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Molecular Mechanism of δ-Selectivity of Indole Analogs of Nonpeptide Opioids

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SUMMARY

A combined experimental and computational approach was used to understand the mechanism of δ-receptor selectivity of a series of nonpeptide opioids. Six pairs of fused ring opioids/indole derivatives were studied. Receptor-binding assays using [^{3}H][D-Ala^2-MePhe^4-Gly-ol]-enkephalin (μ), [^{3}H][D-Pen^2-D-Pen^5]-enkephalin (δ), and [^{3}H]U-69593 (κ) were performed in guinea pig whole-brain membranes. Agonist activity was determined in norbinaltorphimine- or β-funaltrexamine-treated guinea pig ileum (μ and κ) and β-funaltrexamine-treated mouse *vas deferens* (δ). Steric and electronic properties were calculated for each compound. Although the parent compounds were selective for the μ-receptor, the indole analogs displayed selectivity for the δ-site because of a decrease in μ-affinity accompanied by an increase in δ-affinity. The indole analogs displayed little or no activity at the δ-receptor. The role of the indole in enhanced δ-recognition is likely interaction with a lipophilic site in the receptor. The diminished μ-affinity of the indole analogs is a result of the loss of the carbonyl oxygen as the proton-accepting center, which we have previously determined to be important for recognition of the μ-receptor.

For several years, Portoghese (1) has been exploring the possibility that opioid receptor selective ligands could result from modifications of the structure of the opioid antagonist, naltrexone. One of the first such successful compounds was norBNI (2), a selective κ receptor antagonist. In a continuing effort, an indole was fused to naltrexone (3), producing the selective δ-receptor antagonist, naltrindole. Subsequently, the same investigators made additional naltrexone-derived antagonists with significant δ-selectivity that should be useful pharmacologic tools (4).

In this study we have continued to explore the effect of the addition of an indole to other members of the fused ring family represented by naltrexone, focusing particularly on the effect of this addition to parent agonist compounds. In this way, the effect of this variation not only on receptor recognition and selectivity but also on activation could be assessed. To this end, we have chosen six pairs of compounds shown in Fig. 1, each pair identical except for the addition of an indole moiety: two antagonist pairs based on naloxone and naltrexone, and four pairs based on the agonists hydrocodone, oxycodone, hydromorphone and oxymorphone.

We have determined the receptor binding and *in vitro* agonist profiles at the μ-, δ-, and κ-opioid receptors of these six parent/indole pairs. Affinity and selectivity of each compound for the μ-, δ-, and κ-receptors was determined in competitive binding studies using [^{3}H]DAMGO (μ), [^{3}H]DPDPE (δ) and [^{3}H]U-69593 (κ). The pharmacologic activity of the compounds at the three receptors was assessed in the following bioassays: GPI pretreated with norBNI (μ) (2) or β-FNA (κ) (5) and MVD pretreated with β-FNA (δ) (6).

We also have characterized the steric and electronic properties of these compounds using the techniques of computational chemistry, comparing molecular properties with those found to be, in our most recent effort, key molecular determinants for μ-receptor recognition (7). The results of this and other comparisons were used to determine the role of the indole moiety in δ-receptor recognition and the effect of the addition of this group on μ-receptor recognition.

Experimental Procedures

Materials

The following compounds were kindly provided by National Institute on Drug Abuse, Bethesda, MD: U-69593, DAMGO, DPDPE, normorphine, [^{3}H]DAMGO, and [^{3}H]DPDPE. [^{3}H]U-69593 was purchased from New England Nuclear (Boston, MA). Oxymorphone, hydromorphone, naloxone, oxycodone, and hydrocodone were purchased from Sigma Chemical Company (St. Louis, MO). Naltrindole, β-FNA and

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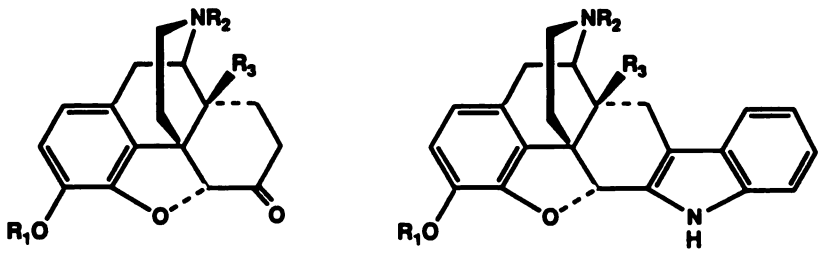
ABBREVIATIONS: DAMGO, [D-ala^2-MePhe^4-Gly-ol]-enkephalin; DPDPE, [D-Pen^2-D-Pen^5]-enkephalin; β-FNA, β-funaltrexamine; norBNI, norbinaltorphimine; GPI, guinea pig ileum; MVD, mouse *vas deferens*; BNTX, 7-benzylidenenaltrexone.



