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Development of concepts on the interaction of drugs with opioid receptors

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Abstract. The development of concepts on the molecular mechanisms of the action of medicinal drugs on the opioid receptors is briefly surveyed. The modern point of view on the mechanism of activation of opioid receptors is given based on the data from chimeric and site-directed mutagenesis of the cloned opioid receptors and the computer-aided simulations of the reception zone and ligand – receptor complexes. Three-dimensional models of the opioid pharmacophore derived by both conventional methods and a comparative analysis of molecular fields are described in detail. The bibliography includes 307 references.

I. Introduction

The challenges of modern pharmacology include the quest for ligands of opioid receptors with desired ratios of agonistic and antagonistic properties. Opioid analgesics are the most efficacious of all analgesics used in practical medicine. They form the main group of pain-killers efficient at injuries, surgical operations, internal diseases, neoplastic processes, in the removal of pain shock and acute alcoholic intoxication, *etc*. Allergy, schizophrenia, insomnia, asthma, head strokes, headaches and migraines, high pressure — this is an incomplete list of diseases that can be relieved and cured by opioid analgesics. Their main drawback is the narcotic action; hence, at present, the active search for new opioid analgesics with improved analgesic properties, wider therapeutic efficacy [the higher ratio of lethal and therapeutic (efficacious) doses LD_{50}/ED_{50}], prolonged action and a considerably weaker ability to induce the dependence is being carried out.

The search for new analgesics, opioid receptor agonists, is in line with the quest for opioid receptor antagonists and compounds with both agonistic and antagonistic properties. This is explained by the fact that the opioid receptor antagonists are the most efficient drugs for curing alcohol and drug dependences. The aggravation of drug addiction and alcoholism observed all over the world as well as the necessity of treating patients by the replacement therapy to reduce the drug and alcohol addiction has heightened the interest in opioid receptor antagonists.

At present, the targeted search for new drugs based on opioid receptor agonists and antagonists involves the use of computer-aided molecular simulations. The design of structural prototypes of future drugs would be impossible without studying the structural peculiarities of both opioid receptor and their ligands, the mechanisms of ligand – receptor interactions and the biological response formation.

The main views on the mechanism of ligand – opioid receptor interaction and the formation of intracellular biological response were established in the 1970s. To a large extent, they relied on the indirect data obtained in analyzing *in vivo* the effect of a drug on the receptor. This involved studying the dependences of the biological effect of a ligand on its structure, dosage or concentration in the organism as well as on the presence of antagonists; thus, the ligand affinity for the opioid receptor was assessed, despite the chemical nature of the opioid receptor and the mechanism of its operation remained unknown. Since the 1990s, in line with the development and the introduction of modern instrumental methods, it became possible to carry out the *in vitro* studies of the mechanism of biological response formation. Experimental data obtained over the past 20 years have greatly changed the views on the reception developed in the second half of the 20th century and favoured the modification of many concepts.

The present review is aimed at the analysis of the evolution of concepts on the mechanisms of the interaction between ligands and opioid receptor and the biological response formation. Attention is focused on the following

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problems: the ligand position with respect to binding sites in the opioid receptor and the structure of the reception zone of the opioid receptor; the mechanism of opioid receptor activation; the pharmacophore concept and the opioid pharmacophore models.

II. The position of an opioid receptor ligand with respect to the receptor binding sites. Reception zone structure

Prior to embarking on the discussion of possible mechanisms of ligand–receptor interaction, let us briefly consider the main notations and terms the interpretation of which remained unchanged since the 1970s.

According to the IUPAC recommendations in the field of pharmaceutical chemistry, a receptor represents a molecule or a polymeric structure in the cell capable of specifically recognizing and binding a molecule that serves as a molecular messenger (neuromediator, hormone, lymphokinine, lectin, drug, *etc.*).[1] The receptors located on the cell cytoplasmic membrane or inside the cell are capable of specific recognition of a ligand, reversible or irreversible interaction with the latter and transmission of a specific signal on this event to the cell.[2] The biologically active substances capable of interacting with specific receptors are called ligands of these receptors.[2] From the viewpoint of pharmacology, the wide spectrum of ligands can be divided into two groups, namely, agonists and antagonists. An agonist is an endogenic substance or a drug that can interact with the receptors and provoke their physiological or pharmacological responses.[1] An antagonist is a drug or a compound that also interacts with the receptor but exerts the opposite physiological effects (as compared with the agonist). From the viewpoint of quantitative assessment of the biological response, the agonists were conventionally divided into complete agonists (invoke the maximum response on the scale from 0 to 1) and partial agonists that are incapable of inducing the maximum activation of the receptor irrespective of the amount of used substance.[1]

Opioid receptors were discovered in the early 1970s in the central nervous system (CNS) of animals.[3] In the first steps of studying the opioid receptor location and functions, it was found that they were distributed in the spinal cord and brain structures related to the transmission and reception of pain pulses. However, later, opioid receptors were observed outside the CNS (in the respiratory system and the digestive tract), which provided grounds to believe that their functions are more diverse. Conventionally, the following three types were assigned to opioid receptors: μ-, κ- and δ-receptors.[4–12] As regards their structure, these opioid receptors share 60% amino acid identity. Their activation accompanied by similar intra- and intercellular processes could provoke different biological responses.[13–15]

It is conventionally assumed that the μ-opioid receptors in the CNS were preferentially involved in modulating the pain sensitivity, their activation caused supraspinal analgesia, miosis, respiratory depression, bradycardia, reduction of the activity of vascular baroreceptors, enteroparesis, euphoria, catalepsy, hypothermia, physical dependence. The κ-opioid receptor activation initiated the development of the sedative effect, spinal analgesia, minimum respiratory depression, dysphoria, psychotomimetic effect, depression of learning and memory processes; insignificant dependence could arise. The activation of δ-opioid receptors is accompanied by hallucinations, mydriasis, tachycardia and nausea. Receptors of this kind mainly control the emotional sphere, their agonists could exert antidepressant and anxiolytic effects.

In 1995, the nociceptin-orphanin receptors (Nopioid receptors) involved also in the regulation of pain sensitivity were discovered.[16]

Orphanin receptors are predominantly localized in the spinal chord and brain. These neuron-expressed receptors could block the activity of adenylate cyclase and Ca^{2+} channels and, like opioid receptors, activate the K^+-channels. They do not react with the majority of opioid receptor ligands including Naloxone (**1a**).† Despite the partial antagonism of Nopioid receptors with respect to classical opioid receptors as regards both pain regulation and some other physiological effects, their functions are deeply intertwined; for this reason and also due to their high homology with respect to κ-opioid receptor, Nopioid receptors are now considered as the fourth class of opioid receptors.[17]

Ligands of opioid receptors were divided into two types, namely, selective (*i.e.*, interacting with a single type of receptors) and non-selective. The majority of core morphine-like agonists pertain to non-selective ligands, although their affinity for μ-opioid receptors is slightly higher than to κ- and δ-opioid receptors. The non-selective antagonists are represented by Naloxone (**1a**) and Naltrexone (**1b**), whereas *N*-phenylpropyl-4β-methyl-5-(3-hydroxyphenyl)morphane represents a selective pure κ-antagonist and Naltriben (NTB) and Naltrindol (NTI) (**2a**) are selective β-antagonists. It should be mentioned that ligands with agonistic–antagonistic properties can be selective with respect to different opioid receptor types and act as both agonists and antagonists. Thus the majority of benzomorphane derivatives can act as κ-opioid receptor agonists and μ-opioid receptor antagonists.

Studies of the opioid receptor structure date back to the mid-1970s when their direct involvement in the mechanism of pain pulse transmission was discovered. To date, it is known that opioid receptors structurally pertain to the family of metabotropic G protein-coupled receptors (GPCR, the rhodopsin group).[18–23] The transfer of information to postsynaptic structures occurs by the modulation of different systems of secondary mediators (messengers), namely, adenylate cyclase, sodium-calcium channels, the phosphoinositide cascade.[2,24–26] Based on experimental data (electron cryomicroscopy, polypeptide chain sequencing), it was found that opioid receptors of all types had similar structures differing only in amino acid sequences of certain polypeptide chain fragments.[27–30] The opioid receptor molecule included the extracellular N-terminal region followed by seven transmembrane domains (TM-I–TM-VII) with three extracellular (EL-I–EL-III), three intracellular (IL-I–IL-III) loops in between and the intracellular C-terminus.[24,25,31–33] Figure 1 shows a two-dimensional model of the κ-opioid receptor.[34]

Seven transmembrane domains are hydrophobic (consist of polar amino acid residues by less than 30%) and have the α-helical secondary structure. The transmembrane domains and intracellular loops of δ-, μ- and κ-opioid receptors are characterized by a high degree of homology (73%–76% and 63%–66%, respectively[35,36]), whereas the greatest

† The structures of the numbered ligands can be found in the Appendix.

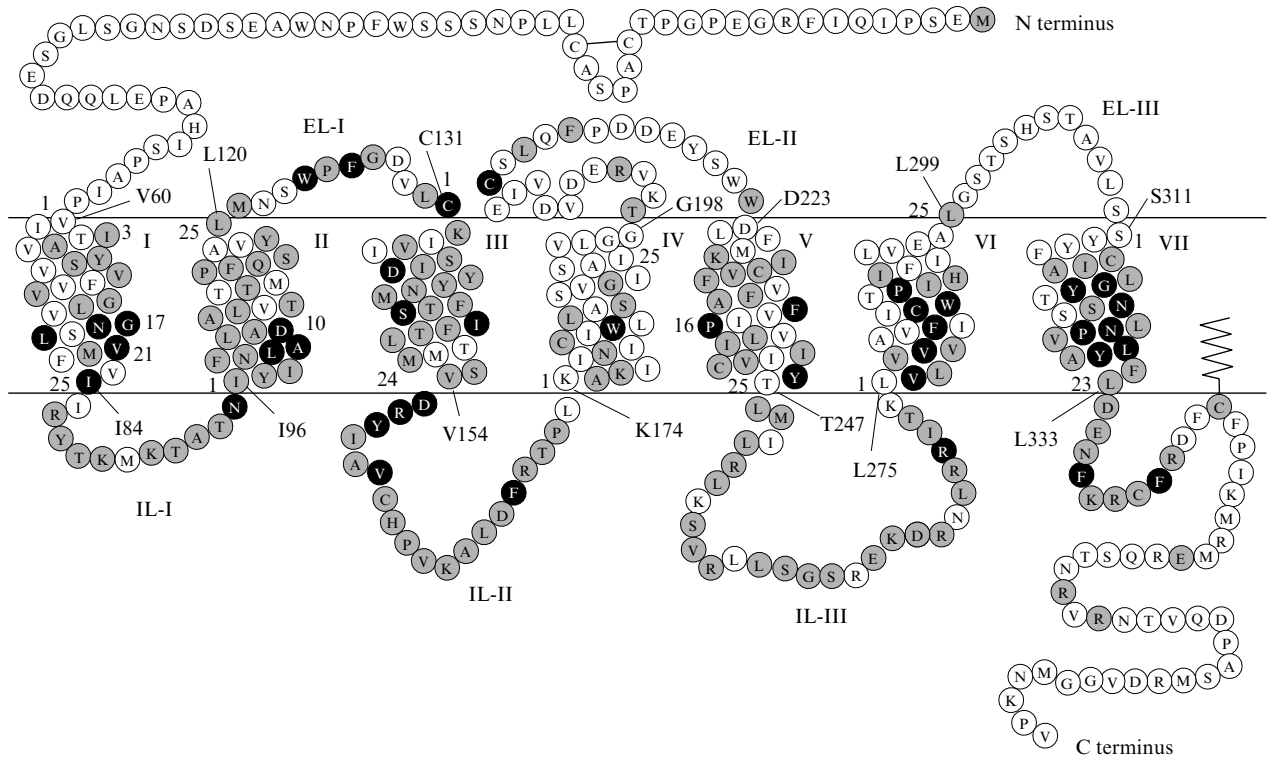


Figure 1. Two-dimensional model of the κ-opioid receptor.[34]
Roman numerals indicate the transmembrane domains, Arabic numerals correspond to amino acid residues. Amino acid residues contained in opioid receptors of all types are grey. The residues present in all GPCR of the rhodopsin family are black.

differences were observed in the amino acid sequences in external loops EL-II and EL-III and terminal fragments (34% – 40% identity).[15, 35] Structural fragments of opioid receptors were found to closely interact with one another.[15, 37]

Binding of the ligands to opioid receptors occurs in a certain site, the so-called reception zone. The presence of binding sites in the reception zone is responsible for the high degree of opioid receptor affinity to ligands. The latter sites are most often formed by the carboxyl groups of dicarboxylic acids, amino groups of diamino acids, hydroxyl groups of hydroxy amino acids, SH groups of cysteine, the indole fragment of tryptophan and the hydrophobic moieties of different amino acids.[2]

In the early stage of studies (in the 1970s – 1980s) on the structures of opioid receptors and the ligand – receptor complexes, no experimental data on the identification of ligand – opioid receptor binding sites were available; the reception zone was assumed to be located on the outer side of the cytoplasmic membrane. Moreover, the agonist and the antagonist were believed to interact with the same site in the reception zone, *i.e.*, the theory of competitive ligand binding with the opioid receptors dominated.[38 – 41] Dukhovich *et al.*[42] still held to this viewpoint in their much later publication. It was suggested that the anionic domain of the receptor is located on the external surface of the cytoplasmic membrane and have a shape of a bowl with hydrophobic walls and the anionic group at the bottom. This bowl also contains water molecules; the electrostatic interaction between the receptor anionic group and the protonated nitrogen atom of the ligand could occur without complete displacement of water molecules from the cavity in the anionic fragment.[42] The extent of bowl opening (conformational modifications of the receptor) and the degree of water molecule displacement from the interaction sphere of ions were different for agonists and antagonists.

