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ACCELERATED COMMUNICATION

The Human δ-Opioid Receptor: Genomic Organization, cDNA Cloning, Functional Expression, and Distribution in Human Brain

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SUMMARY

We have used the mouse δ-opioid receptor (mDOR) cDNA to isolate the mDOR gene and its human homologue. In both species the coding region is interrupted by two introns with conserved exon-intron boundaries located after transmembrane domains 1 and 4. Using the polymerase chain reaction and primers based on the sequence of the cloned human δ-opioid receptor (hDOR) gene, we have obtained a full length cDNA encoding the hDOR from SH-SY5Y neuroblastoma cells. The cDNA sequence is 100% identical to the cloned human genomic sequence and 94% identical to the mouse sequence at the protein level. When expressed in COS cells, hDOR displays nanomolar affinities for δ-selective ligands, whereas the affinities for μ- and κ-selective ligands are in the micromolar range. The δ agonists [D-Ala^2, D-Leu^5]enkephalin, cyclic [D-penicillamine^2,D-penicillamine^5]enkephalin, and BW373U86 efficiently decrease forskolin-induced cAMP levels in hDOR-expressing COS cells, indicating functional coupling of the receptor. The distribution of hDOR mRNA in human brain was investigated using δ-selective reverse transcription-polymerase chain reaction amplification, followed by Southern hybridization with a δ-specific probe. The transcript is found in cortical areas, including olfactory bulb, hippocampus, and amygdala, as well as in basal ganglia and hypothalamus. No expression is detected in internal globus pallidus, thalamus, any investigated brainstem structure, or pituitary gland. Taken together, our results indicate similar structural, pharmacological, functional, and anatomical properties for the hDOR and the mDOR and therefore support the use of rodent models for the study of these receptors in opioid function.

Opioid receptors mediate the analgesic action and addictive properties of opiate drugs. The existence of three classes of receptors, referred to as μ, δ, and κ, is well documented. They are most abundant in the central and peripheral nervous systems and are natural targets for a family of endogenous opioid peptides involved in pain control, neuroendocrine physiology, and affective behavior (1). Opioid receptors act differentially in opioid function. The three opioid receptor types are involved in the mediation of analgesia; however, they have distinct neuroanatomical distributions (2) and pharmacological profiles (3). There is now substantial evidence that δ receptors can mediate antinociception at both the spinal and supraspinal levels (4). Moreover, in the central nervous system δ agonists have been shown to modulate μ receptor-mediated analgesia, suggesting possible cross-talk between μ and δ receptors (5). At present, clinically used opiates for pain treatment act through μ receptors. However, their use is limited by severe side effects such as respiratory depression, constipation, and dependence (6, 7). Interestingly, δ-agonists have analgesic properties but induce weaker opiate physical dependence (8, 9). With regard to the abuse liability of opiates, μ receptors are considered to be the primary target for opiate addiction, whereas the participation of δ receptors in the reinforcing properties of alkaloid compounds is less well documented (10). On the other hand, κ agonists exert an opposing action on mood control, because they have aversive properties, and their clinical use is limited by their strong dysphoric and psychotomimetic action (11). δ receptors therefore remain useful therapeutic targets for the development of novel analgesic agents (12).

The molecular cloning of a mDOR from NG108-15 cells (13, 14) has provided the first insight into the molecular structure of an opioid receptor. The availability of this sequence has allowed the isolation of homologous cDNAs that were identified

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ABBREVIATIONS: mDOR, mouse δ-opioid receptor; hDOR, human δ-opioid receptor; BW373U86, (±)-4-[(α-*R**)-α-[(2*S**,5*R**)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-hydroxybenzyl]-*N*,*N*-diethylbenzamide; DADLE, [D-Ala^2,D-Leu^5]enkephalin; DAGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; DPDPE, cyclic [D-penicillamine^2,D-penicillamine^5]enkephalin; DTLET, Tyr-D-Thr-Gly-Phe-Leu-Thr; RT, reverse transcription; PCR, polymerase chain reaction; U50488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide; bp, base pair(s); kb, kilobase(s); PBS, phosphate-buffered saline.

as the μ and κ receptors, revealing a high degree of homology between the three receptor subtypes. Although pharmacological studies performed on tissues, organs, or whole animals suggest several subtypes for each opioid receptor class (15), multiple molecular forms of μ, δ, and κ receptors have not been found.