Parent compound Indole analog

		R_1	R_2	R_3
Hydrocodone	(1)	CH_3	CH_3	H
Hydromorphone	(2)	H	CH_3	H
Naloxone	(3)	H	$CH_2CH{=}CH_2$	OH
Naltrexone	(4)	H	$CH_2CH(CH_2)_2$	OH
Oxycodone	(5)	CH_3	CH_3	OH
Oxymorphone	(6)	H	CH_3	OH

Fig. 1. Structures of compounds studied.

norBNI were purchased from Research Biochemicals Inc. (Natick, MA). The indole analogs of oxymorphone, hydromorphone, naloxone, oxycodone, and hydrocodone were synthesized as described below. All other chemicals were from standard commercial sources.

Receptor binding assays

General assay procedure. Membranes were prepared from frozen whole guinea pig brains (including cerebella) (Pel Freeze, Rogers, AZ) as previously described (8) and incubated in triplicate with 0.5 nM [^{3}H] DAMGO, 0.5 nM [^{3}H]DPDPE, or 1.0 nM [^{3}H]U-69593 and 15 concentrations of competing drug in a total volume of 2 ml at 25° until steady state was reached (8). Nonspecific binding was determined in the presence of 1 μM unlabeled ligand (DAMGO, DPDPE, or U-69593). The reaction was terminated by filtration (Brandel cell harvester) through glass fiber filters (Whatman GF/B, presoaked in 0.1% polyethylenimine) followed by three 5-ml washes with ice-cold buffer. Radioactivity bound to the filters was quantitated by liquid scintillation with ReadySafe (Beckman) after 6 hr at room temperature.

Data analysis. Data obtained from competitive binding assays were analyzed by a modified version (9) of the program LIGAND (10), which calculates the binding affinities (K_i) and receptor densities (B_{max}) using weighted, nonlinear, least squares regression analysis.

In vitro model tissue studies

Preparation of tissue. GPI and MVD were prepared as previously described (8). β-FNA (200 nM), administered as a single injection into the tissue baths, was used to inactivate μ-receptors in both GPI (11) and MVD (5) assays. The κ-antagonist norBNI (20 nM in Krebs solution) was used to antagonize κ-receptors in the GPI (2).

General assay procedure. All compounds were allowed to incubate in the tissue baths until full effect was reached. Then they were washed at regular intervals until predosage twitch strengths were recovered or no further change was observed. Dose-response curves were constructed from at least three points, but as many data points as possible were gathered. All points used in the calculations fell between 10 and 90% inhibition.

Calculation of IC_{50}. A plot of the percentage of predose contraction strength versus log dose was constructed for each compound. From the linear regression, the IC_{50} was calculated as the dose that produced 50% inhibition. The values reported are means ± standard error. Antagonism of δ-activity was tested by determining the IC_{50} before and after treatment with 10 nM naltrindole.

Computational methods

The semiempirical quantum mechanical method AM1 (12) was used to calculate diverse electronic and structural properties of the compounds that could be relevant to the recognition and activation of opioid receptors. Among the properties analyzed for all the compounds were the frontier orbitals, as a measure of their relative ability to donate and accept electrons. Proton affinities at different positions on selected molecules were computed as the difference in energy between the protonated and unprotonated molecule. Octanol-water partition coefficients were computed using a novel method developed in our laboratory (13). In this procedure, each atom was assigned an atomic hydrophobicity index determined from its contribution to the total van der Waals area and its AM1 net atomic charges. Then the logarithm of the partition coefficient is estimated as the sum of these individual contributions. The molecular volumes and areas were computed using the atomic van der Waals radii provided by Gavezzotti (14).

