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Pharmacology

Cloning and pharmacological characterization of a rat κ opioid receptor

(guanine nucleotide-binding protein-coupled receptor/dynorphin/cAMP/*in situ* hybridization)

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ABSTRACT **A full-length cDNA was isolated from a rat striatal library by using low-stringency screening with two PCR fragments, one spanning transmembrane domains 3–6 of the mouse δ opioid receptor and the other unidentified but homologous to the mouse δ receptor from rat brain. The novel cDNA had a long open reading frame encoding a protein of 380 residues with 59% identity to the mouse δ receptor and topography consistent with a seven-helix guanine nucleotide-binding protein-coupled receptor. COS-1 cells transfected with the coding region of this clone showed high-affinity binding to κ opioid receptor-selective ligands such as dynorphin A and U-50,488 and also nonselective opioid ligands such as bremazocine, ethylketocyclazocine, and naloxone. Not bound at all (or bound with low affinity) were dynorphin A-(2–13), enantiomers of naloxone and levophanol [i.e., (+)-naloxone and dextrorphan], and selective μ and δ opioid receptor ligands. Activation of the expressed receptor by κ receptor agonists led to inhibition of cAMP. Finally, *in situ* hybridization revealed a mRNA distribution in rat brain that corresponded well to the distribution of binding sites labeled with κ-selective ligands. These observations indicate that we have cloned a cDNA encoding a rat κ receptor of the κ_1 subtype.**

Several lines of evidence have established the presence of at least three major opioid receptor types in the central nervous system (CNS) and periphery. These receptors are referred to as μ, δ, and κ and have distinct pharmacological profiles, anatomical distributions, and functions (1–4). The μ receptors bind morphine-like drugs as well as several endogenous opioid peptides and have a widespread distribution in the CNS with particularly high levels in the striatal patches, thalamus, nucleus tractus solitarius, and spinal cord. The μ receptors are thought to mediate the opiate phenomena classically associated with morphine, including analgesia, opiate dependence, cardiovascular and respiratory functions, and several neuroendocrine effects. The δ receptors are also abundant in the CNS, particularly in forebrain areas such as the striatum and cortex, bind enkephalin-like peptides, and are thought to mediate analgesia, gastrointestinal motility, and a number of hormonal functions.

The κ receptors, the third family of opioid receptors, are expressed at low levels in rat brain but are abundant in guinea pig and higher primates including human. They have a widespread distribution in the CNS and are particularly enriched in cortex, hypothalamus, and cerebellum. These receptors exhibit a high affinity for the products of prodynorphin, one of three endogenous opioid peptide precursors that are posttranslationally processed to give rise to several endogenous opioid peptides. In addition, the κ receptors interact with several pharmacological agents, including benzomorphans such as bremazocine, and arylacetamides such as U-50,488 and U-69,593—*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate and D-(5α,7α,8β)-(+)-*N*-methyl-*N*-{7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl}benzeneacetamide, respectively (5); in turn, the arylacetamides exhibit a high degree of selectivity for κ over μ or δ receptors (5). The κ receptors mediate a spectrum of unique and distinctive functions, including the modulation of drinking, water balance, food intake, gut motility, temperature control, and various endocrine functions (6–9). Interestingly, they produce analgesia but they are not primary reinforcers. Furthermore, in man, activation of κ receptors has been found to produce dysphoria and psychotomimesis (10), leading to suggestions regarding a potential role in psychosis. The κ receptors have been subtyped into κ_1 and κ_2 sites (11), with κ_1 sites being more strictly defined as those sites that bind the arylacetamides, while κ_2 sites do not. There are further proposed subdivisions within these two broad categories, based on pharmacological profile and interactions with various endogenous ligands (12, 13).