Most pharmacological and behavioral studies have been conveniently performed on rodent models, and very little information is available concerning the hDOR. Determination of the molecular structure, binding, and functional properties of the hDOR, as well as a better knowledge of the expression pattern of the hDOR in human tissues, is necessary for the rational design of specific δ agonists as new therapeutic agents. We used our recently isolated cDNA encoding the mDOR (13) for the cloning and characterization of the human counterpart. Here we describe the genomic organization of the mDOR and hDOR genes, the cloning of a cDNA encoding the hDOR, and the pharmacological and functional characterization of the hDOR expressed in COS cells. We also report a description of the distribution pattern of the hDOR transcript in human brain.

Materials and Methods

Genomic and cDNA cloning. Protocols were derived from standard procedures (16), unless otherwise stated. Subcloning of genomic fragments for sequencing was into the pBluescript SK(±) plasmid (Stratagene). Sequencing reactions were performed using the Sanger dideoxy nucleotide chain-termination method (Taq dideoxy terminator cycle sequencing kit; Applied Biosystems) and analyzed on an automated DNA sequencer (373A DNA; Applied Bioystems), using fluorescently labeled nucleotides. Most sequences were also confirmed by manual sequencing (Sequenase kit; Unites States Biochemicals). Two clones, encoding exon 1 and exon 2 plus exon 3 of the mouse gene, were isolated by screening a mouse genomic library with a 976-bp *Pst*I-*Not*I fragment of the mDOR cDNA (13). Intron-exon boundaries were identified after subcloning and partial sequencing. A clone encoding exons 2 and 3 of the human gene was obtained by screening a human genomic library, using the same probe, under high stringency conditions (40% formamide). A 10-kb *Not*I-*Not*I fragment and a 1-kb *Pst*I-*Not*I fragment were then subcloned into pBluescript and the coding regions were sequenced. Similarly, a clone encoding exon 1 of the human gene was isolated using a 260-bp *Pst*I-*Bal*I fragment of the same mouse cDNA as a probe. A 2.5-kb *Sac*I-*Eco*RI fragment was subcloned and sequenced. A cDNA encoding the hDOR was obtained by RT-PCR from SH-SY5Y neuroblastoma cells, which were kindly provided by R. Schulz (Institüt fur Pharmakologie, Toxikologie, und Pharmazie, Munich, Germany). Ten micrograms of total RNA were reverse transcribed in the presence of 200 ng of random hexamer oligonucleotide and 400 units of Moloney murine leukemia virus reverse transcriptase (BRL). One tenth of the reaction was then amplified using the forward primer 5′-ACGGTGGAGAGGGACGCGGC-3′ and the reverse primer 5′-GCTCTAGAGAATCGGGGCCGGATGGCCTGGTCA-3′, for 40 cycles (1 min at 94°, 1 min at 55°, and 1 min at 72°), with *Thermus aquaticus* polymerase (1 unit; Cetus). PCR products ranging from 1 to 1.3 kb were isolated and purified (Gene Clean) after 1% agarose gel electrophoresis. A second amplification was performed on this DNA preparation using a new forward primer, 5′-GAGAGCTCGCGG-CCGCATGGAACCGGCCCCCTCCGC-3′, together with the reverse primer under the same conditions, except that the annealing reaction was performed at 60°. The final product, 1.2 kb in size, was purified, blunt-ended in the presence of Klenow, and inserted into *Eco*RV sites of pcDNA/Amp (Invitrogen). The cDNA (hDOR) was sequenced and found to be identical to the genomic sequence.

