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Differentiation of δ, μ, and κ opioid receptor agonists based on pharmacophore development and computed physicochemical properties

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Summary

Compounds that bind with significant affinity to the opioid receptor types, δ, μ, and κ, with different combinations of activation and inhibition at these three receptors could be promising behaviorally selective agents. Working on this hypothesis, the chemical moieties common to three different sets of opioid receptor agonists with significant affinity for each of the three receptor types δ, μ, or κ were identified. Using a distance analysis approach, common geometric arrangements of these chemical moieties were found for selected δ, μ, or κ opioid agonists. The chemical and geometric commonalities among agonists at each opioid receptor type were then compared with a non-specific opioid recognition pharmacophore recently developed. The comparison provided identification of the additional requirements for activation of δ, μ, and κ opioid receptors. The distance analysis approach was able to clearly discriminate κ-agonists, while global molecular properties for all compounds were calculated to identify additional requirements for activation of δ and μ receptors. Comparisons of the combined geometric and physicochemical properties calculated for each of the three sets of agonists allowed the determination of unique requirements for activation of each of the three opioid receptors. These results can be used to improve the activation selectivity of known opioid agonists and as a guide for the identification of novel selective opioid ligands with potential therapeutic usefulness.

Introduction

Identification of behaviorally selective analgesics without major side effects is still an important research topic, because of their potential therapeutic usefulness. In spite of many years of research on pain management, opioids continue to be widely recognized as the best analgesics known in a clinical setting. However, their multiple side effects, including potential for physical dependence and abuse, respiratory depression, euphoria, sedation, muscle rigidity, changes in thermoregulation, and inhibition of gastrointestinal motility [1–3], have seriously impaired their use.

The lack of better targets for the management of pain has led to a continuous re- evaluation of the opioid receptors in the search for new analgesics with limited or no side effects. Despite the long-standing effort of diverse research groups [4–6], including our laboratory [7–14], potent opioid analgesics without major side effects such as respiratory depression and addiction liability, are still unknown. For many years, progress in this area was impaired by a lack of understanding of the molecular bases of opioid action. The discovery of three different opioid receptor types, δ, μ, and κ [15–23], provided a source for speculation on the primary events that led to each of the multiple *in vivo* effects of opioid ligands. Towards the design of more selective ligands, our current working hypothesis is that compounds that bind with significant affinity to all cloned opioid receptors, but with different combinations of activation and inhibition properties at each, could be the most promising behaviorally selective agents.

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Unfortunately, the lack of a consistent number of opioid ligands with mixed agonists/antagonists profiles for different receptor subtypes has not allowed a complete validation of the hypothesis put forward. Generation of such activation-selective compounds, cannot easily be approached using structure based computational methods because of the lack of experimental 3D structure of these receptors at atomic resolution. Like other G-protein coupled receptors (GPCRs) [24, 25], the only 3D structures of the three opioid receptor types at atomic resolution that are so far available in the literature come from different computational strategies [7, 10, 26–29]. Although these 3D models offer insights into the molecular pharmacology of these receptors, they still lack sufficient detail for purposes of drug design.

An alternative strategy to search for new classes of ligands with different combinations of activity at the different receptor types is the analysis of the structural properties of the known analogs, using a ligand based design approach or indirect design [30]. Using such an indirect approach, common chemical and geometric requirements for the non-specific recognition of the three δ, μ, and κ opioid receptors were recently identified in our laboratory [31]. Twenty-three different non-specific opioid binders from diverse chemical families were used as a database for computational procedures based on a distance analysis approach and embedded in an in-house pharmacophore-generating program, MOLMOD [32]. The result was a common 3D-recognition pharmacophore at δ, μ, and κ opioid receptors consisting of a protonated amine, two hydrophobic groups, and the centroid of an aromatic ring in a geometric arrangement common to all binders at the three opioid receptors.

In the present work, the common 3D pharmacophore previously developed for non-specific recognition of δ, μ, and κ opioid receptors [31] was used as a basis to identify the chemical and geometric determinants involved in activation of each opioid receptor type. Three different subsets of the non-specific binders that consisted of ligands with high values of agonistic activity at δ, μ, or κ opioid receptors [31], were used as separate data sets for the development of unique requirements for activation. Five potent agonists at the δ-opioid receptor, nine potent agonists at μ and five potent agonists at κ were used. A systematic distance analysis approach allowed the identification of chemical and geometric commonalities between all agonists acting at each opioid receptor type. Comparison between these chemical and geometric commonalities of all δ, μ, or κ opioid agonists and the molecular determinants previously identified as responsible for non-specific recognition at the three opioid receptor types [31] allowed discrimination between the requirements for recognition and activation at each receptor. In addition, this distance-analysis based study provided a means to differentiate the features responsible for the selective activation of κ-opioid receptor from those involved in activation at δ and μ opioid receptors. The calculation of diverse global molecular properties provided discrimination of the requirements for selective activation of δ- and μ-opioid receptors. The combined results led to the determination of a unique combination of structural and physicochemical requirements for activation of the δ, μ and κ opioid receptor types.

These results, reported in the present study, should aid in the discovery of more selective opioid agonists. They can also serve as a guide to selection of new compounds or new probes to elucidate the molecular mechanisms of analgesia mediated via the δ, μ, and κ opioid receptors. In addition, the models derived can serve as a rational guide for the discovery of novel activation selective opioids that are candidates for new therapeutic agents.