Synthesis

Naloxone indole was prepared by the method of Portoghese *et al.* (15) and converted to its HCl (compound 3, Fig. 1). Oxycodone indole

HCl (5) and hydrocodone indole HCl (1) were prepared as follows. Phenylhydrazine HCl (0.8 g, 6 mmol) and concentrated HCl (1.1 ml, 12 mmol) were added to a solution of either oxycodone HCl or hydrocodone bitartrate (3 mmol) in methanol (20 ml) at room temperature. The solution was heated under reflux for 3 hr. The indole HCl, which precipitated on cooling, was collected and recrystallized. Oxymorphindole HCl (6) and hydromorphone indole HCl (2) were prepared from (5) and (1), by a modification of the *O*-dealkylation procedure reported by Rice (16).

All five compounds showed indolyl protons in the ^1H spectra, in region 6.9 to 7.5 ppm, absent from the starting ketone. For example, oxycodone indole HCl, run in dimethylsulfoxide/D_2O, gave doublets at 7.42 and 7.34 (J = 8.1 Hz) for indole protons 4 and 7, and at 7.2 (t, H-5) and 7.02 (t, H-6). The two other aromatic protons resonated at 6.2 and 6.55 ppm as *o*-coupled doublets (J = 8.0 Hz). The *O*-dealkylated indoles (4) and (5) also showed the loss of 3-OMe singlets at d 3.4 and 3.8 ppm, respectively.

Results

Receptor binding assays were carried out under steady state conditions. The time required to reach steady state was determined experimentally for each radioligand (not shown). Nonspecific binding was typically 6, 15, and 25% of the total [^{3}H] U-69593, [^{3}H]DAMGO, and [^{3}H]DPDPE binding, respectively. Data were best fit to a one-site model for each receptor. The results from binding studies are given in Table 1. In each case, the parent opiate is selective for the μ- over the δ- (range 36- to 108-fold) or κ- (3- to 216-fold) receptor. The addition of the indole moiety dramatically transforms each μ-selective parent compound into a δ-selective analog by enhancing δ-affinity (36- to 125-fold), significantly diminishing μ- affinity (55- to 282-fold), and modestly decreasing κ-affinity (1.5- to 21-fold).

The ability of each of the compounds to inhibit electrically stimulated muscle contractions through each of the opiate receptors was assayed in the GPI (μ- and κ-receptors) and MVD (δ-sites). Preliminary assays using MVD revealed the presence of μ-receptors in relatively low concentration (not shown). Because of the μ-selectivity of the parent compounds (Table 1), it was necessary to ensure that μ-receptors were blocked sufficiently to accurately measure the activity at the κ- (GPI) and δ- (MVD) receptors. Blocking was achieved by pretreating with β-FNA, which irreversibly inactivates the μ- receptor by alkylation (17). To accurately measure the μ-activity, the κ-receptors in the GPI were blocked reversibly by norBNI (2), which was present throughout the assay system.

Results from *in vitro* model tissue studies are shown in Table 2, with the analogs in the same order as in Table 1. Of the 12 compounds tested, only hydromorphone indole exhibited activity in the MVD that could be antagonized by naltrindole. The indole analogs of oxycodone and hydrocodone (at a concentration of 1 μM) were able to antagonize the effect of DPDPE in MVD, producing a 2-fold (19.3 ± 1.8 nM) and 4.6-fold (44 ± 23 nM) shift in the IC_{50} for DPDPE (9.5 ± 2.4 nM), respectively. Only oxymorphone and hydromorphone produced sufficient activity at the κ-receptor to allow the calculation of an IC_{50}. Hydromorphone indole and parent compounds hydromorphone, oxymorphone, oxycodone, and hydrocodone caused significant μ-receptor-mediated reduction in twitch height. The rank order of μ-agonist activity for these compounds paralleled their binding affinity.

Table 3 shows the results obtained for different molecular properties computed for the indole congeners and their parent compounds. The reported data include the computed octanol/water partition coefficient, the total molecular volume, and the monoelectronic energies of the highest occupied molecular orbitals and lowest unoccupied molecular orbitals.