Ligand binding. COS-1 cells (1.5×10^6 cells/140-cm dish) were transfected with purified plasmid (35 μg/dish) by the DEAE-dextran method. After 72 hr of growth in Dulbecco's modified Eagle's medium with 10% fetal calf serum, the cells were washed twice with PBS and scraped from the plates. Membranes were prepared as described previously (13) and stored at −80° until used. Binding assays were performed with 40 μg of membrane protein/tube and incubations were carried out in Tris·HCl, pH 7.4, for 2 hr at 25°. The membrane suspension was then rapidly filtered on 0.1% polyethylenimine-pretreated GF/B filters and washed three times with the same cold Tris buffer, using a Brandel cell harvester. For saturation experiments, various concentrations of [^{3}H]DADLE (47 Ci/mmol; Amersham) and [^{3}H]diprenorphine (37 Ci/mmol; Amersham) were used. For competition studies, the [^{3}H]diprenorphine concentration was 1 nM. DTLET, DADLE, DPDPE, deltorphin II, naltrindole, DAGO, dynorphin A, and U50488H were from Sigma Chemical Co., etonitazen and bremazocine were provided by B. Ilien (Faculté de Pharmacie, Strasbourg, France), and BW373U86 was obtained from K. J. C. Chang (Burroughs Wellcome Co., Research Triangle Park, NC). Nonspecific binding was determined in the presence of 10^{-6} M naloxone (Sigma). K_d and K_i values were calculated using the EBDA/LIGAND program (G. A. McPherson, Biosoft, UK).

cAMP assays. COS-1 cells were seeded in 12-well plates (3×10^4 cells/well) and transfected as described above, using 1 μg of plasmid DNA/well. After 48 hr, cells were washed twice with PBS at room temperature and incubated for 8 min at 37° in 1 ml of PBS containing 100 μM 3-isobutyl-1-xanthine, 3 μM forskolin (Sigma), and the opioid agonist, in the presence or absence of 10^{-5} M naloxone. Intracellular cAMP was then extracted with cold ethanol (0.5 ml/well), lyophilized, and measured using a radioimmunoassay (cAMP kit; Immunotech, Marseille, France).

RT-PCR detection of hDOR transcripts in brain. Three human brains were taken from 50–70-year-old normal Caucasian subjects. The postmortem period varied from 5 to 20 hr. Structures of interest were dissected out and immediately frozen in liquid nitrogen. Total RNA was prepared (17) and cDNA was synthesized from the RNA as described above. For the specific amplification and detection of the hDOR transcript, the following oligonucleotides were used: oligonucleotide 1, 5′-GAGAGCTCGCGGCCGCAAGTACCTGATGGAGACGT-3′ (forward primer); oligonucleotide 2, 5′-GGAAGCTTGAATTCCT-GAAGCTGCTGGGGTCTGGGC-3′ (reverse primer); oligonucleotide 3, 5′-GGACATCGACCGGCGCGACC-3′ (primer for detection). One tenth of the cDNA was amplified using *T. aquaticus* polymerase and oligonucleotides 1 and 2 for 40 cycles (1 min at 94°, 1 min at 55°, and 1 min at 72°). One tenth of the PCR product was then separated by 1.5% agarose gel electrophoresis and transferred onto Hybond N nylon membranes, and specific PCR products were revealed using hybridization with ^{32}P-labeled oligonucleotide 3, followed by autoradiography.

Results

Genomic organization of the mDOR and hDOR genes. We screened mouse and human genomic libraries with a probe from the mDOR cDNA (13) to isolate coding regions of the δ-opioid receptor genes from both species and to determine their genomic organization. Two genomic clones were isolated from the mouse genomic library, using the coding part of mDOR as a probe. Sequence analysis indicated that one clone contained the 5′ coding region (exon 1). Two downstream exons (exon 2 and exon 3), separated by a 3-kb intron, were found in the second clone, encoding the 3′ coding region of the gene. The entire coding sequence is therefore interrupted by two introns in the mouse genome, with intron-exon junctions located after transmembrane domains 1 and 4 (Fig. 1B). The sequence of mouse exons is 100% identical to that in the previously cloned mouse cDNA. A similar organization was found in the human gene (Fig. 1B). The human genomic library was screened with the same mouse probe, and several overlapping clones that encode the human counterparts of exons 2 and 3 of the mouse

