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Molecular Determinants of Non-Specific Recognition of δ, μ, and κ Opioid Receptors

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Abstract—Identification of the molecular determinants of recognition common to all three opioid receptors embedded in a single three-dimensional (3D) non-specific recognition pharmacophore has been carried out. The working hypothesis that underlies the computational study reported here is that ligands that bind with significant affinity to all three cloned opioid receptors, δ, μ, and κ, but with different combinations of activation and inhibition properties at these receptors, could be promising behaviorally selective analgesics with diminished side effects. The study presented here represents the first step towards the rational design of such therapeutic agents. The common 3D pharmacophore developed for recognition of δ, μ, and κ opioid receptors was based on the receptor affinities determined for 23 different opioid ligands that display no specificity for any of the receptor subtypes. The pharmacophore centers identified are a protonated amine, two hydrophobic groups, and the centroid of an aromatic group in a geometric arrangement common to all 23, non-specific, opioid ligands studied. Using this three-dimensional pharmacophore as a query for searching 3D structural databases, novel compounds potentially involved in non-specific recognition of δ, μ, and κ opioid receptors were retrieved. These compounds can be valuable candidates for novel behaviorally selective analgesics with diminished or no side effects, and thus with potential therapeutic usefulness.

Introduction

Despite the presence in the literature of a large body of information on the multiple in vivo effects of opioids, including analgesia, euphoria, sedation, respiratory depression, muscle rigidity, potential for physical dependence and abuse, changes in thermoregulation, and inhibition of gastrointestinal motility, the mechanisms that lead to each of these end points are not yet fully understood.[1–3]

Identification of the three different opioid receptor types δ, μ, and κ by molecular cloning experiments[4–12] suggested a possible explanation for the multiple activities of opioids. These three opioid receptors have been shown to belong to the G-protein coupled receptor (GPCR) superfamily,[13,14] which is characterized by a common structural motif of seven transmembrane spanning helices connected by intracellular and extracellular loops. Unfortunately, an experimental 3D structure of these receptors at atomic resolution is still unknown. However, diverse 3D models of the three opioid receptors have been proposed during the last few years using different computational strategies.[15–20] While the models offer insights into the molecular pharmacology of these receptors, they are still lacking detail for purposes of drug design.

Despite the long-standing effort of different research groups, including our laboratory,[15,20–36] to characterize both opioid ligands and ligand/receptor complexes, the molecular basis of the opioid action is still unclear. Since the beginning of their clinical use, the search for opioid analgesics with limited or no side effects led to the synthesis and evaluation of numerous peptide and non-peptide analogues in many chemical families. However, potent opioid analgesics without side effects such as, for example, respiratory depression and addiction liability, are still unknown.

In order to design behaviorally selective analgesics with diminished side effects, our working hypothesis is that ligands that bind with significant affinity to all three cloned opioid receptors, δ, μ, and κ, but with different combinations of agonism and antagonism at these receptors, offer a promising avenue towards behaviorally selective analgesics. A first step towards that goal requires the identification of the molecular determinants of recognition common to all three opioid receptors. This is a strategy that departs from prior efforts in the opioid field where the emphasis has been on the identification of highly selective ligands.

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In the present work, the existence of common 3D pharmacophoric elements for the recognition of the three δ, μ, and κ opioid receptors is explored. Towards this end, a pharmacophore has been developed using 23 different non-specific opioid binders, from diverse chemical families including fentanyls, morphinans, dihydromorphones, etc. Recent computational studies[37] suggest a unique binding mode of fentanyl-based compounds within the μ opioid receptor. However, there is no experimental evidence that fentanyls and classic opiates cannot display a common recognition pattern within the receptor, although they can bind in different orientations within the receptor. Accordingly, both fentanyl-based and classic opiate compounds have been considered to develop a common 3D pharmacophore for recognition at δ, μ, and κ opioid receptors in the present work. Systematic procedures embedded in an in-house software[34] for the determination of 3D pharmacophores, recently implemented in our laboratory,[38] were used for this purpose. Specifically, this software requires two inputs: i) a conformational library for each of the compounds to be used in the pharmacophore development, and ii) user identification of all chemical moieties potentially responsible for recognition and/or activation, i.e., identification of the pharmacophore components. The computer program then systematically searches for common spatial arrangements of all combinations of the candidate chemical moieties selected for each compound that show appreciable affinity for the opioid receptors. Taken together, these common chemical and geometric determinants constitute the developed 3D pharmacophore.