Discussion

The goal of this work was to determine the extent of universality of the observed μ- to δ-selectivity change, which was produced by the addition of the indole moiety to the morphinan structure, and to investigate whether the intrinsic activity of the agonist parent compounds at the μ-receptor would be preserved at the δ-receptor in the indole analogs. These questions were addressed by determining the binding affinities and agonist activities of the six pairs of compounds (four parent agonists and two parent antagonists) at the μ-, δ-, and κ-receptors.

In each case, the parent compound was selective for the μ- over the δ-receptor, but the addition of the indole moiety conferred δ-selectivity (Table 1). The addition of the indole group seemed to produce a nearly constant shift to lower μ-affinities for all six compounds. This observation indicates a common detrimental effect of the presence of the indole moiety on μ-recognition by all these compounds. In a similar fashion, the δ-affinities are shifted relatively uniformly to higher affin-

TABLE 1
Binding affinities at μ, δ and κ receptors for parent/indole pairs

	K_i (nM)			Selectivity	
	[a]μ	[b]δ	[c]κ	[d]δ/μ	[e]μ/δ
Naltrexone	0.27 ± 0.03	10.7 ± 2.5	0.93 ± 0.09		39
Naltrindole	33.9 ± 4.9	0.10 ± 0.01	19.6 ± 2.3	349	
Naloxone	1.10 ± 0.10	39.9 ± 9.2	5.27 ± 0.50		36
Naloxone indole	60.8 ± 8.3	0.32 ± 0.04	70.3 ± 9.9	190	
Oxymorphone	0.81 ± 0.07	87.8 ± 9.7	119 ± 11		108
Oxymorphindole	111 ± 17	0.73 ± 0.09	228 ± 31	152	
Hydromorphone	0.47 ± 0.04	18.5 ± 4.3	24.9 ± 2.3		39
Hydromorphone indole	29.7 ± 2.7	0.51 ± 0.13	103 ± 11	58	
Oxycodone	20.1 ± 1.7	1170 ± 290	4350 ± 530		58
Oxycodone indole	5660 ± 1433	23.03 ± 2.66	6530 ± 950	246	
Hydrocodone	23.8 ± 2.0	1080 ± 290	1480 ± 170		45
Hydrocodone indole	2220 ± 320	27.5 ± 3.0	4060 ± 570	81	

Inhibition of [a][^{3}H][D-Ala^2-MePhe^4-Gly-ol]-enkephalin, [b][^{3}H][D-Pen^2-D-Pen^5]-enkephalin, and [c][^{3}H]U-69593 binding to guinea pig whole brain membranes, 25°.
[d] δ/μ selectivity—K_i (μ)/K_i (δ).
[e] μ/δ selectivity—K_i (δ)/K_i (μ).

TABLE 2
In vitro model tissue activity for parent/indole pairs

	IC_{20} μ[a]	IC_{20} δ[b]	IC_{20} κ[c]
Naltrexone	n.a. (30 nM) [4][d]	n.a. (2 μM) [4][d]	n.a. (10 μM) [2][d]
Naltrindole	n.a. (10 μM) [4][d]	n.a. (1 μM) [4][d]	n.a. (10 μM) [4][d]
Naloxone	n.a. (60 nM) [4][d]	n.a. (2 μM) [4][d]	n.a. (10 μM) [2][d]
Naloxone indole	n.a. (2 μM) [4][d]	n.a. (30 nM) [4][d]	n.a. (10 μM) [4][d]
Oxymorphone	29.4 ± 4.4 nM [4][e]	23 ± 1% (10 μM) [7][f,g]	3300 ± 1100 nM [3][e]
Oxymorphindole	n.a. (3 μM) [4][d]	3800 ± 500 nM [3][e,g]	n.a. (10 μM) [4][d]
Hydromorphone	10.9 ± 1.3 nM [3][e]	n.a. (10 μM) [3][d]	4700 ± 200 nM [3][e]
Hydromorphone indole	3500 nM [3/dose][e]	2300 ± 200 nM [3][e]	32 ± 8% (10 μM) [4][f]
Oxycodone	342 ± 76 nM [3][e]	n.a. (10 μM) [3][d]	n.a. (10 μM) [3][d]
Oxycodone indole	n.a. (10 μM) [4][d]	4900 nM [4/dose][e,g]	n.a. (20 μM) [3][d]
Hydrocodone	623 ± 56 nM [3][e]	n.a. (10 μM) [3][d]	31 ± 1% (10 μM) [2][f]
Hydrocodone indole	24 ± 5% (10 μM) [4][f]	20 ± 3% (10 μM) [3][f,g]	34 ± 4% (10 μM) [4][f]