A

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     1          *          *                    50        I                               II   100
hDOR MEPAPSAGAE LQPPLFANAS DAYPSACPSA GANASGPPGA RSASSLALAI AITALYSAVC AVGLLGNVLV MFGIVRYTKM KTATNIYIFN LALADALATS
mDOR --LV---R-- --SSPLV-L- -AF---F--- G-----S--- ---------- ---------- ---------- ---------L ---------- ----------
rDOR --PV---R-- --FSLLA-V- -TF---F--- S-----S--- ---------- ---------- ---------- ---------L ---------- ----------

     101                          III        150                                 IV                  200
hDOR TLPFQSAKYL METWPFGELL CKAVLSIDYY NMFTSIFTLT MMSVDRYIAV CHPVKALDFR TPAKAKLINI CIWVLASGVG VPIMVMAVTR PRDGAVVCML
mDOR ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------Q --------M-
rDOR ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------Q --------T-

     201                   V                  #     250     #    #                VI                  300
hDOR QFPSPSWYWD TVTKICVFLF AFVVPILIIT VCYGLMLLRL RSVRLLSGSK EKDRSLRRIT RMVLVVVGAF VVCWAPIHIF VIVWTLVDID RRDPLVVAAL
mDOR ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------N ----------
rDOR ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------N ----------