It has long been suspected that the opioid receptors belong to the guanine nucleotide-binding protein (G protein)-coupled receptor family (14, 15), although their exact structural identity proved elusive. The first cloned putative opioid binding protein was a cell-surface adhesion molecule belonging to the immunoglobin superfamily. Because of difficulties in expressing the cloned molecule, indirect evidence was used to characterize it as a binding site of opiate alkaloids (16). Xie *et al.* (17) reported the cloning of a putative opioid receptor that is a member of the seven-helix receptor family and that has a high degree of sequence homology to the neuromedin K receptor. This clone had an absolute requirement for the presence of tyrosine in position 1 of opioid peptides for any binding to occur and a stereospecificity expected of classical opioid receptors. However, this receptor had only moderate affinity toward opioid ligands and did not show any selectivity among the μ, δ, and κ opioid compounds. Most recently two groups, Kieffer *et al.* (18) and Evans *et al.* (19), independently cloned a mouse δ opioid receptor from the NG108 neuroblastoma–glioma cell line, using an expression cloning strategy. The sequences of the cloned receptors were different in the second extracellular

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Abbreviations: DAGO, [D-Ala², *N*-MePhe⁴, Gly-ol⁵]enkephalin; DPDPE, cyclic [D-Pen², D-Pen⁵]enkephalin, where Pen = penicillamine; Dyn, dynorphin; E-2078, [*N*-MeTyr¹, N^{α}-MeArg⁷, D-Leu⁸]dynorphin A-(1–8) ethylamide; M_r 2034, (−)-(1*R*,5*R*,9*R*,2′*S*)-5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan D-tartrate; nBNI, norbinaltrophine hydrochloride; U-50,488, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate; U-69,593, D-(5α,7α,8β)-(+)-*N*-methyl-*N*-{7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl}benzeneacetamide; CNS, central nervous system; G protein, guanine nucleotide-binding protein; IP_3, inositol 1,4,5-trisphosphate.

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loop but were otherwise identical. Whether this represents the existence of δ receptor variants within the same cell line remains to be determined. The cloned receptors exhibited high affinity and selectivity toward δ opioid ligands, and Evans *et al.* (19) showed that activation of their receptor with selective δ agonists could decrease cAMP levels in transfected cells. The mouse δ receptors were found to be highly homologous to several members of the G protein-coupled receptor family, particularly the somatostatin receptors. The cloning of the mouse δ receptors paved the way for cloning of other members of the opioid receptor family by homology. The present study describes the cloning and characterization of a rat κ receptor,[§] which also appears to be a member of the seven helix G protein-coupled family of receptors and is negatively coupled to adenylate cyclase.

MATERIALS AND METHODS

Isolation of Opioid Receptor Fragments. Oligonucleotide primers complementary to regions encoding transmembrane domains 3, 4, 6, and 7 of the mouse δ opioid receptor DNA sequence were designed and used in PCR amplification. First strand cDNA from NG108 cell mRNA and rat olfactory bulb mRNA was used as template. The PCR products were subcloned into a T-tailed vector system (Invitrogen) and sequenced by using Sequenase (United States Biochemical). Two fragments were identified by this procedure. The first had a 99% homology to the mouse δ opioid receptor cDNA sequence reported by Evans *et al.* (19) from transmembrane domain 3 to the end of transmembrane domain 6. The second clone isolated from rat olfactory bulb was ≈66% homologous to the mouse δ opioid receptor (19) from transmembrane domain 3 to 6.

Library Screening and Sequencing. A rat striatal Lambda ZAP II cDNA library was purchased and plated according to the manufacturer's recommendations (Stratagene). The cDNA fragments from NG108 and rat olfactory bulb were labeled with [^{32}P]dCTP (ICN) by using a random-primed DNA labeling kit (Boehringer Mannheim). Approximately 1.5×10^6 cDNA clones were plated, and replicate nitrocellulose membranes were screened with the radiolabeled cDNA fragments. Prehybridization and hybridization were in 30% formamide/0.75 M NaCl/0.075 M sodium citrate/50 mM potassium phosphate buffer, pH 7.0/0.1% polyvinylpyrrolidone/0.1% Ficoll/0.1% bovine serum albumin/0.1% disodium pyrophosphate/0.1% SDS/100 μg of salmon sperm DNA per ml at 37°C for 16–18 hr. Positive clones were replated and rescreened until they were pure. DNA inserts were rescued into the pBluescript plasmid with M13 helper phage. Restriction enzyme mapping and Southern blot analysis were performed on rescued plasmid DNA. One clone, renamed as rK1OR after pharmacological characterization, showed a strong hybridization signal on plasmid Southern blot analysis and contained a cDNA insert of ≈4.8 kb. Various restriction enzyme digests of rK1OR were subcloned into pBluescript and completely sequenced. DNA sequencing results were analyzed for homology with other known sequences in the GenBank data base by using the Genetics Computer Group program (20).

