Cloning and Characterization of Multiple Opioid Receptors

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THE CLONING OF THE THREE MAJOR OPIOID RECEPTOR TYPES

Until recently, the study of opioid receptors, while greatly profiting from the rich array of synthetic peptides and alkaloids with high affinity and specificity, has been hampered by the absence of opioid receptor clones. Matters were changed, however, in December 1992, when two groups, Evans' and Kieffer's, independently cloned the mouse delta receptor from the NG-108 cell line (Evans et al. 1992; Kieffer et al. 1992). This work opened the door for rapid advances in this area. Several groups, including the authors, subsequently cloned the remaining two major types of opioid receptors from various rodent species (Chen et al. 1993; Fukuda et al. 1993; Kong et al. 1993; Meng et al. 1993; Minami et al. 1993; Thompson et al. 1993; Wang et al. 1993; Xie et al. 1994; Yasuda et al. 1993). The authors have independently cloned the three prototypical mu, delta, and kappa opioid receptors from rat brain, as well as the kappa receptor from guinea pig brain. The authors also have partial delta and mu receptor clones from guinea pig brain, which are currently being fully cloned and characterized (Fickel et al. 1994). In addition, in searching for other opioid receptor subtypes from rat and guinea pig brain, the authors have isolated several clones, also obtained by other laboratories (Mollereau et al. 1994), which appear to encode a protein closely related to the opioid family, but fails to bind a number of opiate alkaloids or opioid peptides. Thus, the question of the existence of opioid receptor subtypes at the molecular level remains to be fully elucidated.

SELECTIVITY OF THE CLONED RECEPTORS TOWARD THE ENDOGENOUS OPIOID LIGANDS

Since the endogenous opioid system is particularly rich in endogenous ligands, comprising three separate genes each giving rise to multiple active peptides, the issue of the exact relationship between

these endogenous ligands and the opioid receptors is of great importance. Previous studies in tissue homogenates led the authors to suspect that there is no one-to-one correspondence between any given precursor and any given receptor. Now that these receptors can be expressed indi-vidually, it is possible to carefully ascertain the selectivity profile of each of the endogenous ligands toward the individual receptors. The question of whether each precursor has at least one ligand that "sees" each of the receptors, or whether there is any exclusivity, can be addressed. For example, researchers already know that the mu receptor can interact with members of each of the families, but is kappa truly a "dynorphin" receptor, or can it be accessed by proenkephalin (proEnk) products? By comparing all three receptors and all the ligands side by side, a much more complete picture can be obtained than was had before. An equally important issue is that of efficacy at the receptors. While it may be assumed that all endogenous ligands are agonists, there is evidence that naturally occurring opioids, such as beta-endorphin1-27, can be antagonists in behavioral studies (Bals-Kubik et al. 1988; Tseng and Li 1986). This needs to be confirmed at the cellular level, and leads to the question of whether any of the other endogenous ligands can serve as antagonists or as partial agonists at one or more of the receptors.

A set of studies that is currently being performed in the authors' laboratory involves the evaluation of the selectivities of the endogenous opioid peptides for the cloned mu, delta, and kappa receptors. A series of proEnk, prodynorphin, and pro-opio-melanocortin peptides has been evaluated for affinity to the mu, delta, and kappa receptors that have been transiently transfected into COS-1 cells. Thus far, the data suggest that prodynorphin peptides have a high affinity for all three opioid receptor types, with approximately a tenfold selectivity for the kappa receptors. A surprising finding that is being further explored is that extended proEnk peptides such as Methione-Enkephalin-Arginine-Phenylalanine or Methione-Enkephalin-Arginine-Glycine-Leucine also have high affinity for kappa receptors in addition to mu and delta. Interestingly, Leu-enkephalin emerges as the most selective endogenous ligand, exhibiting a more than hundredfold preference for delta over kappa binding. On the other hand, the kappa receptor appears to have the widest range of selectivity vis-a-vis endogenous ligands, whereas mu and delta show relatively less discrimination (within one order of magnitude).