     301        VII                                  350 #          #      372
hDOR HLCIALGYAN SSLNPVLYAF LDENFKRCFR QLCRKPCGRP DPSSFSRARE ATARERVTAC TPSDGPGGGA AA
mDOR ---------- ---------- ---------- ----T---RQ E-G-LR-P-Q --T------- ---------- --
rDOR ---------- ---------- ---------- ----A---GQ E-G-LR-P-Q --A------- ---------- --
```

B

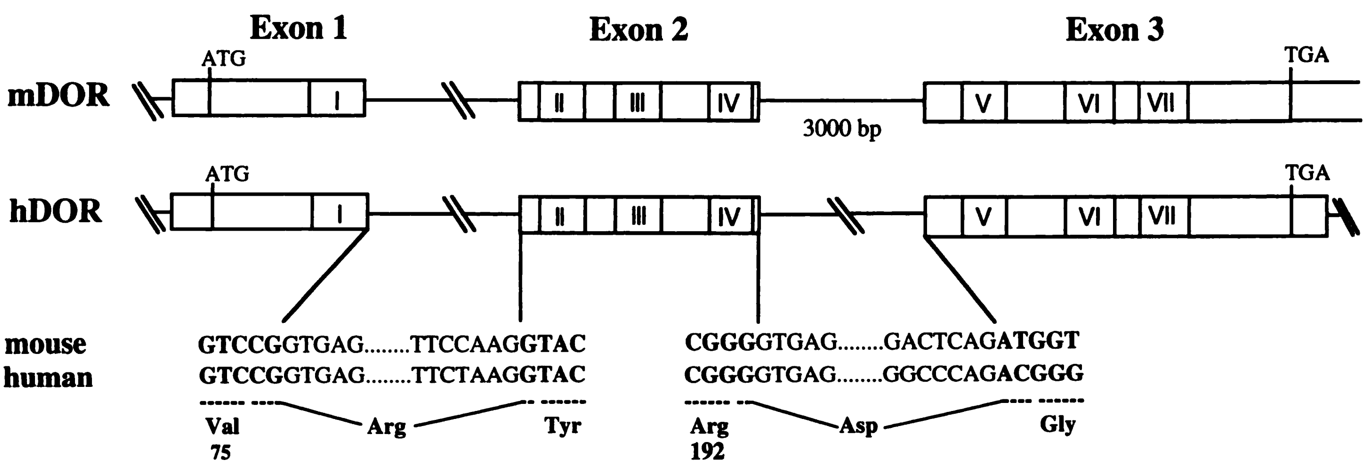

Fig. 1. A, Deduced protein sequence of the hDOR. The hDOR sequence is aligned with the mDOR and rat δ-opioid receptor (*rDOR*) sequences, in which amino acid residues that are different from those in the hDOR sequence are represented. *I-VII*, putative transmembrane domains. *, Consensus *N*-linked glycosylation sites; #, potential phosphorylation sites. The hDOR sequence is 94% identical to both the mouse and rat sequences. The sequence for the hDOR cDNA has been submitted to GenBank (accession number U10504). B, Genomic organization of coding regions in mDOR and hDOR genes. *Boxes*, the three coding exons. The localization of the initiation codon (*ATG*), termination codon (*TGA*), and putative transmembrane domains (*I-VII*) is indicated. Introns are shown as *lines* and are not on the same scale as exons. The sequences of intron-exon boundaries for both mouse and human genes are detailed with protein translation of the coding regions. Amino acid numbers correspond to their positions in the sequence shown in A.

gene were obtained. None of these clones contained the 5′ region; therefore, we rescreened the library using a 260-bp probe encoding a 5′ fragment of the mouse probe. A single clone encoding exon 1, but not exons 2 and 3, was isolated. The comparison of the organization of the mouse and human genes indicates variable intron-exon boundaries in the 5′ and 3′ untranslated regions but conserved junctions within the coding sequence of the genes, with donor-acceptor splice sites fitting the GT/AG consensus. Intronic sequences close to the junction are well conserved between species (Fig. 1B).

Primary structure of the hDOR. SH-SY5Y cells are human neuroblastoma cells that express μ- and δ-opioid receptors (18). We prepared total RNA from these cells and synthesized cDNA by RT. We used human sequences, derived from the analysis of genomic clones, for the design of specific primers located at the 5′ and 3′ ends of the coding region. A nested PCR strategy was used and led to the cloning of a full length cDNA that encodes the human homologue of the mDOR (the hDOR), as suggested by the high degree of similarity between the mouse and human sequences. The deduced protein sequence is 94% identical to both the mouse and rat sequences, with greatest diversity being located in the amino- and carboxyl-terminal regions (Fig. 1A). We found potential post-translational modification signals (which are also present in the mDOR and rat δ-opioid receptor sequences), i.e., two putative *N*-glycosylation sites at the amino terminus and five consensus sequences that are potential targets for kinases in the third intracellular loop and in the cytoplasmic region. The murine sequences contain an additional potential target for protein kinase C at position 344, which is not present in the human

sequence. Studies performed on other G protein-coupled receptors have demonstrated the critical role of phosphorylation in receptor desensitization (19), and such a difference might be responsible for distinct receptor regulation in humans.

Binding characteristics of the hDOR. The hDOR and mDOR were transiently expressed in COS cells. Expression levels were comparable, with B_{max} values ranging from 3.5 to 6.6 pmol/mg of membrane protein. No specific radiolabel binding was observed with control mock-transfected COS cells. The pharmacological profiles of the hDOR and mDOR were compared (Table 1). No discrepancy was found between the mDOR and hDOR in the binding potencies of all ligands tested. Both [^{3}H]DADLE and [^{3}H]diprenorphine displayed high affinity, saturable, specific binding to a single site (data not shown), with K_d values of 1.80 ± 0.01 nM and 3.09 ± 0.15 nM, respectively. δ-Selective agonists such as DTLET, DPDPE, deltorphin II, and BW373U86, as well as the selective antagonist naltrindole, competed efficiently with [^{3}H]diprenorphine binding. The receptor did not bind the highly μ-specific ligands DAGO and etonitazen or the κ-selective agonist U50488H. The poorly selective ligands dynorphin A, naloxone, and bremazocine exhibited affinities similar to those for the mDOR.