The appearance of experimental data on the identification of opioid receptor binding sites with agonists and antagonists changed the views on the reception zone position with respect to the outer boundary of the cytoplasmic membrane and its structure. The introduction of methods of cloning, site-directed mutagenesis, preparation of chimeric opioid receptors favoured deeper insight into the structure of the opioid receptor reception zone.[16, 43 – 48] Experiments on the site-directed mutagenesis and the synthesis of chimeric receptors were aimed at the elucidation of the role of opioid receptor segments or particular amino acid residues in the ligand recognition by a receptor and the biological response generation. A chimeric opioid receptor results from exchange of segments between different opioid receptor types. Studies with chimeric opioid receptors made it possible to consider in detail the involvement of extracellular loops and transmembrane domains in binding agonists and antagonists of μ-, δ- and κ-opioid receptors and reveal the segments responsible for the formation of selectivity with respect to different opioid receptor ligands.[25]

Experiments with chimeric opioid receptors favoured the development of a theory of polycomplementary binding of agonists and antagonists to the opioid receptors. This idea was put forward by Portoghese[49, 50] in the mid-1970s when analyzing the peculiarities of the three-dimensional structure of ligands with different ratios of agonistic and antagonistic properties.[50] However, up to the 1990s, the theory of polycomplementary binding of ligands to opioid

receptors failed to gain wide acceptance (as opposed to the theory of competitive binding). According to this theory, an opioid receptor contains several complementary sites capable of interacting with the ligand. In turn, a ligand contained several specific functional regions or groups that were 'recognized' by the corresponding complementary site of the receptor; agonists and antagonists were bound to different complementary sites of the receptor. The unambiguous and conclusive evidence of the polycomplementary agonist and antagonist binding to opioid receptors was obtained in studying the binding of radioligands to chimeric receptors.[16,51] Thus some experiments [16] dealt with chimeric receptors, which were based on δ- and κ-opioid receptors. These receptors contained the N-terminal fragment and a part of the TM-I receptor both belonging to one receptor type and also the C-terminal fragment, external and internal loops and six transmembrane helices belonging to the another opioid receptor type: $\kappa_{1-78}/\delta_{70-372}$ and $\delta_{1-69}/\kappa_{79-380}$ (the numbers in indices correspond to amino acid sequences in the original δ- and κ-opioid receptors). Using radioligand binding methods, the κ-selective antagonist [^{3}H]naloxone (**1a**) was found to bind to the chimeric receptor $\kappa_{1-78}/\delta_{70-372}$ and failed to bind to the chimeric receptor $\delta_{1-69}/\kappa_{79-380}$. The opposite situation was observed for κ-selective agonist [^{3}H]U-69.593 (**3**) which interacted with the chimeric receptor $\delta_{1-69}/\kappa_{79-380}$ and failed to interact with $\kappa_{1-78}/\delta_{70-372}$. These results suggested that κ-selective agonists and antagonists interact with different binding sites in κ-opioid receptors.

Studies of chimeric opioid receptors failed to determine the key amino acids involved in the ligand–receptor interactions. Hence, the next step was the investigation of contributions of individual amino acids by using mutations of one or several amino acid residues in cloned receptors. Studies along this direction were aimed at inducing such mutations as to weaken the receptor affinity for selective ligands (agonists, antagonists and ligands with agonistic–antagonistic properties) as compared with the original receptor. As a rule, the functional characteristics of receptors were assessed (based on the parameter of radioligand binding, the activity of transduction systems).

It deserves mention that the comparative analysis of results of site-directed mutagenesis obtained by different authors is difficult to accomplish due to the non-uniformity of the analyzed subjects (human and animal δ-, μ-, κ-opioid receptors differed in amino acid sequences) and the existence of a wide diversity of nomenclatures of G protein coupled receptors. The nomenclatures differ in the numeration of amino acid residues designated by their own letter codes. The numerations used were either throughout the whole amino acid sequence starting from the extracellular N-terminus or individual for each transmembrane helix. For example, aspartate that occupies the seventh position on the third transmembrane helix of human δ-, μ- and κ-opioid receptors, was designated as Asp^{128}, Asp^{147} and Asp^{138}, respectively, in the former nomenclature and as Asp(III:7) in the latter nomenclature.[52] In our review, we keep the authors particular nomenclatures.

The problem of determination of amino acid residues involved in the ligand–receptor interactions was studied in more detail for the binding of agonists of the peptide and non-peptide nature.[15,45,47,51,53–60] The alkaloid μ-agonists and δ-agonists of the peptide nature were found to bind to amino acid residues TM-V–TM-VII and EL-III, whereas μ-agonists of the peptide nature were observed to bind to amino acid residues EL-I and TM-III.[15,45,47,51] For the δ-agonist binding, the residues were classified as follows: Asp^{95} (TM-I),[45,51,57] Asp^{128} (TM-III),[51,52] Trp^{284}, Val^{296}, Val^{297} in EL-III,[51,53] Leu(VII:03),[46] Lys^{108} in EL-I.[58] In the selective binding of the κ-agonists, the amino acid residues of the second extracellular loop were involved.[51,59,60] It should be noted that the involvement of the second extracellular loop in the binding of κ-agonists is typical of only opioid receptors, it is not observed for other members of the GPCR family.[51]

Thus, based on experiments with chimeric and mutant receptors it was shown that the external loops of the μ-, κ- and δ-opioid receptors are involved in binding to selective agonists. Insofar as structural fragments of these opioid receptors had the lowest homology, it was hypothesized that the divergence in the amino acid sequence of external loops is responsible for the functional differences of the main opioid receptor types.[51]

At the same time, information has also been accumulated sufficient for elucidation of binding sites of opioid receptor antagonists. It was shown that the specific binding of Naloxone and Naltrexone (**1a**,**b**) weakened upon replacement of the following amino acids in the third–seventh transmembrane domains: Tyr^{129} (TM-III), Trp^{173} (TM-IV), Trp^{274} (TM-VI), Tyr^{308} (TM-VII), Trp^{318} (TM-VII), Tyr^{326} (TM-VII).[15,32] The important role of aromatic amino acids in Naloxone binding was confirmed. Thus the replacement of Tyr^{129} (TM-III) by another aromatic acid, namely, phenylalanine, had an insignificant effect on the antagonist binding, whereas its replacement by alanine was accompanied by a decrease in the affinity by a factor of several tens.[32]

In the selective binding of κ-antagonists, a significant role belongs to amino acid residues of the third extracellular loop and also to Glu^{297} in TM-VII.[34,51,61–64] For example, the substitution of the negatively charged Glu^{297} for the positively charged Lys reduced hundredfold the affinity of norbinaltorphimine (nor-BNI, **4**) for the κ-opioid receptors.[63] It should be noted that the affinity of nor-BNI for the μ-opioid receptor, which contains Lys^{303}, an analogue of Glu^{297}, was also 100-times weaker than its affinity for the κ-opioid receptor.[63] In several studies, the third extracellular loop (EL-III) was referred to as the key fragment of the opioid receptor responsible for the different affinity of NTI (**2a**), nor-BNI (**4**) and GNTI (**2b**) for μ-, κ- and δ-opioid receptors.[53,63–68] For example, the analysis of binding sites in chimeric μ/κ- (see Ref. 63) and δ/κ- (see Ref. 64) opioid receptors has shown that only the presence of EL-III from the κ-opioid receptor could provide the efficient nor-BNI binding to the opioid receptors. Moreover, the δ-selective ligand NTI was observed to bind with a high degree of affinity to the δ/κ-opioid receptor (see Ref. 64) and δ/μ-opioid receptor [66] both containing EL-III from the δ-opioid receptor.

The following amino acid residues were suggested [35] to be essential for binding to the opioid receptor ligands:‡ Asp(3.32), Tyr(3.33), Lys(5.39), Phe(5.47), Trp(6.48), Ile(6.51), His(6.52), Ile(6.53), Ile(7.39), Tyr(7.43) as inferred

‡ The designations of amino acid residues correspond to those shown in original publications.

from the experimental data on chimeric and site-directed mutagenesis.[15, 31, 32, 57, 69–74]

Using site-directed mutagenesis, it was established that the key role in the opioid receptor recognition by ligands (both agonists and antagonists) is played by the following three charged amino acid residues in the transmembrane domains of the opioid receptors: Asp^{114} (TM-II), Asp^{128} (TM-III) and His^{297} (TM-VI) (see Refs 15, 43, 47, 51, 52, 57, 73, 75, 76). These residues form the central hydrophilic domain in the reception zone. It was assumed that Asp^{114} was responsible for the recognition of the receptor by a ligand and also for its binding to sodium ions, which regulated the antagonistic binding to the receptor and the G protein activation.[43,75] The most important role in the ligand–receptor recognition and binding is played by Asp^{128} (in the μ- and κ-opioid receptors, it corresponds to Asp^{147} and Asp^{138}, respectively [52]). It is this amino acid residue that is involved in the hydrogen bonding to the protonated nitrogen atom of all opioid receptor ligand types (both agonists and antagonists), which is also accompanied by the electrostatic interaction. This most important conclusion was made by many scientists who studied the opioid receptor reception zone by the method of site-directed mutagenesis.[15, 47, 57, 73, 76] It deserves mention that for the other representatives of the GPCR family, the amino acid residue homologous of Asp^{128} (TM-III) was found to interact with the protonated nitrogen atom of ligands (the data from site-directed mutagenesis).[15, 75–82]

Mutations of His^{297} substantially weakened the affinity of agonists and antagonists to the opioid receptors.[74] It is generally accepted that His^{297} took part in the intramolecular ion–ion interaction with the carboxyl group of Asp^{147} in TM-III.[57] Moreover, the imidazole ring of His^{297} is involved in the donor–acceptor interaction with the hydroxyl group in the aromatic ring of ligands containing the morphine core.[62]

At present, the reception zone of the opioid receptor ligands is arbitrarily divided into a selectivity region and a 'binding pocket' as inferred from the data on site-directed mutagenesis. The former is largely located above the membrane outer surface, it is formed by amino acid residues of intracellular loops and the transmembrane domain vertices V–VI.[16, 44, 45, 53, 58, 59, 63, 83, 84] The 'binding pocket' is located below the membrane external surface and is confined by the helices of transmembrane domains III–VII.[31, 32, 35] The reception of the peptide ligands involves both the segments of the selectivity region and the 'binding pocket'. Alkaloids and their derivatives preferentially interact with the 'binding pocket' region.[31, 32, 43, 53, 57, 63, 65, 73, 84] The tyramine fragment of opioid peptides and alkaloids interacts with the amino acid residues of the 'binding pocket'.

It should be noted that the three-dimensional structure of the reception zone cannot be inferred based on the experimental data from electron cryomicroscopy, site-directed mutagenesis and synthesis of chimeric receptors. The most complete information on the spatial structure of macromolecules can be obtained by X-ray diffraction analysis, but the respective data are absent so far. Hence, the three-dimensional structure of opioid receptors is being studied theoretically by methods of computer-aided simulation. The theoretical methods are based on the abundant experimental data on the intra- and intermolecular interactions involving different amino acid residues of the opioid receptors acquired by studies on site-directed mutagenesis.

The modelling of the three-dimensional opioid receptor structure involved different approaches.[52, 85–95] The most widely used are the methods of structural similarity and distance geometry. The methods of structural similarity involve 'tuning' of a modelled receptor to the template. The data from electron cryomicroscopy or XRD on the structures of close homologues, *e.g.*, for rhodopsin or bacteriorhodopsin, which also pertain to the GPCR family, serve most often as the template.[28, 85, 96–101] In generating the known structural templates and the models of structural homologues, the computer programs MODELLER,[102, 103] web-servers SWISS-MODEL, EsyPred3D, Robetta, CPHmodels, SDSC1 (see Refs 104–106) and databases ModBase,[107] Swssprot,[108] GenBank,[109] PIR,[110] GCRDb,[111] PRINTS (see Refs 112, 113) are used most often. Thus Filizola *et al.*[86] constructed 3D models of the binding pocket of μ-, κ- and δ-opioid receptors using the structures of rhodopsin α-helices found by the electron cryomicroscopy as the starting models. The spatial structure of a transmembrane helix bundle was modified in the course of unconstrained energy optimization of the intramolecular inter-helix interactions. The stability of the obtained three-dimensional transmembrane domain structures was assessed by a molecular dynamics method.

The XRD data suggest that the mutual orientation of GPCR helices is non-uniform (in rhodopsin, the transmembrane helices are parallel to one another and in bacteriorhodopsin they are in bundles); thus, the results of simulations substantially differ depending on the template chosen.

From our point of view, the procedure of distance geometry developed by Pogozheva *et al.*[52, 114] is the most promising method. It consists of developing a three-dimensional opioid receptor model in which the maximum number of inter-helix interactions (hydrogen bonds, electrostatic and S···S interactions, π-complexes, *etc.*) can be realized with minimum steric hindrance. The construction of such a model makes use of experimental data on the amino acid sequences of opioid receptors and peculiarities of the three-dimensional structures of GPCR α-helices. The three-dimensional models of reception zones of μ-, δ-, κ-opioid receptors [52] can illustrate the use of the distance geometry algorithm. The reception zone was simulated in such a way as to involve the side chains of transmembrane helices in the extensive network of hydrogen bonds. The relationship between the numbers of intra- and intermolecular hydrogen bonds was assumed [115] to be the main criterion in optimization of the reception zone structure. A characteristic feature of the reception zone models built [52] for different types of opioid receptors is the presence of the inner consereved region of 18 amino acid residues that are identical for all types of the opioid receptors and of the peripheral variable region of 19 amino acid residues that are different in δ-, μ- and κ-opioid receptors. This result agreed well with the division of the reception zone to the selectivity domain and the 'binding pocket', which has been done based on the data of site-directed mutagenesis.

