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[54] **METHOD OF DETECTING EXPRESSION OF PROTEINS CLOSELY RELATED TO OPIOID RECEPTORS**

[75] Inventors: **C. Mark Eppler**, Langhorne; **Bradley A. Ozenberger**, Yardley, both of Pa.; **Jeffrey D. Hulmes**, Ringwood, N.J.

[73] Assignee: **American Cyanamid Company**, Wayne, N.J.

[21] Appl. No.: **454,549**

[22] Filed: **May 30, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 185,360, filed as PCT/US95/00939 Jan. 20, 1995, abandoned.

[51] **Int. Cl.**[6] **C12Q 1/68**; C12P 19/34; C07H 19/00; C07K 1/00

[52] **U.S. Cl.** **435/6**; 435/91.1; 435/91.2; 435/91.21; 536/221; 536/24.3; 530/350

[58] **Field of Search** 435/6, 91.1, 91.2, 435/91.21; 536/22.1, 24.3; 530/350

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Primary Examiner—Ardin H. Marschel
Assistant Examiner—Jezia Riley
Attorney, Agent, or Firm—Darby & Darby

[57] **ABSTRACT**

Isolated DNAs encoding opiorph receptor polypeptides are provided. Recombinant cloning vectors which include these DNA sequences and cells which include these vectors are also provided. Methods for detecting the expression, in a tissue, of mRNA encoding a polypeptide encoded by this DNA are encompassed as well as methods for producing these polypeptides. These isolated polypeptides and antibodies to these polypeptides are also contemplated.