Functional properties of the hDOR. The hDOR expressed in COS cells was functionaly coupled to adenylate cyclase, as previously observed for the cloned mDOR (14, 20). The addition of the δ-selective agonists DADLE, DPDPE, or BW373U86 to COS cells expressing hDOR decreased the forskolin-induced production of cAMP by 41.6%, 45.7%, and 47.3%, respectively (Fig. 2). BW373U86, a recently developed nonpeptide δ-selective agonist (21), appeared most potent, because lower concentrations (10 nM) were needed to reach the maximal inhibitory effect. Cyclase inhibition is reversed by the antagonist naloxone when the receptor is activated by DADLE or DPDPE. Naloxone, however, failed to reverse the BW373U86-induced cyclase inhibition under our experimental conditions. The absence of naloxone competition at the receptor might be due to the slow rate of dissociation of BW373U86 (22).

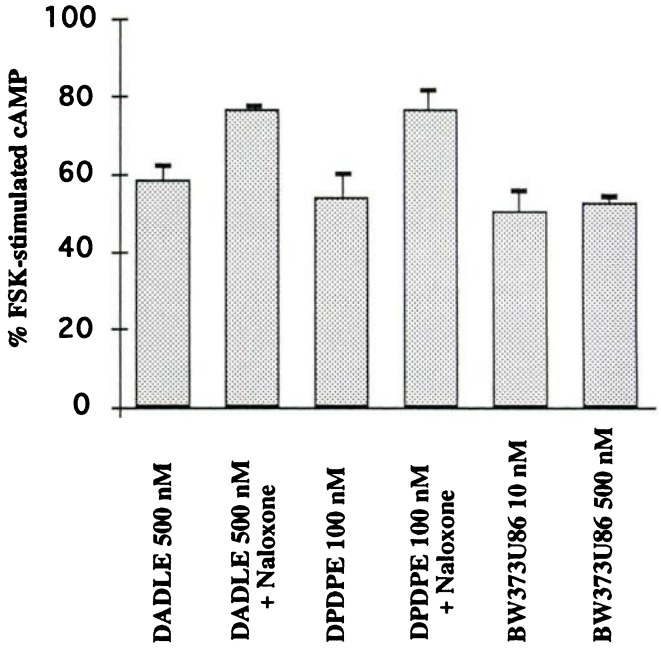

Fig. 2. Functional coupling of the hDOR to adenylyl cyclase. COS cells were stimulated with forskolin (*FSK*) to elevate adenylyl cyclase activity above basal levels. The treatment was performed in the absence (100%) or presence of δ-selective agonists at the indicated concentrations, as described in Materials and Methods. Cellular cAMP levels were determined. Data are means ± standard errors from three experiments. Reversal of the effect was tested in the presence of 10^{-5} M naloxone and was observed for DADLE and DPDPE.

TABLE 1

Ligand binding to the hDOR and mDOR

Affinities of opioid ligands were determined from saturation (K_d) or competition (K_i) binding studies. Binding experiments were performed on membrane preparations of COS cells transiently expressing the cloned hDOR or mDOR. Saturation experiments were performed in triplicate using [^{3}H]diprenorphine and [^{3}H]DADLE, with nonspecific binding being determined in the presence of 10^{-6} M naloxone. In competion studies, [^{3}H]diprenorphine (1 nM) displacement was measured in the presence of various concentrations of unlabeled ligands. Experiments were done in duplicate and repeated two or three times for each compound. K_d and K_i values were determined using the EBDA/LIGAND program.

Ligands	K_d or K_i	
	hDOR	mDOR
	nM	
Saturation K_d		
[^{3}H]Diprenorphine	1.80	0.60
[^{3}H]DADLE	3.09	2.18 (K_i)
Competition K_i		
δ-selective		
DTLET	3.24	1.04 (K_d)
DPDPE	10.1	12.75
Deltorphin II	2.08	8.3
BW373U86	0.65	0.72
Naltrindole	0.23	0.295
μ-selective		
DAGO	>1000	>1000
Etonitazen	>1000	>1000
κ-selective		
U50488H	>1000	>1000
Nonselective		
Bremazocine	3.38	5.62
Dynorphin A-(1–17)	21.3	26.5
Levorphanol	16.4	35.8
Naloxone	88.9	29.75

Expression of the hDOR in human brain. Total RNA was prepared from human brain structures and from SH-SY5Y cells. For each sample, RNA integrity was confirmed by agarose gel electrophoresis, followed by ethidium bromide staining (data not shown). No RNA degradation was observed within the 5–20-hr postmortem period. Northern analysis indicated the presence of a transcript of large size (≥9 kb) in the cell line only (data not shown). No signal was found in brain samples, presumably due to low expression levels of the hDOR, compared with SH-SY5Y cells. We therefore used RT-PCR methodology to increase the sensitivity of detection of the transcript. We established RT-PCR conditions, with regard to number of PCR cycles and the amount of input cDNA, using SH-SY5Y cells as an external standard. Signal intensity appeared well correlated with specific cDNA abundance when the reaction was performed with 1/1 to 1/100 serial dilutions of SH-SY5Y cDNA (data not shown). The primers used for the amplification were designed from human opioid receptor sequences specific to the δ subtype, with no possible cross-hybridization to μ and κ receptor cDNAs. Mismatches to the human μ- and κ-homologous sequences were between 6 and 17 over 20 bases.[1] The amplification produced a DNA fragment of the expected size (755 bp), spanning transmembrane domains 2–7. This fragment

[1] F. Simonin and C. Gavériaux-Ruff, unpublished observations.