Inhibition of electrically stimulated muscle contractions in [a] GPI + nor BNI, [b] MVD + β-FNA and [c] GPI + β-FNA as described in Materials and Methods.
[d] n.a. (no activity): < 20% inhibition at the concentration noted in parentheses.
[e] IC_{20} values are mean ± standard error—the number of experiments is noted in the brackets.
[f] % inhibition at the concentration noted in parentheses.
[g] Not antagonized by 10 nM naltrindole.

TABLE 3
Structural and electronic properties of parent/indole pairs

	LogP[a]	V[b]	HOMO[c]	LUMO[d]
Naltrexone	0.61	305.4	−8.90	−0.026
Naltrindole	2.78	391.4	−8.30	0.089
Naloxone	0.46	291.2	−8.90	−0.031
Naloxone indole	2.69	377.1	−8.31	0.085
Oxymorphone	−0.38	262.0	−8.88	0.000
Oxymorphindole	1.26	329.8	−8.43	−0.030
Hydromorphone	0.70	255.9	−8.77	0.160
Hydromorphone indole	2.31	324.4	−8.30	0.099
Oxycodone	0.16	280.3	−8.78	0.075
Oxycodone indole	1.87	348.4	−8.32	0.072
Hydrocodone	1.26	274.2	−8.67	0.210
Hydrocodone indole	2.91	348.4	−8.19	0.214
BNTX	3.14	403.9	−8.60	−0.530

[a] LogP is the logarithm of the octanol/water partition coefficient.
[b] V is the molecular volume in Å³.
[c] HOMO and [d] LUMO are the monoelectronic energies of the highest occupied and lowest unoccupied molecular orbitals, respectively, in eV.

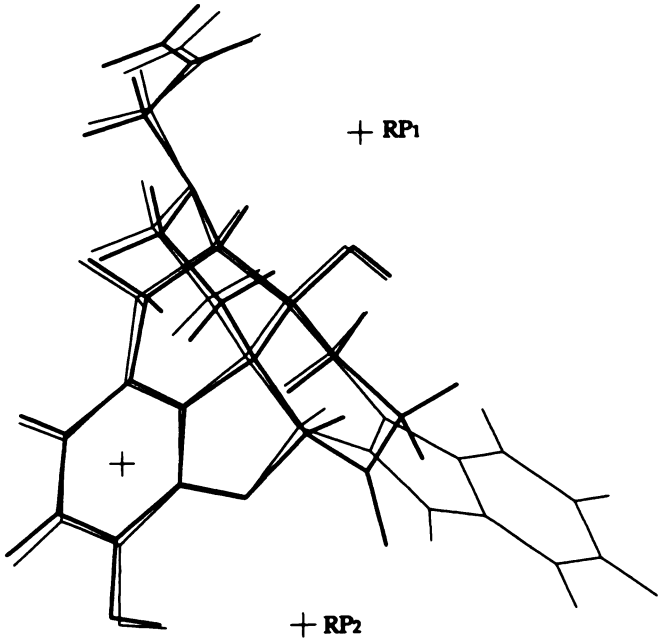


Fig. 2. Superimposition of naloxone (*bold*) and naloxone indole (*light*). RP_1 and RP_2 are defined as theoretical interaction points within the μ receptor binding site. RP_1 is located 3 Å from the amine nitrogen (PA_1) in the direction of protonation. RP_2 is located 3 Å from PA_2 (the carbonyl oxygen in naloxone and the furan oxygen in naloxone indole), also in the direction of protonation. These receptor points, as well as the center of the phenoxy ring, were used as points of overlap.

ities. This result indicates a common role of the indole group in enhancing δ-recognition in these compounds.

The addition of the indole moiety to a parent compound with agonist activity led to significantly reduced activity, as well as affinity, at the μ-receptor (Table 2). Although the significant decrease in μ-activity paralleled a decrease in μ-affinity in the indole derivatives, the enhancement of δ-affinity was not reflected in a similar increase in their δ-activity. Thus, it seems that although addition of an indole moiety enhances recognition of the δ-receptor, it does not enhance its activation.