2 Claims, 13 Drawing Sheets

MESLFPAPYW EVLYGSHFQG NLSLLNETVP HHLLLNASHS AFLPLGLKVT
IVGLYLAVCI GGILGNCLVM YVILRHTKMK

TATNIYIFNL ALADTLVLLT LPFQGTDILL GFWPFGNALC KTVIAIDYYN
MFTSTFTLTA MSVDRYVAIC HPIRALDVRT

SSKAQAVNVA IWALASVVGV PVAIMGSAQV EDEEIECLVE IPAPQDYWGP
VFAICIFLFS FIIPVLIISV CYSLMIRRLR

GVRLLSGSRE KDRNLRRITR LVLVVVAVFV GCWTPVQVFV LVQGLGVQPG
SETAVAILRF CTALGYVNSC LNPILYAFLD

ENFKACFRKF CCASSLHREM QVSDRVRSIA KDVGLGCKTS ETVPRPA

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FIG. 1A

GCGGCCGCCT TTCTGCTAAG CATTGGGGTC TATTTTGGCC CAGCTTCTGA
AGAGGCTGTG TGTGCCGTTG GAGGAACTGT

ACTGAGTGGC TTTGCAGGGT GACAGCATGG AGTCCCTCTT TCCTGCTCCA
TACTGGGAGG TCTTGTATGG CAGCCACTTT

CAAGGGAACC TGTCCCTCCT AAATGAGACC GTACCCCACC ACCTGCTCCT
CAATGCTAGT CACAGCGCCT TCCTGCCCCT

TGGACTCAAG GTCACCATCG TGGGGCTCTA CTTGGCTGTG TGCATCGGGG
GGCTCCTGGG GAACTGCCTC GTCATGTATG

TCATCCTCAG GCACACCAAG ATGAAGACAG CTACCAACAT TTACATATTT
AATCTGGCAC TGGCTGATAC CCTGGTCTTG

CTAACACTGC CCTTCCAGGG CACAGACATC CTACTGGGCT TCTGGCCATT
TGGGAATGCA CTCTGCAAGA CTGTCATTGC

TATCGACTAC TACAACATGT TTACCAGCAC TTTTACTCTG ACCGCCATGA
GCGTAGACCG CTATGTGGCT ATCTGCCACC

FIG. 1B

CTATCCGTGC CCTTGATGTT CGGACATCCA GCAAAGCCCA GGCTGTTAAT
GTGGCCATAT GGGCCCTGGC TTCAGTGGTT

GGTGTTCCTG TTGCCATCAT GGGTTCAGCA CAAGTGGAAG ATGAAGAGAT
CGAGTGCCTG GTGGAGATCC CTGCCCCTCA

GGACTATTGG GGCCCTGTAT TCGCCATCTG CATCTTCCTT TTTTCCTTCA
TCATCCCTGT GCTGATCATC TCTGTCTGCT

FIG. 1C

ACAGCCTCAT GATTCGACGA CTTCGTGGTG TCCGTCTGCT TTCAGGCTCC
CGGGAGAAGG ACCGAACCT GCGGCGTATC

ACTCGACTGG TGCTGGTAGT GGTGGCTGTG TTTGTGGGCT GCTGGACGCC
TGTGCAGGTG TTTGTCCTGG TTCAAGGACT

GGGTGTTCAG CCAGGTAGTG AGACTGCAGT TGCCATCCTG CGCTTCTGCA
CAGCCCCTGGG CTATGTCAAC AGTTGTCTCA

ATCCCATTCT CTATGCTTTC CTGGATGAGA ACTTCAAGGC CTGCTTTAGA
AAGTTCTGCT GTGCTTCATC CCTGCACCGG

GAGATGCAGG TTTCTGATCG TGTGCGGAGC ATTGCCAAGG ATGTTGGCCT
TGGTTGCAAG ACTTCTGAGA CAGTACCACG

GCCAGCATGA CTAGGCGTGG ACCTGCCCAT GGTGCCTGTC AGCCCACAGA
GCCCATCTAC ACCCAACACG GAGCTCACAC

AGGTCACTGC TCTCTAGGTT GACCCTGAAC CTTGAGCATC TGGAGCCTTG
AATGGCTTTT CTTTTGGATC AGGATGCTCA

GTCCTAGAGG AAGACCTTTT AGCACCATGG GACAGGTCAA AGCATCAAGG
TGGTCTCCAT GGCCTCTGTC AGATTAAGTT

CCCTCCCTGG TATAGGACCA GAGAGGACCA AAGGAACTGA ATAGAAACAT
CCACAACACA GTGGACATGC CTGGTGAGCC

CATGTAGGTA TTCATGCTTC ACTTGACTCT TCTCTGGCTT CTCCCTGCTG
CCCTGGCTCT AGCTGGGCTC AACCTGAGGT

ATTGTAGTGG TCATGTAGTC ACTCTTGTGA CTACATGTTG TGTGCTGTTG
CTCTCGGCCT TTCAGTATTT CCACAGGACT

FIG. 1D

GCTGAACATA CCTGGTATTG CAGTGGGGAG CATTAATTTT CTTTTAAAGT
GAGACTGGCC CTTAAGCTTG GCGTTGCCTT

GGAGCGTCTT CTACTTCTGA CTTCACTGAT GCAGTCAGAT TACCCGAGGG
TGAGCATCAG TGGTTTCTTG GATGGCTGTT

TTCTGAAGAT TCTTCCCATC CAGTACATGG AGTCTATGAA GGGGAGTCAC
AATTCATCTG GTACTGCCAC TACCTGCTCT

FIG. 1E

ATAATCCTGG GCTATCTTCT TGGCAAGATG ACAGTGGGGG AGACAAGACA
CAGAGCTTCC CTAAGGCTCT TTCCCTCCAA

AACCACTGTG AACTCTTATC CTACAGACTG TTCGGCAAGC ACTGCTTCTA
GGTGTGTGGG AGGTAATCAG GAGAAAGCTT

TGTGGCCTCT GTAGGCTGCT CACAACATGG AGGCACCACA TGCTGGTCTT
GCCTGCTTAG TACAGGCAGG ACAGAGCAGA

ATATGCTCTC TCTCGATTCT CTACAAACTC CCTCAGTTCT CCAGCAGAGT
CTCTTTTACT TGCTATCAGA GGTCAGGAGT

TGTACTGCTA GAAGCATACT TGTAGCTTGG GAAGAGTGGC AGTCAGGATG
TGTTCTACTC TATATCCACA GTGACCACCT

GCTTCATATA TAGGGTTAGG ACATATCTGA GTAAGGCCTG AGTGTGCTGC
CAAATTGGAG GTTGGTATGA GAGCTGATGC

CTAAAGTGGC TCATTTGCAA GGACTATTAT GGTTTGGAAT AGCAATGGGG
GGCATGGGAA GAAGAGTCTA TACCTTGGAG

FIG. 1F

ATCTATTTGA TGGTTCACAG AAGAGGTTTT GTAAACGCCC TTTCTATGGG
TCAGATATCA AAATACCAGC AACGTTGGAT

AGATTCTGAC CTTTTACTGA GACCTCGGTC AGATGGTTTC ATGTCATGCA
GAGAACCTAG GCTGGTTCCT GTGTCAGAGA

GACCTGGGCT TCTGGGGAGG CCAGGGTTCT TCCTTTGACA CTTGTGCGGG
AGCCGTTAGC TCTAGA

FIG. 2

MESLFPAPYW EVLYGSHFQG NLSLLNETVP HHLLLNASHS AFLPLGLKVT
IVGLYLAVCI GGILGNCLVM YVILRHTKMK

TATNIYIFNL ALADTLVLLT LPFQGTDILL GFWPFGNALC KTVIAIDYYN
MFTSTFTLTA MSVDRYVAIC HPIRALDVRT

SSKAQAVNVA IWALASVVGV PVAIMGSAQV EDEEIECLVE IPAPQDYWGP
VFAICIFLFS FIIPVLIISV CYSLMIRRLR

GVRLLSGSRE KDRNLRRITR LVLVVVAVFV GCWTPVQVFV LVQGLGVQPG
SETAVAILRF CTALGYVNSC LNPILYAFLD

ENFKACFRKF CCASSLHREM QVSDRVRSIA KDVGLGCKTS ETVPRPA

FIG. 3A

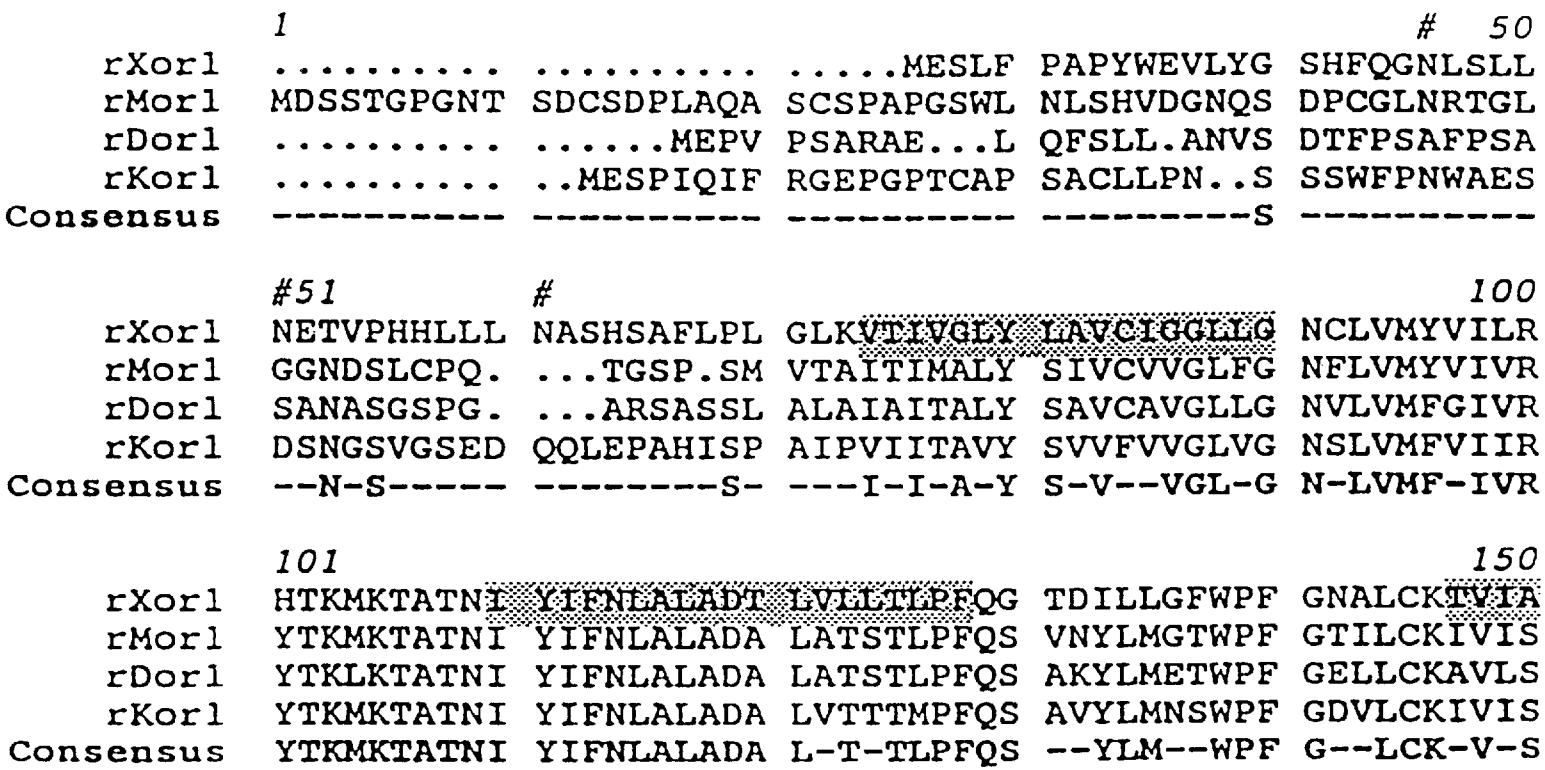

1
50
rXor1 ....MESLF PAPYWEVLYG SHFQGNLSLL
rMor1 MDSSTGPGNT SDCSDPLAQA SCSPAPGSWL NLSHVDGNQS DPCGLNRTGL
rDor1 ....MEPV PSARAE...L QFSLL.ANVS DTFPSAFPSA
rKor1 ....MESPIQIF RGEPGPTCAP SACLLPN..S SSWFPNWAES
Consensus ---------- ---------- ---------- ---------S ----------
#51 # 100
rXor1 NETVPHHLLL NASHSAFLPL GLKVTIVGLY LAVCIGGLLG NCLVMYVILR
rMor1 GGNDSLCPQ. ...TGSP.SM VTAITIMALY SIVCVVGLFG NFLVMYVIVR
rDor1 SANASGSPG. ...ARSASSL ALAIAITALY SAVCAVGLLG NVLVMFGIVR
rKor1 DSNGSVGSED QQLEPAHISP AIPVIITAVY SVVFVVGLVG NSLVMFVIIR
Consensus --N-S----- --------S- ---I-I-A-Y S-V--VGL-G N-LVMF-IVR
101 150
rXor1 HTKMKTATNI YIFNLALADT LVLLTLPFQG TDILLGFWPF GNALCKTVIA
rMor1 YTKMKTATNI YIFNLALADA LATSTLPFQS VNYLMGTWPF GTILCKIVIS
rDor1 YTKLKTATNI YIFNLALADA LATSTLPFQS AKYLMETWPF GELLCKAVLS
rKor1 YTKMKTATNI YIFNLALADA LVTTTMPFQS AVYLMNSWPF GDVLCKIVIS
Consensus YTKMKTATNI YIFNLALADA L-T-TLPFQS --YLM--WPF G--LCK-V-S

FIG. 3B

```
           151                                                    200
rXorl      IDYYNMFTST FTLTAMSVDR YVAICHPIRA LDVRTSSKAQ AVNVAIWALA
rMorl      IDYYNMFTSI FTLCTMSVDR YIAVCHPVKA LDFRTPRNAK IVNVCNWILS
rDorl      IDYYNMFTSI FTLTMMSVDR YIAVCHPVKA LDFRTPAKAK LINICIWVLA
rKorl      IDYYNMFTSI FTLTMMSVDR YIAVCHPVKA LDFRTPLKAK IINICIWLLA
Consensus  IDYYNMFTSI FTL--MSVDR YIAVCHPVKA LDFRTP--AK -INIC-W-L-

                                                  #
                        GQWVVLLPDSLVSHGFLLVPLPPNPSPA
           201                                                    250
rXorl      SVVGVPVAIM GSAQVED..E EIECLVEIPA PQ.DYWGPVF AICIFLFSFI
rMorl      SAIGLPVMFM ATTKYRQ..G SIDCTLTFSH PTW.YWENLL KICVFIFAFI
rDorl      SGVGVPIMVM AVTQPRD..G AVVCTLQFPS PSW.YWDTVT KICVFLFAFV
rKorl      SSVGISAIVL GGTKVREDVD VIECSLQFPD DEYSWWDLFM KICVFVFAFV
Consensus  S-VG-----M --T--R---- -I-C-L-F-- --W-YWD--- KICVF-FAFV

           251                                                    300
rXorl      IPVLLISVCY SLMIRRLRGV RLLSGSREKD RNLRRITRLV LVVVAVFVGC
rMorl      MPVLIITVCY GLMILRLKSV RMLSGSKEKD RNLRRITRMV LVVVAVFIVC
rDorl      VPILIITVCY GLMLLRLRSV RLLSGSKEKD RSLRRITRMV LVVVGAFVVC
rKorl      IPVLIIIVCY TLMILRLKSV RLLSGSREKD RNLRRITKLV LVVVAVFIIC
Consensus  -PVLII-VCY -LM-LRL-SV RLLSGS-EKD R-LRRIT-MV LVVV--FIVC
```