Use of the methodology in the present study permitted the development of a four-point pharmacophore common to all non-specific ligands that bind to δ, μ, and κ opioid receptors. A data set of binding affinities of twenty-nine different opioid ligands at these three receptor types was used in the present work. Among these 29 compounds were 23 non-specific binders and six low affinity molecules at all three opioid receptor types. The presence in the six low affinity compounds of extra bulky groups that protrude in an area that is not occupied in all 23 non-specific binders at δ, μ, and κ opioid receptors was suggested as responsible for the lack of affinity of these six compounds. The 3D pharmacophore developed in the present work was first assessed using an in-house database of mixed compounds having different CNS activities and then used as a query for searching for novel compounds in 3D structural databases. These novel compounds are potential non-specific opioid ligands that may be optimized into behaviorally selective analgesics with diminished or no side effects. Experiments on these compounds are currently under investigation in our laboratory.

Methods

Structures of all the compounds selected were obtained using the MSI/Quanta package (MSI-Quanta, Biosym/MSI, San Diego, CA). Initial atomic coordinates for ligands like metazocine,[39] morphine,[40] nalorphine,[41] naltrexone,[42] butorphanol,[43] dezocine,[44] etorphine,[45] fentanyl,[46] and lofentanyl[47] were obtained from X-ray crystallographic data available in the literature. Initial structures for all other compounds were built by combining features of the crystal structures of their known analogues. The Quanta/CHARMm force field[48] was used to energy minimize the initial structures, as well as for the subsequent conformational searches. During the minimization procedure, a dielectric constant of 80 and no cut-off were used. Specifically, energy minimization was performed using 200 steps of steepest descent followed by 2000–3000 steps of conjugate gradient method until the root mean square deviation (rmsd) changes were less than 0.01 Å.

Conformational searches were performed for all the 29 compounds selected for the present work. The compounds were studied in their protonated form. The conformational space of all compounds was explored using different strategies that depended on the number of significant rotatable bonds present in the molecule. Systematic nested rotations of each selected dihedral angle were done for compounds with four or less significant rotatable bonds, employing 30° increments and energy minimizing the resulting conformations. When the molecules had more than four significant rotatable bonds, systematic nested rotations become impractical. Therefore, a hybrid genetic algorithm (GA)/minimization procedure (CCEMD, Sandia, CA) was used for the more flexible ligands. Three separate steps were performed for each GA run: i) generation of an initial population of low energy conformers using a genetic algorithm step; ii) clustering of the generated population into families of unique conformers, using a 5° rms torsion criterion; and iii) energy minimization of the resulting unique conformers. The procedure was repeated until no new unique conformers were obtained within 3 kcal/mol from the lowest energy conformer found, after five consecutive cycles of the procedure reported above. A conformation was considered unique if at least one of its dihedral angles differed by 30° or more from all other conformations identified in the conformational search.

The conformational libraries of the different selected compounds within 3 kcal/mol of the lowest energy conformation constitute one of the inputs for the in-house pharmacophore-generating program, Molmod.[38] This program is an improved version of our previous in-house computer program Distcomp.[34] The selection of the 3 kcal/mol energy cut-off is based on recent works that suggest that, for a majority of ligand–protein complexes, the bioactive conformations are within such a threshold.[49,50] Nevertheless, the sensitivity of the pharmacophore detection was checked and it was verified that an identical pharmacophore is obtained using a larger energy window of 5 kcal/mol.

The software Molmod requires as a second input a set of candidate chemical moieties common to each ligand with high affinity to all the three opioid receptors δ, μ, and κ. Specifically, the selection of candidate pharmacophoric

components is initiated by the identification of the chemical moieties common to the most similar ligands within the set of compounds. Subsequently, corresponding moieties are identified in the remaining more heterogeneous ligands. Using these two inputs, Molmod performs systematic pairwise comparisons between the selected candidate chemical moieties of all the low energy conformers characterized for each ligand. The program uses principles of clustering and distance matrix comparisons as those found in the methods of previously development computer programs for pharmacophore identification like Disco[51] and Distcomp.[34] The algorithm involves computing the similarities in distance matrix elements between dissimilar ligands. In the Molmod implementation, the possibility for the development of pharmacophores that include points, which are receptor based, is included. In addition, the uncertainties in the geometric descriptors of the 3D pharmacophores are calculated as sum of the variances of each interpharmacophore distance.