Three-dimensional models of ligand–receptor complexes were constructed by docking the ligands to the reception zone, *i.e.*, the establishment of matching the ligand to its binding sites on the receptor. Several programs based on different algorithms, such as

DOCK,[116] AUTODOCK,[117,118] GOLD,[119] FlexX,[120] FDS,[121] Glide,[122] LigandFit,[123] ICM,[124] allow automatic docking of small molecules into the protein cavity. These and other docking programs have critically been assessed.[125] The performance of the overwhelming majority of modern docking programs is based on the principle of flexible docking that has earlier been described in detail.[126] It takes into account the ligand conformational mobility but ignores the conformational mobility of the receptor molecule. In essence, the flexible docking approach is reduced to the automated item-by-item examination of ligand conformations and orientations in the binding site and to the estimation of the interaction energy. The most promising and sophisticated docking methods made it possible to take into account the conformational mobility of not only the ligand but the receptor, too. To date, there are only few programme packages specially designed for docking where such a possibility is implemented. The simplest version of completely flexible docking took into account the conformational mobility of side chains in amino acid residues located directly in the binding site.

Various methodological approaches allowed generating a series of three-dimensional models of the opioid receptor reception zone, which differed in the mutual orientation of transmembrane helices, extracellular loops and terminal fragments, and of ligand–opioid receptor complexes involving the latter fragments.[36,85,87–89,91,127–152] Despite differences in these models, the following general features could be revealed:

1) the reception zone is a fairly extended region in which the agonist and antagonist molecules can occupy different positions without substantial steric hindrance;

2) structural fragments of molecules common to the selective and non-selective ligands interact with the sites in transmembrane helices; the structural fragments that provide selectivity of the ligands (the indole ring in NTI (**2a**), the oligopeptide ring in DPDPE (**5**), *etc.*) are localized in a region between the extracellular loops and the vertices of TM-VI and TM-VII;[46,145]

3) in the majority of cases, the receptor binding sites common to agonists and antagonists are represented by polar amino acid residues [Asp(III:7), Tyr(III:8), His(VI:20)];

4) the structural fragment common to ligands (the tyramine fragment common to alkaloids and Tyr common to cyclic peptides) binds in the same way to the transmembrane domains of receptors of all three types;[46,52] the protonated nitrogen atom and the OH group of the tyramine or tyrosine fragment forms hydrogen bonds with the Asp(III:7) and His(VI:20) residues, respectively.

Thus, according to the recent experimental data and the results of computer-aided simulations, the reception zone represents an intramolecular cavity of the opioid receptors, its largest part being located within the cytoplasmic membrane. It is formed by amino acid residues of transmembrane helices (TM-III–TM-VII) and extracellular loops. Neither the extracellular N terminus, the intracellular C terminus nor the intracellular loops are involved in the reception zone.[16,57] Agonists and antagonists bind to different sites in the reception zone. However, the protonated nitrogen atom of both agonists and antagonists interacts with the same anionic group of the opioid receptor, namely, the side-chain carboxyl group of Asp (Asp^{128}, Asp^{147} and Asp^{138} for δ-, μ- and κ-opioid receptors, respectively), which is located within the central part of the TM-III transmembrane domain rather than on the surface of the cytoplasmic membrane.

III. Mechanism of initiation of the cell biological response to the opioid receptor–ligand interaction

In the first stages of studying the reception processes, the pharmacological effect intensity was usually assumed to depend on the concentration of ligand–receptor complexes; hence, studies of how the ligands affect the cell biological response have been limited to consideration of the mechanism of the ligand–receptor interaction. The first attempt to quantitatively describe the pharmacological effect of a cell as a function of the ligand concentration was undertaken by Clark in 1926.[153] According to Clark, the interaction of ligands with receptors can be described as the following equilibrium:

$$L + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} LR,$$

where L is the ligand, R is the receptor, LR is the ligand–receptor complex, k_1 and k_{-1} are rate constants for the formation and dissociation of the ligand–receptor complexes.

The formation of a ligand–receptor complex is characterized by the association or the affinity constant (K_a)

$$K_a = \frac{[LR]}{[L][R]} = \frac{k_1}{k_{-1}}.$$

However, a value opposite to K_a, *i.e.*, the dissociation constant of the complex (K_d), is used more often. The use of the latter equilibrium constant allowed the changes in the Gibbs free energy upon complexation to be determined. For a temperature of 37.5 °C at which K_d is usually measured in the tests with isolated animal muscles, the equation $G = 1.42 \log K_d$ (kcal mol^{-1}) is true.[42]

As a rule, for ligand–receptor systems, the absolute value of G does not exceed 14–15 kcal mol^{-1} and K_d does not drop below 10^{-11} mol litre^{-1} in the order of magnitude. For example, for the carfentanyl (**6b**)–opioid receptor complex, the values of K_d and G were 3.6×10^{-11} mol litre^{-1} and -14.2 kcal mol^{-1}, respectively.[42]

Clark assumed[153] the cell response to be directly proportional to the concentration of ligand–receptor complexes. In the excess of the ligand with respect to the receptors, the dependence of the cell biological response on the concentration of the added ligand is described by the Langmuir isotherm:[2]

$$[LR] = \frac{[L_0][R_0]}{[L_0] + K_d}\left\{1 - \exp[-(k_1[L_0] + k_{-1})t]\right\},$$

where [LR], [L_0] and [R_0] are the concentrations of the ligand–receptor complex, the ligand and the receptor (initial), respectively.

To date, considerable number of publications are known that deal with the dependence of the biological effect of the opioid receptor ligand on the ligand dose.[2,42] The majority of studies assumed the biological effect to be determined by the degree of occupancy of the receptors by this physiologically active substance. However, with the accumulation of

experimental data, the following three basic factors that could not be explained in terms of the Clark theory were revealed:

1) the maximum biological response of a cell is observed in many cases where only insignificant part of receptors was bound to the ligands;

2) in many cases, the dependence of the cell biological response on the added ligand concentration is bell-shaped rather than follows the Langmuir isotherm (Fig. 2);

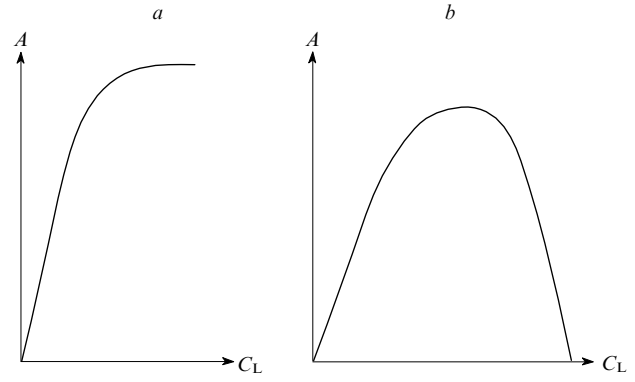


Figure 2. Dependences of the magnitude of the cell biological response (A) on the added ligand concentration (C_L): the Langmuir isotherm (a), bell-shaped dependence (b).[2]

3) the difference in the mechanisms of action of agonists and antagonists of one type is as follows: agonists did cause a biological response and antagonists did not.

These data served as the grounds for the formulation of concepts more complicated that the Clack theory on the mechanisms of cell biological response to its interaction with an opioid receptor. In this context, the Clark theory is probably of only historical interest; however, it did not lose its importance in describing the processes of ligand binding to receptors.

In 1956, Stepherson [154] assumed that the magnitude of the biological response to the ligand–receptor interaction is a certain function of the number of occupied receptors and, moreover, that the maximum biological response is observed when only a part of receptors are bound to the ligand. However, the Stepherson theory failed to explain the bell-shaped dependence of the cell biological response on the concentration of the added ligand as well as the difference in the effects of agonists and antagonists. To explain the difference in the mechanisms of action of agonists and antagonists, Paton [155] put forward in 1961 a theory now called the rate theory. According to the Paton theory, the ligand efficacy is determined by the rate of ligand association with the receptor. The antagonist is a substance that reacts at a high rate with the receptor to form a short-lived ligand–receptor complex. An antagonist is a substance, which interacts with a receptor at a low rate; however, this interaction produces a long-lived ligand–receptor complex. The main observation that contradicted the rate theory is the experimentally determined association rates of agonists and antagonists with the receptors of the same type. It was found that in many cases the interaction rates of agonists and antagonists did not differ substantially; moreover, the association rate of an antagonist with a receptor could be higher than the antagonist association rate with the same receptor.[2] For example, the lifetimes of complexes formed by the agonists of morphine (**7b**) and carfentanyl (**6b**) with the μ-opioid receptor [62 s (Ref. 156) and 560 s,[157] respectively] were higher as compared with that of the Naloxone (**1a**) antagonist [16 s (Ref. 158)].

In line with the Paton theory is the Dukhovich theory, which explained the differences in the performance of agonists and antagonists of all receptors including opioid receptors by the peculiarities of the ion–ion interaction of ligands with the receptors.[42] According to the latter theory, the nature of the opioid activity, *i.e.*, the ratio of agonistic and antagonistic properties, was determined by the contribution of the ion–ion component in the ligand–receptor interaction. The protonated nitrogen atom of the ligand reacts with the receptor anionic group localized at the bottom of the bowl-shaped anionic region with hydrophobic walls (Fig. 3). The substituents at the nitrogen atom larger than the methyl group bind to the opioid receptor hydrophobic regions and restrict the mutual approach of ionic groups of the ligand and the receptor, thus decreasing the energy of the ion–ion interaction (Table 1). None of such limitations were characteristic of morphine-like analgesics containing the *N*-methyl fragment and for normorphine (**7a**), which is an agonist. A common feature of the *N*-substituents of the morphinane derivatives that behave as partial agonists and antagonists is that their 'anchor' fragments (vinyl, cyclopropyl, cyclobutyl) were linked to the

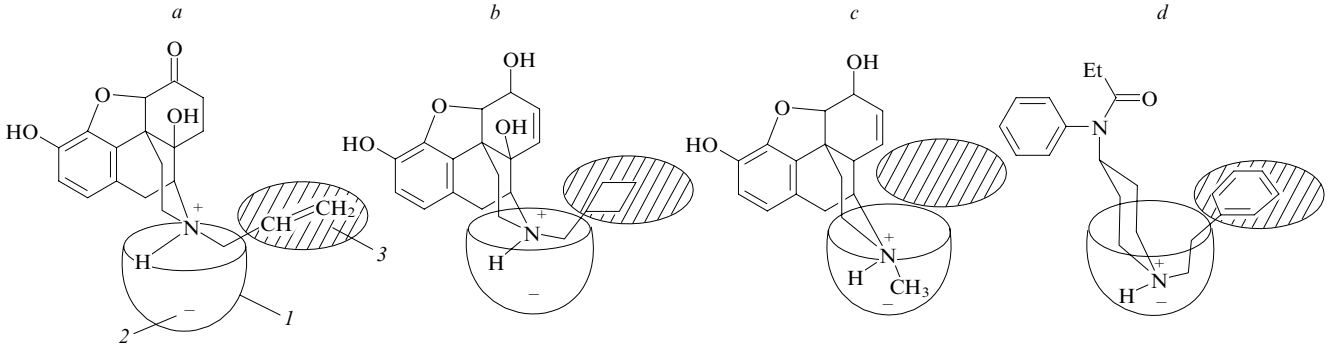


Figure 3. Hypothetical scheme of the binding of Naloxone (**1a**) (*a*), Nalbuphine (*b*), morphine (**7a**) (*c*) and Fentanyl (**6a**) (*d*) to the anionic domain of the opioid receptor.[42]
(*1*) the anionic domain of the receptor; (*2*) the anionic group; (*3*) the area adjacent to the anionic domain.

Table 1. Calculated shifts of the protonated nitrogen atom in compounds towards the anionic group of the receptor.[42]

Compound	Shift of protonated N atom /Å	Activity type
Naloxone (**1a**)	0	antagonist
Naltrexone (**1b**)	0.78	antagonist
Nalbuphine	1.37	partial agonist
Fentanyl (**7a**)	2.56	agonist

Note. Calculations were performed within the framework of the model shown in Fig. 3.

nitrogen atom not directly but through a methylene unit. Rigid binding of the 'anchor' fragments to the area adjacent to the anionic region in the receptor prevented the maximum shift of the nitrogen atom deep into the 'bowl' of the opioid receptor reception region. For Naltrexone (**1b**), this shift was deeper than for Naloxone (**1a**) because the methylene group could occupy the axial position with respect to the planar cyclopropyl fragment. The non-planar structure of the cyclobutyl group allowed the nitrogen atom to 'immerse' deeper into the cavity in the opioid receptor anionic region. Morphine (**7b**), as opposed to Naloxone (**1a**), Naltrexone (**1b**) and Nalbuphine, is devoid of such a barrier to the nitrogen atom mobility, which shortened the distance between the nitrogen atoms and the opioid receptor anionic group. Thus, due to substituents at the nitrogen atom, the barrier (2–3 kcal mol^{-1}) to the ion–ion interaction between the protonated N atom in the ligand and the anionic group in the opioid receptor was shown to be responsible for the nature of the ligand opioid activity (agonist, partial agonist, antagonist).[42] The extremely strong agonistic effect of Fentanyl (**6a**) and its derivatives as compared with morphine was explained by Dukhovich *et al*.[42] by closer contacts of the Fentanyl ionic groups due to the presence of an additional methylene group in its phenethyl substituent, which lift restrictions to the mobility of the cationic group.