FIG. 3C

```
           301                                                350
rXorl      WTPVQVFVLV QGL.GVQPGS ETAVAILRFC TALGYVNSCL NPILYAFLDE
rMorl      WTPIHIYVII KALITI.PET TFQTVSWHFC IALGYTNSCL NPVLYAFLDE
rDorl      WAPIHIFVIV WTLVDINRRD PLVVAALHLC IALGYANSSL NPVLYAFLDE
rKorl      WTPIHIFILV EALGSTSHST A.VLSSYYFC IALGYTNSSL NPVLYAFLDE
Consensus  W-PIHIFV-V --L------- --------FC IALGY-NS-L NPVLYAFLDE

           351                                                400
rXorl      NFKACFRKFC LLSSLHREMQ VSDRVRSIAK DVGLGCKTSE TVPRPA....
rMorl      NFKRCFREFC IPTSSTIEQQ NSTRVRQNTR EHPSTANTVD RTNHQLENLE
rDorl      NFKRCFRQLC RAPCGGQEPG SLRRPRQATA RERVTACTPS ......DGPG
rKorl      NFKRCFRDFC FPIKMRMERQ STNRVR.NTV QDPASMRDVG GMNKPV....
Consensus  NFKRCFR-FC -------E-- ---R-R--T- ---------- ----------
```

FIG. 4

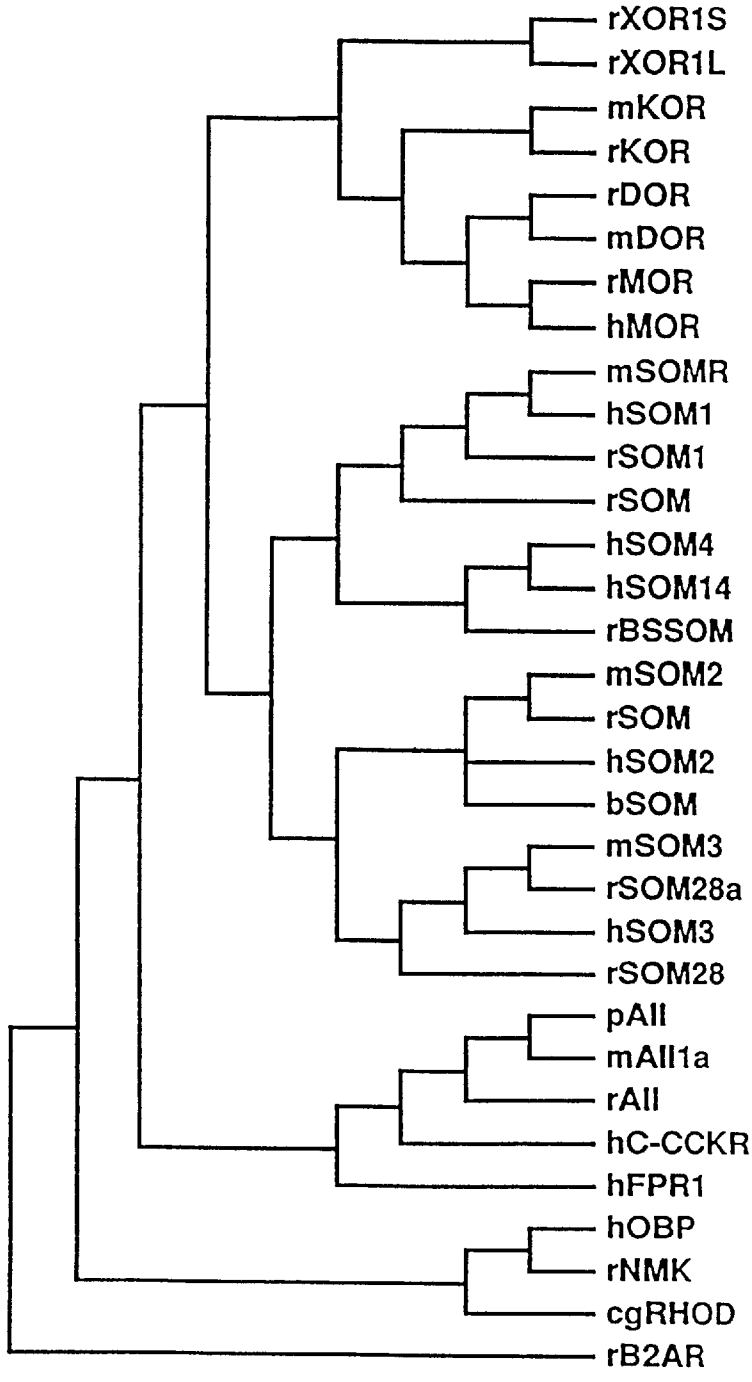
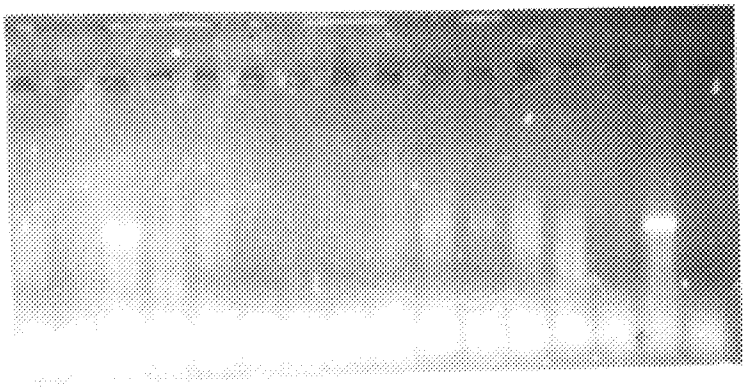

rXOR1S
rXOR1L
mKOR
rKOR
rDOR
mDOR
rMOR
hMOR
mSOMR
hSOM1
rSOM1
rSOM
hSOM4
hSOM14
rBSSOM
mSOM2
rSOM
hSOM2
bSOM
mSOM3
rSOM28a
hSOM3
rSOM28
pAII
mAII1a
rAII
hC-CCKR
hFPR1
hOBP
rNMK
cgRHOD
rB2AR

FIG. 5



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

RNAS

1 = BRAIN
2 = PITUITARY
3 = GH_4C_1 CELLS
4 = THYMUS
5 = LUNG
6 = HEART
7 = LIVER
8 = KIDNEY
9 = SPLEEN
10 = STOMACH
11 = MUSCLE
12 = FAT
13 = OVARY
14 = TESTIS

CONTROLS

15 = GENOMIC DNA
16 = NO TEMPLATE

FIG. 6A

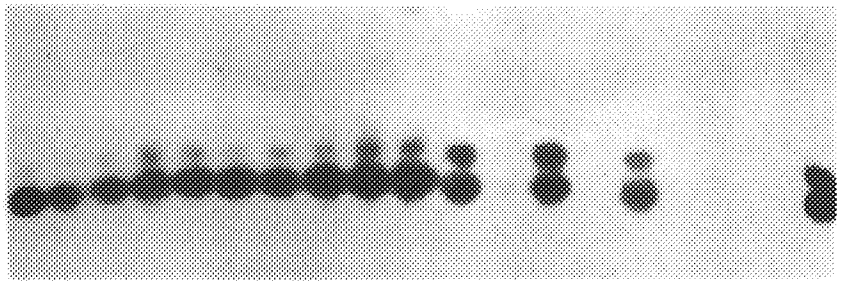


FIG. 6B

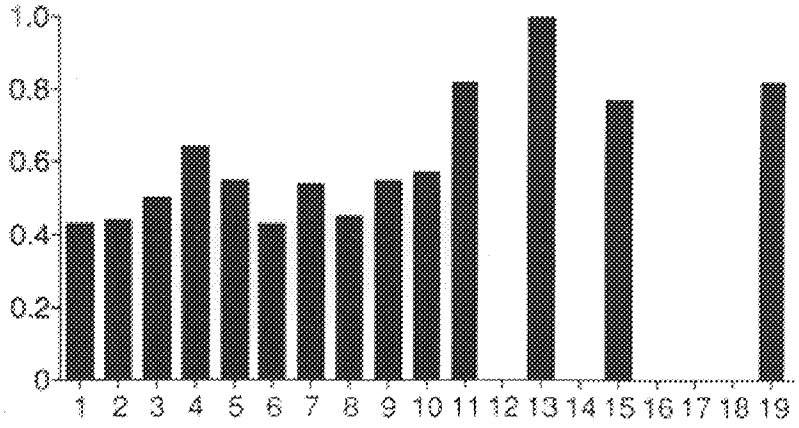
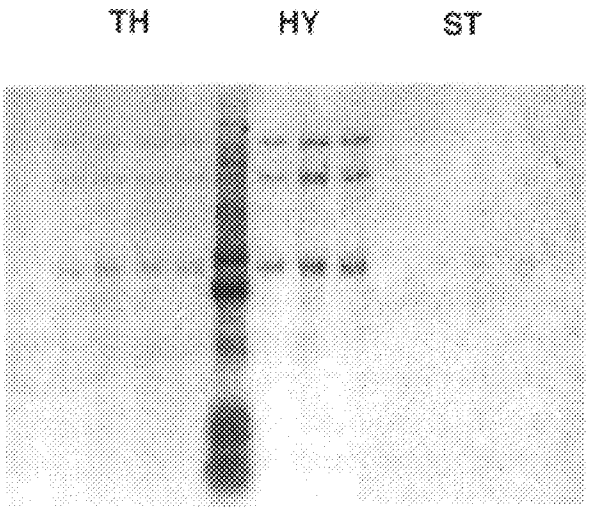


FIG. 7



METHOD OF DETECTING EXPRESSION OF PROTEINS CLOSELY RELATED TO OPIOID RECEPTORS

This application is a divisional application of International Application No. PCT/US95/00939, filed Jan. 20, 1995, which is a continuation-in-part of U.S. patent application Ser. No. 08/185,360, filed Jan. 21, 1994, now abandoned.