Database searches using the pharmacophore developed in the present work as a query were performed using the Tripos Inc. SYBYL/UNITY package (SYBYL/UNITY, Tripos Associates, Inc., St. Louis, MO, 1999) and Cambridge, Maybridge, NCI, and Chapman-Hole 3D-Structural Databases.

Results and Discussion

A database of 29 different compounds was selected for the present work. Figure 1 shows the chemical structures of all these compounds. As can be seen from this figure, the selected compounds belong to diverse chemical families. The families include benzomorphans (metazocine, SFK10047, cyclazocine, WIN4441, and MR2266), dihydromorphones (hydromorphone, naltrexone, and nalburphine), aminotetralines (dezocine), morphinans (xorphanol and butorphanol), oripavines (etorphine), fentanyls (fentanyl, lofentanyl, carfentanyl, *N*-methylfentanyl, fentanyl #13, fentanyl #28), dihydromorphindoles (oxymorphindole, naltrindole, nor-BNI, compounds **15**, **16**, and **17**), 4,5-epoxymorphinans (morphine, nalorphine, and SIOM), and ethenomorphinans (compounds **24** and **26**).

Figure 1 also shows in different colors the four chemical moieties A, B, C, and D common to all the compounds with high affinity to δ, μ, and κ opioid receptors that were selected as potential components of the 3D pharmacophore, according to the procedure reported in the Methods section. Specifically, A refers to a protonated amine, B and C indicate two hydrophobic groups, and the letter D marks the centroid of an aromatic ring.

The selection of receptor ligands was based on the results of Table 1, that lists the binding affinities to the three opioid receptor types δ, μ, and κ of all the compounds shown in Figure 1. Most of the experimental data employed have been obtained over the last few years in our laboratory.[24,30,33] Specifically, affinities at δ receptors were determined in competitive binding assays using [^{3}H]DPDPE, at μ receptors using [^{3}H]DAMGO, and at κ receptors using [^{3}H]U69593. In contrast, binding affinity data for compounds **24**, **26**, **15**, **16**, and **17** in Table 1 were retrieved from the literature.[52,53]

Among the compounds shown in Figure 1, whose binding affinities are reported in Table 1, are 23 binders ($K_i < 1\,\mu M$) at all the three opioid receptors δ, μ, and κ and six non-binders ($K_i > 1\,\mu M$). The cut-off of 1 μM chosen to distinguish binders from non-binders is somewhat arbitrary. However, the reported K_i values of all the opioid ligands selected for the present work are sufficiently different from the cut-off value, in a way that any uncertainty in the relative definition of binders and non-binders has low probability.

Table 2 gives the number of rotatable bonds considered in the conformational analysis for each of the opioid ligands. The total number of conformers characterized computationally, and the number of low energy conformers within 3.0 kcal/mol of the lowest energy conformation found, are also reported. As reported in the Methods section, the use of the nested rotation or GA/minimization procedures as computational strategies to characterize the conformational space of the opioids selected for the present work depended on their number of significant rotatable bonds. Specifically, the nested rotation method was applied to all the selected opioid ligands except the most flexible ones. Among these last ones were WIN44441, fentanyl, lofentanyl, carfentanyl, fentanyl #13, and fentanyl #28. The total number of conformers reported in Table 2 for these six compounds refers to the total number of unique conformations, i.e., the ones with variations of more than 30° in their selected rotatable bonds. This criterion for uniqueness was also used as a convergence criterion for the GA/minimization procedure carried out for searching all the energetically possible conformations of the flexible opioid ligands studied in the present work. Specifically, this procedure was repeated until no new unique conformers were obtained within 3 kcal/mol of the lowest energy conformer found, during five consecutive GA/minimization cycles.