It should be noted that the Dukhovich theory did not agree with the experimental data on site-directed mutagenesis. According to the latter, the carboxyl group of an opioid receptor bound to the nitrogen atom of any ligand by the electrostatic interaction and through hydrogen bonding is located inside the cytoplasmic membrane in the TM-III central part rather than on its surface. Moreover, the formation of the NH···O hydrogen bond, which involves the protonated nitrogen atom of the ligand and the carboxyl group of the receptor is impossible where the *N*-substituent at the N atom rather than a proton is directed towards the carboxyl group. This theory also failed to explain the following experimental data:

1) the presence of antagonistic properties in bulky morphine-like compounds containing an *N*-methyl substituent [*e.g.*, *N*-methylbenzomorphanes containing 6-Alk and 11-Alk substituents (Alk = Me, Et, Pr^n)];[159]

2) the disappearance of the 'anchor' ability of typical *N*-antagonistic derivatives for molecules of other chemical classes that, like the morphine-type structures, contain the piperidine ring, for example, for analogues of meperidine, prodine, ketobimedone, which proved to remain 'pure' agonists even with *N*-antagonistic substituents;[160]

3) the transformation of morphine-like antagonists containing typical *N*-antagonistic substituents (allyl, cyclopropylmethyl, cyclobutylmethyl, propyl) to 'pure' agonists upon replacement of the hydroxyl group in the *meta*-position of the aromatic ring by bulkier (anchor) groups;[161]

4) the different ratios of agonistic and antagonistic properties in enantiomers of opioid receptor ligands with the same orientation of *N*-substituents.[50, 162–175]

A common feature of the Paton and Dukhovich theories is that they invoked the idea of two receptor conformations (active and inactive) to explain the differences in the mechanisms of action of agonists and antagonists. This idea was first formulated by Karlin in 1967.[176] According to his theory, which was called allosteric, the receptors exist in two states, active and inactive. The transition between these states is reversible, *i.e.*, $R \rightleftarrows R^*$, where R and R* are the inactive and active receptors, respectively. The ligand preferentially interacting with the inactive receptor form is called antagonist and the ligand that interacts with the active receptor is called agonist.

The idea of the existence of active and inactive conformations of the receptor and, correspondingly, active and inactive ligand–receptor complexes proved to be very fruitful. It was used in virtually all later theories of the generation of a biological response.

An attempt to combine the Stepherson, Paton and Karlin theories into a whole was undertaken by Ariëns in the late 1970s–early 1980s.[177–180] According to his conception, the interaction of a ligand with the receptor induced the formation of an inactive ligand–receptor complex, which could later reversibly transform into the active state. The rate of activation of the ligand–receptor complexes formed by antagonists with receptors is much lower than the rate of association of agonists with receptors. The biological response is proportional to the number of active ligand–receptor complexes rather than to the total number of bound receptors. In the general form, according to this theory, the kinetics of ligand–receptor interaction can be written as follows:

$$L + R \rightleftharpoons LR \rightleftharpoons LR^* \rightleftharpoons L + R^* \rightleftharpoons L + R.$$

It should be noted that the Ariëns scheme failed to provide any rigorous description of the processes of ligand–receptor binding (affinity) and the processes that induce the biological response (efficacy). To date, it was experimentally shown that the receptor activation can proceed both spontaneously (constitutional activity) and under the action of a ligand.[181–184] The first description of the spontaneous activation of receptors of the GPCR family was given back in 1989 by the example of the δ-opioid receptors.[185] In terms of a concept that receptors can be activated spontaneously in the absence of ligands and also that agonists activate the receptors, the antagonists were divided into the following two types:

— inverse agonists that lift the effect of agonists and block the spontaneous activation of the receptor;[181, 182, 186]

— neutral antagonists that lift the action of both agonists and inverse agonists.[181, 182]

According to these experimental results, a model of the two-state activation of a receptor was proposed.[187] It schematic illustration is as follows (Fig. 4). The receptor can exist in two conformations, namely, inactive (R) and active (R*). In the absence of an agonist, the existence of

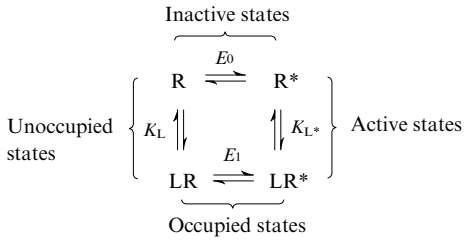


Figure 4. Two-component model of the receptor activation.[183]

these two conformations is determined by the equilibrium constant (E_0), which is the measure of the constitutional activity of the receptor.[183] Binding of the agonist to the inactive receptor form produces the inactive ligand – receptor complex and the corresponding equilibrium constant determines the agonist affinity for the receptor inactive form (K_L). An analogous equilibrium constant K_{L*} determines the affinity of agonists for the receptor active form.

The equilibrium constant (E_1) that characterizes the transition from the inactive agonist – receptor complex (LR) to the active complex (LR*) is the measure of the agonist efficacy. The efficacy is a property that allows an agonist to induce a biological response.[1] The magnitude of the biological response generated upon the formation of the ligand – receptor complex is directly proportional to the number of occupied receptors in their active conformations (R*).

Varfolomeev and Gurevich[2] who described the generation of the analgesic effect upon introduction of opioids assumed that the analgesic reaction to the action of opioid agonists (L) in the receptor microenvironment was developed according to the following scheme:

$$L + R \underset{K_d}{\rightleftharpoons} LR \overset{E(K)}{\rightleftharpoons} LRE \overset{X}{\rightleftharpoons} \text{biological response.}$$

They assumed that the biological response to the action of compounds is directly proportional (with the proportionality factor X) to the concentration of the ligand – receptor – effector (LRE) complex, where E is the effector through which the analgesic effect is formed. As a rule, the constant K is considerably smaller that the dissociation constants of ligand – receptor complexes. This apparently explains the fact that the maximum biological response is manifested only when a small fraction of opioid receptors is occupied.[2]

At present, many scientists assume that the interaction of the ligand – receptor complex with the effector could also occur indirectly, where the G-protein localized on the inner side of the cytoplasmic membrane served as the mediator[2, 22, 38, 181, 188 – 194] (Fig. 5). G-Protein contains α-, β- and γ-subunits. The α-subunit binds glycosyltransferase (GTF), while β- and γ-subunits provide the 'fixation' of G-protein to the cytoplasmic membrane. The α-subunit is bound to the C-terminus and the third inner loop of GPCR, while β- and γ-subunits are attached only to the C-terminus.[2, 195] A free receptor is not bound to the G-protein. After binding an agonist, the receptor conformation changes and the receptor becomes capable of interacting with the GTF-free G-protein. The addition of GTF to the receptor-coupled G-protein results in the dissociation of the G-protein to α- and βγ-subunits. An activated GTF-coupled α-subunit stimulates different systems in secondary mediators.[2] The XRD data on three-dimensional structures of the G-protein complex with activated GPCR, the GTF-coupled activated α-subunit and the βγ-subunit were published[196, 197]. This structural information is of fundamental importance for understanding the mechanism of GPCR activation and intracellular signal generation.

It was shown experimentally that all three classes of opioid receptors could activate α-subunits of G_i- and G_0-proteins.[2, 198 – 203] Activation of G_0-proteins was observed to suppress the voltage-dependent calcium channels[198 – 203] and stimulate the potassium channels.[204, 205] Activation of G_i-proteins had an inhibiting effect on adenylate cyclase.[2, 203, 206 – 209]

Three-state receptor models for activation that involves the G-protein are known.[188 – 194, 210, 211] For example, Fig. 6 shows the cubic ternary complex model proposed by Weiss *et al.*[192 – 194] It is noteworthy that some three-state models of the receptor activation considered the possibility for the

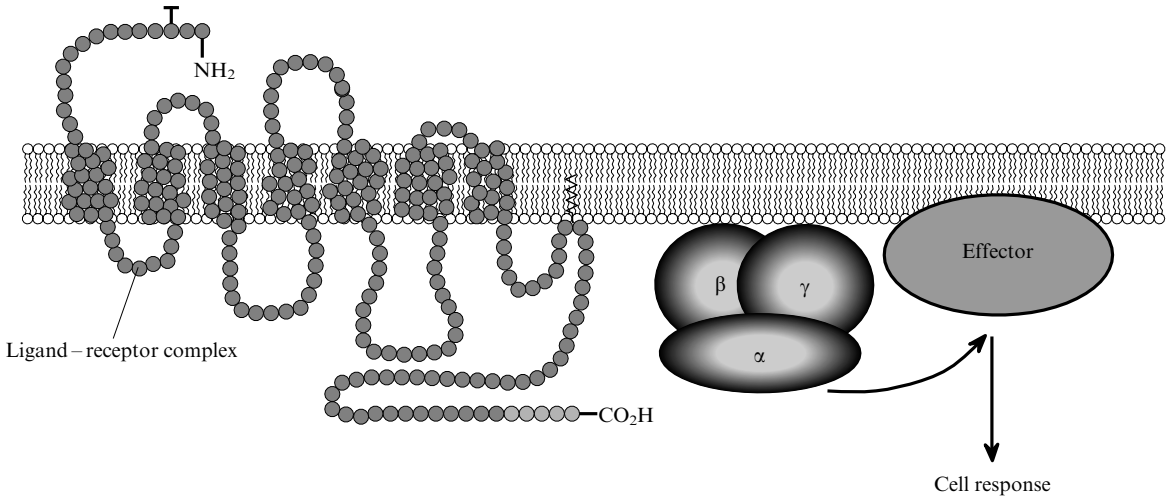


Figure 5. Illustration of the process of biological response formation.[181] α, β and γ are G-protein subunits.

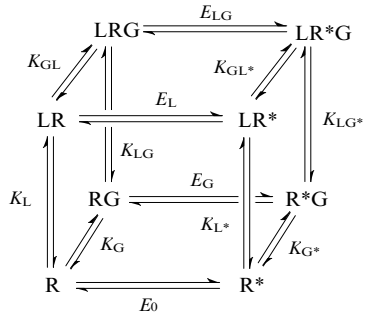


Figure 6. Three-state model of receptor activation.[193]

activated ligand–receptor complex (LR*) to interact with different G-proteins, which gives rise to different signal transduction (transmission) pathways.[210]

When an antagonist interacts with the receptor, no changes in the receptor conformation occurs and the receptor cannot interact with the G-protein. Hence, the signal on the ligand–receptor interaction of antagonists with the opioid receptors does not transform into the intracellular signal.

Thus, the modern concepts on the mechanisms of intracellular biological response formation upon the interaction of opioid receptors with ligands are tightly linked with the formation of an active ligand–receptor complex. At present, studies in this field are concentrated on the following problems:

1) how the inactive conformation transforms into the active one?

2) why the agonist does provoke changes in the opioid receptor conformation and the antagonist does not?

The mechanism of GPCR activation was studied using the methods of site-directed mutagenesis and computer-aided modelling.[69, 152, 190, 212–216] For example, the point mutations of adrenergic receptors clearly demonstrated that GPCR structural modifications could induce activation of the receptor independently of the agonist.[31, 216] For rhodopsin, adrenergic, muscarin and melacortin receptors, the constitutional activity is the result of mutations of amino acid residues in transmembrane domains II, III, VI and VII. Based on these experimental data, the transmembrane domains were assumed to be involved in activation of receptors of the GPCR family.[212–215] Using the method of site-directed mutagenesis, it was found[217, 218] that the constitutional activity of all opioid receptor sites can be increased upon structural modifications of the amino acid sequence Asp–Arg–Tyr in TM-III. Using the same method, it was shown[219] that the modifications in TM-III and TM-VII were responsible for the δ-opioid receptor activation. Befort *et al.*[31] identified the amino acid residues in TM-III and TM-VII mutations of which led to spontaneous activation of the δ-opioid receptor of mice. These residues were Asp^{128}, Tyr^{129} (TM-III) and Tyr^{308} (TM-VII). Earlier, these amino acid residues were shown to form a part of the reception zone and to be involved in the recognition of δ-opioid receptor ligands.[31, 32]

In later studies, it was noted that for all GPCR, one common mechanism operates, which involves the amino acid residues of TM-III, TM-VI, TM-VII.[22, 220, 221] It deserves mention that these TM are localized in the most 'shielded' zone of the transmembrane domain inaccessible for the lipid membrane environment.[27, 28] A receptor in its active state could later bind to the G-protein by non-valence interactions involving amino acid residues of intracellular loops and the C-terminus.[188]

Based on the generalized results of studies of the receptors of the GPCR family (rhodopsin, opioid, muscarinic and adrenergic receptors), several hypotheses of the GPCR activation were put forward. According to one hypothesis, the inactive receptor conformation is retained due to a certain system of hydrogen bonds formed between transmembrane helices; modification of these hydrogen bonds is a part of the activation process. It was assumed that Asp^{128}, Tyr^{129} (TM-III) and Tyr^{308} were involved in these intramolecular hydrogen bonds.[90, 222, 223] The structural modifications, namely, the substitution of asparagine (D128N) and alanine (D128A) for Asp^{128}, and also of phenylalanine (Y129F and Y308F) for Tyr^{129} and Tyr^{308}, modified or destroyed the hydrogen bonds inside the transmembrane domain, thus favouring the higher conformational flexibility of the receptor. A result of the conformational flexibility of a receptor was the possibility of tuning the receptor to the G-protein, *i.e.*, its pre-organization required for binding the G-protein to the receptor.