1. Field of the Invention

This invention pertains to DNA sequences that encode opiorph receptor polypeptide(s). Opiorph receptor polypeptides are highly related to known opioid receptors. The invention also encompasses the opiorph receptors and antibodies directed against these polypeptides.

2. Background to the Invention

Opioid receptors are members of the receptor superfamily of polypeptides that typically have seven transmembrane domains and that are functionally coupled to G proteins. cDNAs encoding several types of opioid receptors have been cloned, including the mu, delta, and kappa opioid receptors (Wang et al., (1983), *Proc. Natl. Acad. Sci., USA,* 90:10230; Chen et al., (1993), *Mol. Pharmacol.,* 44:8; Evans et al., (1992), Science, 258:1952; Kieffer et al., *Proc. Natl. Acad. Sci., USA,* 89:12048; Yasuda et al, (1993), *Proc. Natl. Acad. Sci., USA,* 90:6736.)

It is believed that the proteins encoded by these cDNAs mediate many of the physiological effects of endogenous opioid agonist peptides, such as, for example, met- and leu-enkephalin, beta-endorphin, and dynorphin, as well as opiate alkaloids such as morphine (Jaffe and Martin, in *The Pharmacological Basis of Therapeutics,* A. G. Gilman et al., eds., MacMillan, New York, 1985, pages 491–531). These physiological effects, which occur in both the central and peripheral nervous system, include analgesia, drowsiness, mood changes, respiratory depression, decreased gastrointestinal mobility, nausea, vomiting, and other alterations in the endocrine and autonomic nervous system.

Another family of opioid receptors, the epsilon receptors, have been studied in brain and immune tissue (Nock et al., (1993), *J. Pharm. Expl. Therap.,* 264:349; Sibinga et al., (1988), *Ann. Rev. ImmunoL,* 6:219). Epsilon receptors, in the immune system, appear to mediate the effects of beta-endorphin on the cytotoxicity of monocytes, on conversion of precursor cells into killer cells, and on chemotaxis.

It has been found that some opioid effects may be mediated by receptors other than the known mu, delta, and kappa receptors. This indicates the existence of subtypes of each of these receptor classes. For example, two subtypes of mu-receptor, two subtypes of delta receptor, and three subtypes of kappa receptor have been identified pharmacologically (Pasternak, *Clin.Neuropharm.* 16:1, 1993).

New opioid receptor polypeptides have now been identified by isolating cDNAs that are homologous to known receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is an illustration of DNA sequences encoding rat opiorph receptor polypeptides (SEQ ID NO:1).

FIG. **2** is an illustration of the predicted amino acid sequences of rat opiorph receptor polypeptides (SEQ ID NO:2).

FIG. **3** illustrates a comparison among an opiorph receptor polypeptide sequence of FIG. **2** (OR7) (rXor1) (SEQ ID NO:2) and the amino acid sequences of rat delta opioid receptor polypeptide (rDor1), (SEQ ID NO:4)rat mu opioid receptor polypeptide (rMor1) (SEQ ID NO:3), and rat kappa opioid receptor polypeptide (rKor1) (SEQ ID NO:5). Putative transmembrane domains are shaded. The extra amino acids encoded by the large splice variant of the opiorph receptor polypeptides are shown as an insert (SEQ ID NO:6).

FIG. **4** is a dendrogram illustrating the evolutionary relatedness of the opiorph receptor polypeptides of FIG. **2** and other G-protein-linked receptor polypeptides.

FIG. **5** is an illustration of an autoradiogram showing the tissue distribution of mRNA encoding an opiorph receptor polypeptide, as determined by reverse transcription-polymerase chain reaction (RT-PCR) using, as a template, RNA derived from different rat tissues and cell lines. The RT-PCR products were resolved in an agarose gel. The left lane contains molecular mass markers, after which the lanes are numbered sequentially 1–16 from left to right.

FIG. **6A** is an illustration of an autoradiogram showing the tissue distribution of mRNA encoding an opiorph receptor polypeptide, as determined by RT-PCR using as a template RNA derived from different rat tissues. The RT-PCR products were resolved in an agarose gel, transferred to nylon membranes, and hybridized with an opiorph receptor polypeptide-specific radiolabelled DNA probe. The tissues used as sources of RNA were as follows: Lane 1, cerebellum; lane 2, cerebral cortex; lane 3, striatum; lane 4, midbrain; lane 5, hippocampus; lane 6, brainstem; lane 7,. olfactory bulb; lane 8, spinal cord; lane 9, thalamus; lane 10, hypothalamus; lane 11, intestine; lane 12, skeletal muscle; lane 13, vas deferens; lane 14, esophagus; lane 15, liver; lane 16, kidney; lane 17, testis; lane 18, adrenal; and lane 19, spleen.

FIG. **6B** is an illustration of the ratio between the RT-PCR products derived from the small and large splice variants, respectively. The tissues are as in FIG. **6A**. The Y-axis represents the ratio of labelled hybridization probe recognizing the short variant to that recognizing the long variant.

FIG. **7** is an illustration of a Northern blot of RNA derived from rat thalamus (TH, lanes 11–4), hypothalamus (HV, lanes 5–8), and striatum (ST, lanes 9–12) hybridized to a radiolabelled opiorph receptor polypeptide DNA probe.

SUMMARY OF THE INVENTION

Isolated DNAs encoding opiorph receptor polypeptides are provided. These DNAs include:

(A) nucleotides 367–918 of the DNA sequence of FIG. **1**; (SEQ ID NO:1)

(B) nucleotides 368–916 of the DNA sequence of FIG. **1**; (SEQ ID NO:1)

(C) DNA encoding amino acid residues 88–269 of the amino acid sequence of FIG. **2**; (SEQ ID NO:2)

(D) sequence-conservative variants, function-conservative variants, and sequence- and function-conservative variants of any of (A), (B), or (C);

(E) intronless DNA encoding an amino acid sequence selected from the group consisting of amino acid residues 88–269 of the amino acid sequence of FIG. **2** (SEQ ID NO:2) and function-conservative variants thereof; and

(F) DNA wherein exons of the DNA encode an amino acid sequence selected from the group consisting of amino acid residues 88–269 of the amino acid sequence of FIG. (SEQ ID NO:2) **2** and function-conservative variants thereof.

Recombinant cloning vectors comprising these DNA sequences and cells comprising these vectors are provided as well.

Also contemplated by the present invention are methods for detecting the expression, in a tissue, of MRNA encoding a polypeptide having an amino acid sequence selected from the group consisting of amino acid residues 88–269 of the amino acid sequence of FIG. **2** (SEQ ID NO:2) and function-conservative variants thereof. These methods comprise:

(A) selecting at least one oligonucleotide sequence unique to the polypeptide, wherein the sequence comprises from about 15 to about 30 nucleotides;

(B) synthesizing the oligonucleotides;

(C) hybridizing the oligonucleotide to total MRNA isolated from the tissue under stringent conditions; and

(D) detecting the hybridization.

Further contemplated are methods for producing a polypeptide selected form the group consisting of amino acid residues 88–269 of the amino acid sequence of FIG. **2** SEQ ID NO:2 and function-conservative variants thereof. These methods include

(A) culturing the cells above in a medium and under conditions suitable for expression of the polypeptide;

(B) expressing the polypeptide; and

(C) optionally, isolating the expressed polypeptide.

Isolated polypeptides selected from the group consisting of amino acid residues 88–269 of FIG. **2** (SEQ ID NO:2) and function-conservative variants thereof, as well as antibodies to these polypeptides are also contemplated.