Expression of Receptor and Binding Assay. A *Kpn* I fragment of rK1OR containing the whole coding region was subcloned into a cytomegalovirus pCMV-neo expression vector. For binding assays, 25 μg of plasmid DNA was transfected into each 100-mm dish of COS-1 cells by the method of Chen and Okayama (21). Receptor binding of the membrane preparation of the transfected cells was performed as described by Goldstein and Naidu (5). [^{3}H]U-69,593 (58 Ci/mmol; NEN; 1 Ci = 37 GBq), tritiated [D-Ala^2, *N*-Me-Phe^4, Gly-ol^5]enkephalin ([^{3}H]DAGO; 55 Ci/mmol; NEN), and tritiated cyclic [D-Pen^2, D-Pen^5]enkephalin (Pen = penicillamine) ([^{3}H]DPDPE; 34.3 Ci/mmol; NEN) were used in the characterization of the receptor. Nonspecific binding was defined as the binding of radioligand in the presence of 1 μM U-50,488, naloxone, and [D-Ser^2, Leu^5]enkephalin-Thr (DSLET), respectively. [^{3}H]U-69,593 (2 nM) was used to label the receptor in competition studies. All assays were conducted in 50 mM Tris buffer (pH 7.4) at room temperature. Receptor binding results were analyzed with the LIGAND program (22). In determining K_d and K_i values, free concentrations were not determined, possibly altering the accuracy of some of these measures.

cAMP and Inositol 1,4,5-Trisphosphate (IP_3) Assays. Plasmid DNA was transfected by electroporation into COS-1 cells at 330 μF, 275 V, and a low-resistance setting by using the BRL Cell-Porator System. For the cAMP assay, ≈10^5 cells were incubated in Krebs–Hepes buffer with appropriate opioid drugs 72 hr after transfection in the presence of 1 μM forskolin in a final volume of 200 μl at 37°C for 20 min (23). cAMP levels were measured by radioimmunoassay with an antibody from Sigma (lot 97F4822). The IP_3 measurements were performed under similar conditions but without forskolin. An IP_3 assay system (Amersham) was used in the determination of IP_3 levels, and the trichloroacetic acid method was used to extract IP_3 according to the manufacturer's protocol.

***In Situ* Hybridization.** The tissue-specific expression of this receptor was studied in the brain of adult male Sprague–Dawley rats by using a standard *in situ* hybridization procedure (24). A *Bam*HI–*Hin*dIII fragment of the clone, which overlapped the last 45 nucleotides of the coding region and 728 nucleotides of the 3′ untranslated region, was used to prepare antisense riboprobes labeled with uridine 5′-[α-(^{35}S)thio]triphosphate. To ensure specificity, sense-strand and RNase pretreatment controls were performed. In addition, brain sections were hybridized to complementary RNA probes generated from different domains of the clone to ascertain that the mRNA distribution was the same.

RESULTS

DNA and Protein Structure. Two PCR-generated probes, one corresponding to transmembrane domains 3–6 of the mouse δ opioid receptor and the other unidentified but homologous to the δ receptor, were used to screen a rat striatal cDNA library. One of the positive clones containing a 4.8-kb insert was completely sequenced. A GenBank data base search revealed that this clone encoded a previously unreported sequence most closely related to the mouse δ opioid receptor and to the somatostatin receptor family. Its longest open reading frame had an in-frame stop codon at nucleotide 155, upstream of the first ATG, which began at nucleotide 257 (see the nucleotide sequence in GenBank). It encoded a protein with 380 amino acid residues that exhibited 59% and 37% identity in amino acids to the mouse δ receptor and rat somatostatin receptor subtype 1, respectively. Protein motif analysis suggested that it is a member of the seven-helix G protein-coupled receptor family. Furthermore, its protein sequence possessed other features shared by many receptors of this family—e.g., two possible N-glycosylation sites at Asn-25 and Asn-39 in the N-terminal domain and two putative palmitoylation sites at Cys-340 and Cys-345. In addition, it contained three putative target sites for protein kinase C phosphorylation (Ser-255, Thr-357, and Ser-369), two for Ca^{2+}/camodulin-dependent kinase (Ser-260 and Thr-357), one putative target site for cAMP-dependent protein

[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. U00442).