Methods

Conformational searches carried out on all compounds included in the present work were done as described in our previous study [31]. The initial structures of morphine [33], nalburphine [34], butorphanol [35], dezocine [36], etorphine [37], fentanyl [38], and lofentanyl [39], were obtained from X-ray crystallographic data available in the literature. Initial structures for all other compounds were built in their cationic form by combining features of the crystal structures of their known analogs and using the MSI/Quanta package (MSI-Quanta. Biosym/MSI, San Diego, CA). Using the Quanta/CHARMm force field [40], a dielectric constant of 80, and no cutoff, energy minimization of the initial structures was performed. Specifically, 200 steps of steepest descent followed by 2000 steps of conjugate gradient method were carried out until the root mean square deviation (rmsd) changes were less than 0.01 Å. A conformational search for each molecule was then performed using the same force field.

Conformational searches of all opioid agonists considered in the present and previous work [31]

were done using different strategies selected on the basis of the number of significant rotatable bonds present in each molecule. For molecules with four rotatable bonds or less a nested rotation search was made using 30 degrees intervals of each torsion angle. Molecules with more than four significant rotatable bonds were subjected to a hybrid genetic algorithm (GA)/minimization procedure (CCEMD, Sandia, CA) to explore their conformational space. Briefly, this procedure consisted of (1) generating an initial population of low energy conformers using a genetic algorithm step, (2) clustering the generated population into families of unique conformers, using a 5-degrees rms torsion criterion; and (3) energy minimizing the resulting unique conformers. The minimized conformers from the previous cycles resulted in GA inputs for second and subsequent cycles. Convergence was achieved when no new unique conformers were obtained within 3 kcal/mol from the lowest energy conformer found, after five consecutive cycles of the hybrid genetic algorithm (GA)/minimization procedure. A conformation was considered unique if at least one of its dihedral angles differed by 30-degrees or more from all other conformations identified in the process.

Systematic pairwise comparisons of the distances between the chemical moieties common to all the agonists at a given opioid receptor type were done using the in-house computer program MOLMOD. The details of this software are described elsewhere [32]. Briefly, MOLMOD is an automated unbiased method that creates alignments between different molecules, using two inputs: (i) the conformational libraries within 3 kcal/mol from the lowest energy conformation found for each agonist at δ, μ, or κ opioid receptors, and (ii) a set of candidate chemical moieties common to each ligand with agonistic activity at each opioid receptor type. The result of applying the computer program MOLMOD to the sets of agonists at δ, μ, or κ opioid receptors consisted of the identification of common chemical moieties in specific geometric arrangements common to all active compounds. For this analysis only conformers within 3 kcal/mol from the lowest energy conformation found for each compound were used. Such a cut-off appears justified based on the analysis of the conformations observed for small molecule ligands in crystallographic studies [41, 42].

Global molecular properties were calculated for the lowest energy conformation of each δ- or μ-agonist that was present in the geometric arrangement common to all agonists at δ or μ opioid receptors, respectively. To this end, semiempirical calculations were carried out using the MOPAC7 package [43]. The selected conformers for each compound were optimized using a PM3 method, until the changes in the norm of the gradient were less than 0.1 Å. Structures optimized showed little variation from the initial geometries. Properties computed for each compound included orbital energies for the highest occupied (HOMO) and lowest empty (LUMO) molecular orbitals, total entropy, rotational entropy, total enthalpy and heat capacity. The proton donating and accepting abilities of candidate ligand moieties were also calculated as the difference in heat of formation of the neutral and protonated molecules using PM3 in vacuum. Steric parameters were computed using the in-house computer program GRAPHA. Among them were total volume, total area, total solvent-accessible area, globularity and the sterimol parameters (L, B1, B2, B3 and B4) [44]. The program was also used to determine group hydrophobicities. Additionally, measurements of free energy of solvation in water were computed for each of the selected agonists at their in-vacuum geometries using the AMSOL 6.5.3/PM3 package [45].

The S-PLUS 3.4 program (StatSci Division, MathSoft Inc., Seattle, WA) was used for property analysis and to generate graphs.

Results and discussion

Selection of potent agonists at δ, μ, and κ opioid receptors

Only potent opioid agonists acting at δ, μ, and κ opioid receptors were considered for the present study. Compounds were classified as agonists if they displayed an IC_{50} of 110 nM or better at any opioid receptor type from the set of previous experimental results obtained in our laboratory using model tissues (unpublished results). Briefly, in these experimental studies, inhibition of muscle contractions in guinea pig ileum (GPI) treated with nor-binaltorphimine (nor-BNI) or b-funaltrexamine (B-FNA) was used to assess activation of μ and κ receptors, respectively, and of mouse vas deference (MVD) treated with B-FNA was used to assess activity at δ. Table 1 lists the five agonists at the δ-opioid receptor, the nine agonists at μ and the five agonists at κ selected for this present work, together with their corresponding activation data.

There have been recent suggestions in the literature that fentanyls may bind in a different way than other

Table 1. Model tissue agonistic activity of the compounds selected in the present work.