DETAILED DESCRIPTION OF THE INVENTION

DNA has been isolated that encodes opiorph receptor polypeptide(s). These opiorph receptor polypeptide(s) are related to, but distinct from, known opioid receptor polypeptides. The opiorph receptor polypeptide(s) has been characterized, establishing the differences between it and other members of the opioid receptor family. Accordingly, the opiorph receptor polypeptide(s) is an important target for the development of new opioid or opioid-like agonists and antagonists, which are psychotropic, analgesic, anti-emetic, immunomodulatory, growth hormone-releasing, and growth-promoting agents. Agonists or antagonists of the invertebrate homologue(s) of the opiorph receptor polypeptide(s) are believed to be pesticides. The DNA, opiorph receptor polypeptide(s), and antibodies of the present invention can be used, for example, for the detection and manipulation of pharmacological phenomena that are mediated by opioids and opioid-related molecules.

Opiorph Receptor Nucleic Acids

The DNA sequence set forth in FIG. **1** (SEQ ID NO:1) corresponds to the cDNA sequence encoding the seven transmembrane domain opiorph receptor polypeptide (OR7). The 3.2 kb sequence comprises a 5' untranslated region of 128 bp, an open reading frame of 1,101 bp, and a 3' untranslated region of 2 kb that includes a polyadenylation consensus site. The sequence also includes a splice donor site and a splice acceptor site. When the intervening sequence is excised by splicing, the resulting sequence encodes a smaller form of opiorph receptor polypeptide. The sequence between nucleotides 367 and 918 and preferably between nucleotides 368 and 916 encodes a five transmembrane-domain polypeptide (OR-5) (amino acid residues 88–269 of FIG. **2**) (SEQ ID NO:2).

FIG. **2** (SEQ ID NO:2) illustrates the amino acid sequence of the opiorph receptor polypeptides OR5 and OR7 including a long splice variant (OR7L) and a short splice variant (OR7S), i.e. the polypeptide encoded by the DNA sequence of FIG. **1**(SEQ ID NO:1). Because of the degeneracy of the genetic code in that multiple codons encode for certain amino acids, DNA sequences other than that shown in FIG. **1** (SEQ ID NO:1) can also encode the opiorph amino acid sequences shown in FIG. **2**(SEQ ID NO:2). Such other DNAs include those containing "sequence-conservative" variation in which a change in one or more nucleotides in a given codon results in no alteration in the amino acid encoded at that position.

Additionally, a given amino acid residue in a polypeptide can be changed without altering the overall conformation and function of the native polypeptide. Such "function-conservative" varants include, but are not limited to, replacement of an amino acid with one having similar physico-chemical properties, such as, for example, acidic, basic, hydrophobic, and the like.

The opiorph receptor(s) DNAs within the scope of the present invention are those of FIG. **1**(SEQ ID NO:1), sequence-conservative variant DNAs, DNA sequences encoding function-conservative variant polypeptides, and combinations thereof.

Generally, nucleic acid manipulations according to the present invention use methods that are well known in the art, as disclosed in e.g. *Molecular Cloning, A Laboratory Manual* (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor), or *Current Protocols in Molecular Biology* (Eds. Aufubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc., Wiley-Interscience, NY, N.Y., 1992).

The present invention encompasses cDNA and RNA sequences and sense and antisense sequences. The invention also encompasses genomic opiorph receptor polypeptide DNA sequences and flanking sequences, including, but not limited to, regulatory sequences. Nucleic acid sequences encoding opiorph receptor polypeptide(s) may also be associated with heterologous sequences, including promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'- noncoding regions, and the like. Transcriptional regulatory elements that may be operably linked to opioiph receptor polypeptide DNA sequence(s) include, without limitation, those that have the ability to direct the expression of genes derived from prokaryotic cells, eukaryotic cells, viruses of prokaryotic cells, viruses of eukaryotic cells, and any combination thereof. Other useful heterologous sequences are known to those skilled in the art.

The nucleic acids of the present invention can be modified by methods known to those skilled in the art to alter their stability, solubility, binding affinity, and specificity. For example, the sequences can be selectively methylated. The nucleic acid sequences of the present invention may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, biotin, and the like.

Vectors and Transformants

The present invention also provides vectors that include nucleic acids encoding the opiorph receptor polypeptide(s). Such vectors include, for example, plasmid vectors for expression in a variety of eukaryotic and prokaryotic hosts. Preferably, vectors also include a promotor operably linked to the opiorph receptor polypeptide encoding portion. The encoded opiorph receptor polypeptide(s) may be expressed by using any suitable vectors and host cells as explained herein or otherwise known to those skilled in the art.

Vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host such as, for example, antibiotic resistance, and

one or more expression cassettes. The inserted coding sequences may be synthesized, isolated from natural sources, prepared as hybrids, or the like. Ligation of the coding sequences to the transcriptional regulatory sequences may be achieved by methods known to those skilled in the art. Suitable host cells may be transformed/transfected/infected by any suitable method including electroporation, $CaCl_2$ mediated DNA uptake, fungal infection, microinjection, microprojectile, or the like.

Suitable vectors for use in practicing the present invention include without limitation YEp352, pcDNAI (InVitrogen), and pRC/CMV (InVitrogen). Suitable host cells include *E. coli,* yeast, COS cells, PC12 cells, CHO cells, GH4Cl cells, and amphibian melanophore cells.

Nucleic acids encoding the opiorph receptor polypeptide (s) may also be introduced into cells by recombination events. For example, such a sequence can be microinjected into a cell, effecting homologous recombination at the site of an endogenous gene encoding the polypeptide, an analog or pseudogene thereof, or a sequence with substantial identity to an opiorph receptor polypeptide-encoding gene. Other recombination-based methods such as non-homologous recombinations, and deletion of endogenous gene by homologous recombination, especially in pluripotent cells, may also be used.

Opiorph Receptor Polypeptides

Opiorph receptor polypeptides OR5 and OR7 are shown in FIG. **2**(SEQ ID NO:2). Sequence analysis using Genetics Computer Group software revealed the presence of an open reading frame encoding 367 amino acids, containing seven candidate hydrophobic membrane-spanning domains of 20–24 amino acids that are homologous to those in other, G-protein-linked transmembrane receptors (see FIGS. **3** (SEQ ID NO:2),(SEQ ID NO:3), (SEQ ID NO:4), (SEQ ID NO:5), and (SEQ ID NO:6) and **4**). Additionally, the sequence contains four consensus sequences for asparagine-linked glycosylation, as well as serine and threonine residues that are contained in possible intracellular domains and are present within local sequence contexts favorable for phosphorylation by protein kinases A and C. The smaller polypeptide encoded by the splice variant lacks 28 amino acids, (SEQ ID NO:6) including a glycosylation consensus sequence, but is otherwise identical to the larger polypeptide.

Several features of the OR7 structure are consistent with specific functional implications. The size of the third putative intracellular loop predicted by the cDNA is modest, consistent with sizes of the homologous segments in the seven transmembrane domain receptors that do not couple to adenylate cyclase stimulating G proteins. Although many residues lying in transmembrane regions are conserved, the OR7 sequence (SEQ ID NO:2) contain a glutamine at position 305 instead of the histidine that lies in comparable positions in the mu, kappa, and delta opiate receptor sequences. The 28 additional amino acids (SEQ ID NO:6) encoded by the longer splice variant separate a number of negatively charged residues in the putative third extracellular segment from each other.

The present invention also encompasses function-conservative variants as explained above of the amino acid sequences in FIG. **2** (SEQ ID NO:2). Furthermore, fragments of the polypeptide greater than 20 amino acids in length may also exhibit functional properties characteristic of the intact native molecule, for example, the capacity to bind particular ligands.

Opiorph receptor polypeptides may be isolated from any source, such as, for example, native sources in rat tissues or heterologous cells programmed to produce the polypeptide by recombinant DNA methods. Alternately, the polypeptide (s) or peptide fragments thereof can be synthesized in a cell-free context. Peptides of up to 50 amino acids can be chemically synthesized, and larger polypeptides can be synthesized using cell-free translation systems.

Opiorph receptor polypeptides may be modified by methods known in the art. For example, the polypeptides may be phosphorylated or dephosphorylated, glycosylated or deglycosylated, acylated or deacylated, and the like.

In addition, opiorph receptor polypeptides may be expressed as fusion proteins incorporating heterologous sequences. Appropriate fusion partners include sequences useful for immobilization and purification. For example, sequences derived from glutathione-S-transferase (GST) provide a binding site for immobilized glutathione, and sequences that form an epitope recognized by an available monoclonal antibody (e.g. 12CA5 monoclonal antibody) provide a binding site for the immobilized antibody.

Opiorph Receptor Antibodies

Antibodies that are specific for the opiorph receptor polypeptide(s) are provided. These antibodies may be polyclonal or monclonal, and may distinguish the opiorph receptor polypeptide(s) from other opioid receptors or other transmembrane proteins, discriminate opiorph receptor polypeptide (s) from different species, identify associational or other functional domains, and the like.