STRUCTURAL PROPERTIES OF THE OPIOID RECEPTORS—BINDING TO PEPTIDES VERSUS SMALL MOLECULES

The three opioid receptors cloned to date are all members of the guanosine triphosphate binding protein (G-protein)-coupled family of receptors containing seven transmembrane alpha helixes. While they exhibit significant homology to the somatostatin receptor family, they are particularly similar among themselves, exhibiting 61 percent level of identity at the amino acid level. The fact that opioid receptors belong to the seven transmembrane family of G-protein-coupled receptors tells a fair amount about their general topography based on analogy to bacterial rhodopsin, which has been visualized by electron microscopy (Findlay and Pappin 1986; Henderson et al. 1990). The seven alpha-helical transmembrane segments are thought to be arranged in a circular manner allowing the macromolecule to form a ligand binding cavity, and exposing three intracellular loops and the carboxy terminus to the cytoplasmic milieu and three extracellular loops and the amino terminus to the outside environment (Humblet and Mizadegen 1992). The intra-cellular loops, particularly the second and third loop, along with the carboxy terminal domain, are thought to be the site of interaction with G-proteins. The N-terminal domain and extracellular loops are in a position to play a role in receptor selectivity. It has been shown for several small neurotransmitter receptors that actual ligand binding takes place via specific interactions within the pocket formed by the trans-membrane domains. A great deal of this information derives from mutants and chimeras that have been constructed for many members of this superfamily, particularly the beta-adrenergic receptor (Dixon et al. 1987; Dohlman et al. 1987). The structure/function relationship of the dopamine receptors by site-directed mutagenesis has been studied in the authors' laboratory (Mansour et al. 1992), as well as by constructing chimeras between dopamine type 1 (D1) and dopamine type 2 (D2) (Meng et al. 1992).

In spite of the general model presented above, a great deal remains to be learned about the family of G-protein-coupled receptors in terms of actual three-dimensional arrangement, means of achieving ligand selectivity, molecular basis of drug efficacy and receptor-effector coupling, require-ments for selective G-protein interactions, and mechanisms of receptor regulation such as desensitization. For example, the homology of the mammalian receptors to rhodopsin, even in the transmembrane helixes, appears imperfect when examined in greater detail (Pardo et al. 1992). In addition, while they are discussed as a superfamily, recent studies have pointed to differences

as well as similarities between these receptors, especially between some inhibitory G-protein (Gi)-coupled receptors and the prototypical stimulatory G-protein (Gs)-coupled ß-adrenergic receptor (Dixon et al. 1987; Dohlman et al. 1987). Most importantly, while researchers' understanding of the interactions of small ligands with G-protein receptors is reasonable, understanding how peptide ligands bind and activate these structures is more limited.

Studying the opioid receptors at this structural level is of interest for several reasons. Such an analysis might help scientists understand the cellular basis of tolerance by describing mechanisms of receptor desensitization (e.g., phosphorylation), internalization, and down-regulation. Equally important, however, this level of analysis will allow researchers to understand, at the molecular level, the issue of receptor heterogeneity and of ligand selectivity in the context of the opioid system, which is unusually rich in endogenous ligands and in pharmacological probes. It is particularly advantageous that, in the opioid system, multiple peptides and multiple alkaloids interact with one family of receptors. A first-order question is, do they interact at the same sites (i.e., with the same critical residues) in the receptor? A related question is, do these receptors achieve selectivity toward peptide ligands in the same way that they achieve selectivity toward alkaloid ligands? The authors' working hypothesis is as follows: Both peptide and alkaloid agonists are likely to interact with the same region of the "binding pocket" of the receptor (though not necessarily in exactly the same way) to trigger a chain of allosteric events, which leads to a change in the interaction with G-proteins, thereby initiating the signal transduction cascade. However, it is possible that the selectivity between opioid peptides and opioid receptors is achieved through mechanisms quite distinct from those at play in alkaloid selectivity.