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  1 MESPIQIFRG EPGPTCAPSA CLLPNSSSWF PNWAESDSNG SVGSEDQQLE
                          *                 *
 51 PAHISPAIPV IITAVYSVVF VVGLVGNSLV MFVIIRYTKM KTATNIYIFN
                        TM 1
101 LALADALVTT TMPFQSAVYL MNSWPFGDVL CKIVISIDYY NMFTSIFTLT
          TM 2                                    TM 3
151 MMSVDRYIAV CHPVKALDFR TPLKAKIINI CIWLLASSVG ISAIVLGGTK
                                                TM 4
201 VREDVDVIEC SLQFPDDEYS WWDLFMKICV FVFAFVIPVL IIIVCYTLMI
                                                TM 5
251 LRLKSVRLLS GSREKDRNLR RITKLVLVVV AVFIICWTPI HIFILVEALG
        Δ    o            ◊          TM 6
301 STSHSTAVLS SYYFCIALGY TNSSLNPVLY AFLDENFKRC FRDFCFPIKM
                          TM 7                  #    #
351 RMERQSTNRV RNTVQDPASM RDVGGMNKPV
        o     •    Δ
        Δ
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FIG. 1. Deduced amino acid sequence from rat κ_1 cDNA coding region. Putative transmembrane domains are underlined and labeled, although the exact assignments of these domains are somewhat arbitrary. Potential sites for posttranslational modification are marked by various signs under the corresponding amino acid residue: *, N-glycosylation; ◇, cAMP-dependent phosphorylation; ○, Ca^{2+}/calmodulin-dependent protein kinase; △, protein kinase C; ●, casein kinase II; and #, palmitoylation site(s).

kinase (Thr-273), and one for casein kinase II (Thr-363) (Fig. 1).

Binding Characteristics. Initial binding assays of COS-1 cells transfected with a pCMV-neo expression vector containing the whole coding region of rK1OR showed that [^{3}H]U-69,593 bound with high affinity (K_d = 1.46 nM), but [^{3}H]DAGO and [^{3}H]DPDPE did not (Fig. 2). To further characterize this clone, [^{3}H]U-69,593 was used as a labeling ligand, and various opioid and nonopioid ligands were tested for competition (Table 1). The expressed protein bound prodynorphin products with high affinity. From available information on the structure–function relationship of opioid peptides (25), the Tyr-1 residue should be critical for dynorphin (Dyn) A to interact with the cloned receptor. Indeed, the affinity of Dyn A-(2–13) was three orders of magnitude lower than that of Dyn A-(1–13). All other κ opioid receptor-selective ligands tested bound with high affinity. The rank order of these compounds in decreasing order of affinity is Dyn A = Dyn A-(1–13) > Dyn B = U-63,640 = nBNI > β-neoendorphin > α-neoendorphin = M_r 2034 = E-2078 > U-50,488 = U-69,593. The expressed receptor also bound several nonselective opioid ligands such as bremazocine,

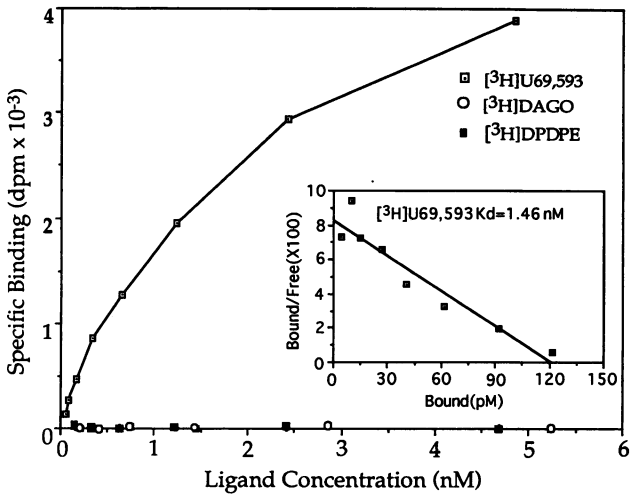

FIG. 2. Specific binding of [^{3}H]U-69,593, [^{3}H]DAGO, and [^{3}H]DPDPE to the membrane of COS-1 cells transfected with the rK1OR clone. (*Inset*) Scatchard analysis of [^{3}H]U-69,593 binding. K_d was 1.46 nM for the expressed receptor.