Such antibodies are conveniently made using the methods and compositions disclosed in Harlow and Lane, *Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988, as well as immunological and hybridoma technologies known to those in the art.

Where natural or synthetic opiorph receptor-derived peptides are used to induce a specific immune response, the peptides may be conveniently coupled to an suitable carrier such as KLH and may be administered in a suitable adjuvant such as Freunds. Preferably, selected peptides are coupled to a lysine core carrier substantially according to the methods of Tam (1988) *Proc Natl Acad Sci USA* 85, 5409–5413. The resulting antibodies may be modified to a monovalent form, such as, for example, Fab, FAB', or FV. Anti-idiotypic antibodies, especially internal imaging anti-idiotypic antibodies, may also be prepared using known methods.

For example, purified opiorph receptor polypeptide(s) can be used to immunize mice. Subsequently; the mice spleens are removed. Splenocytes are used to form cell hybrids with myeloma cells and to obtain clones of antibody-secreted cells according to techniques that are known in the art. The resulting monoclonal antibodies are screened for their ability to bind immobilized opiorph receptor(s) or peptide fragments thereof.

In another example, peptides corresponding to different extracellular domains of the opiorph receptor polypeptide(s) are used as immunogens, and the resulting monoclonal antibodies are screened for their activity in inhibiting the binding of ligands to cells expressing the opiorph receptor polypeptide(s).

Anti-opiorph receptor polypeptide antibodies can be used to identify, isolate, and purify opiorph receptor polypeptide (s) from different sources and to perform subcellular and histochemical localization studies.

Applications

The polypeptides and nucleic acids sequences above can be used in the discovery, design, and development of pharmacologically useful opioid or opioid-like agonists and antagonists or unrelated non-opioid ligands. They can also be used in the design of diagnostic tests for pathological

conditions influenced by the presence or absence of opioiph receptor polypeptide function.

For example, the cloned receptor polypeptide(s), or fragments thereof, can be expressed in a heterologous cell in which it can achieve a proper transmembrane orientation and an appropriate localization in the plasma membrane. Examples of suitable cells include COS cells, PC12 cells, CHO cells, Xenopus oocytes, and amphibian melanophore cells. The ability of the expressed polypeptide(s) to bind different ligands can be assessed either by measurement of binding of radiolabelled ligand directly using methods that are standard in the art followed by analysis by, for example, Scatchard analysis or by measurement of the ability of a ligand to alter forskolin-stimulated adenylate cyclase activity. For example, morphine (an exemplary opioid) inhibits the forskolin-stimulated adenylate cyclase activity of the rat or human mu-OR1 opioid receptor and also inhibits IP_3 production. Alternatively, in amphibian melanophore cells, a number of G-protein-regulated activities can be easily assessed by visually monitoring the effect of ligands on melanophore distribution within the cells (Jayawickreme, C. K. et al., (1994), *Proc. Natl.Acad. Sci. USA* 91:1614–1618).

In another embodiment, nucleic acid probes are prepared that are specific for the opiorph receptor polypeptide(s) and are used to measure the level of expression of opiorph receptor polypeptide mRNA in different tissues and under different physiological and/or pathological situations. The probes are labelled using a radioactive, fluorescent, or enzymatic label, and are used as direct hybridization probes in a Northern blot. Alternately,. the probes can serve as primers for coupled reverse transcription-polymerase chain reaction, using RNA from the tissue as a template. This results in selective amplification of opiorph receptor related polynucleotide sequences only in tissues in which they are expressed.

Additionally, mutations can be introduced into the sequence of the opiorph receptor polypeptide(s). The mutated sequences are then expressed in a heterologous cell and the structure and function of the variants can be tested. Mutations in the predicted extracellular domains of the polypeptide should alter the opiorph receptor polypeptide(s) ability to bind ligands, while mutations in the predicted intracellular domains, including particular serine and threonine residues, will alter its ability to respond to ligand binding by initiating a biochemical signalling cascade within the cell.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples illustrate the invention without limitation.

Example 1

Cloning and Sequencing of Opiorph Receptor cDNA

A. Polymerase Chain Reaction

Two degenerate oligonucleotide primers were prepared using an automated oligonucleotide synthesizer. The first, 5'-ACGATGAA(GC)AC(TGA)GCCACCACCA-3'(SEQ ID NO:7), was derived from the unique amino acid sequence VLVVVAVFIV (SEQ ID NO:8) corresponding to amino acids 325–334 of the rat brain mu opioid receptor. The second primer, 5'-CTTCAA(TC)CTGGC(TC)TTGCCTGAT-3'(SEQ ID NO:9), corresponds to amino acids 89–95 derived from the predicted second transmembrane domain, of the murine delta opioid receptor.

PCR reactions were carried out using rat genomic DNA as a template and the Taq polymerase PCR kit (Perkin-Elmer/Cetus, Inc.). The reactions included 20 ng of genomic DNA and 1 μg of each primer. The thermal cycling protocol was as follows: 94° C., 1 minute, followed by 35 cycles of 94.5° C., 20 seconds; 49° C., 45 seconds; 72° C., 45 seconds. This was followed by incubation at 72° C. for 10 minutes, after which the samples were placed on ice.

Resolution of the PCR products on a 1 % agarose gel revealed the presence of products in the range of 500–600 bp in length.

The PCR products from the first reaction were then re-amplified, using identical primers and conditions as above. The products of the second PCR reaction were separated by electrophoresis in a 1% agarose gel, and discrete products were excised and purified on glass beads using the Gene-Clean kit (Bio-101). The purified fragments were then subcloned into the pCR-II vector (InVitrogen) and amplified in *E. coli.*

Bacterial colonies transformed with the pCR-II vector were subjected to alkine lysis to isolate plasmid DNA. The DNAs were then sequenced using the dye primer automated sequencing system (Applied Biosystems, Model 373A). Sequence analyses and alignments were performed using the MacVector software package (I.B.I.).

This approach identified an unspliced opiorph receptor-encoding sequence corresponding to OR5 (see FIG. **1**)(SEQ ID NO:1). This sequence contains the 84 nucleotides that are absent from the smaller splice variant.

B. Library screening

pPCR4A is a 700 base pair (bp) pPCRII (InVitrogen) subclone of a partial mu opiate receptor cDNA amplified from single stranded rat brain CDNA. The 700 bp pPCR4A insert. was excised with EcoRI, radiolabelled by random priming, and used to isolate cDNAs from a size-selected rate cerebral cortex lambda ZAP CDNA library. Sequence analyses of the inserts from autoexcised plasmids revealed apparent partial sequences with substantial homology to other cloned opiate receptors, including a 2.8 kb cDNA, from which a 5' 500 bp fragment was isolated using HindIII. This fragment was radiolabeled by random priming and was used to isolate other more 5' cDNAs including a 3 kb cDNA. Inserts from the two clones were cut and ligated to form a fused clone encoding the smaller, splice-variant form of the opiorph receptor (SEQ ID NO:2) i.e. lacking the internal 28 amino acids encoded by the spliced-out oligonucleotide (SEQ ID NO:6)(see FIG. **3**).

The present cDNAs add substantially to the diversity of the gene subfamily that contains opiate receptors. The splice variant documented for this receptor represents the first example of differential splicing in this receptor gene subfamily, and suggests an intron-exon border likely to be conserved in several opiate receptor subfamily genes.

Example 2

Tissue Distribution of Opiorph Receptor mRNA

A. Reverse Transcription-Polymerase Chain Reaction

Two oligonucleotide primers were prepared corresponding to nucleotides 51–71 and 546–566 of the sequence of FIG. **1**, consisting of 5'-AGGGCACAGACATCCTACTGG-3' (SEQ ID NO:10) and 5'-AGCCTGAAAGC AGACGGACAC-3'(SEQ ID NO:11).

RNA was prepared from rat tissues that were rapidly dissected and frozen at −70° C. and from rat cell lines. The RNAs served as templates for combined reverse-transcriptase-polymerase chain reactions (RT-PCR). The reactions were carried out using an RT-PCR kit (Perkin-Elmer/Cetus) employing rTth bifunctional polymerase. Synthesis of single-stranded cDNA was performed using 100–200 ng of RNA and 2 μg of the 3' primer. After

incubation at 65° C. for 10 minutes, chelating buffers, $MgCl_2$, and 0.75 μg of the 5' primer were added. The thermal cycling sequence Was as follows: 94° C., 1 minute, followed by 35 cycles of 94.5° C., 20 seconds; 60° C., 20 seconds; and 72° C., 60 seconds. The reaction mixtures were then chilled, and the products were analyzed on a 1% agarose gel in tris-borate-EDTA buffer.