Compounds	Model tissue agonistic activity IC_{50} (nM)		
	δ	μ	κ
Morphine	n.e.	110	n.e.
Hydromorphone	n.e.	11	n.e.
Nalburphine	n.e.	n.e.	10
Xorphanol	n.e.	93	n.e.
Butorphanol	n.e.	88	90
Dezocine	n.e.	106	n.e.
Etorphine	7.8	0.4	1.2
Fentanyl	n.e.	3.6	n.e.
Lofentanyl	1.0	0.18	1.3
Carfentanyl	17	0.019	59
SIOM	19	n.e.	n.e.
Comp1*	0.25	n.e.	n.e.

n.e. = non-significant agonistic effect.
Nagase, H., Kawai, K., Hayakawa, J., Wakita, H., Mizusuna, A., Matsuura, H., Tajima, C., Takezawa, Y., Endoh, T., Rational Drug Design and Synthesis of a Highly Selective Nonpeptide δ-Opioid Agonist, (4aS, 12aR*)-4a-(3-Hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-b]acridine (TAN-67) Chem. Pharm. Bull., 1998, 46, 1695–1702.

Table 2. Calculated relative heats of protonation* for each opioid ligand using the PM3 method.

Opioid ligands	Protonation site (cf. Figure 1)			
	E	E′	E″	E‴
Morphine	9.4	0	–	5.9
Hydromorphone	17.8	0	–	12.1
Nalburphine	19.7	0	45.2	14.6
Xorphanol	0	–	–	–
Butorphanol	0	–	15.1	–
Dezocine	0	–	–	–
Etorphine	25.6	8.8	0	26.4
Fentanyl	0	–	–	–
Lofentanyl	0	0	15.4	3.4
Carfentanyl	0	0	15.0	3.4
SIOM	22.6	0	42.4	14.3
Comp1	58.2	0	–	–

*The heats of protonation of all sites have been expressed relatively to the site with the smallest heat of protonation value.

classic opiates [46]. However, there is no evidence that they bind to a completely different receptor site. Since fentanyl-based and classic opiate compounds could display a common recognition pattern in the interaction with their receptors, we considered both classes of opioid ligands in both previous [31] and present studies.

Selection of common candidate chemical moieties

Figure 1 shows the chemical structures of the potent agonists at δ, μ, and κ opioid receptors selected. The chemical moieties that were found in a common geometric arrangement in all agonists at each receptor type are indicated by colored squares. The squares surrounding the moieties labeled by A, B, C, and D, indicate the non-specific recognition motifs embedded in the non-specific 3D recognition pharmacophore at δ, μ, and κ opioid receptors previously developed in our laboratory [31]. Label A refers to a protonated amine, B and C are the labels for two generic hydrophobic groups, and D corresponds to the centroid of an aromatic ring. All additional candidate chemical groups that are present in each δ, μ, and κ agonist besides the four recognition moieties A, B, C, and D, were considered as potential determinants of activation at each opioid receptor type.

Other proton acceptor atoms are found in the compounds selected and could be potential points of interaction with the receptor in all agonists acting at δ, μ, and κ opioid receptors. These candidate proton acceptor atoms are indicated by a light blue color in Figure 1 and labeled by E, E′, E″, or E‴. However, not all of them are common to all agonists. In addition to the four recognition moieties A, B, C, and D and to the candidate proton acceptor centers labeled E–E‴, all κ-agonists have also a common hydrophobic moiety labeled by F in Figure 1.

The heats of protonation of each of the points labeled E to E‴ provide some valuable insights into the role these centers may play. Table 2 reports the relative values of the heats of protonation at each candidate center E, E′, E″, or E‴ compared to the center with the smallest value. Values of zero indicate that that is the strongest proton acceptor among the candidate centers. For all molecules, but etorphine, we selected the strongest proton accepting center common to all agonists at δ, μ, or κ opioid receptors as the favored additional point (light blue squares in Figure 1) that may be involved in the activation of the different opioid receptor types. In the case of etorphine, E or E′ were selected instead of the center E″, which possesses the lowest value of heat of protonation. Selection of points E or E′ instead of E″ for etorphine is mainly dictated by the necessity of this