All known mammalian opioid peptides begin with the sequence Tyr-Gly-Gly-Phe- (YGGF), followed by Leu or Met. Clearly, this sequence, sometimes termed the "message," is critical to ensure interaction with the binding pocket of all opioid receptors, but the selectivity rests in the remaining sequence, the carboxy terminal extension beyond the penta-peptide (ranging from 2 to 26 residues in length), which provides the "address" (Schwyzer 1986). This carboxy terminal domain, at least when it is long, is likely to interact with the N-terminal domain or extracellular loops in a distinctive manner that may contribute to selectivity and that cannot be achieved by the much smaller alkaloids. The authors have proposed this model for kappa selectivity (Robinson and Berridge 1993) as the unique presence of

negative charges were noted on the second extra-cellular loop of the receptor, which the authors proposed to be interacting with the positive charge of the Arg-Arg residues in the kappa-selective prodynorphin products. The authors have already obtained some evidence in support of this model (see below). Interestingly, a similar model has been subsequently suggested for the thrombin receptor (Gerszten et al. 1994). This model for opioid selectivity does not exclude the possibility that opioid peptides, short or long, may also achieve discrimination by other means (e.g., by interacting with residues or sequences within the binding cavity).

The authors have embarked on a series of studies using the construction of chimeric receptors and the use of site-directed mutagenesis in order to examine the structural basis of receptor selectivity and specificity. In particular, the authors are very interested in the question of how this set of receptors achieves selectivity toward the family of endogenous ligands. A related question is whether peptides and opiate alkaloids bind to the receptor in the same way, or whether the complex structure of a peptide endows it with unique ways of interacting with the receptor protein.

Structure Function Analysis of the Receptors: The Chimera Approach

The authors have identified specific locations in each of the three opioid receptors that are good candidates for introducing mutations in order to make cassettes for the construction of chimeras. The mutations have been engineered into all three receptors, and the authors have constructed over 20 of the possible chimeras. Each receptor can now be divided into seven distinct domains, labeled "a" through "g" moving from the N- to the C-terminal domains. One type of study carried out to date has revolved around delta/kappa chimeric receptors constructed by using native restriction sites in these two receptors. Because both the rat kappa and delta receptors contain an Afl3 restriction site in the middle of transmembrane 3 (TM3) and a Bgl2 site at the beginning of TM5, the authors were able to construct six chimeras directly from the wild-type receptors. Two of the chimeric receptors did not show any binding to [3H]ethylketo. The arrangement of their DNA fragments appears to be correct as judged by several restriction enzyme digests. Their inability to bind is probably due to a disruption of some subtype-specific interactions among the different domains in a receptor. At this stage, it was not possible to localize these interactions because the domains involved were relatively large; this issue is being addressed with single-segment-exchanged chimeric receptors. However, the authors were able to

obtain complete binding profiles for the four remaining chimeras along with the wild-type kappa and delta receptors when they are labeled by 1 nano-molar (nM) [3H]ethylketocyclazocine (EKC). Multiple scatchard plot analyses were carried out using several classes of ligands, including nonselective ligands, highly selective kappa and delta ligands, and endogenous ligands, particularly members of the prodynorphin family. The following conclusions can be derived from these data:

1. The authors had hypothesized that high-affinity binding of the prodynorphin peptides to the kappa receptor was related to the presence of the highly negatively charged N-terminal and extra-cellular loop 2 in that receptor (Meng et al. 1993). The present results seem consistent with this hypothesis. A domain that includes extracellular loop 2 (negatively charged) appears to be particularly critical. When replaced by the delta sequence, the resulting chimeric receptor shows very low affinity for prodynorphin products while retaining excellent affinity for EKC, naloxone, or naltrexone. On the other hand, when these kappa domains are preserved but the regions C-terminal to them are replaced with delta fragments, the resulting receptor exhibits excellent kappa affinity. Thus, extracellular regions, particularly extracellular loop 2, may be critical for both the high affinity and high selectivity of DynA for the kappa receptor.