This hypothesis agreed well with the results of three-dimensional modelling of the reception zone and the ligand–receptor complexes. For example, in a model developed for the δ-opioid receptor reception zone,[148] the transmembrane helices were spatially oriented in such a way that hydrogen bonds linked Asp^{128} (TM-III) to Tyr^{308} (TM-VII) and Tyr^{129} (TMC-III) to His^{278} (TM-VI). A similar system of hydrogen bonds between transmembrane domains III, VI and VII was also present in the three-dimensional δ-opioid receptor model proposed in another study.[149] The intramolecular hydrogen bond between Asp^{128} (TM-III) and Tyr^{308} (TM-VII) was present in the three-dimensional δ-opioid receptor model put forward by Pogozheva *et al.*[52] In the three-dimensional models of μ- and κ-opioid receptors proposed in this work, the $Asp^{147}\cdots Tyr^{326}$ and $Asp^{138}\cdots Tyr^{323}$ bonds in the μ- and κ-opioid receptors, respectively, corresponded to the aforementioned $Asp^{128}\cdots Tyr^{308}$ intramolecular bond in the δ-opioid receptor. In the models of ligand–opioid receptor complexes, Asp^{128}, Tyr^{129}, Tyr^{308} (δ-opioid receptor) and their amino acid analogues in μ- and κ-opioid receptors were involved in the hydrogen bonding of different agonists docked to the reception zone.[52] For example, in the morphine–μ-opioid receptor complex, the protonated nitrogen atom of morphine interacted with Asp^{147} (TM-III) and the 3-OH group of the tyramine fragment interacted with His^{297} (TM-VI).

According to yet another hypothesis,[86, 223–225] a substantial contribution to the stabilization of inactive μ-, κ- and δ-opioid receptor conformations was made by the electrostatic interactions supplemented by hydrogen bonding between the side chains of neighbouring amino acid residues, namely, Asp···Arg in TM-III ($Asp^{145}\cdots Arg^{146}$, $Asp^{164}\cdots Arg^{165}$ and $Asp^{155}\cdots Arg^{156}$ for the δ-, μ- and κ-opioid receptors, respectively). The break of this salt bridge enhanced the constitutional activity of all classes of the opioid receptor.[86, 223–225] This hypothesis also relied on the results of site-directed mutagenesis and computer-aided modelling of receptors of the GPCR family.[217, 218, 224, 226–230] Within the framework of this hypothesis, the interaction of an agonist with the opioid receptor

did result in the break of the Asp···Arg salt bridge in TM-III, whereas its interaction with an antagonist did not.

The conclusion of the important role the Asp···Arg salt bridge in TM-III played in the stabilization of the inactive opioid receptor conformation agreed well with the results of three-dimensional modelling of Etorphine (**8**) – δ-opioid receptor and Naltrexone (**1b**) – δ-opioid receptor complexes.[86] In designing the starting conformations for each complex, the following two structures of the δ-opioid receptor transmembrane domain were modelled: with retention of the salt bridge in the Asp-Arg fragment of the receptor (inactive complex) and with its break (active complex). The energetically stable conformations of all four complexes were determined by molecular dynamics methods. The analysis of results revealed considerable differences in the structures of active and inactive complexes, which corresponded to the potential energy minima. Thus in inactive Etorphine – δ-opioid receptor and Naltrexone – δ-opioid receptor complexes, the hydrogen bond between Asp^{145} and Arg^{146} in TM-III remained intact. During stabilization, the active Naltrexone – δ-opioid receptor complex was converted into its inactive form (a hydrogen bond formed between Asp^{145} and Arg^{146} in TM-III), whereas the stabilization of the active Etorphine – opioid receptor complex did not involve the formation of bonds between these amino acid residues, and the complex remained active.

It deserves mention that the intramolecular electrostatic interaction and hydrogen bonding analogous to the Asp^{145}···Arg^{146} interactions in TM-III and Asp^{128} (TM-III)···Tyr^{308} interactions (TM-VII), were also observed in other GPCR. Thus the salt bridge destruction in the structural fragment Asp – Arg TM-III led to the activation of GnRH receptors (GnRH is the gonadotropin-releasing hormone)[86] and $\alpha_{1\beta}$-adrenergic receptors.[218] For rhodopsin, a hydrogen bond between Glu^{113} (TM-III) [Asp^{128} (TM-III) homologue] and Lys^{296} (TM VII) [Tyr^{308} (TM-VII) homologue], which is involved in fixation of the free receptor inactive conformation, was revealed.[231–233] In $\alpha_{1\beta}$-adrenergic receptors, the amino acid homologue of Asp^{128} (TM-III) was coupled with the homologue of Tyr^{308} (TM-VII), and the break of their bond initiated the activation of the $\alpha_{1\beta}$-adrenergic receptor.[80] Furthermore, for the other GPCR members, no hydrogen bonding was observed between amino acid residues homologous to Tyr^{129} (TM-III) and His^{278} (TM-VI), *i.e.*, this interaction was apparently intrinsic of the opioid receptors.

Thus, in the modern literature, a viewpoint is accepted that the inactive receptor conformation is stabilized by a system of strong intramolecular interactions (electrostatic and hydrogen bonding) between transmembrane helices. For the δ-opioid receptor, the key role in the stabilization of an inactive conformation belongs to the hydrogen bonds between the carboxylate anion of Asp^{128} (TM-III) and the phenolic hydroxyl group of Tyr^{308} (TM-VII) and between the phenolic hydroxyl group of Tyr^{129} (TM-III) and the nitrogen atom of the imidazole ring in His^{278} (TM-VI) as well as to the electrostatic interaction supplemented by hydrogen bonding between the carboxylate anion of Asp^{145} and the protonated amino group of the guanidine fragment of Arg^{146} (TM-III). The amino acid residues Asp^{128}, Tyr^{129}, His^{278} and Tyr^{308} in the δ-opioid receptor correspond to amino acid residues of Asp^{147}, Tyr^{148}, His^{297} and Tyr^{326} in the μ-opioid receptor and to Asp^{138}, Tyr^{139}, His^{291} and Tyr^{320} in the κ-opioid receptor.[52] Mutations of these amino acid residues mimic the effect of the opioid receptor agonists. In both cases, changes were observed in the system of intramolecular interaction between transmembrane helices; thus, the opioid receptor conformation transformed from inactive to active. This point of view agrees with the experimental evidence according to which the amino acid residues in the receptor responsible for the receptor activation are also involved in its interaction with ligands.[15, 32, 57, 73, 74, 76] Thus Asp(III:08) interacts with the protonated nitrogen atom of the ligand and His(VI:17) interacts with its phenolic group.

The opioid receptor agonists and antagonists possess similar structures, they interact similarly with the same receptor amino acid residues, which makes it possible to associate the different opioid activity of the opioid receptor agonists and antagonists not only with the chemical affinity between the components of the ligand – receptor complex but also with the differences in their geometric complementarity. Within the framework of this assumption, Pogozheva *et al.*[35, 52, 114] put forward a theory according to which the ability of a ligand to modify or destroy the system of intramolecular interaction in a transmembrane domain (correspondingly, the ratio of its agonistic and antagonistic properties) is associated with the ligand position in the reception zone. Based on the results of site-directed mutagenesis and computer-aided three-dimensional modelling of the reception zone and ligand – receptor complexes, it was assumed that in the reception zone, a ligand molecule can occupy two positions different with respect to Asp(III:7). One position ('upper') is closer to the upper boundary of the cytoplasmic membrane and intracellular loops and farther from Asp(III:7); the other ('lower') is shifted deeper into the membrane bulk as compared with the 'upper' position and located near Asp(III:7). The distance between the 'upper'and 'lower' positions is *ca.* 2 Å. The ligand molecules are embedded into these positions like interlocked puzzle pieces; hence, the possibility for a molecule to occupy a particular position is determined by its shape. The incorporation into the 'lower' position is more difficult due to the presence of a rather narrow hydrophobic region, the so-called hydrophobic 'slot'. *N*-Substituents of definite length (allyl, propyl, cyclomethylpropyl, cyclobutylmethyl) oriented equatorially with respect to the morphine core could fit the mentioned 'slot' between the side chains Met(III:11), Trp(VI:16), Ile(VII:7), Gly(VII:10), Tyr(VII:1) without any steric hindrance. More bulky substituents either at the N atom (*e.g.*, phenetyl) or in other positions of the piperidine ring prevented the incorporation of a ligand molecule into the 'lower' position.

If a molecule occupies the 'upper' position [more remote from Asp(III:7)], the implementation of electrostatic interaction or a hydrogen bond between the NH^+ groups of the ligand and COO^- of Asp(III:7) required that the side chain in Asp(III:7) was turned. The change in the Asp(III:7) conformation initiated the break of the hydrogen bond Asp(III:7)···Tyr(VII:11), which resulted in the TM-III shift from its initial position. The shift of the transmembrane helix entails the break of the Tyr(III:8)···His(VI:20) hydrogen bond and the Asp···Arg (TM-III) salt bridge. Thus, the turn of the Asp(III:7) side chain, which is due to the impossibility of the ligand molecule to approach the amino acid residue, triggers the opioid receptor transition from its inactive conformation to active; hence, a ligand that occu-

pied the 'upper' position in the reception zone exhibits the agonistic properties.

If a ligand occupies the 'lower' position in the reception zone, which is closer to the central region of the transmembrane fragment and to Asp(III:7), the efficient interaction between the protonated nitrogen atom of the ligand and the carboxyl group of Asp(III:7) in the opioid receptor requires no turn of the side chain of aspartate. The inactive conformation of the opioid receptor is thus retained and the ligand exhibits the antagonistic properties. It should be noted that the ligand occupying the 'lower' position in the reception zone induces steric hindrance for the side chain rotation in the aspartate fragment, *i.e.*, not only changes the receptor inactive conformation but also fixes it.

The Pogozheva theory agreed well with the results of our structural studies.[234] For compounds of the benzomorphane series with typical *N*-antagonistic substituents, it was found that if the substituent atoms were at a distance more than 2 Å from the aromatic ring plane, the antagonistic properties of opioid receptor ligands disappeared.

The theory that the opioid efficacy of a ligand depends on its position in the reception zone seems to be very attractive because makes it possible to explain the following experimental facts:

1) considerable exceed in the number of agonists over the number of antagonists (inaccessibility of the 'lower' position in the reception zone);

2) the transformation of 'pure' antagonists with typical *N*-antagonistic substituents into 'pure' agonists upon the introduction of bulky substituents into their molecules;

3) the different effect of the introduction of typical *N*-antagonistic substituents into the piperidine ring of molecules of different chemical classes;

4) the different opioid efficacy of enantiomers and diastereomers of the opioid receptor ligands.

The assumption that the ligand position in the reception zone is determined only by the conformation of its molecule could not explain the agonistic properties of morphine and other scaffold compounds with *N*-methyl substituents. Advancing the Pogozheva theory, we assumed that the shape of a molecule is not the only factor that fixes it in the 'upper' position. Other intermolecular interactions exist that prevent penetration of a molecule inside the membrane and transform it into the 'pure' agonist.[235–237] From this standpoint, all ligand–receptor interactions can be divided into the following three types:

1) agonistic interactions that occur in the 'upper' (agonist) position in the opioid receptor reception zone and fix the ligand molecule in this position thus transforming it into a 'pure' agonist;

2) antagonistic interactions that occur only in the 'lower' (antagonist) position of the opioid receptor reception zone, fix the ligand molecules in this position and transform it into a 'pure' antagonist;

3) affinity interactions that occur irrespective of the position occupied by the ligand molecule in the opioid receptor reception zone; the interaction of the protonated nitrogen atom with the Asp(III:7) carboxylate group can serve as the example.

The proposed classification agrees well with the results of computer-aided three-dimensional modelling of ligand–receptor complexes, because the ligand–receptor interactions comprised those common for agonists and antagonists as well as those inherent to only agonists or antagonists.[52, 86]

Thus, based on the currently available information, the appearance of the cell biological response to the ligand–receptor interaction can be associated with the appearance of an active ligand–receptor complex formed not directly but *via* the following system: agonist–receptor–G-protein. The transition from the inactive receptor conformation to its active conformation (activation), which favoured its tuning to match the G-protein, is associated with the destruction of the system of strong intramolecular interactions (electrostatic, hydrogen bonds) that fix the inactive conformation. The receptor activation is initiated by a turn of the Asp(III:7) side chain. Ligand-induced conformational changes in Asp(III:7) are determined by the ligand position in the reception zone, which in turn is determined by both the chemical affinity of the ligand and the peculiarities of its spatial structure. This conclusion demonstrates the importance of studying the three-dimensional structure of ligands and elucidating its structural elements responsible for its position in the reception zone.

IV. The concept of a pharmacophore. Models of opioid pharmacophores

Numerous investigators of the ligand–receptor interaction divided the reception process into the following stages: recognition, preassembling, ligand binding and transformation of the signal of the receptor–ligand interaction into the biological response of the cell. Interpretations of the term 'pharmacophore' proposed at different times differed in both the functional characteristics (pharmacophore functions in different stages of the reception process) and the degree of specification of pharmacophore features or characteristics (electronic, spatial, *etc.*) necessary for its involvement in the reception process.

Historically, the term 'pharmacophore' was implicitly used by Paul Ehrlich[238] in 1909. In the 1960s–1970s, with the deeper insight into the reception process and the relationship between the ligand biological effect and its structure, the scientists became aware of a fact that the ligand–receptor interaction is a multistage process and that it is the whole molecular scaffold rather than structural fragments of the molecule (functional groups and atoms) that is involved in the interaction with the receptor. The scaffold ensures the required spatial position of these structural components. In 1977, Gund updated the former definition to 'a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity'.[239] In this definition, we are dealing with two functions of structural features, namely, a function of recognition and a function of triggering the biological response (a change in the cell biological activity).