Results are shown in FIG. **5** and indicate that opiorph receptor is expressed in brain, pituitary, thymus, stomach, muscle, and fat tissues.

In another experiment, reverse transcription-PCR was performed using as template 5 μg of total RNA extracted from different tissues and oligonucleotide primers 5'-ACCCTGGTCTTGCTAACA-3' (SEQ ID NO:12) and 5'-CAGCACCAGTCGAGTGAT-3' (SEQ ID NO:13). Single-stranded cDNA was amplified by 35 cycles of 94° C. for 1 minute, 55° C. for 1 minute, and 92° C. for 1 minute), with separation of PCR products by 2% agarose gel electrophoresis, transfer to nylon membranes, hybridization overnight with a ^{32}P-labeled opiorph cDNA probe at 42° C., followed by phosphorimaging.

Results are shown in FIGS. **6**A and 6B. FIG. **6**A indicates that two splice variant products were detected in various brain regions, as well as in several peripheral tissues such as intestine, skeletal muscle, vas deferens and spleen. FIG. **6**B indicates that the ratio between the two splice variants also varies among the brain regions and peripheral tissues examined.

B. Northern Analysis

Total RNA was prepared from rat tissues that were rapidly dissected and frozen at −70° C. 20 μg of each RNA were resolved in agarose-formamide gels. The separated RNA species were then transferred to nylon membranes. Blots were hybridized with opiorph receptor cDNA radiolabelled with ^{32}P by random priming. Hybridizations were carried out in 50% formamide, 5×SSC, 50 mM $NaPO_4$, 1% SDS, 2.5×Denhardt's solution, and 200 μg/ml salmon sperm DNA at 42° C. overnight. The filters were then washed twice in 0.1×SSC/0.1% SDS for 30 minutes at 65° C. Radioactive patterns were identified using a phosphorimaging device (Molecular Dynamics) following overnight exposures.

Results are illustrated in FIG. **7**. This analysis revealed that the highest levels of opiorph receptor expression are in the hypothalamus. At least three hybridizing mRNA species are observed in this brain region and in brainstem, midbrain, cerebral cortex, thalamus and hippocampus, but not in striatum or cerebellum.

Conceivably, two of these three mRNAs could represent products of different genes closely related to OR7 in sequence. Alternately, mRNA splicing and/or polyadenylation site usage events in the gene's untranslated regions could yield the significant differences in transcript molecular mass noted in Northern analyses.

Example 3

Heterologous Expression of Opiorph Receptor in COS cells

COS cells were transfected by electroporation with 20 4g/10^7 cells of opiorph cDNA which had been cloned into the pcDNAI vector (InVitrogen). Transfected cells were plated in Dulbecco's modified minimal essential medium (GIBCO, Grand Island, N.Y.) containing 10% fetal bovine serum and maintained at 37° C. in a humidified atmosphere containing 5 % CO_2.

Expression of opiorph receptor polypeptide(s) was assessed by measurement of specific ligand binding. Alternatively, expression of opiorph receptor polypeptide(s) may be assessed by RNA extraction and RT-PCR according to the procedure of Example 2 above or immuno assay with antibodies specific to the opiorph receptor(s).

Example 4

Analysis of Ligand Binding Characteristics of Opiorph Receptors

COS cells transfected with opiorph receptor cDNA or, as a control, rat or human mu opiate receptor cDNA were harvested. Membranes were prepared by homogenization at 4° C. in 50 mM Tris buffer and centrifugation at 1000×g for 10 minutes. The supernatant was then recovered and subjected to centrifugation at 46,000×g for 30 minutes. The membrane-containing pellet was recovered, and fractions corresponding to 50 μg of protein were resuspended in 0.5 ml of Tris buffer and incubated with different radiolabelled ligands.

The ligands were: [^{3}H]bremazocine (29.2 Ci/mmol, NEN), [^{3}H]naloxone (47.2 Ci/mmol, NEN), [^{3}]diprenorphine (29 Ci/mmol, NEN), [^{3}H]DAMGO ([D-Ala2,N-Methyl-Phe4,Glyol[5]enkephalin; 60 Ci/mmol, Amersham), [^{3}H]DPDPEpCl ([D-Pen2,4'-Cl-Phe4,D-Pea5] enkephalin; 51 Ci/mmol, NEN), [^{3}H]DADLE (D-Ala2,D-LeU5 enkephalin; 37 Ci/mmol, NEN), [^{3}H] ethylketocyclazocine (28.5 Ci/mmol, NEN), [^{3}H]etorphine (38.7 Ci/mmol, NEN), [^{3}]buprenorphine (13.4 Ci/mmol, RBI). [^{125}]β-endorphin (2,000 Ci/mmol, Amersham) and [^{3}]U-69,593 (57 Ci/mmol, Amersham).

Incubations were for 150 minutes at 22° C., after which the reactions were filtered through GFB filters (Whatman). The filters were washed three times with Tris buffer at 4° C. Radioactivity associated with the filters was determined by liquid scintillation counting, and data were analyzed using EBDA and LIGAND (Munson et al., *Anal. Biochem.* 107:220, 1980).

Under conditions in which robust binding to rat or human μOR1 polypeptides was observed, no definitive binding of the above ligands to opiorph receptor polypeptide(s) was observed. No specific radioligand binding above background levels was observed in eight of ten experiments using cells expressing the smaller splice variant of the opiorph receptor or in four of four experiments using cells expressing the larger splice variant. In two experiments, modest naloxone-displacable diprenorphine, bremazocine, and β-endorphin binding above background values was noted in cells expressing the smaller splice variant. However, intermittent naloxone-displacable binding of naloxone and β-endorphin was also observed in mock-transfected COS cells in several negative control experiments. Neither radiolabeled diprenorphine, bremazocine, not β-endorphin displayed specific binding in eight additional experiments. Neither ethylketocyclazocine, naloxone, DAMGO, DPDPE, U,69,693, ctorphine, buprenorphine, not DADLE resulted in specific binding in any experiment.

COS cells transfected with either the large or small splice variant of OR7 failed to display consistent opiate-induced alteration in forskolin-stimulated adenylate cyclase activity. In 14 experiments in which morphine-inhibited adenylate cyclase activity in COS cells expressing rat or human mu opiate receptor cDNAs served as positive controls, eight of 10 experiments revealed no opiate-mediated inhibition of forskolin-stimulated cyclase activity in cells expressing the smaller splice variant of OR7, and four of four experiments revealed no opiate-mediated inhibition of forskolin-stimulated cyclase activity in cells expressing the larger splice variant of OR7. In two experiments, bremazocine, buprenorphine, etorphine and β-endorphin did elicit modest naloxone-reversible inhibition of forskolin-stimulated cyclase activity in cells expressing the smaller splice variant. However, intermittent naloxone-reversible β-endorphin effects were also noted in some experiments in mock-

fransfected cells. Neither bremazocine, buprenoiphine, etorphine nor endorphin altered forskolin-stimulated cAMP levels in eight additional experiments; neither DADLE, Dynorphin A, morphine, nor U50,488 altered cAMP levels in any experiment.

Deposit of Biological Materials

The following biological materials were deposited with the American type Culture Collection, 12301 Park Lain Drive, Rockville, Md. 20857 as follows:

Strain OZ86 deposited Dec. 23, 1993,
Accession Number ATCC 69525.

All patents, applications, articles, publications, and test methods mentioned above are hereby incorporated by reference.

Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. Such obvious variations are within the full intended scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 13

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2706 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(v i) ORIGINAL SOURCE:
(F) TISSUE TYPE: Rat brain