Figure 2 shows the energy distribution obtained for the opioid ligand WIN44441 after 9 cycles of the GA/minimization procedure. As can be seen, in the last five computational cycles of the GA/minimization procedure, the number of unique conformations within the 0–3 kcal/mol energetic range remains the same, suggesting the convergence of the computational method used. Regarding the other flexible molecules studied in the present work, convergence was achieved after 45, 8, 10, 12, and 27 cycles for fentanyl, lofentanyl, carfentanyl, fentanyl #13, and fentanyl #28, respectively.

Using the low energy conformers within 3.0 kcal/mol of the lowest energy conformation found for all the opioid ligands with high affinity at the three opioid receptor types δ, μ, and κ (Table 1), together with the selected moieties A, B, C, and D shown in Figure 1, as inputs to the in-house computer program described in the Methods section, a single common geometrical arrangement of

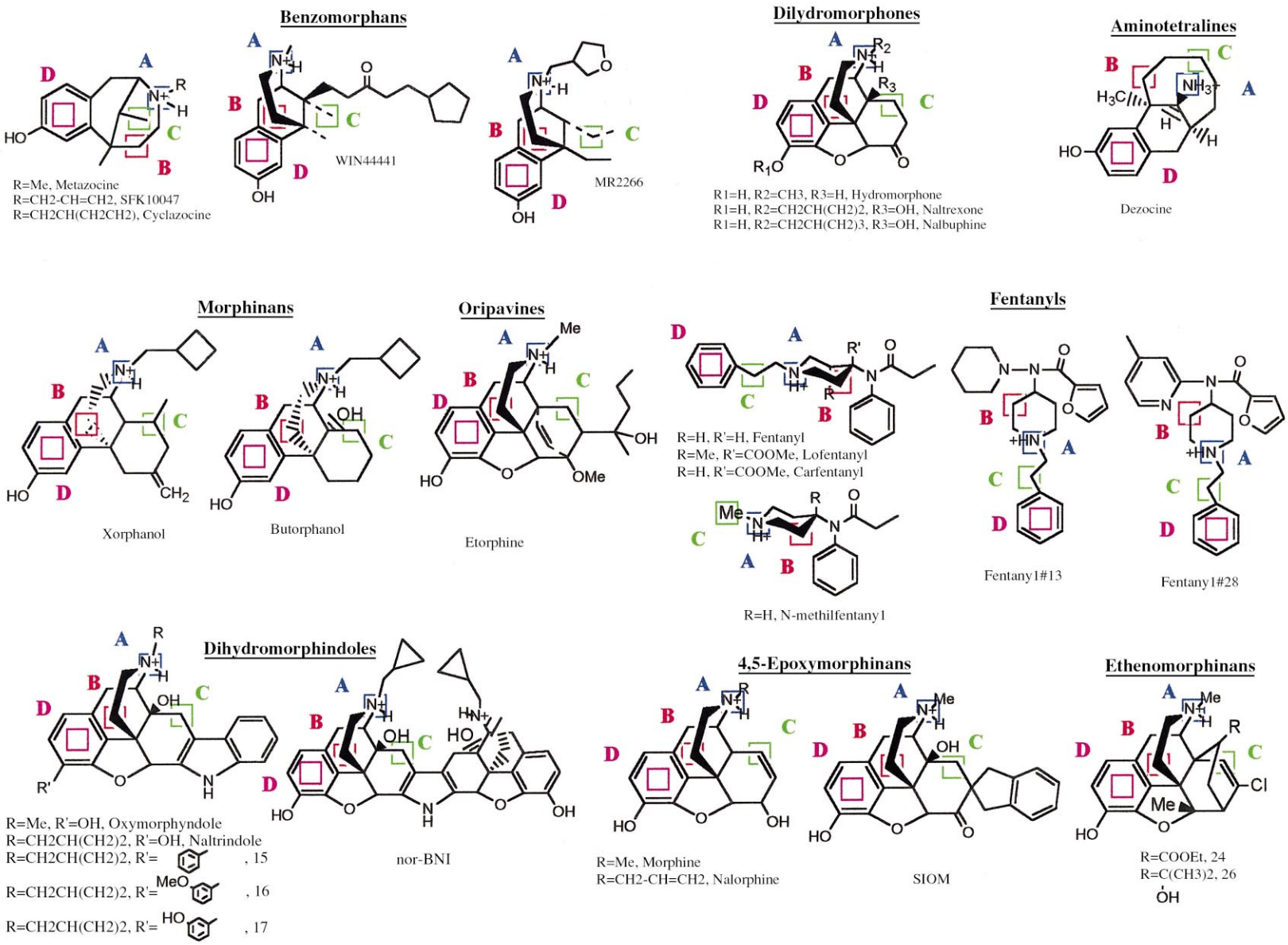

Figure 1. Chemical structures of the 29 opioid ligands included in the present work. Shown in different colors are the four candidate chemical moieties (A, B, C, and D) selected for each compound as possible components of the 3D-recognition pharmacophore. A (blue color) corresponds to a protonated amine, B and C (red and green colors, respectively) are two hydrophobic groups, and D (magenta color) indicates the centroid of an aromatic ring.

Table 1. Receptor binding affinities of the compounds used for the development of a non-specific recognition pharmacophore at the δ, μ, and κ opioid receptors

Compounds	Receptor binding affinity		
	K_i (nM)		
	δ	μ	κ
Metazocine	44.3	3.8	13.3
SKF10047	4.1	2.7	3.2
Cyclazocine	1.0	0.2	0.5
WIN44441	1.14	0.05	0.09
MR2266	3.0	1.0	0.16
Morphine	90	1.8	137