Several of these compounds have been studied by other groups, but the parent and indole analogs have not been directly compared previously. For the indole analogs of naltrexone, naloxone, and oxymorphone, the affinities reported here for the μ- and δ-receptor are very close to the values reported by Portoghese *et al.* (15), although we detected much higher κ-affinity than previously reported. This may be a result of the differences in the method used to assess the binding to this receptor. Although Portoghese *et al.* (15) observed all subclasses of κ-receptors using [³H]ethyl-ketocyclazocine + DAMGO, we studied only the κ_1-subtype using [³H]U-69593. This difference in methodology could mask possible κ-subtype selectivity. In a recent study, several opioids, including naltrindole and oxymorphindole, were investigated at four subtypes of κ-receptors (18). Both naltrindole and oxymorphindole were found to have highest affinity for the κ_{2b}-site (1 and 21 nM, respectively).

Four of the six indole analogs reported here have been studied previously in model tissue systems. They are naltrindole (3, 15), oxymorphindole (15, 19, 20), naloxone indole (15), and hydromorphone indole (20). Naltrindole and naloxone indole were shown to be antagonists in MVD (3, 15, 21) with K_e values of 0.13 nM and 1.1 nM, respectively. Oxymorphindole and hydromorphone indole have been reported as partial agonists in MVD, producing maximal responses of 65 and 40% (15, 20). Although the activity of oxymorphindole was reported to be antagonized by naltrindole (3, 15), in our hands, this was not the case. As a partial agonist, oxymorphindole was shown to act as an antagonist in the MVD, with a K_e of ≥8.8 nM (15).

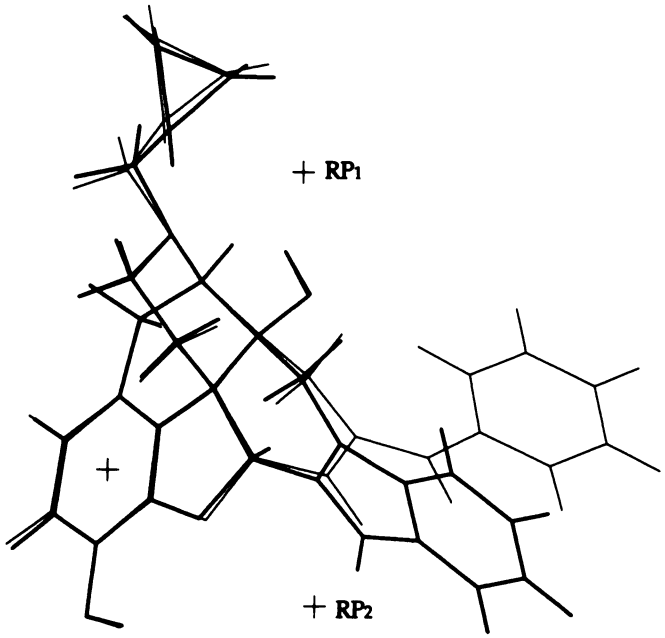


Fig. 3. Superimposition of naltrindole (*bold*) and BNTX (*light*). RP_1 and RP_2 are defined as described in Fig. 2.

The only indole analog that seemed to have δ-receptor-mediated agonism was hydromorphone indole; its activity was antagonized by naltrindole. In addition, although very weak, hydromorphone indole did not seem to be a partial agonist, as it produced >80% inhibition at the highest concentration examined (10 μM), with no evidence of a plateau. In addition, oxycodone indole and hydrocodone indole were able to antagonize the effect of DPDPE in MVD. It is clear, in general, that the indole analogs of these potent μ-agonists do not retain that agonism at the δ-receptor.

The most challenging question, addressed using the techniques of computational chemistry, was to understand the mechanism by which the addition of an indole moiety significantly increases the affinity for the δ-receptor while decreasing the μ-affinity. Both the μ- and δ-receptors have similar requirements regarding the nature of the substituents R_1, R_2, and R_3 (Fig. 1). The presence of a methoxy group at R_1 significantly reduces the affinity of the ligands for both μ- and δ-receptors. This phenomenon has been described previously for the μ-receptor as determined by binding assays using [^{3}H]naloxone (22). Changes in R_2 produce less pronounced effects on affinity than those observed in R_1, with the δ-affinities showing a larger dependence on the nature of this substituent. The parent compounds and indole analogs with the more lipophilic R_2 substituents have higher affinities (smaller K_i) for the μ- and δ-receptor. Finally, substituents at R_3 reduce affinity relative to the unsubstituted analogs.