To date, wealth of studies were devoted to the investigation of structural fragments (atoms or functional groups) of opiate receptor ligands that can be involved in the ligand–receptor interaction. These structural fragments are called the pharmacophore components or the pharmacophore elements.[35, 150, 240] The molecules of opiate receptor ligands of different chemical classes were shown to contain various pharmacophore elements. Among the latter, the key elements that were found in virtually all opiate receptor ligands irrespective of their chemical class and selectivity

could be distinguished, namely, the protonated nitrogen atom and the aromatic ring.[15, 31, 49–52, 163, 241–244]

The mechanism of interaction of key pharmacophore elements of opiate receptor ligands was studied by many authors [15, 31, 73, 243, 244] who have shown that the protonated nitrogen atom is responsible for the electrostatic interaction with the carboxyl group of the opiate receptor segment supplemented by hydrogen bonding. The aromatic ring was shown to be involved in the π–π-interaction with a certain complementary segment in opiate receptors.[32, 245]

The hydroxyl group in the *meta*-position of the aromatic ring is conventionally assumed to be an additional binding site of opiate receptor ligands. For scaffold ligands, namely, derivatives of meperidine, 4-alkyl-4-arylpiperidines and 5-arylmorphanes, it was experimentally shown that the introduction of a hydroxyl group into the *meta*-position of the aromatic ring increased the opioid efficacy.[159, 160] Conventionally it is assumed that this substituent forms a hydrogen bond with the opiate receptor as the proton donor.[159, 160, 246]

A typical model of the opioid pharmacophore complying with the Gund concept of pharmacophore represented an ensemble of certain pharmacophore features. For example, Beckett and Casy [3] proposed to use the three-point opiate receptor model (anionic and phenolic binding sites and a hydrophobic region) for estimating roughly the ligand–receptor interactions by assuming that the anionic and phenolic opiate receptor sites interact with the tyramine fragment of morphine-like opioid ligands, while the hydrophobic moiety interacts with the C and E rings of the carbon scaffold (For the structure, see Appendix) (Fig. 7). Later, the same authors assumed that the opioid pharmacophore can be described as 4-phenylpiperidine in the chair conformation with the axially oriented phenyl ring.[163] Later, Krylov [247] proposed that the analgesic pharmacophore could be a tyramine fragment present in structures of many narcotic analgesics. In many studies devoted to opioid peptides exhibiting δ-antagonism, the pharmacophore was identified with 2′,6′-dimethyl-L-tyrosyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**9**) (Dmt-Tic).[248–257] In another study, [258] the 2-amino-7-hydroxy-1,1-dimethyltetraline fragment was proposed as the antagonistic pharmacophore.

Many explorers of the ligand–receptor interactions noted that different pharmacophore elements were functionally inequal and took part in different stages of the reception process (recognition, preassembling, binding and bioresponse generation). Thus Schwyzer [259] has developed a concept of the address message, according to which all pharmacophore elements were conventionally divided into 'address' and 'message' elements. The message represents a pharmacophore element that serves for binding the ligand to the biological target; the address is a pharmacophore element responsible for the selectivity or the biological effect formation. Portoghese noted that the message represents a molecular-structural feature common to a series of ligands and recognizable by all receptor classes, while the address is recognized by the binding site intrinsic to only one class of receptors.[260] Thus in the 7-(spiroindano)oxymorphone (**10**) molecule, which was a δ-selective antagonist, Portoghese singled out the oxymorphone fragment as the message and the benzene ring of the spiroindane fragment, which was indeed responsible for the selective antagonistic effect, as the address.[260] In the κ-selective antagonists of the indolomorphinane series, the address fragment responsible for the κ-selective antagonism was identified with the second positively charged nitrogen atom.[62, 261] In opioid peptides, the message and the address were identified with the animo acid residues Tyr^1 and Phe^4, respectively.[262–264]

In many publications, the tyramine fragment was referred to as the message fragment of δ-ligands with the morphine-like structure, while the aromatic ring corresponding to the enkephalin Phe^4 residue performs as the address fragment.[241, 265, 266] Several authors singled out particular functional groups in the tyramine fragment as responsible for the affinity of opiate receptor antagonists. These were the substituent at the N atom and the phenolic hydroxyl group.[266–269] Filisola *et al.*[87] identified the same structural fragments with the pharmacophore elements-messages inherent to all opiate receptor ligands.

In δ-selective peptides containing the Dmt-Tic fragment, Dmt was identified with the pharmacophore element-message, while the Tic fragment acted as the address pharmacophore element that provided selective antagonism.[254]

Based on the Schwyzer concept of the address, Metzger *et al.*[46] put forward a hypothesis according to which the opiate receptor reception zone also contained a message region that interacted with the pharmacophore elements-messages as well as an 'address' region that could be coupled with the address pharmacophore elements of the ligand. Within the framework of this hypothesis, the address region of the opiate receptor reception zone contained sites that differed for κ-, μ- and δ-opiate receptors. The selectivity of ligands with respect to a particular opiate receptor type was assumed to be the consequence of the interaction between the address pharmacophore elements of the ligand and the address regions in the reception zones of all three classes, being determined by the combination of the following two factors:

1) the phamacophore ligand elements that act as the address with respect to a certain opiate receptor class (*e.g.*, κ) interact with complementary binding κ-sites in the address region of the reception zone; as the result, the ligands affinity for this particular opiate receptor class increases;

2) at the same time, due to steric hindrance, the address κ-pharmacophore elements inhibit the interaction of other pharmacophore elements with the μ- and δ-address regions in the reception zone thus decreasing the ligand affinity for the μ- and δ-opiate receptors.

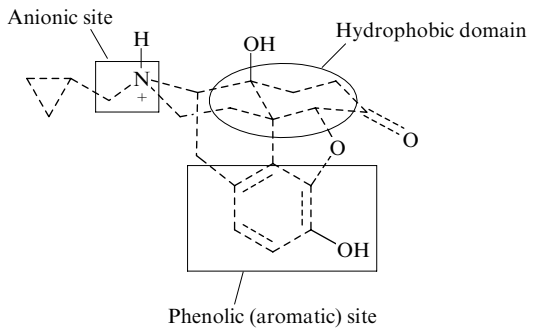


Figure 7. Three-point model of an opioid receptor.[3]

Within the framework of this hypothesis, the δ-selectivity of NTI (**2a**) is the result of the enhanced affinity for the δ-opiate receptor and the reduced affinity for the μ- and κ-opiate receptors.

A somewhat different explanation of the selective effect of ligands was proposed by Blake *et al.*[51] Analysis of the binding sites of cloned κ-, μ- and δ-opiate receptors with the selective agonists and antagonists has led to a conclusion that the address regions in the reception zone were located very close to one another and partly spatially overlapped. Binding to one address opiate receptor site triggered conformational modification in the opiate receptor, which prevented the ligand binding to another address site in the opiate receptor.

Studies were carried out on the identification of address regions in the reception zone. For example, the amino acid residue Glu^{297} (TM-VI) was suggested to serve as the address site of the κ-opiate receptor.[34,46,62,63]

With the accumulation of experimental data on the pharmacophore elements of the opiate pharmacophore, it became evident that they should have particular spatial orientation to efficiently interact with the opiate receptor. The experimentally found data on the difference in the selectivity of enantiomers and distereomers of the opiate receptor ligands and on their agonistic and antagonistic properties served as the evidence for the important role of geometric complementarity of the pharmacophore elements.[50,162–175]

The comprehension of the important role of the ligand three-dimensional structure and the structural correspondence between the interacting opiate receptor and ligand molecules led to the refinement of the pharmacophore definition. In 1986, Dixon *et al.*[270] put forward the following pharmacophore definition: a particular three-dimensional ensemble of certain chemical groups common to biologically active molecules and recognizable by a certain biological target is the pharmacophore of this biological target. It should be noted that this definition dealt exclusively with the recognition function rather than with the biological response formation function, because the concept of proportionality between the biological response and the number of ligand–receptor complexes formed dominated in the literature of that time. Insofar as the recognition function of a receptor was similar for agonists and antagonists, the pharmacophore in Dixon's terms was also the same for agonists and antagonists.

A definition proposed by Dukhovich[42] in 2004 seems to be a sort of hybrid between the definitions put forward by Gund and Dixon. According to this definition, a pharmacophore is a set of common structural features that allow the recognition of a given receptor. In this interpretation, the pharmacophore is no longer a three-dimensional object (similar to the Gund interpretation), performs the function of receptor recognition and hence is the same for agonists and antagonists (similar to the Dixon interpretation).

To characterize the three-dimensional opioid pharmacophore, three-dimensional models were used. The traditional method of designing three-dimensional pharmacophore models was based on the elucidation of the structural similarity of ligands[271] and included several stages.

1. Selection of molecules for the analysis (reference molecules). The following main requirement was imposed on the reference molecules: the latter should interact with the opiate receptor in the similar fashion. The larger number of molecules in the reference series the more accurate the three-dimensional model.

2. Selection of atoms or functional groups that can be involved in the ligand–receptor interaction, *i.e.*, the potential pharmacophore elements.

3. Selection of biologically active conformations of reference molecules, *i.e.*, the conformations in which pharmacophore elements demonstrated the strongest interaction with the biological target.

4. Superposition of individual molecules in their active conformations involving pharmacophore elements.

5. Elucidation of general patterns in the spatial arrangement of potential pharmacophore elements in biologically active conformations and of the general features in the geometry of pharmacophore elements of different ligands.

As a rule, the active conformations were chosen according to the principle of structural similarity. The real conformations of conformationally rigid ligands with the rigidly fixed mutual arrangement of the protonated nitrogen atom and the aromatic ring served as the templates (morphine (**6b**) and its analogues are used most often). For conformationally flexible molecules, the starting models designed using structural templates were optimized by the iteration procedure of searching the local minima on the potential energy surface. For this purpose, the approximation of conformationally sampled pharmacophore (CSP) was used,[272] which consisted of considering all accessible conformers of molecules rather than only those corresponding to the global energy minimum. As a criterion of the sampled nature of the conformational field, the dihedral angle of 30° was conventionally used. In molecules with a larger number of degrees of freedom, the number of possible conformers sharply increased because in the cases where all accessible conformations were generated, the limitations or energy filters (the chosen deviations in the energies of conformations under consideration from the global energy minimum) were imposed. For example, the energy filter of 3 kcal mol^{-1} was used,[240] which meant that not all energetically stable conformations but only those with energies deviating from the most stable state by 3 kcal mol^{-1} were considered.

Conventionally, the determination of geometric similarity is reduced to finding the total set of distances, angles and dihedral angles that would unambiguously characterize the overall spatial arrangement of the pharmacophore elements in molecules of different ligands.

Thus, a conventional three-dimensional pharmacophore model represents a set of geometrical parameters that reflect the similarity in the spatial arrangement of similar pharmacophore elements. As a rule, this is the geometrical generality for the three (more rarely, four) similar pharmacophore elements. Hence, the models of a pharmacophore common to the biologically active molecules recognizable by a certain biological target are usually three-point[273,274] or four-point.[271]

Today, automated procedures are used for simulating the three-dimensional pharmacophore of a biological target. In designing 3D pharmacophore models, different computer programs are used (for example, Chem-X,[275] Tripos Sybyl,[276] CATALYST,[277,278] PHASE,[279] MOE,[280] GALAHAD,[281,282] LIGANDSCOUT[283]) and the databases of three-dimensional ligand structures for different biological targets (CONCORD,[284] CAMBRIDGE,[285] PDB,[286] *etc.*). The strategy of designing 3D-pharmaco-

phore models based on the method of exhaustive search in databases of three-dimensional ligand structures (3D database search) is believed to be the most promising.[287–290] The theoretical grounds for simulating a 3D pharmacophore have been described [278, 291–294].

According to experimental data (NMR spectroscopy, XRD), the regular features in the spatial arrangement of similar pharmacophore elements of opiate receptor ligands can only be observed for individual chemical classes, because the mutual orientation of the protonated nitrogen atoms and the aromatic ring is different in different classes of conformationally rigid ligands. For example, in the derivatives of 4,5-epoxymorphines, morphinanes, morphinan-6-ones, benzomorphinanes, dihydromorphone, oripavine, buprenorphine, pyrrolomorphinanes, the aromatic ring is in the axial position with respect to the piperidine ring, whereas in 5-arylmorphane molecules, it occupies the equatorial position.[49, 50, 241, 295] In line with this, a problem of orientation of the aromatic ring and the protonated nitrogen atom in a pharmacophore has been disputed for a long time. Thus Beckett and Casy [163] believed that in the analgesic pharmacophore containing the 4-phenylpiperidine fragment the phenyl ring should be oriented axially with respect to the piperidine ring. Fries and Portoghese [296] assumed that it is the aromatic ring located in any (axial or equatorial) orientation that enables interaction of a narcotic analgesic with the opiate receptor. Andrews and Lloyd [297] noted that an assumption of the equatorially oriented phenyl ring in the 4-phenylpiperidine pharmacophore appears to be unreasonable in view of the three-dimensional structure similarity. These authors proposed to use morphine as the conformational standard in studying the general analgesic pharmacophore. From their point of view, a morphine-like ligand conformation should favour the maximum analgesic activity. Lawson *et al.*[298] suggested that the phenyl ring orientation determined the ratio of agonistic and antagonistic properties in a ligand and proposed the 4-phenylpiperidine fragment with the equatorially oriented phenyl ring as the antagonistic three-dimensional opiate receptor model. This assumption agreed with the hypothesis by Casy *et al.*,[299, 300] according to which the antagonistic properties of compounds of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine series were associated with the presence of a piperidine ring with the equatorially oriented phenyl ring.