Nalorphine	7.2	1.8	7.7
Oxymorphindole	0.7	111	228
Hydromorphone	18.5	0.47	24.9
Naltrexone	6.6	1.0	8.5
Nalburphine	170	3.8	35
Xorphanol	1.6	0.2	0.2
Butorphanol	10	1.3	2.0
Dezocine	290	3.6	460
Etorphine	0.6	1.0	0.2
Fentanyl	180	1.2	290
Lofentanyl	0.24	0.023	0.60
Carfentanyl	3.3	0.024	43
Fentanyl #13	152	1.5	43
Fentanyl #28	68	1.3	2.7
Naltrindole	0.1	34	20
NorBNI	13	22	1.2
SIOM	1.4	10.6	588
N-Methylfentanyl	> 5000	> 5000	> 5000
24	> 10000	3963	> 10000
26	> 10000	> 10000	> 10000
15	10588	> 5682	> 6803
16	> 6667	> 5682	> 6803
17	1605	> 5682	> 6803

Table 2. Computational results of the conformational searches carried out on the 29 different ligands used for the development of a 3D pharmacophore for non-specific recognition of δ, μ, and κ opioid receptors

Compounds	No. of significant rotatable bonds	Total no. of conformations	No. of conformations within 3 kcal/mol
Metazocine	None	1	1
SKF10047	2	144	135
Cyclazocine	2	144	123
WIN44441	6	602[a]	72
MR2266	4	1296	232
Morphine	None	1	1
Nalorphine	2	144	97
Oxymorphindole	None	1	1
Hydromorphone	None	1	1
Naltrexone	2	144	42
Nalburphine	2	144	20
Xorphanol	2	144	28
Butorphanol	2	144	26
Dezocine	None	1	1
Etorphine	4	1296	205
Fentanyl	7	663[a]	379
Lofentanyl	9	603[a]	114
Carfentanyl	9	931[a]	305
Fentanyl #13	7	611[a]	87
Fentanyl #28	7	704[a]	395
Naltrindole	2	144	42
NorBNI	4	1296	38
SIOM	None	1	1
N-Methylfentanyl	4	1296	20
24	3	432	37
26	1	12	4
15	3	432	131
16	4	1296	274
17	3	432	131

[a]Number of unique conformations obtained using the GA/minimization procedure and the criterion for uniqueness reported in the Methods section.

these moieties (i.e., a 3D pharmacophore) was identified. Note that multiple pharmacophores could be found in principle. However, in this case only one overlay was possible when the four centers were considered simultaneously.