Table 1. Pharmacological profile of the cloned rat κ_1 receptor

Ligand	K_i, nM	Ligand	K_i, nM
κ-Selective		μ/δ-Selective	
Dyn A	0.11	DPDPE	280
Dyn A-(1–13)	0.12	DAGO	1,200
Dyn B	0.29	β-Endorphin (human)	150
α-Neoendorphin	1.4	[Leu]Enkephalin	930
β-Neoendorphin	0.77	[Met]Enkephalin	1,000
U-69,593	2.0	Morphine	94
U-50,488	1.9		
U-63,640	0.22	Enantiomeric ligand pairs	
nBNI	0.26	(−)-Naloxone	9
M_r 2034	1.3	(+)-Naloxone	19,000
E-2078	1.5	Levorphanol	12
		Dextrorphan	3,000
Nonselective alkaloids			
EKC	1.6	Other peptides	
Bremazocine	0.11	Dyn A-(2–13)	680
Naltrexone	1.1	Somatostatin	4900

The K_i values were calculated by using the LIGAND program with an algorithm of fitting the entire range of binding data to the equation for a single homogenous class of binding sites. EKC, ethylketoclazocine; M_r 2034, (−)-(1*R*,5*R*,9*R*,2′*S*)-5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan D-tartrate; nBNI, norbinaltrophine hydrochloride; E-2078, [*N*-MeTyr^1, *N*$^{\alpha}$-MeArg^7, D-Leu^8]-dynorphin A-(1–8) ethylamide.

ethylketoclazocine, and naltrexone with high affinity. Furthermore, it showed high stereospecificity with good affinity toward (−)-naloxone and levorphanol but very poor binding of their enantiomers (+)-naloxone and dextrorphan. Finally, the expressed receptor bound neither μ- nor δ-selective compounds with high affinity. Based on this binding profile, and the high affinity of opioids of the acrylacetamide family, we identify this clone as a κ receptor of the κ_1 subtype.

Signal Transduction Properties of the Cloned κ Receptor. To determine whether this protein is functionally coupled to a second messenger system, we studied its ability to induce changes in cAMP and IP_3 levels upon activation by opioid ligands. The results of the cAMP assay showed (Table 2) that U-50,488 can inhibit the forskolin-induced cAMP increase by about 30% and that this effect can be blocked by the presence of 1 μM nBNI, a specific κ receptor antagonist. DAGO and DPDPE did not have a significant effect on the cAMP level in COS-1 cells transfected with this plasmid. IP_3 assays were also performed on the transfected COS-1 cells but no significant alteration in IP_3 levels was observed after incubation with different opioid ligands (data not shown).

Distribution of the κ Receptor mRNA. The rK1OR mRNA distribution was widespread in the CNS (Fig. 3) with particularly high levels in the claustrum, nucleus accumbens, olfactory tubercle, endopiriform nucleus, parietal cortex,

Table 2. Inhibition of cAMP levels in COS-1 cells transfected with the rat κ_1 clone

Opioid ligand (1 μM)	cAMP level, pmol per tube		Inhibition, %
	Exp. 1	Exp. 2	
None (forskolin only)	96	87	0
U-50,488	61	68	30
nBNI	84	88	7
+ U-50,488	79	84	11
DAGO	79	84	11
DPDPE	88	82	8

Forskolin (1 μM) was used to stimulate the cAMP level in COS-1 cells during the incubation with the opioid ligands. Baseline cAMP levels without the presence of any drugs were 13.5 and 17.0 (pmol per tube). All cAMP measures were from two independent assays.