The following radically new concept was formulated by Portoghese: the phenyl rings in equatorial and axial orientations with respect to the piperidine ring interact with different binding sites of the receptor, *i.e.*, were essentially different pharmacophore elements.[49, 50, 241] Studying the same modifications of *N*-substituents in the ligand series with axial and equatorial phenyl ring orientations with respect to the piperidine ring, Portoghese arrived at a conclusion that identical structural fragments in ligand molecules of different chemical classes made different contributions to the opioid activity.[49, 50, 241, 296] Recent studies confirmed the validity of this conclusion. A wealth of information can be found in a review [301], which generalized the results of experimental and theoretical studies of opiate receptor binding sites to the structural fragments of selective and non-selective ligands of different chemical classes (morphine-like scaffold compounds, diarylpiperazines, arylacetamides, 4-anilidopiperidines).

Advancing the Portoghese ideas, Carroll [302] assumed that opiate receptor ligands of the 4-phenylpiperidine class interacted with the receptor in two different modes conditionally named 'phenyl-axial' and 'phenyl-equatorial'. The phenyl-axial mode (agonistic) realized in scaffold morphine-like structures was associated with the agonistic activity and the *N*-substituent-induced antagonistic activity. The phenyl-equatorial (antagonist) mode realized in *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines was associated with the antagonistic activity unrelated to the *N*-substituent. Figure 8 illustrates the opiate receptor regions that interact with different structures of ligands of the 4-phenyliperidine class in the agonistic and antagonistic modes of interaction with the opiate receptors. In both cases, the phenyl ring interacted with the same receptor regions (T region). For the phenyl-axial and phenyl-equatorial interactions, the ammonium proton occupied different spatial positions with respect to the phenyl ring but interacted with one and the same anionic region in the opiate receptor (A region). The *N*-substituents of piperidine interacted with different opiate receptor regions, namely, with N1 for the phenyl-axial mode and with N2 for the phenyl-equatorial mode of interaction with the opiate receptors. It deserves mention that this hypothesis was consistent with the Pogozheva theory on the agonistic and antagonistic positions of ligands in the reception zone. According to Carroll, the ammonium proton could occupy different positions for the agonistic and antagonistic modes of ligand binding to the same receptor region and the difference between these positions was not great.

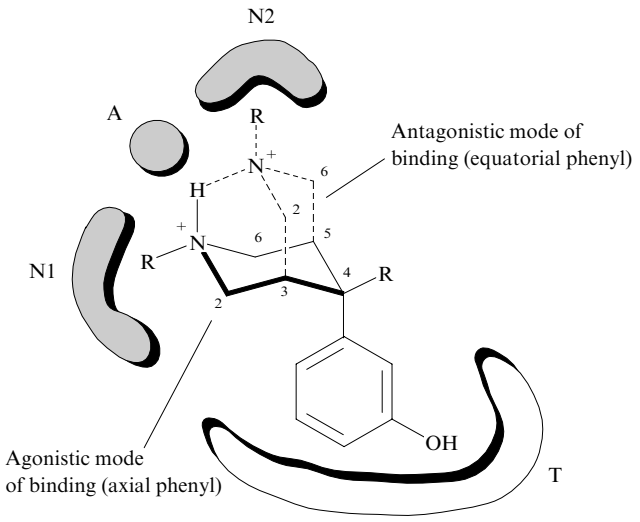


Figure 8. Illustration of the agonistic and antagonistic interactions of ligands of the 4-phenylpiperidine type with opioid receptors (see explanation in the text).[302]

Due to the different spatial orientation of key pharmacophore elements in different chemical classes of ligands, three-dimensional pharmacophore models were developed for structures with the phenyl ring and the protonated nitrogen atom oriented in the same way with respect to one another (as a rule, the axial orientation of the aromatic ring with respect to the piperidine ring). The models developed by Loew,[303] Coop and Jacobson,[304] Filizola *et al.*[240] can serve as the examples.

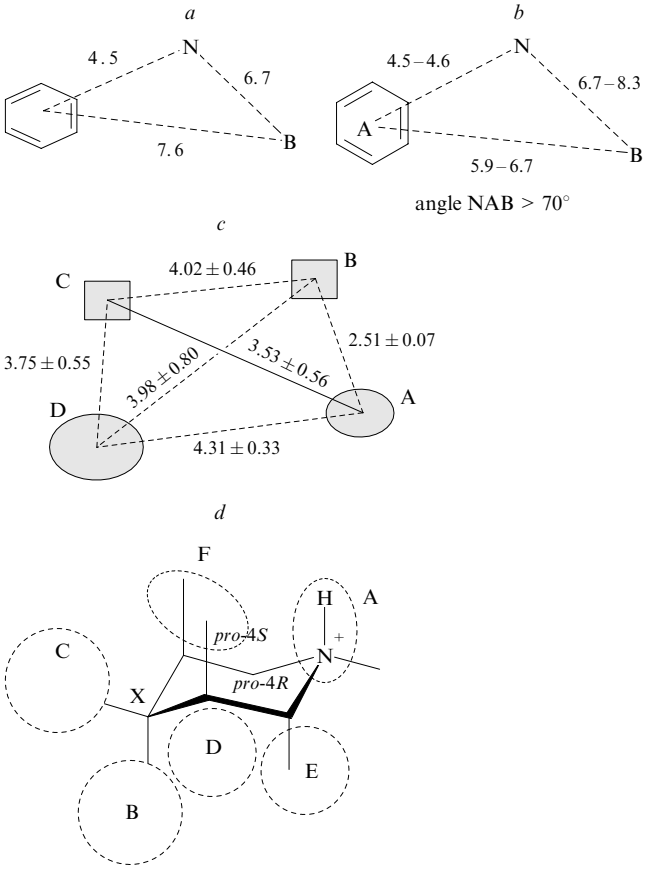


Figure 9. Three-dimensional models of the opioid pharmacophore proposed by Loew (*a*),[303] Coop (*b*),[304] Filizola (*c*) [240] and the authors of the present review (*d*) [235–237] (see explanation in the text). Distances are shown in Å.

Loew [303] have developed a three-dimensional opiate receptor model general for peptide and non-peptide ligands (Fig. 9 *a*). The learning sampling involved conformationally rigid morphine-like peptides, conformationally flexible analogues of fentanyl, cyclic enkephalin-like peptides and linear peptide antagonists. The morphine conformation was taken as the template of the active conformation. The Loew model contained the following three pharmacophore elements necessary for the recognition of the receptor: a protonated nitrogen atom, a phenyl ring (A) and an abstract hydrophobic region (B). The distances between the pharmacophore elements were used as the geometrical characteristics in the three-dimensional model (see Fig. 9 *a*).

Coop and Jacobson [304] put forward a three-dimensional model of the pharmacophore recognition of the δ-opiate receptor (Fig. 9 *b*). As the learning sample, they used Naltrexone (**1b**) derivatives containing the Ar–CH= substituent in position 7 (Ar is 1-naphthyl, 2-naphthyl, 9-anthryl, phenyl, biphenyl-4-yl). Similar to the Loew model, the Coop model contained the following three pharmacophore elements: a protonated nitrogen atom (N), a phenyl ring (A) and a region with an excess electron density (B). Presumably, the angular descriptor correlated with the δ-selectivity (the high selectivity with respect to the δ-opiate receptor required that the angle NAB was >90°), and the distance descriptors correlated with the affinity for the opiate receptors. Based on oxymorphindole (**11**) and SNC80 (**12**) as the reference sample compounds, the main limitation of the proposed model was formulated as follows: the model was valid only for ligands of similar structural classes containing a scaffold core. In the SNC80 molecule, the N–A distance and the NAB angle substantially differed from those in the pharmacophore.

The Filizola model [240] (Fig. 9 *c*) was developed for the non-selective recognition of μ-, κ- and δ-opiate receptors. Twenty three non-selective ligands with the high affinity for all opiate receptor types (4,5-epoxymorphinanes, benzomorphanes, dihydromorphones, aminotetralines, morphinanes, ethenomorphinanes, oripavines, dihydromorphindoles and derivatives of fentanyl) and six inactive compounds were used as the learning sample. The active conformations of all compounds of the learning sample contained the axially oriented aromatic ring. This three-dimensional model included four pharmacophore elements, namely, a protonated nitrogen atom (A), two hydrophobic regions (B, C) and an aromatic ring (D).

It should be noted that the Loew, Coop and Filizola three-dimensional models, in addition to the pharmacophore elements representing particular atoms and functional groups, employed abstract regions with certain electronic properties (hydrophobic regions, a region with excess electron density). The necessity of introducing abstract pharmacophore elements with similar electronic properties was determined by the wide diversity of opiate receptor ligands containing different and sometimes different in principle structural elements. To the end of the 1990s, new classes of opiate receptor ligands were discovered, which strongly differed in their structures from conventional opiates and opioids. Thus molecules of the discovered opiate receptor ligands contained none of the key pharmacophore elements [for example, natural diterpenoid salvinorin A (**13**), the strong selective κ-opiate receptor agonist].[305]

The extension of the range of known ligands of receptors and understanding that the appearance of the cell biological response was related only indirectly to the formation of the ligand–receptor complex led to refinement of the term 'pharmacopore'. The IUPAC defines the pharmacophore to be an 'ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response'.[1] In the Glossary of Terms Used in Medicinal Chemistry (1998), it was noted that the pharmacophore is not a real molecule or a real association of functional groups, but an abstract concept that considers the general molecular interaction capacities of a group of compounds towards their target structure. In the IUPAC definition, as in the Gund definition, the pharmacophore performs the following two functions: the binding to the receptor and the generation of a biological response. Agonists and antagonists differ in their ability to invoke the biological response; hence, according to IUPAC definition of the term 'pharmacophore', the latter may be the same for agonists and antagonists. In the ensemble of pharmacophore elements, the following two groups can be singled out: common for agonists and antagonists (perform only the function of binding to the receptor) and different for agonists and antagonists (perform two functions, namely, the binding and the biological response formation). For example, Pogozheva [35] analyzed the results of flexible docking of the peptide and non-peptide agonists and antagonists

into different types of three-dimensional opiate receptor models and distinguished the pharmacophore elements general and different for agonists and antagonists. The common pharmacophore elements involved in both the agonist and antagonist opiate receptors comprised a protonated nitrogen atom, an aromatic ring of the tyramine fragment (A) and the hydrophobic region (C) (Fig. 10). An agonistic pharmacophore contained an additional pharmacophore element (F) with the electronic properties that correlated with the selectivity. For the μ-opiate receptor agonists, F was the second aromatic ring; for the κ-opiate receptor agonists, F represented a polar region. An antagonistic pharmacophore contained, along with the general pharmacophore elements, an additional hydrophobic region (D) in the vicinity of the protonated nitrogen atom.

Virtually all three-dimensional opiate receptor models put forward in the past decade contained abstract regions with certain electronic properties as the pharmacophore elements. Moreover, it was implied that these regions were located in the three-dimensional space in a definite fashion, *i.e.*, their spatial arrangement was an intrinsic characteristic of a pharmacophore element like their electronic properties. A term '3D-pharmacophore element' has been proposed.[240] To characterize the steric features necessary for providing the optimal interactions with the receptor, we proposed to use of a concept of the pharmacophore domain. A pharmacophore domain represents a spatial region in which the ligand–receptor interactions are manifested to the maximum degree. Depending on certain functions of ligand–receptor interactions, the pharmacophore domains were divided into affinity, agonistic and antagonistic. In the affinity pharmacophore domains, the interactions were responsible only for binding to the receptor, *i.e.*, common to agonists and antagonists (for example, the interactions of the protonated nitrogen atom with Asp(III:7) and of an aromatic ring with His(VI:20)). In agonistic pharmacophore domains, the interactions inherent to agonists were realized. They fixed the ligand molecule in the 'upper' position of the reception zone thus modifying the receptor conformation and triggering the biological response. In antagonistic pharmacophore domains, the interactions intrinsic to antagonists occurred. They bonded the molecule to the receptor in the 'lower' position of the reception zone and fixed the inactive receptor conformation. The pharmacophore domain size, shape and centre are determined by the