Shown in Figure 3 is a schematic representation of the four-component 3D pharmacophore obtained for the non-specific ligand recognition at δ, μ, and κ opioid receptors. As in Figure 1, the label A refers to a protonated amine, B and C indicate two hydrophobic groups, and D corresponds to the centroid of an aromatic group. Also shown in Figure 3 are the pairwise distances between the above defined four components common to the 23 ligands that bind with significant affinity to all the three opioid receptor types. The standard deviations of the mean values of these distances are also reported in this figure and they correspond to a measure of the uncertainties in the geometric descriptors of the 3-D pharmacophore.

Figure 4 shows the superposition of the conformers selected as the bioactive form for each of the 23 opioid ligands with high affinity to the δ, μ, and κ opioid receptors that conformed to the 3D pharmacophore. The superposition was obtained by spatial overlap of the four common recognition moieties, A, B, C, and D identified in each of the 23 high-affinity opioid ligands used in this work.

As reported in Table 1, six compounds of the database of opioid ligands used for the development of the 3D pharmacophore reported here have no affinity ($K_i > 1$ μM) at all δ, μ, and κ opioid receptors. As seen in Figure 1, the first of these six non-binders at all the three opioid receptor types, *N*-methylfentanyl, does not fit the pharmacophore because of the lack of a pharmacophore component corresponding to the one labeled as D. This component corresponds to the centroid of an aromatic group. Although such a moiety is present in *N*-methylfentanyl (Fig. 1), it is not in the right spatial position to comply with the pharmacophore distance requirements reported in Figure 3.

In contrast, the remaining five opioid ligands **24**, **26**, **15**, **16**, and **17**, with no affinity to all δ, μ, and κ opioid receptors, do satisfy all the requirements of the 3D pharmacophore developed in the present work. However, when the four pharmacophore moieties A, B, C, and D that conform to the 3D pharmacophore are overlapped with the corresponding moieties of the 23 high-affinity binders (Fig. 5), bulky groups of these compounds (black squares in Figure 5) protrude into an area that is not occupied by any of the high-affinity compounds. The steric constraints resulting from the presence of these groups in the compounds with no affinity at the δ, μ, and κ opioid receptors could be

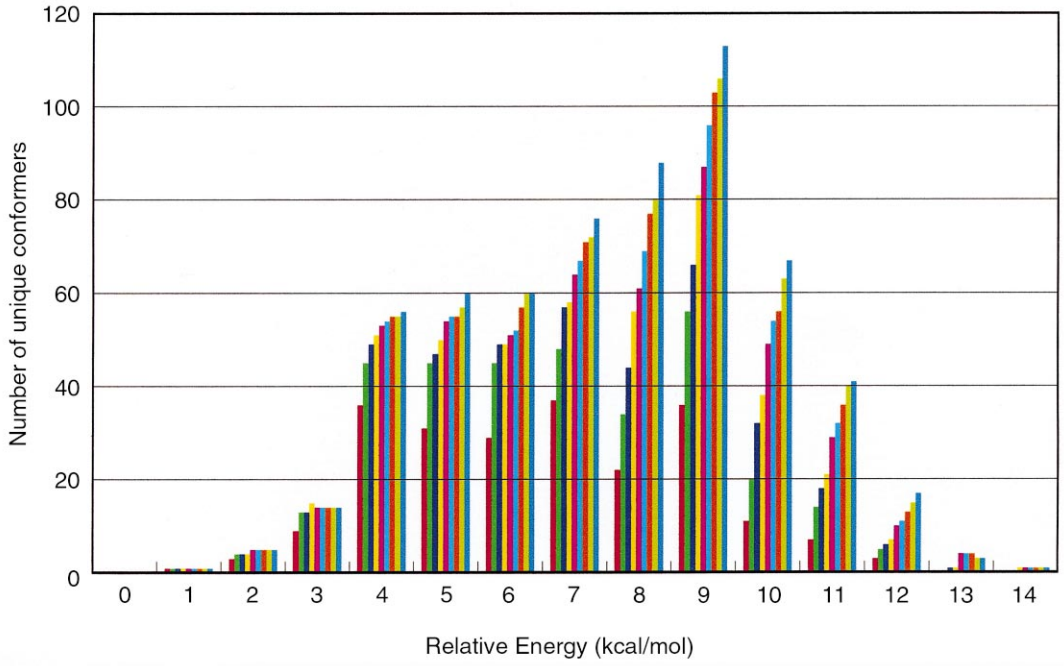

Figure 2. Energy distribution of the number of unique conformations of the opioid ligand WIN44441 obtained after 9 cycles of the GA/minimization procedure.