2. There is a high-affinity Tyr-Gly-Gly-Phe binding pocket in the delta receptor. This pocket is likely localized in the TM5-TM7 region in the delta receptor. This can be seen by comparing the binding of delta-selective ligands (peptides or alkaloids) to a chimeric receptor that contains domains TM5-TM7 of delta versus one that does not (i.e., delta receptor with kappa TM5-TM7). Whenever the C-terminal domain of delta is preserved, high delta affinity is maintained, but replacing it with its kappa equivalent abolishes this high-affinity delta binding.

3. Taken together, these results suggest that, in general, the delta recep-tor binds its selective ligands differently from the kappa receptor. Thus, the N-terminal half appears more critical for kappa binding and the C-terminal part for delta binding. This is revealed by the fact that a receptor with a kappa N-terminus and a delta C-terminus binds almost all ligands tested with an affinity comparable to wild type, whereas its mirror image (delta-N-terminus/kappa-C-terminus) binds all the specific ligands with low affinity but still binds the nonspecific ligands with good affinity, showing that the protein is being expressed and a generic opiate binding pocket is formed.

4. Several other observations can be made regarding the binding of alkaloids versus peptides, and of highly selective versus nonselective ligands. There are also some interesting exceptions to the general rules that the authors have tried to derive, suggesting that certain ligands with unusual structures have unique modes of interfacing with the receptor proteins.

Several mutants have been constructed. In the case of kappa, the authors examined the effect of simultaneously mutating the three negative charges on extracellular domain 2 to test the possible role of this region in interacting with the positive charges found in dynorphin. These mutations resulted in a small decrease in the affinity of Dyn A, Dyn B, and alpha neoendorphin by less than one order of magnitude. This finding suggests that while the negative charges may play a small role in the binding of prodynorphin products, other features of the extracellular loop may be even more critical for kappa selectivity. Binding of EKC and nor-binaltorphimine (nor-BNI) was not significantly altered by any manipulations of this region, supporting the notion that the extracellular loop may be particularly important in interaction with the peptides.

Structure Function Analysis of the Receptors: The Modeling Approach

The authors have used as a starting point the fact that all three cloned opioid receptors interact with the Tyr-Gly-Gly-Phe sequence. The most likely sites of interactions within this tetrapeptide are the NH3 + of the N-terminal Tyr, the OH group on the phenyl ring of this same Tyr1, possibly the NH group of Gly3, and the potential aromatic interactions with the phenyl ring of Tyr1 and Phe4. These sites are likely to bind to the receptors through hydrogen bonds, charge interactions, or hydro-phobic interactions. Therefore, it is presumed that there are comple-mentary sites on opioid receptors that interact with these active groups. As a working hypothesis, it can be assumed that these are among the amino acid residues that are conserved across the three opioid receptors. Some of these residues should be unique to the opioid receptor family, and distinctive from sites found on other members of the G-protein super-family. However, this latter criterion should not be used to exclude important residues such as the Asp in TM3, since this negatively charged residue is used by other receptors for ionic interactions and may also be recruited in opioid receptors to perform a similar function. Thus, the authors sought to

identify residues within the TM domains that are common to delta, mu, and kappa receptors and which, by their charge, their hydrogen bonding potential, or their potential for interaction with aromatic nuclei, may be involved in binding the opioid core sequence.

With these notions in mind, two models of the binding pocket of opioid ligands have been developed, one within the authors' group and the other in collaboration with Dr. Henry Mosberg and colleagues. The details of these models and their similarities and differences are beyond the scope of this paper. Suffice it to say that although independently derived, they share some common amino acids as key in the binding pocket, but they differ in their orientations of the ligands within the pocket. The advantage of having two working models is to force researchers to consider alternatives in interpreting empirical results. Mutation studies will first examine the residues chosen by both models as being important in the binding. Once so-called critical residues have been ascertained, focus will then shift toward testing orientation of ligand within the pocket. Testing has already begun on the proposed critical sites in the mu receptor. Preliminary results with several mutants created based on modeling are encouraging and show the usefulness of this modeling approach, which has led to specific residues of the receptors in domains not previously seen as important anchor points in the monoaminergic receptors.