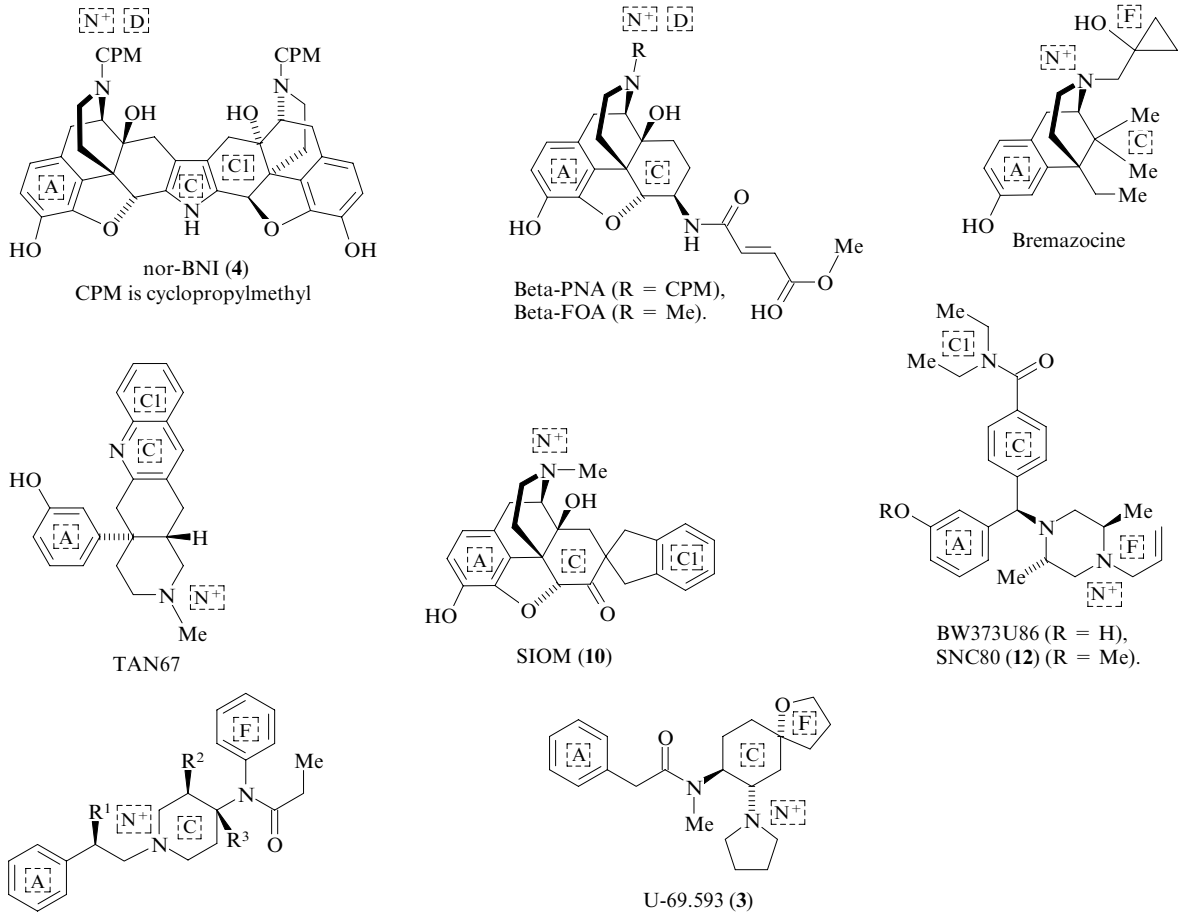


Figure 10. Elements of agonistic and antagonistic opioid pharmacophores in ligands of different chemical classes (see explanation in the text).[35] Pharmacophore elements are shown in dashed-line squares.

size, the shape and the centre of gravity of a structural fragment of the ligand molecule occupying this domain.

The spatial characteristics of a pharmacophore domain can unambiguously be determined by a set of distances, angles and dihedral angles. The electronic characteristics of pharmacophore domains can be found by considering the general features in the properties of different structural fragments that interact with a receptor and occupy the similar spatial positions, the same pharmacophore domain. The electronic characteristics are determined by the type of ligand – receptor interactions.

The authors of the present review developed a three-dimensional model of a non-selective opiate receptor that contained six pharmacophore domains A – F (see Fig. 9 *d*).[235–237] In the affinity domain A occupied as a rule by a protonated nitrogen atom, the electrostatic interaction supplemented by hydrogen bonding is realized. In the affinity domains B and C, the electron density transfer supplemented by hydrogen bonding is observed. In agonistic domains D and E, the hydrophobic interactions occurred. In antagonistic domain F, either hydrophobic interactions or hydrogen bonding were realized and the type of the antagonistic interaction determined the selectivity. The organization of pharmacophore domains B – F towards the pharmacophore domain A was characterized by the following geometrical parameters (the Z-matrix analogue): two distances 'pharmacophore domain centre – nitrogen atom' (R_{CentN}) and 'pharmacophore domain centre – ammonium hydrogen atom' (R_{CentH}), the angle 'nitrogen atom – pharmacophore domain centre – ammonium hydrogen atom' (NCentH) and the dihedral angle 'pharmacophore domain centre – bridge atom X the closest to the pharmacophore domain – nitrogen atom – ammonium hydrogen atom' (∠CentXNH). The virtual atom (X) was introduced for simplifying the dihedral angle computations in the intrinsic coordinate system. For an affinity pharmacophore domain in which the structural fragments are linked to the protonated nitrogen atom in a single common carbon chain, an aliphatic chain atom the closest to these fragments was taken as the X atom. For example, in 4-phenylpiperidines and in scaffold morphine-like molecules, X corresponded to the C(4) atom in the piperidine ring. In methadone and its analogues, X corresponded to the C(3) atom of the propylamine fragment. For the affinity pharmacophore domains of a ligand molecule in which the structural fragments were connected with the protonated nitrogen atom in region A by different aliphatic chains, the X atom was identified as the atom in the molecule that maximally coincided with the X atom if this molecule was superimposed with the 3D-opiate receptor model.

In the determination of spatial and electronic features of a pharmacophore domain, the conformationally rigid ligands (4,5-epoxymorphinanes, dihydromorphones, morphinanes, benzomorphanes, oripavines, spiro(benzofuran-3(2*H*),4′-piperidines), 5-arylmorphanes, derivatives of tropane, isoquinoline and 4-phenylpiperidines in the fixed ring conformation and the chiral centre configuration), were considered as the learning sample. The reference sample included the conformationally flexible ligands (4-anilinopiperidines, diphenylpropylamines, arylacetamides, peptides, *etc.*). A three-dimensional model of a non-selective opioid receptor developed in our studies formed the basis of the computational method of assessing the agonistic and antagonistic properties of opioid receptor ligands.[306] Table 2 shows the geometrical parameters of pharmacophore domains.

Table 2. Geometrical parameters characterizing the mutual arrangement of pharmacophore domains A – F in a three-dimensional opioid pharmacophore model.[235–237]

Pharmacophore domain	R_{CentN} /Å	R_{CentH} /Å	NCentH /deg	∠CentXNH /deg
B	4.4 – 5.0	5.1 – 5.7	7 – 9	178 – 195
C	5.2 – 5.7	5.4 – 5.7	7 – 10	−7 – 12
D	4.2 – 4.7	4.9 – 5.3	8 – 10	from −117 to −137
E	2.4 – 2,6	3.3 – 3.5	10 – 11	from −139 to −158
F	2.6 – 3.0	2.3 – 2.7	18 – 21	−40 – 40

In the past decade, in addition to conventional opioid receptor models, the models built by the comparative molecular field analysis (CoMFA) method were widely used. The classical CoMFA relates the biological activity of drugs to dummy variables 'yes/no, 1/0, true/false', which determine the particular structural features.[307] The dummy variables were taken to reflect the electronic properties, lipophilicity, polarizability, *etc.* The CoMFA method involves the comparison of molecules by aligning them in space and mapping their molecular fields to three-dimensional grids. The comparison of molecules is carried out by taking into account the functionally equivalent groups identified with the pharmacophore groups either manually or by using a corresponding comparison technique, by taking into account the field amplitude in a series of similar substances. After mutual positioning of molecules, a box was constructed around them in such a way that the distance from its sides to any atom was not smaller than several Ångström. Next, the grid distance is defined for identifying the points in crossings. Depending on the box dimensions and the chosen grid spacing, several thousand points are usually preset. Next, the partial charges on atoms are determined (mainly by semiempirical methods) either before or after the generation of different molecular conformations. The found charges are used for calculating (separately for each molecule) the electrostatic field intensity in all grid points by using a charged probe atom. Furthermore, the steric fields are calculated with a neutral probe atom. In addition to these fields, the hydrophobic fields are calculated. Mapping the molecular fields to three-dimensional grids results in the corresponding tables, which often contained several thousand columns. In the next stage, the regression analysis of molecular fields mapped to three-dimensional grids and of the data on the biological activity (*e.g.*, experimental data on binding constants) is carried out.

In the CoMFA method, the three-dimensional pharmacophore model represents a set of coloured contour maps. These maps present the regions around ligands in which the variation of molecular field amplitudes is associated with the maximum difference in the ligand – receptor interaction, namely, the unfavourable and favourable steric regions around the molecule, the favourable and unfavourable zones for electropositive and electronegative substituents, for hydrophilic and lipophilic substituents, *etc.* Selective CoMFA models of δ-, μ- and κ-opiate receptor antagonists can serve as the example of three-dimensional opiate receptor models.[266] A hundred of antagonists with the morphine,

morphinane and 5-arylmorphane cores were used as the learning sample. In the CoMFA models, coloured polyhedra corresponded to special regions around ligand molecules in which the variations in the steric or electrostatic fields caused the maximum deviations in the opioid receptor properties. Thus, the regions were selected in which an intensification of steric strains was desirable (undesirable) or the regions where an increase in the electropositive (electronegative) nature was associated with the increase in the ligand affinity.

Thus, the current literature describes different conventional and CoMFA models of opioid receptors that reflect the electronic and spatial properties of ligand molecules necessary for the selective and non-selective recognition of the opioid receptors and the formation of the biological response. The choice of one or another model is determined by its goals and problems and by the strategy chosen for searching for new drugs.

* * *

The concepts of the opioid receptor mechanisms depend on the level of knowledge and undergo considerable modifications with the refinement of instrumental research methods, the development of new methodological approaches to process information, the sophistication of computer technologies, which make it possible to carry out fundamental studies of opioid reception processes by considering the whole spectrum of effects. In the past two decades, considerable progress in the field of opioid reception was largely associated with the identification of binding sites in the ligand – opioid receptor complexes responsible for the selectivity and opioid efficacy of the ligand. It is natural to assume that the next breakthrough in this direction will be associated with elucidation of the spatial structure of opiate receptor and ligand – receptor complexes. It is hoped that intense studies in the field of opioid reception with help to interpret the processes of pain appreciation, the formation of emotional status, the reaction to stress, *etc*. on the molecular level.

V. Appendix

Table. Ligands of opioid receptors.

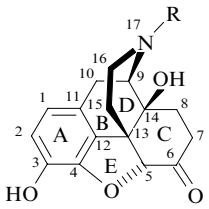
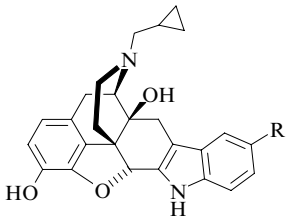
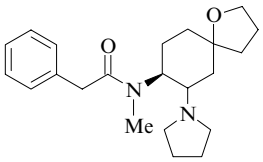
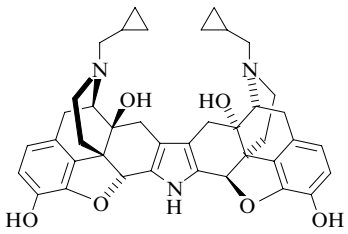
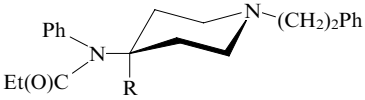
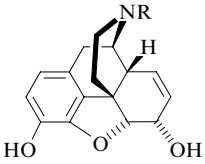
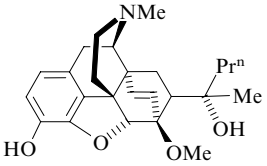
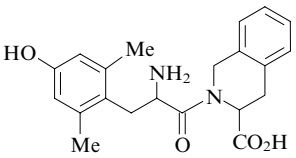
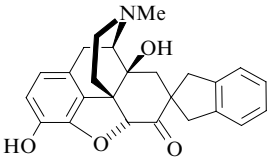
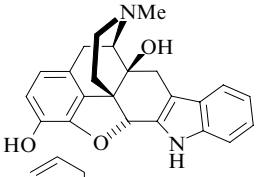
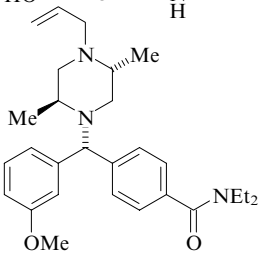
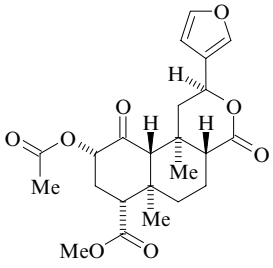
Compound	Structure	Activity type	Ref.
Naloxone (**1a**) Naltrexone (**1b**)	 R = $CH_2CH{=}CH_2$ (**1a**), cyclo-$C_3H_5CH_2$ (**1b**).	non-selective antagonists	46
Naltrindole (NTI) (**2a**) 5′-Guanidinonaltrindole (GNTI) (**2b**)	 R = H (**2a**), NHC(=NH)NH_2 (**2b**).	δ-selective antagonists	46, 240
U-69.593 (**3**)		κ-selective agonist	14
Norbinaltorphimine (nor-BNI) (**4**)		κ-selective antagonist	35, 46, 240

Table (continued).

Compound	Structure	Activity type	Ref.
Peptide DPDPE (**5**)	H_2NTyr-D-Pen-Gly-Phe-D-PenCO_2H	δ-selective agonist	51
Fentanyl (**6a**) Karfentanyl (**6b**)	 R = H (**7a**), CO_2Me (**7b**).	the same	240
Normorphine (**7a**) Morphine (**7b**)	 R = H (**6a**), Me (**6b**).	μ-selective agonists	52, 240
Etorphine (**8**)		non-selective agonist	240
2′,6′-Dimethyl-L-tyrosyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (H-Dmt-Tic-OH) (**9**)		δ-selective antagonist	254
7-(Spiroindano)oxymorphone (SIOM) (**10**)		δ-selective agonist	46
Oxymorphindole (**11**)		the same	240, 304
SNC80 (**12**)		δ-selective agonist	304
Salvinorin A (**13**)		κ-selective agonist	305

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