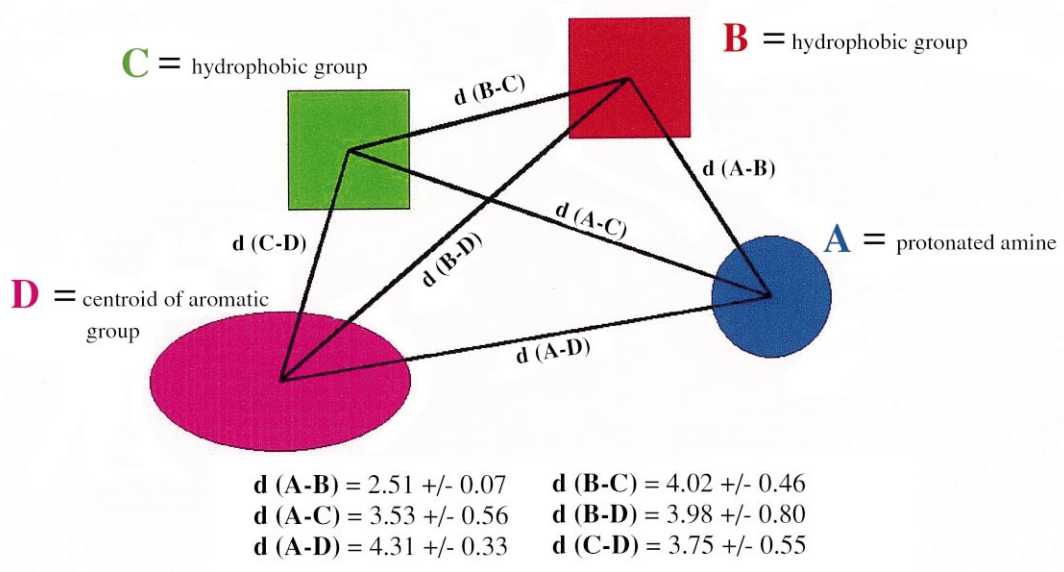

Figure 3. The four-component 3D pharmacophore developed for non-selective recognition of the δ, μ, and κ opioid receptors. A, B, C, and D follow the definition of Figure 1. Also given in this figure are the pairwise distances and the variance values between these four pharmacophore components.

responsible for their lack of affinity. Otherwise, the protruding groups could interfere with the proper positioning of the pharmacophore components C and D in the binding site.

Assessment of the 3D pharmacophore developed in the present work was done by performing a controlled computational experiment. This experiment consisted of using this 3D pharmacophore as query for searching an in-house database of compounds with reported central nervous system activities. Specifically, this database included 80 diverse non-opioid compounds with different CNS activity, including analgesics, antidepressants, anesthetics, anticonvulsants, anxiolytics, antipsychotics, etc. Several conformations for each compound were stored. A 3D search was performed using the same criterion for compliance, 1.5 Å, of distance similarities between the selected recognition moieties as in the development of the pharmacophore. In addition, excluded volumes corresponding to the groups of the five non-binders indicated by black squares in Figure 5 were considered in the database search. A low false positive rate of 5% was

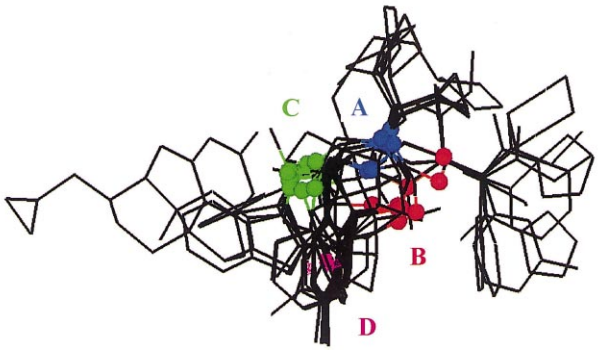

Figure 4. Spatial overlap of the four common recognition moieties A, B, C, and D of the lowest energy conformer of the 23 opioid ligands with high affinity to all the three opioid receptor types, which comply with the non-specific recognition pharmacophore. The definition of moieties A, B, C, and D is the same as in Figure 1.