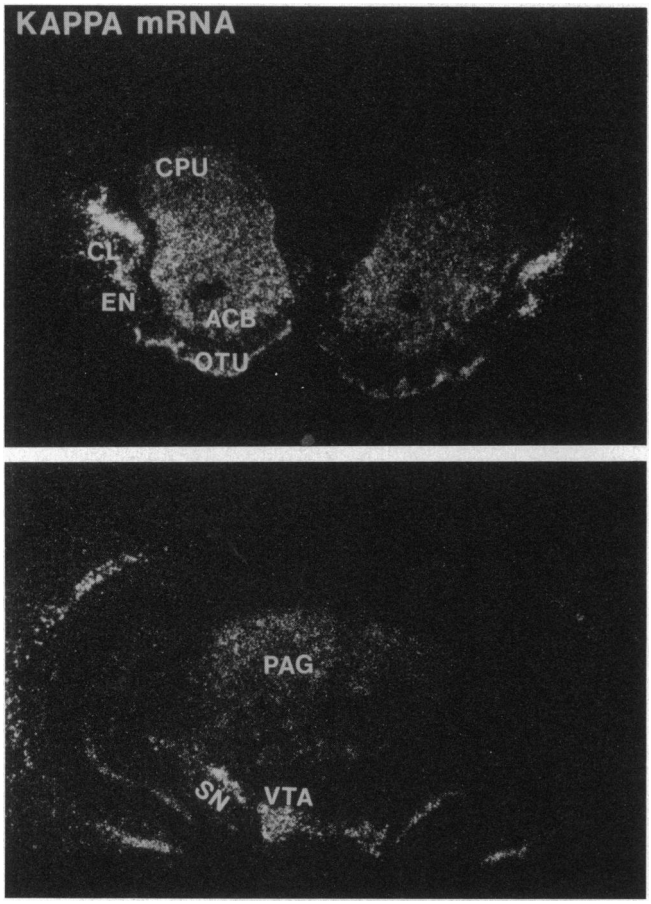

FIG. 3. Dark-field autoradiograph demonstrating the distribution of rK1OR mRNA at two levels of the rat brain. The rK1OR mRNA was localized in the nucleus accumbens (ACB), olfactory tubercule (OTU), endopiriform nucleus (EN), claustrum (CL), periaqueductal area (PAG), parietal cortex, substantia nigra (SN), and ventral tegmental area (VTA).

hypothalamus (preoptic, supraoptic, paraventricular, dorsomedial, and ventromedial nuclei), amygdala (basolateral, medial, cortical), paraventricular thalamus, zona incerta, substantia nigra, and ventral tegmental area. Comparatively lower mRNA levels were observed in the inferior colliculi, periaqueductal gray, locus coeruleus, and spinal cord. Within the nigrostriatal and mesolimbic dopamine systems, rK1OR mRNA appeared to be selectively distributed in the mesolimbic system with a localization primarily in the medial substantia nigra, pars compacta and medial caudate-putamen as well as the nucleus accumbens and ventral tegmental area. Scattered cells containing rK1OR mRNA were also present in the substantia nigra, pars reticulata. Sense probe of rK1OR and RNase pretreatment controls failed to produce any *in situ* hybridization signal. cRNA probes directed to different regions of the rK1OR either to transmembrane domains 3–6 or the 3′ untranslated region generated the same mRNA distribution.

DISCUSSION

The results of the present study demonstrate that we have cloned a member of the G protein-coupled receptor family that can be identified as the rat κ_1 opioid receptor. This conclusion is based on the following observations. (*i*) Binding data showed that the protein encoded by rK1OR has very high (subnanomolar) affinity toward Dyn A and other products of the prodynorphin gene, while it has very low affinity for β-endorphin, [Met]enkephalin, DAGO, and DPDPE. Given that μ sites are typically defined with high-affinity binding for DAGO and δ sites are defined with high-affinity binding for DPDPE, this pattern indicates a κ classification. (*ii*) Binding of Dyn A showed an absolute requirement for the presence of tyrosine at the N terminus. It should be noted that Dyn peptides lacking the N-terminal tyrosine or having a chemically altered Tyr-1 are known to be biologically active, but their effects are mediated through nonopioid sites (25, 26). The requirement for the N-terminal tyrosine is characteristic of opioid binding and rules out the possibility that the cloned receptor may represent a nonopioid Dyn site. (*iii*) The cloned receptor interacts with several classical opioid ligands, such as naltrexone, naloxone, levorphanol, and bremazocine, confirming its opioid nature. (*iv*) The receptor exhibits the predicted stereospecificity, recognizing (−)-naloxone and levorphanol with affinities several orders of magnitude higher than for their respective enantiomers, (+)-naloxone and dextrorphan. (*v*) The cloned receptor also interacts with high affinity with the arylacetamides U-50,488 and U-69,593, a criterion that defines κ_1. (*vi*) Agonist activation of this site leads to a decrease in cAMP levels, as would be expected for the κ receptor based on several reports (27, 28). (*vii*) The anatomical distribution of the mRNA coding for this receptor is consistent with the distribution of κ_1 binding as defined with pharmacological ligands. Taken together, these results provide compelling evidence that the present clone encodes a κ_1 rat opioid receptor.