Taken together, structural studies of the opioid receptors carried out to date, along with the findings of others in the field, strongly reinforce the view that these receptors are much more complex than previously anticipated, that multiple domains are used for multiple types of inter-actions, and that small molecules versus large peptides may interact very differently with these receptors. It is anticipated that continued efforts in this arena will lead to a better understanding not only of the opioid receptors but also of peptide receptors in general.

ANATOMICAL STUDIES

Regardless of their relative preferences at the pharmacological level, opioid signaling in a particular region or circuit depends primarily on the local opioid anatomy. For example, even if Leu-Enk exhibits a preference for the delta receptor, if no delta sites are found in the vicinity, but mu sites are, Leu-Enk may act as a mu agonist. When both mu and delta sites are present, then the difference in selectivity becomes a way in which to code the presence and concentration of

ligand in a more com-plex way than would be possible with one site. If this logic is extended to multiple endogenous ligands deriving from one or more precursor, and is interacting with multiple opioid receptors, it can be seen how this system changes a binary signal (receptor bound or unbound) to an analog communication mechanism. Thus, ideally, to describe a system under-lying a given behavior or function, it would be necessary to delineate the local complement of receptors and endogenous ligands with their range of affinities, selectivities, and efficacies.

The location of opioid receptors vis-a-vis their endogenous ligands has been the subject of much interest and discussion—reports of "mismatch" between the two (Herkenham and McLean 1986) have described several types of lack of concordance between the distribution of receptors and ligands. While this can be seen as the basis of nonsynaptic communi-cation, with diffusion of the ligands to distant sites, there are a number of alternative interpretations, including the fact that there is not a one-to-one correspondence between a given receptor and the selectivities of products deriving from a single opioid precursor (not even for kappa and proDyn, as shown above). Thus, ligand-receptor matching needs to include the relation of all opioid receptors to all endogenous ligands. Furthermore, the level of resolution possible with receptor autoradiography does not allow detailed anatomical studies possible with in situ hybridization (ISH) and immunocytochemistry (ICC) with specific antibodies directed at the individual receptors. The use of these approaches has only recently become possible, and these tools can now be used to readdress the ques-tion of the anatomical relationship between opioid peptides and receptors.

Anatomical procedures for identifying the receptor messenger ribonucleic acids (mRNAs) that encode the three classical opioid receptors have been developed. ISH studies demonstrate that cells expressing the mu, delta, and kappa receptor mRNAs are differentially distributed in the central nervous system (CNS) and spinal cord and correspond well to known receptor binding distributions defined by receptor autoradiography. Three separate studies were carried out comparing the individual receptor mRNAs to their respective binding sites using combined ISH and receptor autoradiographic techniques. In addition, a study was completed revealing the overall distribution of kappa receptors, in comparison to the expression of pro-dynorphin, using adjacent sections. This body of work has been published detailing the anatomy of the cells that express the opioid receptor genes in relation to various known anatomical and functional

characteristics of the endogenous opioid system in the brain (Mansour etal. 1993, 1994a, 1994b).

To the authors' anatomical armamentarium have recently been added antibodies that have been raised to nonhomologous regions of the mu and kappa receptors. The mu antibody is the best characterized with fairly complete immunohistochemical maps in both colchicine and non-colchicine-treated animals. The distribution of mu receptor protein corresponds well to mu receptor binding and mRNA expression with high levels of expression in such regions as the striatal patches, medial habenula, interpeduncular nucleus, and the dorsal horn of spinal cord. The importance of developing this and other opioid receptor antibodies lies in the higher cellular resolution that can be achieved and in the visualization of fibers and terminals, which is imperative in under-standing the anatomy of these receptors. Both immunofluoresence and diaminobenzidine (DAB)/nickel chloride visualization procedures have been developed, allowing for the direct co-localization with mRNA probes and antibodies to other molecules of interest.