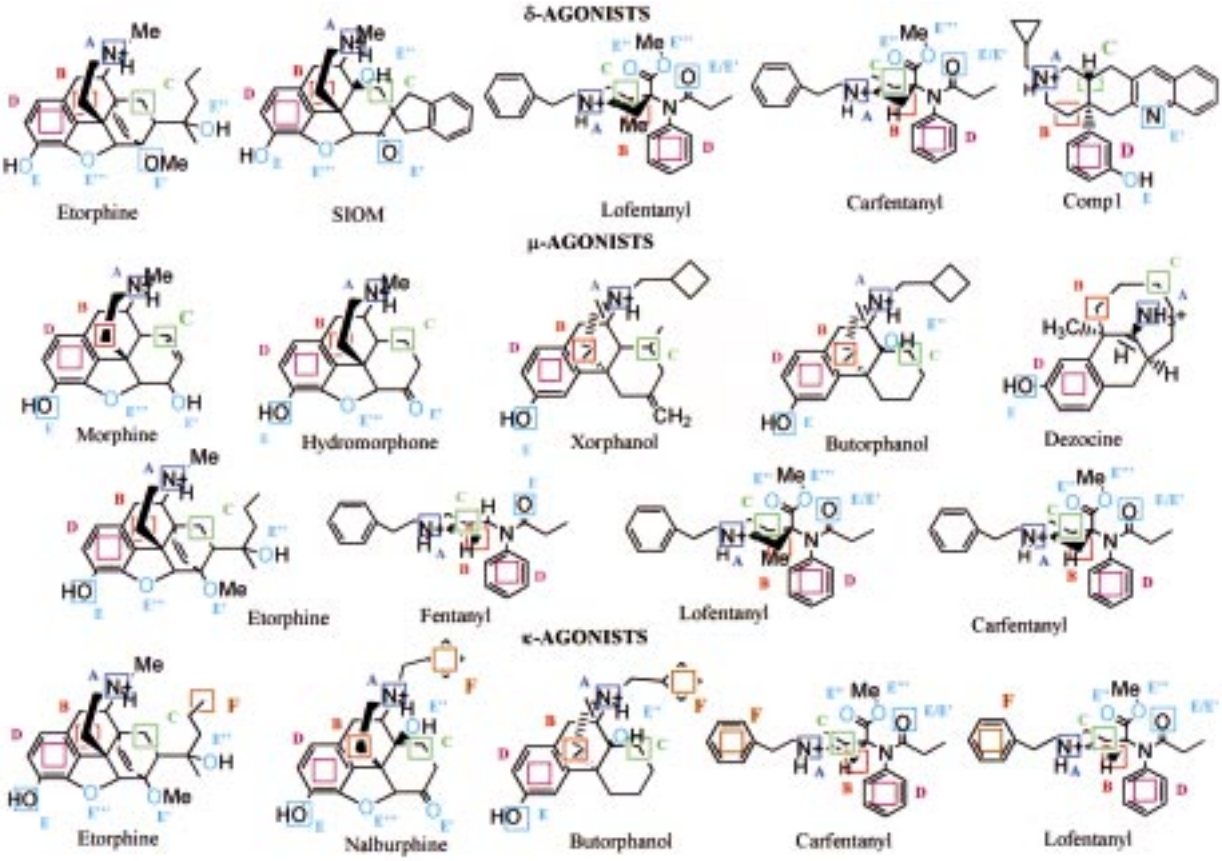

Figure 1. Chemical structures of the compounds selected in the present work as potent agonists at δ, μ, and κ opioid receptors. In different colors are candidate chemical moieties for recognition and activation of δ, μ, and κ-opioid receptors. Specifically, for all of them, A refers to a protonated amine, B, C, D, and F indicate generic hydrophobic groups, while E, E′, E″, and E‴ stand for candidate proton acceptor atoms. The chemical moieties that resulted in common to all agonists at each opioid receptor type and were found in a common geometric arrangement are indicated by light blue squares.

potent 'universal' agonist at δ, μ, and κ opioid receptors to fulfill the distances between pharmacophore points common to all active compounds at each specific receptor type. The relatively small differences between the corresponding absolute values of heats of protonation makes this also a plausible site for interaction.

The chemical moieties common to all δ, μ, or κ agonists as well as the conformational libraries of all the compounds included in the present work were used as input to the in-house software, MOLMOD [32]. This program performs systematic pairwise comparisons between the selected candidate chemical moieties of all the low energy conformers characterized for each ligand. The average distances found in common for each set of agonists at δ, μ, and κ opioid receptors, as well as the distances found between A, B, C and D in the non-specific opioid pharmacophore previously characterized [31] are reported in Table 3.

Chemical, structural, and physicochemical properties of δ-opioid agonists

Analysis of Table 2 suggests that the most favorable proton acceptor atom common to all the δ-agonists corresponds to the oxygen atom labeled by E′ in Figure 1. Etorphine is an exception, because E′ center is the second strongest proton acceptor (Table 2). However, the relatively small difference between the corresponding absolute values of heats of protonation of E′ and the first strongest proton acceptor, i.e., E″, makes E′ a plausible site for interaction.

Slight differences among the heats of protonation, are also observed for a second proton acceptor atom, labeled by E in Figure 1, which may equally play a role

Table 3. Distances between chemical moieties common to all non-specific binders at the three cloned opioid receptor types, and to all selected δ, μ, or κ agonists.

Distances between common moieties*	Non-specific Binders	δ-agonists	μ-agonists	κ-agonists
A-B	2.51 ± 0.07	2.49 ± 0.01 (2.49 ± 0.01)	2.53 ± 0.11	2.49 ± 0.01
A-C	3.53 ± 0.56	3.13 ± 0.68 (3.55 ± 0.48)	2.70 ± 0.33	3.21 ± 0.67
A-D	4.31 ± 0.33	4.47 ± 0.39 (4.47 ± 0.39)	5.32 ± 0.59	5.09 ± 0.83
A-E (A-E′)	–	6.58 ± 0.74 (6.17 ± 0.46)	6.51 ± 0.81	6.22 ± 1.00
A-F	–	–	–	3.78 ± 0.69
B-C	4.02 ± 0.46	3.16 ± 0.70 (3.62 ± 0.46)	2.60 ± 0.24	3.25 ± 0.69
B-D	3.98 ± 0.80	3.53 ± 0.35 (3.53 ± 0.35)	3.70 ± 0.47	3.81 ± 0.37
B-E (B-E′)	–	5.24 ± 0.51 (4.78 ± 0.25)	5.09 ± 0.52	4.79 ± 0.94
B-F	–	–	–	5.61 ± 0.96
C-D	3.75 ± 0.55	4.23 ± 0.70 (3.94 ± 0.07)	4.13 ± 0.58	4.43 ± 0.42
C-E (C-E′)	–	6.19 ± 0.52 (3.61 ± 0.46)	4.79 ± 0.64	5.08 ± 1.66
C-F	–	–	–	5.87 ± 1.63
D-E (D-E′)	–	3.28 ± 1.19 (4.69 ± 0.27)	2.90 ± 0.19	3.66 ± 1.05
D-F	–	–	–	7.86 ± 1.60
E-F	–	–	–	8.96 ± 1.32

*As reported by different colors in Figure 1, A refers to the protonated amine nitrogen, B, C and F indicate three generic hydrophobic moieties, D refers to an aromatic ring, while E and E′ correspond to polar proton acceptors

Table 4. Selected global molecular properties calculated for all agonists acting at δ, μ, and κ opioid receptors.