hybridized to a third δ-specific oligonucleotide, confirming the selective detection of the hDOR transcript. Each brain region was tested at least twice. A consistent pattern was observed and the results are presented in Fig. 3. In telencephalon, the signal was clearly present in several parts of the neocortex, hippocampus, amygdaloid complex, and olfactory bulb, as well as in basal ganglia including nucleus accumbens, caudate nucleus, putamen, and external globus pallidus. In diencephalon, the transcript was found in hypothalamus and mammilary bodies. In contrast, no transcription was detected in internal globus pallidus, thalamus, substantia nigra, ventral tegmental area, locus coeruleus, or the other brainstem structures tested. No transcript was observed in the pituitary gland. The intensity of hybridization signals in brain was usually lower than in the neuroblastoma cell line SH-SY 5Y.

Discussion

Our previous work has indicated the existence of a single locus in the human genome (23) that hybridizes to the previously cloned mDOR cDNA. In this study, we have isolated mouse and human genomic fragments using the mDOR probe. We have investigated the structures of both the mouse and human genes. The high degree of similarity between exonic sequences clearly indicates that we have cloned species homologues. Interestingly, the coding region is interrupted by two introns, with intron-exon boundaries located at conserved positions between the two species. Functional assays have provided evidence for the existence of δ-opioid receptor subtypes (for review, see Ref. 5), and the presence of two introns suggests the possible existence of alternatively spliced forms. The RT-PCR strategy that we developed to detect the hDOR transcript in brain was designed for the amplification of a region spanning the second intron. The reaction, however, did not generate PCR fragments of variable size that would support the hypothesis of several hDOR isoforms generated by alternative splicing from a single gene, at least in human brain.

We have used sequences derived from the analysis of the gene to isolate a cDNA encoding the δ-opioid receptor gene

Human Brain Structures		n	hDOR transcript
TELENCEPHALON			
Cortex frontalis		2	+
Cortex temporalis		3	+
Cortex parietalis		2	+
Hippocampus		2	+
Corpus amygdaloideum		3	+
Bulbus olfactorius		3	+
Nucleus accumbens		3	+
Nucleus caudatus		3	+
Putamen		3	+
Globus pallidus, pars lateralis		3	+
Globus pallidus, pars medialis		3	-
DIENCEPHALON			
Nuclei anteriores thalami		2	-
Nuclei posteriores thalami		2	-
Hypothalamus		3	+
Corpus mamillare		3	+
MESENCEPHALON			
Substantia nigra		2	-
Area tegmentalis ventralis		2	-
Colliculus inferior		2	-
Colliculus superior		2	-
PONS MEDULLA			
Locus coeruleus		2	-
Nucleus olivaris		3	-
Hypophysis		2	-
SH-SY5Y			+

Fig. 3. Distribution of the hDOR transcript in human brain. Total RNA was prepared from SH-SY5Y neuroblastoma cells and a set of dissected human brain structures. A region spanning transmembrane domains 2–7 was amplified by RT-PCR, using two hDOR-specific oligonucleotides. The PCR product (755 bp) was separated by 1.5% agarose gel electrophoresis, blotted onto nylon membranes, hybridized with a third δ-specific ^{32}P-labeled oligonucleotide, and exposed to X-ray film for 1.5 hr (SH-SY5Y cells and frontal, temporal, and parietal cortex) or 8 hr (other brain regions). A representative autoradiogram for a set of brain structures from one subject is presented. Each brain structure has been tested two or three times. +, Transcript was clearly and consistently observed; −, no transcript could be detected. *n*, number of samples tested.

product. We have achieved functional expression of the hDOR and examined the pharmacology of the receptor expressed in COS cells. We have determined the binding potency of a set of δ ligands representative of various opioid classes (peptide/alkaloid, agonist/antagonist, δ-selective or not). Our results indicate similar properties for the hDOR and the mDOR, confirming that we have cloned the human counterpart of the mDOR. Moreover, it suggests that nonconserved amino acid residues between the hDOR and the mDOR are not critical for the interaction of the receptor with commonly used opioid ligands. A similar conclusion can be drawn by comparing our results with binding studies on the cloned rat receptor (24). Nevertheless, it has recently become apparent that equivalent receptors from different species can exhibit distinct pharmacological profiles with respect to some synthetic ligands (25) or that small differences in primary structure can relate to changes in pharmacology (26). In the case of the δ-opioid receptor, we have noted the presence of two charged residues, in the second (arginine-190) and third (aspartate-290) putative extracellular loops, in the hDOR but not in the rat receptor or the mDOR (glutamine and asparagine, respectively). These differences in sequence occur at regions potentially involved in ligand binding, and the possibility remains that other opioid ligands, which have not been tested in this study, may display species selectivity.