The kappa antibody is in the latter stages of development. Specific immunohistochemical staining is observed in such regions as the nucleus accumbens, paraventricular hypothalamus, median eminence, substantia nigra (pars reticulata), and periaqueductal grey. The ideal immuno-histochemical conditions have presently, however, not been achieved, and further studies are in progress. The delta antibodies are in the early stages of development, and peptides are being produced in order to inoculate rabbits. These antibodies when fully characterized will be invaluable in studying the opioid receptor proteins anatomically and in regulatory studies.

SUMMARY

Over the course of 1 to 2 years, the field has moved swiftly to investigate the functional and structural properties of the newly cloned opioid receptors. Achieving a better understanding of these macromolecules is likely to have profound implications for drug design aimed at the production of better analgesic drugs, for a more fundamental under-standing of mechanisms of action of drugs of abuse, and for a more comprehensive knowledge base regarding the biology of opioid peptides in particular, and neuroactive peptides in general.

REFERENCES

Bals-Kubik, R.; Herz, A.; and Shippenberg, T.S. ß-Endorphin-(1-27) is a naturally occurring antagonist of the reinforcing effects of opioids. Naunyn Schmiedebergs Arch Pharmacol 338:392-396, 1988.

Chen, Y.; Mestek, A.; Liu, J.; Hurley, J.A.; and Yu, L. Molecular cloning and functional expression of a µ-opioid receptor from rat brain. Mol Pharmacol 44:8-12, 1993.

Dixon, R.; Sigal, I.; and Candelore, M. Structural features required for ligand binding to the beta-adrenergic receptor. EMBO J 6:3269-3275, 1987.

Dohlman, H.; Caron, M.; and Lefkowitz, R. A family of receptors coupled to guanine nucleotide regulatory proteins. Biochemistry 26:2657-2664, 1987.

Evans, C.J.; Keith, D.E., Jr.; Morrison, H.; Magendzo, K.; and Edwards,R.H. Cloning of a delta opioid receptor by functional expression. Science 258:1952-1955, 1992.

Fickel, J.; Xie, G.; Thompson, R.C.; Watson, S.J.; and Akil, H. Cloning of potential candidates for guinea pig opioid receptors. Regulatory Peptides - Proceedings of INRC'94 54:1, 1994.

Findlay, J., and Pappin, D. The opsin family of proteins. Biochem J 238:625-642, 1986.

Fukuda, K.; Kato, S.; Mori, K.; Nishi, M.; and Takeshima, H. Primary structures and expression from cDNAs of rat opioid receptor d- and µ-subtypes. FEBS Lett 327:311-314, 1993.

Gerszten, R.E.; Chen, J.; Ishii, M.; Ishii, K.; Wang, L.; Nanevicz, T.; Turck, C.W.; Vu, T.-K.H.; and Coughlin, S.R. Specificity of the thrombin receptor for agonist peptide is defined by its extracellular surface. Nature 368:648-651, 1994.

Henderson, R.; Baldwin, J.; Ceska, T.; Zemlin, F.; Beckmann, E.; and Downing, K. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J Mol Biol 213:899-929, 1990.

Herkenham, M., and McLean, S. Mismatches between receptor and transmitter localizations in the brain. In: Boast, C.; Snowhill, E.W.; and Altar, C.A., eds. Quantitative Receptor Autoradiography. New York: Alan R. Liss, 1986.

Humblet, C., and Mizadegen, T. Three dimensional models of G-protein coupled receptors. In: Bristol, T., ed. Annual Reports in Medicinal Chemistry. New York: Academic Press, 1992.

Kieffer, B.; Befort, K.; Gaveriaux-Ruff, C.; and Hirth, C. The delta opioid receptor: Isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci U S A 89:12048-12052, 1992.

Kong, H.; Raynor, K.; Yasuda, K.; Moe, S.T.; Portoghese, P.S.; Bell,G.I.; and Reisine, T. A single residue, aspartic acid 95, in the delta opioid receptor specifies selective high affinity agonist binding. JBiol Chem 268:23055-23058, 1993.