Compound	Total volume ($Å^3$)	Total area ($Å^2$)	Solv. acc. area ($Å^2$)	L (Å)	B1 (Å)	B2 (Å)	B3 (Å)	B4 (Å)	ΔG solv (kcal/mol)	Homo	Lumo	Rotational entropy (cal/k/mol)	Hydrophobic index	ΔHf (cal/k/mol)
SIOM	359.67	383.99	592.23	14.46	8.58	5.35	3.74	4.13	−29.56	−11.46	−4.03	35.94	1.81	150.22
Comp1	377.13	416.09	652.78	17.99	7.63	4.44	3.87	3.14	−37.59	−11.12	−4.02	36.46	4.34	229.37
Hydromorphone	255.69	282.07	457.36	11.58	6.69	4.58	3.28	2.75	−26.16	−11.77	−4.32	33.58	1.23	157.94
Etorphine	385.8	412.02	606.84	14.89	6.29	4.36	3.59	6.1	−20.15	−11.62	−4.15	35.82	1.34	154.88
Lofentanyl	404.16	462.49	671.29	15.93	7.46	6.68	4.64	3.44	−18.48	−12.43	−3.68	36.5	3.51	146.06
Carfentanyl	388.84	449.26	673.68	15.04	8.06	7.2	2.98	2.52	−18.07	−12.45	−3.83	36.4	3.37	147.9
Nalburphine	319.83	350.91	552.27	14.92	6.64	4.72	3.21	2.83	−29.56	−11.77	−3.92	35.22	0.76	147.63
Morphine	258	283.73	460.28	11.48	5.64	3.95	3.82	3.76	−20.14	−11.71	−4.24	33.55	1.07	156.63
Dezocine	249.76	271.37	434.46	10.82	5.27	4.76	3.48	3.57	−20.3	−11.76	−4.26	32.82	2.38	146.42
Fentanyl	345.49	400.44	616.9	15.03	7.12	5.15	2.87	3.55	−15.05	−12.01	−4.11	35.69	4.23	151.85
Xorphanol	345.17	376.96	580.95	14.84	7.12	4.75	3.82	2.96	−7.93	−11.84	−3.84	35.29	3.66	147.84
Butorphanol	323.17	350.32	552.16	14.06	7.13	4.48	3.94	3.46	−14.53	−11.94	−3.71	34.99	1.94	142.09

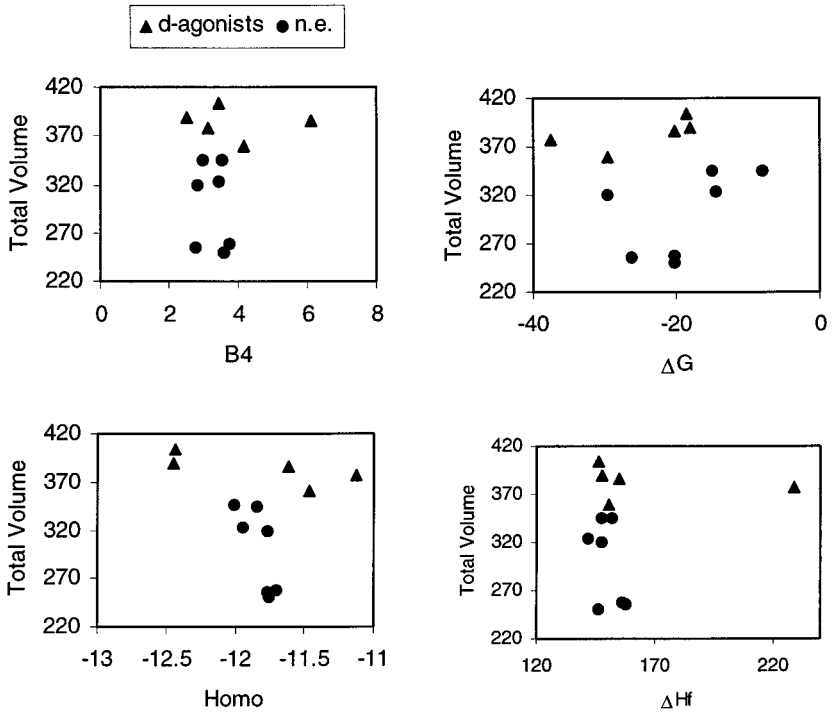

Figure 2. Plots of the molecular property providing the best discrimination between δ-agonists and compounds with non-significant agonistic activity at δ-opioid receptor versus all its non-correlated properties.

as a proton acceptor center as E′. These two additional candidate proton acceptor atoms, E′ and E, are both common to all the five potent δ-agonists considered in the present work, together with the four recognition moieties A, B, C, and D. However, no common geometric arrangement could be found for all six centers that are common to all δ-agonists, in any of the low energy conformers. Common geometric arrangements were possible only if either one or the other of the two acceptor atoms at the time was considered together with the other four recognition moieties A, B, C, and D. In the case of carfentanyl and lofentanyl, the different labels E and E′ were assigned to the same carbonyl group, since this was the only center that could provide common overlap of the recognition moieties A, B, C, and D for all agonists at each receptor type.

