might also result in the loss of the fluorescent moiety. These aspects of the probes are being studied at present. It should be pointed out, however, that an analogous thiosemicarbazone linkage between another biologically active ketone, estrone, and fluorescein in 17-FE (17-fluorescein labeled estrone; 1-(*N*)-fluoresceinyl estrone thiosemicarbazone) as formed by coupling of estrone hydrazone and FITC (24) is extremely resistant to cleavage both *in vitro* and *in vivo* (25, 26). For example, a thin layer chromatography analysis of ethanolic extracts of 17-FE incorporated in tissues and cultured cells showed that over 95% of 17-FE was not metabolized (25).

The hydrophobicity and charge of the fluorescent moiety are also of interest in terms of possible interactions affecting the probes' overall kinetics, other than with the receptor itself.

In the mouse hot-plate tests, the acute antagonism of 6-FN was 16-fold lower than that of naloxone, whereas their binding affinity ratio is about 7. This difference could be due to different pharmacokinetic properties. The effects of naloxonazine 6 h after an intermediate dose appeared to be reversible. However, naloxonazine did show prolonged effects at substantially higher doses, in accordance with other studies (20). This suggests that the extreme long lasting effects of some opioids are complex phenomena, and renders it difficult to test long lasting properties of less soluble antagonistic compounds such as 6-FN that cannot be administered at very high doses.

The excitation-emission wavelengths of the attached fluorescent moiety also affect the usefulness of a probe. Previous studies have shown that a dansyl group is not suitable as a fluorescent moiety since it requires excitation at short wavelength ultraviolet light. Such wavelengths cause problems with tissue autofluorescence and irradiation damage to the opioid receptor and ligands (3, 27). The moieties we have used have high excitation and emission wavelengths.

Although the fluorescent moieties we have used are rather bulky molecules, we found that the capacity to displace dihydromorphine, known to be selective for the mu-type opioid receptor (28), was not much impaired in any compound. Receptor affinity also seems to be related to the physicochemical properties of the fluorescent moiety. Thus, we found a larger decrease in binding affinity of derivatives with more hydrophilic substitution (K_d ratios 6-RN/NAL = 3; 6-FN/NAL = 6-FNX/NALT = 7). Preliminary results with the probes in labeling synaptosomes for fluorescence activated cell sorting trials (in collaboration with Dr. H. F. Feldegg) and after intrastriatal injections (in collaboration with Dr. M. Herrera-Marschitz and Dr. T. Hökfelt) have shown fluorescence labeling, but further study is required before the specificity of this labeling is definitely confirmed. In conclusion, several of the probes with acceptable fluorescent properties showed sufficient retention of binding affinity to make them possible candidates for use as fluorescent probes of opioid receptors.

Acknowledgements

V. M. Kolb is grateful to Drs. Alan A. Rubin and Harold M. Ginzburg for donating naloxone, oxymorphone, and naltrexone. Thanks are expressed to Dr. Duy H. Hua for taking high-resolution NMR spectra, Ingrid Lantz and Per Hallin for packing HPLC columns. This work was supported by grants from the National Institute on Drug Abuse (DA 03 489) and the University Research Foundation, La Jolla, California (to V. M. Kolb), and by the Swedish Medical Research Council (grant no. 04X-3766 – to L. Terenius).

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Lymphatic Transport of Liposome-Encapsulated Drugs Following Intraperitoneal Administration – Effect of Lipid Composition

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Received: March 30, 1985; accepted: June 11, 1985.

Abstract: Tumor cells often metastasize through lymphatic channels. It follows that localization of antitumor agents in the lymphatics may be therapeutically beneficial. This study determines the extent to which lipid composition controls lymphatic transport of a model compound (^{14}C-sucrose) in liposomes following intraperitoneal administration in rats. All liposomes tested had mean diameters of approximately 0.2 μm. Liposomes were administerd to thoracic duct cannulated rats, and ^{14}C was quantified in thoracic lymph, several lymph nodes, blood, urine, and peritoneal wash. Changing liposome composition altered the rate of absorption of ^{14}C from the peritoneal cavity, stability in biological fluids, and the relative ability of liposomes to be retained by lymph nodes. Stability in biological fluids (plasma and lymph) appeared to be a reasonable predictor of observed lymph node recovery. Direct measures of lymph node level alone were poor measures of the ability of liposomes to function as prototypal lymphatic drug carriers. Neutral liposomes were better at reaching the general ciruclation following absorption from the peritoneal cavity.

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Controlled release of drugs in the vicinity of target tissues may lead to improved therapeutic availability. A theoretical analysis (1) of the pharmacology and pharmacokinetics of intravenous versus intraperitoneal drug therapy concluded that the latter route can have major advantages in chemotherapy of cancers confined to the peritoneal cavity. Experimental results support these predictions (2, 3). Such improved therapeutic availability need not be limited to treatment of peritoneal tumors if a drug carrier can gain access to more distant tumors.

The findings of Parker et al. (4–7) and ourselves (8) suggest that liposomes can function both as prototypal lymphatic drug carriers and as vehicles for localized sustained drug release. Subcutaneous or intramuscular administration of anticancer agents entrapped in liposomes can result in both improved delivery to lymph nodes (9–12) and suppression of tumor metastases within the lymphatics (13, 14). The governing mechanisms and the important variables that may control drug availability are not yet known. However, it has been predicted that only drugs with specific physicochemical, pharmacokinetic and mechanistic properties will be good candidates for use with *in vivo* drug carriers such as liposomes (15).