Sequence analysis indicated that this receptor has a high degree of homology to the recently cloned mouse δ opioid receptors. The most homologous regions are the transmembrane domains, the intracellular loops, and the N-terminal region of the C-terminal tail, which may form the fourth intracellular loop after the palmitoylation of a cysteine residue. The high homology of the intracellular loops between the δ and κ_1 receptor implies that they may share similar second-messenger systems, as intracellular loops are thought to be critical for the coupling of this family of receptors to G proteins (29).

The different N-terminal domains and extracellular loops of the κ and the δ receptors are of interest as they may play an important role in determining selectivity of these receptors towards the various endogenous ligands. The notion that these extracellular domains may have a key function in determining ligand binding and ligand selectivity for peptide receptors was elegantly demonstrated in a series of studies on luteinizing hormone and follicle-stimulating hormone receptors (30–32). The same idea was put forth by Xie *et al.* (17) to explain the unique ligand selectivity profile of their putative opioid receptor in comparison to the profile of the neuromedin K receptor, as the two receptors exhibited different N-terminal domains but virtually identical transmembrane spanning domains.

Given the potential importance of extracellular domains in defining peptide receptor binding and selectivity, we have compared these regions in the κ_1 receptor and δ receptors. The most salient feature of the κ_1 receptor is that it has a net charge of −11 to −12 in the extracellular domains, depending on the transmembrane domain assignment. The two reported sequences of the mouse δ receptors, while having totally different second extracellular loops, contain a net charge of −2 in their entire extracellular region. The highly negative charge in the extracellular domains of the κ receptor may play a role in determining the selectivity of Dyn peptides toward this site. Residues 6–13 in Dyn A can be seen as the "address sequence," which determines receptor selectivity (25, 33). This region contains five basic amino acids. It was hypothesized that this highly basic sequence may interact with specific complementary anionic sites on the membrane in the vicinity of the receptor (34). However, the fact that

solubilized κ binding sites still preserve their characteristic binding profile (35) suggests that the selectivity is intrinsic to the receptor and does not rely on the surrounding membrane environment. It is reasonable to propose that the putative anionic sites that can interact with the address sequence of the Dyn A peptide may actually be on the extracellular domains of the κ receptor itself.

The distribution of the mRNA coding for this κ_1 receptor corresponds well to the distribution of κ binding sites labeled with either the selective κ agonist [^{3}H]U-69,593 or [^{3}H]bremazocine in the presence of a 300-fold excess of DAGO and DPDPE (36). The high level of rK1OR mRNA in hypothalamic nuclei and its distribution pattern in the cortex is consistent with the κ receptor distribution revealed by binding studies and argues against any cross-hybridization to δ and μ receptor mRNAs. One region of discrepancy between the localization of rK1OR mRNA and binding sites is the ventral tegmental area and substantia nigra, pars compacta, where relatively high levels of rK1OR mRNA are observed and little or no κ receptor binding is seen. The relatively high levels of κ receptor binding in the nucleus accumbens and medial caudate-putamen suggest that these sites may be synthesized in part in the midbrain and transported to the striatum. This localization may relate to the proposed role of κ receptor agonists in inhibiting the release of dopamine within the nigrostriatal and mesolimbic dopaminergic pathways (37, 38).

The localization of rK1OR mRNA in many regions of CNS including the hypothalamus, limbic areas, periaqueductal gray, and the mesolimbic and nigrostriatal systems is consistent with the wide variety of functions that are thought to be mediated by κ receptors, such as water balance, feeding, analgesia, aversion, and neuroendocrine effects.

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Note Added in Proof. The unidentified PCR DNA fragment described in the *Methods and Materials* section and used in the original screening of the rat striatal cDNA library has recently been identified and characterized as a cDNA fragment of a rat mu opioid receptor (39). After this paper was submitted, Yasuda *et al.* (40) reported the sequence of a mouse κ receptor.

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