Because of the presence of two equally strong acceptor atoms in all the selected δ-agonists, two geometric arrangements were possible for the five centers. These geometries are reported in Table 3 in terms of distances between either the selected chemical moieties A, B, C, D, and E or A, B, C, D, and E′ common to at least one of the lowest energy conformers of all δ-agonists. The only significant difference between these two possible geometric arrangements is related to the distance between the moieties C and E or C and E′. One of the two possible common geometric arrangements, i.e. the one that considers E as additional proton accepting atom, also corresponds to the unique one found for μ-agonists. Thus, there is the possibility for at least some of the agonists acting at δ-opioid receptor to also activate μ-opioid receptors. In fact, three of the five compounds considered in the present work as δ-agonists, i.e. etorphine, carfentanyl and lofentanyl are also agonists at μ-opioid receptors.

Further refinement of the requirements for activation of δ-and μ-opioid receptors was performed by calculation and analysis of diverse global molecular properties of all the compounds considered in the present work. Properties were calculated for the lowest energy conformers that satisfied the geometric requirements for recognition and activation. The values of all calculated properties, including total volume, total area, solvent-accessible area, the sterimol parameters (L, B1, B2, B3 and B4) [44], hydrophobic index [47, 48], HOMO, LUMO, rotational entropy, and free energy of solvation are reported in Table 4 for each of the compounds included in the present work.

Analysis of all these properties calculated for each of the compounds included in the present work in-

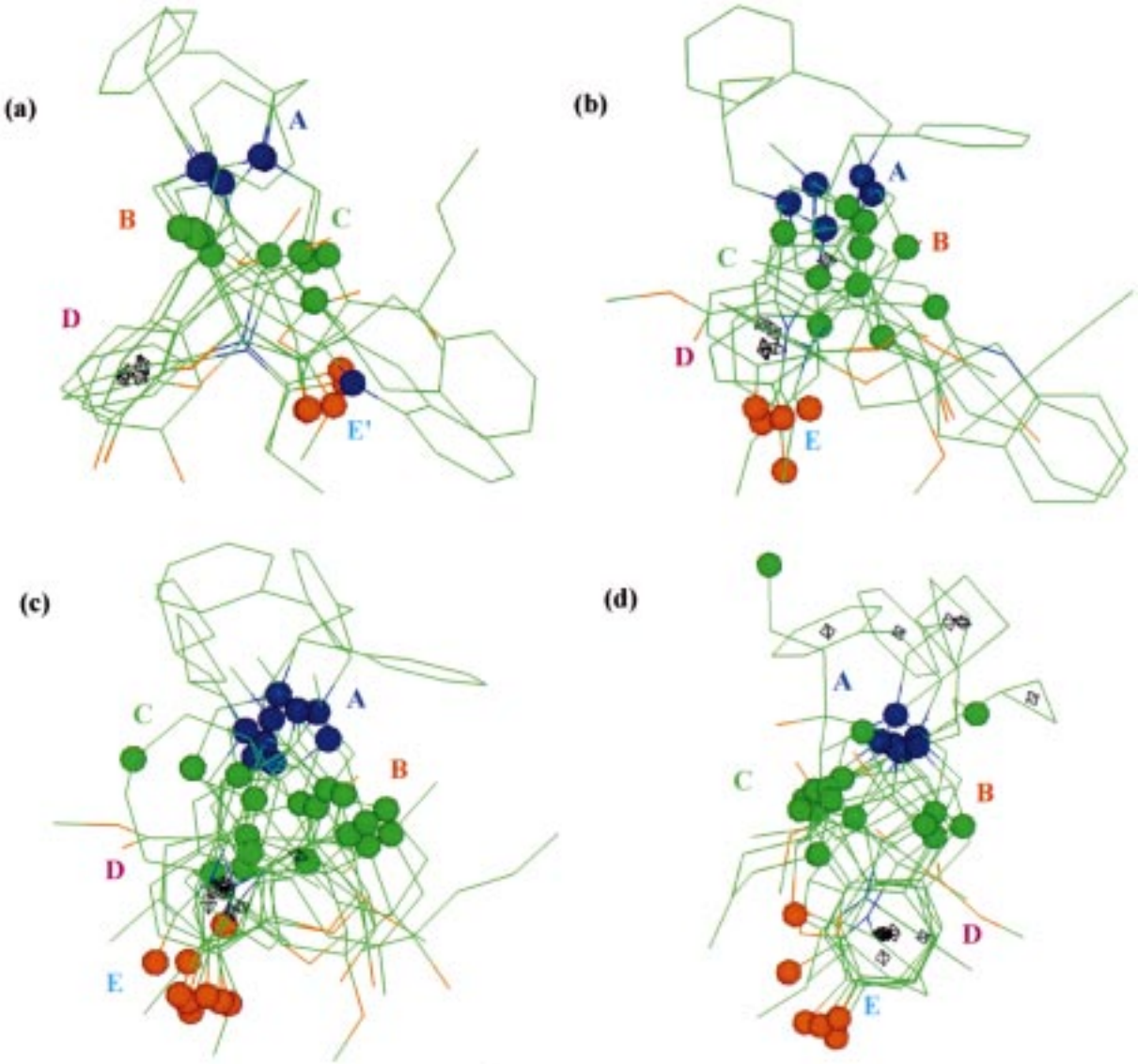

Figure 3. Overlay of the chemical moieties common to all selected δ-agonists (3a and 3b), μ-agonists (3c) and κ-agonists (3d) obtained using the lowest energy conformer of each compound that satisfy the common geometric arrangement of Table 3.