However, the effect of the indole itself is qualitatively different and most striking. In all cases, it decreases μ-affinity while increasing affinity for the δ-receptor. The effect of the addition of the indole on μ-receptor affinity can be probed using the model for μ-receptor recognition we have developed recently using a series of fentanyl analogs (7), and further refined and validated in a comparative study that included members of the fused ring and 4-phenylpiperidine families (23). The model embodies a recognition pattern consisting of at least three molecular determinants in a specific geometrical arrangement: 1) a proton-accepting center (PA_1), which corresponds to an amine nitrogen; 2) a group capable of acting as a second proton-accepting center (PA_2), and 3) an aromatic ring (Ring B) capable of electron transfer or π-π stacking interactions. These determinants have a specific geometric relationship that is determined by defining two proton donating points in the receptor: RP_1 located 3 Å from the amine nitrogen (PA_1) in the direction of protonation and RP_2 located 3 Å from PA_2, also in the direction of protonation.

All of the parent compounds studied in the present work exhibit the molecular determinants required for μ-recognition: the PA_1, located on the amine nitrogen, the PA_2 on the carbonyl group, and Ring B on the phenoxy group. Moreover, geometrical parameters are within the range of the pharmacophore, providing further validation of the μ-recognition model.

Direct comparison of the indole derivatives with the parent compounds can be achieved by superposition of the common atoms of the parent and indole analogs (Fig. 2). The difference between the indole analogs and the parent compounds is that the indole replaces the carbonyl group. Because the carbonyl oxygen acts as PA_2 in the parent compounds, its replacement by the indole moiety could account for the systematic decrease in μ-affinity in the indole analogs.

The question still remains as to the reasons for enhancement of δ-affinity on addition of the indole moiety. The four properties that show the most significant changes when the indole moiety is added are shown in Table 3. The molecular volume increases, as would be expected, because of the addition of the indole group. There is also a significant increase in lipophilicity as shown by the increased octanol/water partition coefficient. Finally, the indole ring enhances the electron-donating capability of these compounds. They have lower ionization potentials, as shown by the larger absolute values associated with the highest occupied molecular orbital, which is localized on the indole.

Based on these results, three plausible functions of the indole in δ-receptor recognition could be deduced: 1) interaction of the indole with a lipophilic pocket in the δ-receptor, 2) formation of a charge transfer complex with an electron-accepting amino acid in the binding site, and 3) formation of a hydrogen bond by the amine hydrogen of the pyrrole ring with a proton-accepting group in the receptor. The compounds studied do not permit us to determine which of these three possibilities is likely to be the primary function of the indole moiety. However, comparison with the recently published analog, BNTX (24), could help in this effort. BNTX is a compound related to naltrexone and naltrindole, with high affinity for the δ-receptor (K_i (δ) = 0.10 nM; K_i (μ) = 13 nM). The compound has an aromatic ring superimposable with the indole moiety of naltrindole (Fig. 3). Nevertheless, it does not have strong electron-donating character (Table 3), nor the ability to form hydrogen bonds. Therefore, in BNTX, the aromatic group can provide only an interaction with a lipophilic pocket. Therefore, the enhanced δ-affinity induced by addition of an indole can be inferred to be produced by the same type of hydrophobic interactions. This result is consistent with the nearly constant shift to higher affinity caused by the addition of the indole moiety.

In summary, the addition of the indole moiety to the fused ring opiates confers δ-receptor selectivity by decreasing μ-affinity and enhancing δ-affinity. It is likely that the reason of

a decreased affinity for μ is the loss of the carbonyl group that is replaced by the indole moiety. In addition, the indole provides a lipophilic region capable of interaction with a specific region within the δ-receptor. This interaction leads to enhanced recognition of the δ-receptor, but it does not lead to activation. Further studies, including analogs with δ-agonist activity, are necessary to develop a more complete model for δ-receptor recognition and activation.

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