Mansour, A.; Fox, C.A.; Burke, S.; Meng, F.; Thompson, R.C.; Akil, H.; and Watson, S.J. Mu, delta and kappa opioid receptor mRNA expression in the rat CNS: An in situ hybridization study. J Comp Neurol 350:412-438, 1994a.

Mansour, A.; Fox, C.; Meng, F.; Akil, H.; and Watson, S.J. Kappa1 receptor mRNA distribution in the rat CNS: Comparison to kappa receptor binding and prodynorphin mRNA. Mol Cell Neurosci 5:124-144, 1994b.

Mansour, A.; Meng, F.; Meador-Woodruff, J.; Taylor, L.; Civelli, O.; and Akil, H. Site-directed mutagenesis of the human D2 receptor. Eur J Pharmacol 227:205-214, 1992.

Mansour, A.; Thompson, R.C.; Akil, H.; and Watson, S.J. Delta opioid receptor mRNA distribution in the brain: Comparison to delta receptor binding and proenkephalin mRNA. J Chem Neuroanat 6:351-362, 1993.

Meng, F.; Mansour, A.; Hoversten, M.; Taylor, L.; and Akil, H. Structure-function relationship of dopamine receptors: A chimeric study. Soc Neurosci Abstr 18(1):662, 1992.

Meng, F.; Xie, G.X.; Thompson, R.C.; Mansour, A.; Goldstein, A.; Watson, S.J.; and Akil, H. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc Natl Acad Sci U-SA 90:9954-9958, 1993.

Minami, M.; Toya, T.; Katao, Y.; Maekawa, K.; Nakamura, S.; Onogi,T.; Kaneko, S.; and Satoh, M. Cloning and expression of a cDNA for the rat k-opioid receptor. FEBS Lett 329:291-295, 1993.

Mollereau, C.; Parmentier, M.; Mailleux, P.; Butour, J.-L.; Moisand, C.; Chalon, P.; Caput, D.; Vassart, G.; and Meunier, J.-C. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 341:33-38, 1994.

Pardo, L.; Ballesteros, J.; Osman, R.; and Weinstein, H. On the use of the transmembrane domain of bacteriorhodopsin as a template for modeling the three-dimensional structure of guanine nucleotide-binding regulatory protein-coupled receptors. Proc Natl Acad Sci US-A 89:4009-4012, 1992.

Robinson, T.E., and Berridge, K.C. The neural basis of drug craving: An incentive-sensitization theory of addiction. Brain Res Rev 18:247-291, 1993.

Schwyzer, R. Molecular mechanism of opioid receptor selection. Biochemistry 25:6335-6342, 1986.

Thompson, R.C.; Mansour, A.; Akil, H.; and Watson, S.J. Cloning and pharmacological characterization of a rat mu opioid receptor. Neuron 11:903-913, 1993.

Tseng, L.F., and Li, C.H. Beta-endorphin (1-27) inhibits the spinal beta-endorphin-induced release of met enkephalin. Int J Pept Protein Res 27:394-397, 1986.

Wang, J.B.; Imai, Y.; Eppler, C.M.; Gregor, P.; Spivak, C.E.; and Uhl,G.R. μ Opiate receptor: cDNA cloning and expression. Proc Natl Acad Sci U S A 90:10230-10234, 1993.

Xie, G.-X.; Meng, F.; Mansour, A.; Thompson, R.C.; Hoversten, M.T.; Goldstein, A.; Watson, S.J.; and Akil, H. Primary structure and functional expression of a guinea pig kappa opioid (dynorphin) receptor. Proc Natl Acad Sci U S A 91:3779-3783, 1994.

Yasuda, K.; Raynor, K.; Kong, H.; Breder, C.D.; Takeda, J.; Reisine, T.; and Bell, G.I. Cloning and functional comparison of kappa and delta opioid receptors from mouse brain. Proc Natl Acad Sci U S A 90:6736-6740, 1993.

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