dicates that the total volume is the best discriminant between active and inactive compounds at the δ-opioid receptor. Figure 2 shows plots of the total volume computed for each compound versus other selected properties. These plots suggest that δ-opioid receptor can accommodate largest molecules in comparison to the other opioid receptor types, since the total volume values of δ-agonists are highest than the ones corresponding to compounds displaying a non-significant agonistic activity at the δ-opioid receptor.

Figures 3a and 3b offer a visual representation of the results obtained for the δ-agonists considered in the present work, using a distance analysis approach. These figures display the best overlay of the chemical moieties common to each selected δ-agonist obtained using the lowest energy conformers of each compound and considering either the chemical moiety E or E′ as a determinant of activation at the δ-opioid receptor. Comparison between the superposition of all δ-agonists shown in Figure 3b and the corresponding superposition of all μ- and κ-agonists shown in Figures 3c and 3d, respectively, suggests that δ-agonists may be discriminated from μ and κ agonists due to the presence of a bulky hydrophobic area located be-

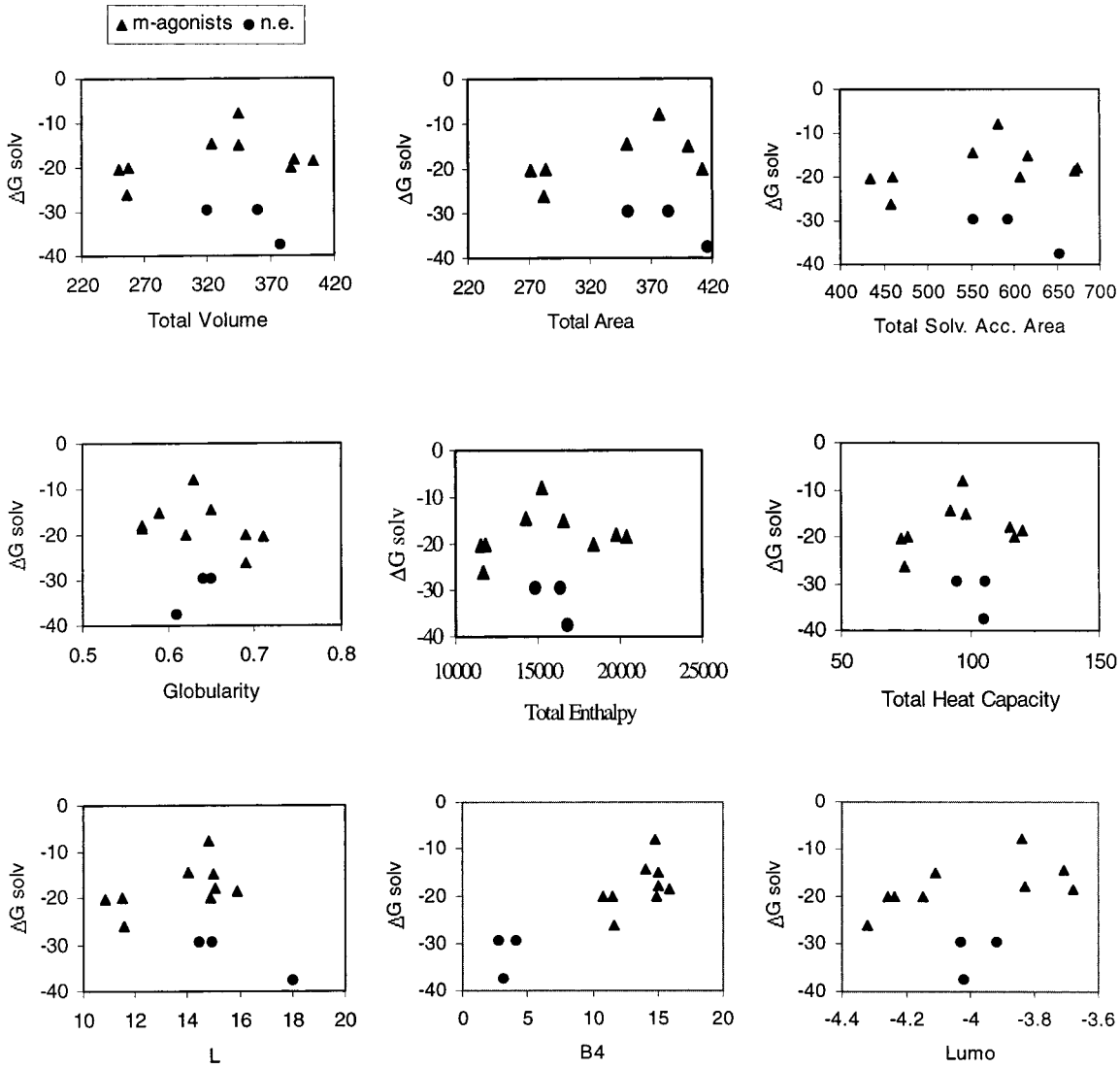

Figure 4. Plots of the molecular property providing the best discrimination between μ-agonists and compounds with non-significant agonistic activity at μ-opioid receptor versus all its non-correlated properties.