The hDOR cDNA was obtained from a neuroblastoma cell line. Our tissue distribution study indicates that this receptor is also well represented in brain. The distribution of the opioid receptors in brain has been extensively studied in rodents, using ligand binding and autoradiographic studies (for review, see Ref. 2). Compared with μ and κ receptors, δ receptor binding sites have been reported to be particularly abundant in forebrain regions, and their distribution is well conserved across mammalian species. Recently, the localization of the δ receptor transcript, determined by *in situ* hybridization, has been reported in rats and mice (27, 28). In accordance with the murine expression pattern, our results indicate the presence of mRNA in cortical areas, including olfactory bulb, and limbic structures such as amygdala and hippocampus, as well as basal ganglia and hypothalamus. The absence of δ receptor expression in thalamus and the investigated brainstem regions is also consistent with the rodent mRNA distribution. In contrast, there is a difference between human and rat transcript distribution in the external globus pallidus. δ receptor mRNA has not been found in this region in rats (28), whereas a weak but clear signal is present in our human study. Our finding is consistent with the description of an enkephalinergic efferent pathway from the striatum towards the external globus pallidus in both rats (29) and humans (30). Otherwise, minor discrepancies might be due to differences in sensitivity and resolution between the two experimental approaches (RT-PCR versus *in situ* hybridization), rather than interspecies variations in mRNA distribution.

Considering human brain exclusively, binding sites have been reported in discrete regions, including those we have investigated (31–33). In most regions, a good correspondence was found between the distribution of the receptor and that of its transcript. There is a coexistence of mRNA and binding sites in neocortex, nucleus accumbens, putamen, external globus pallidus, amygdala, and hippocampus. We were unable to detect the mRNA in thalamus and substantia nigra, which is consistent with the absence of binding sites in these regions (31). In contrast, we detected a high level of hDOR mRNA in the hypothalamus, whereas binding sites have not been found in this region (31). This result suggests that δ-opioid receptors translated locally in hypothalamic cell bodies might be transported to nerve terminals located at distant sites. δ receptors might therefore act presynaptically in hypothalamic target structures (hippocampus and amygdala), in which binding sites have been described. This is consistent with several reports indicating an inhibitory action of opioids on norepinephrine release at nerve terminals in hippocampus (34, 35). The presence of mRNA in hypothalamus but not in the pituitary gland also suggests presynaptic modulation of pituitary function as a possible mechanism for the regulation of endocrine physiology by opioids (1). In conclusion, the general expression pattern obtained in this study underscores the possible involvement of the δ-opioid receptor in cognitive, affective, and neuroendocrine aspects of opioid function in humans.

We have shown that the cloned hDOR exhibits properties similar to those of its rodent homologues with regard to primary structure, pharmacological properties, functional coupling, and tissue distribution of the transcript in brain. This study supports the use of rodent models in the development of new therapeutic compounds. In the future, detailed structure-function studies and further analysis of the hDOR expression pattern will contribute to a better understanding of the specific role of this opioid receptor subtype in pain and addiction.

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