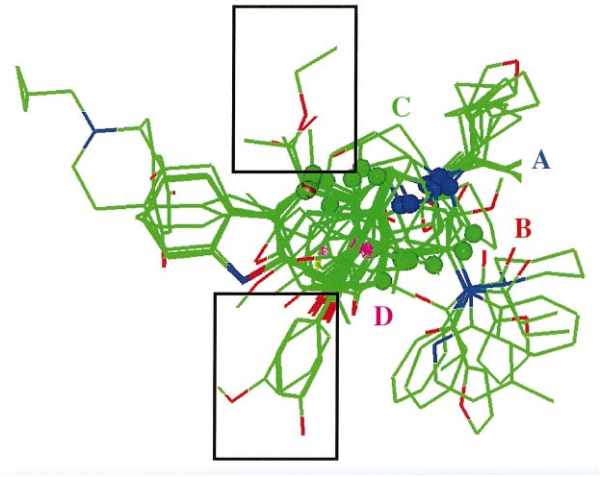

Figure 5. Superposition of all the 29 compounds used as database set for the pharmacophore development. This superposition has been carried out by spatial overlap of the four pharmacophore components A, B, C, and D identified in the lowest energy conformer of each ligand, which comply with the 3D pharmacophore. Same definition as in Figure 1 for A, B, C, and D.

obtained. The four compounds retrieved were the anorexic Mazindol, the anticonvulsant Phenytoin, the anxiolytic Flutazolam, and the antidepressant Aptazapine. However, values of opioid binding affinity have not been reported in the literature for any of them. Experiments on these compounds are presently under consideration in our laboratory, in order to further validate the opioid non-specific pharmacophore developed in the present work. On the other hand, false negative rates could not be determined because of the lack in the literature of non-specific opioid ligands different from the ones used as dataset in the present study for the development of the non-specific pharmacophore at δ, μ, and κ opioid receptors.

In order to identify novel non-specific opioid compounds which could reveal activation selectivity at δ, μ, or κ opioid receptors, additional database searches were performed using the pharmacophore developed in the present work and different 3D structural databases. A number of classical opioid ligands are contained in these databases. A rigid 3D search of them permitted the identification of both opioid ligands known to be binders to all opioid receptor types and novel compounds. Among the opioid ligands found in the database search were morphine, diverse morphinans, butorphanol, metazocine, naltrexone, nalburphine, nalorphine, fentanyl, and fentanyl derivatives. The remaining molecules identified in the database search, which do not correspond to known opioid ligands, can be considered as novel non-specific opioid ligands. These compounds could eventually exhibit activation selectivity at δ, μ, and κ opioid receptors and thus have potential therapeutic usefulness, according to our initial working hypothesis. These potential novel non-specific opioid ligands are currently under experimental investigation in our laboratory.

Conclusions

A database of 29 different opioid ligands has been used to develop a 3D pharmacophore for non-specific recognition of δ, μ, and κ opioid receptors. Conformational libraries were obtained for all compounds using different computational procedures depending on the number of significant rotatable bonds in each molecule. These libraries served as input for the in-house 3D pharmacophore-generating program, Molmod, together with a set of candidate chemical moieties common to each of the 23 compounds with high affinity at all three opioid receptor types. The 3D pharmacophore for non-specific recognition of δ, μ, and κ opioid receptors consisted of a protonated amine, two hydrophobic groups and the centroid of an aromatic ring found in a specific geometric arrangement in all binders at all three receptor types. Using the six compounds with no affinity at the three opioid receptor types as negative controls provided a refinement of this pharmacophore that suggested the existence of excluded volumes. A validation of this pharmacophore was then made by using it to search an in-house 3D database consisting of diverse non-opioid ligands with different CNS activities. Additional searches using different 3D structural databases provided identification of a number of novel compounds that are potentially involved in non-specific recognition of δ, μ, and κ opioid receptors. These novel non-opioid compounds could eventually exhibit activation selectivity at the three opioid receptor types and thus have potential therapeutic usefulness, according to our initial working hypothesis. Experiments on these compounds are currently under consideration in our laboratory.

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