tween the chemical moieties B and E. This bulky hydrophobic area is absent in the nine and five agonists acting at μ-and κ-opioid receptors, respectively, and can be traced to the presence of additional voluminous aromatic groups in the only two selective δ-agonists used in the present work, i.e. SIOM and Comp1 (Table 1). The lack of agonistic activity of these two compounds at both μ and κ receptors leads to the inference of a substantial difference in the binding pockets of μ and κ receptors with respect to δ. Accordingly, activation selectivity at the δ-opioid receptor is found when bulky hydrophobic groups are located between the chemical moieties B and E. This observation is in agreement with the results obtained by molecular property analysis reported in Figure 2, where higher values of total volume are required for selective agonism at the δ-opioid receptor.

Chemical, structural, and physicochemical properties of μ-opioid agonists

The compounds selected in the present work as potent μ-agonists have E as the only additional proton accepting moiety common to all agonists at μ-opioid receptor with the lowest value of heat of protonation (Table 2) and in addition to the four recognition

chemical moieties A, B, C, and D. Using the computer software MOLMOD [32], pairwise distances between these five candidate chemical moieties A, B, C, D, and E were performed for all the lowest energy conformers within 3 kcal/mol from the global minimum identified for each of the selected μ-agonists. The common geometric arrangement of all μ-agonists found by MOLMOD is reported in Table 3. A visual representation of these results is shown in Figure 3c. In this figure, a superposition of the five chemical moieties A, B, C, D, and E is provided using the lowest energy conformer of each agonist at the μ-opioid receptor satisfying the common geometric arrangement reported in Table 3.

From the distance analysis approach, it is not possible to deduce an unequivocal discrimination between δ and μ agonists. Hence, analysis of the global molecular properties was necessary to refine the proposed models. A summary of the results obtained by analysis of all molecular properties calculated for the lowest energy conformers of all μ-agonists satisfying the common arrangement reported in Table 3, is reported in Figure 4. The best discrimination between all μ-agonists and all compounds with non-significant agonistic effect at μ-opioid receptor is provided by the ΔG of solvation. As suggested from the analysis of the plots reported in Figure 4, high values of ΔG of solvation may be required for selective activation of μ-opioid receptor. In summary, highest values of ΔG of solvation and lowest values of total volume differentiate μ- from δ-agonists.

Chemical and structural properties of κ-opioid agonists

Unlike agonists acting at δ and μ opioid receptors, the requirements for selective activation of κ-opioid receptor were uniformly determined by the distance analysis approach applied to the five κ-agonists selected in the present work. Besides the common recognition moieties A, B, C, and D, and the common proton acceptor moiety labeled by E in Figure 1, these five κ-agonists possess an additional unique common chemical center. This seventh common chemical center corresponds to the hydrophobic moiety labeled by F in Figure 1.

The average distances between the seven chemical moieties common to at least one of the low energy conformers identified for each κ-agonist are reported in Table 3. A visual representation of the results obtained for κ-agonists is reported in Figure 3d. Specifically, this figure reports an overlap of the seven common chemical moieties identified in the lowest energy conformer of each κ-agonist that satisfies the common geometric arrangement reported in Table 3.

As can be clearly seen in this figure, the presence of this additional hydrophobic moiety present in all κ-agonists but not to all δ and μ agonists constitutes the unique requirement for selective activation of κ-opioid receptors.

Conclusions

The aim of the present work was to identify the structural and physicochemical properties that are unique for activation of each of the three opioid receptor types. This goal was based on the working hypothesis that ligands that bind with significant affinity to all the three cloned opioid receptor types, δ, μ, and κ, but with different combinations of activation and inhibition at the three receptors could be the most promising behaviorally selective agents. Thus in this study, three subsets of a database of twenty-three non-specific binders were selected as potent δ, μ, and κ agonists. Chemical and geometric determinants common to all δ, μ, or κ opioid receptors were identified using an in-house computer program based on a distance analysis approach. Comparison between these chemical and geometric commonalities of all agonists at a given opioid receptor type with the ones embedded in a non-specific opioid recognition 3D pharmacophore recently developed in our laboratory, provided identification of the requirements for activation at δ, μ, and κ opioid receptors. Additionally, this approach provided identification of the requirements for selective activation of the κ-opioid receptor. Specifically, the presence in all κ-agonists, but not in δ- and μ-agonists, of an additional common hydrophobic moiety labeled by F in the present work, may be responsible for selective activation of this specific opioid receptor type. In order to discriminate between agonists acting at δ and μ receptors, different global molecular properties were calculated for each of these ligands. Analysis of these properties suggested that agonists at δ have the unique requirement of largest volume and agonists at μ have the unique requirement of largest free energy of solvation.

The results reported in the present study can now serve to improve the selectivity of known opioid agonists. They can also be used for the discovery or

design of novel selective opioid ligands with potential therapeutic usefulness.

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