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGGCCGCCT TTCTGCTAAG CATTGGGGTC TATTTTGGCC CAGCTTCTGA AGAGGCTGTG 60

TGTGCCGTTG GAGGAACTGT ACTGAGTGGC TTTGCAGGGT GACAGCATGG AGTCCCTCTT 120

TCCTGCTCCA TACTGGGAGG TCTTGTATGG CAGCCACTTT CAAGGGAACC TGTCCCTCCT 180

AAATGAGACC GTACCCCACC ACCTGCTCCT CAATGCTAGT CACAGCGCCT TCCTGCCCCT 240

TGGACTCAAG GTCACCATCG TGGGGCTCTA CTTGGCTGTG TGCATCGGGG GGCTCCTGGG 300

GAACTGCCTC GTCATGTATG TCATCCTCAG GCACACCAAG ATGAAGACAG CTACCAACAT 360

TTACATATTT AATCTGGCAC TGGCTGATAC CCTGGTCTTG CTAACACTGC CCTTCCAGGG 420

CACAGACATC CTACTGGGCT TCTGGCCATT TGGGAATGCA CTCTGCAAGA CTGTCATTGC 480

TATCGACTAC TACAACATGT TTACCAGCAC TTTTACTCTG ACCGCCATGA GCGTAGACCG 540

CTATGTGGCT ATCTGCCACC CTATCCGTGC CCTTGATGTT CGGACATCCA GCAAAGCCCA 600

GGCTGTTAAT GTGGCCATAT GGGCCCTGGC TTCAGTGGTT GGTGTTCCTG TTGCCATCAT 660

GGGTTCAGCA CAAGTGGAAG ATGAAGAGAT CGAGTGCCTG GTGGAGATCC CTGCCCCTCA 720

GGACTATTGG GGCCCTGTAT TCGCCATCTG CATCTTCCTT TTTTCCTTCA TCATCCCTGT 780

GCTGATCATC TCTGTCTGCT ACAGCCTCAT GATTCGACGA CTTCGTGGTG TCCGTCTGCT 840

TTCAGGCTCC CGGGAGAAGG ACCGAAACCT GCGGCGTATC ACTCGACTGG TGCTGGTAGT 900

GGTGGCTGTG TTTGTGGGCT GCTGGACGCC TGTGCAGGTG TTTGTCCTGG TTCAAGGACT 960

GGGTGTTCAG CCAGGTAGTG AGACTGCAGT TGCCATCCTG CGCTTCTGCA CAGCCCTGGG 1020

CTATGTCAAC AGTTGTCTCA ATCCCATTCT CTATGCTTTC CTGGATGAGA ACTTCAAGGC 1080

CTGCTTTAGA AAGTTCTGCT GTGCTTCATC CCTGCACCGG GAGATGCAGG TTTCTGATCG 1140

TGTGCGGAGC ATTGCCAAGG ATGTTGGCCT TGGTTGCAAG ACTTCTGAGA CAGTACCACG 1200

GCCAGCATGA CTAGGCGTGG ACCTGCCCAT GGTGCCTGTC AGCCCACAGA GCCCATCTAC 1260

ACCCAACACG GAGCTCACAC AGGTCACTGC TCTCTAGGTT GACCCTGAAC CTTGAGCATC 1320

TGGAGCCTTG AATGGCTTTT CTTTTGGATC AGGATGCTCA GTCCTAGAGG AAGACCTTTT 1380

-continued

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AGCACCATGG GACAGGTCAA AGCATCAAGG TGGTCTCCAT GGCCTCTGTC AGATTAAGTT    1440
CCCTCCCTGG TATAGGACCA GAGAGGACCA AAGGAACTGA ATAGAAACAT CCACAACACA    1500
GTGGACATGC CTGGTGAGCC CATGTAGGTA TTCATGCTTC ACTTGACTCT TCTCTGGCTT    1560
CTCCCTGCTG CCCTGGCTCT AGCTGGGCTC AACCTGAGGT ATTGTAGTGG TCATGTAGTC    1620
ACTCTTGTGA CTACATGTTG TGTGCTGTTG CTCTCGGCCT TTCAGTATTT CCACAGGACT    1680
GCTGAACATA CCTGGTATTG CAGTGGGGAG CATTAATTTT CTTTTAAAGT GAGACTGGCC    1740
CTTAAGCTTG GCGTTGCCTT GGAGCGTCTT CTACTTCTGA CTTCACTGAT GCAGTCAGAT    1800
TACCCGAGGG TGAGCATCAG TGGTTTCTTG GATGGCTGTT TTCTGAAGAT TCTTCCCATC    1860
CAGTACATGG AGTCTATGAA GGGGAGTCAC AATTCATCTG GTACTGCCAC TACCTGCTCT    1920
ATAATCCTGG GCTATCTTCT TGGCAAGATG ACAGTGGGGG AGACAAGACA CAGAGCTTCC    1980
CTAAGGCTCT TTCCCTCCAA AACCACTGTG AACTCTTATC CTACAGACTG TTCGGCAAGC    2040
ACTGCTTCTA GGTGTGTGGG AGGTAATCAG GAGAAAGCTT TGTGGCCTCT GTAGGCTGCT    2100
CACAACATGG AGGCACCACA TGCTGGTCTT GCCTGCTTAG TACAGGCAGG ACAGAGCAGA    2160
ATATGCTCTC TCTCGATTCT CTACAAACTC CCTCAGTTCT CCAGCAGAGT CTCTTTTACT    2220
TGCTATCAGA GGTCAGGAGT TGTACTGCTA GAAGCATACT TGTAGCTTGG GAAGAGTGGC    2280
AGTCAGGATG TGTTCTACTC TATATCCACA GTGACCACCT GCTTCATATA TAGGGTTAGG    2340
ACATATCTGA GTAAGGCCTG AGTGTGCTGC CAAATTGGAG GTTGGTATGA GAGCTGATGC    2400
CTAAAGTGGC TCATTTGCAA GGACTATTAT GGTTTGGAAT AGCAATGGGG GGCATGGGAA    2460
GAAGAGTCTA TACCTTGGAG ATCTATTTGA TGGTTCACAG AAGAGGTTTT GTAAACGCCC    2520
TTTCTATGGG TCAGATATCA AAATACCAGC AACGTTGGAT AGATTCTGAC CTTTTACTGA    2580
GACCTCGGTC AGATGGTTTC ATGTCATGCA GAGAACCTAG GCTGGTTCCT GTGTCAGAGA    2640
GACCTGGGCT TCTGGGGAGG CCAGGGTTCT TCCTTTGACA CTTGTGCGGG AGCCGTTAGC    2700
TCTAGA                                                               2706
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 367 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Ser Leu Phe Pro Ala Pro Tyr Trp Glu Val Leu Tyr Gly Ser
1               5                  10                  15

His Phe Gln Gly Asn Leu Ser Leu Leu Asn Glu Thr Val Pro His His
            20                  25                  30

Leu Leu Leu Asn Ala Ser His Ser Ala Phe Leu Pro Leu Gly Leu Lys
        35                  40                  45

Val Thr Ile Val Gly Leu Tyr Leu Ala Val Cys Ile Gly Gly Leu Leu
    50                  55                  60

Gly Asn Cys Leu Val Met Tyr Val Ile Leu Arg His Thr Lys Met Lys
65                  70                  75                  80

Thr Ala Thr Asn Ile Tyr Ile Phe Asn Leu Ala Leu Ala Asp Thr Leu
                85                  90                  95
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Val Leu Leu Thr Leu Pro Phe Gln Gly Thr Asp Ile Leu Leu Gly Phe
100 105 110

Trp Pro Phe Gly Asn Ala Leu Cys Lys Thr Val Ile Ala Ile Asp Tyr
115 120 125

Tyr Asn Met Phe Thr Ser Thr Phe Thr Leu Thr Ala Met Ser Val Asp
130 135 140

Arg Tyr Val Ala Ile Cys His Pro Ile Arg Ala Leu Asp Val Arg Thr
145 150 155 160

Ser Ser Lys Ala Gln Ala Val Asn Val Ala Ile Trp Ala Leu Ala Ser
165 170 175

Val Val Gly Val Pro Val Ala Ile Met Gly Ser Ala Gln Val Glu Asp
180 185 190

Glu Glu Ile Glu Cys Leu Val Glu Ile Pro Ala Pro Gln Asp Tyr Trp
195 200 205

Gly Pro Val Phe Ala Ile Cys Ile Phe Leu Phe Ser Phe Ile Ile Pro
210 215 220

Val Leu Ile Ile Ser Val Cys Tyr Ser Leu Met Ile Arg Arg Leu Arg
225 230 235 240

Gly Val Arg Leu Leu Ser Gly Ser Arg Glu Lys Asp Arg Asn Leu Arg
245 250 255

Arg Ile Thr Arg Leu Val Leu Val Val Val Ala Val Phe Val Gly Cys
260 265 270

Trp Thr Pro Val Gln Val Phe Val Leu Val Gln Gly Leu Gly Val Gln
275 280 285

Pro Gly Ser Glu Thr Ala Val Ala Ile Leu Arg Phe Cys Thr Ala Leu
290 295 300

Gly Tyr Val Asn Ser Cys Leu Asn Pro Ile Leu Tyr Ala Phe Leu Asp
305 310 315 320

Glu Asn Phe Lys Ala Cys Phe Arg Lys Phe Cys Cys Ala Ser Ser Leu
325 330 335

His Arg Glu Met Gln Val Ser Asp Arg Val Arg Ser Ile Ala Lys Asp
340 345 350

Val Gly Leu Gly Cys Lys Thr Ser Glu Thr Val Pro Arg Pro Ala
355 360 365

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 391 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Asp Ser Ser Thr Gly Pro Gly Asn Thr Ser Asp Cys Ser Asp Pro
1 5 10 15

Leu Ala Gln Ala Ser Cys Ser Pro Ala Pro Gly Ser Trp Leu Asn Leu
20 25 30

Ser His Val Asp Gly Asn Gln Ser Asp Pro Cys Gly Leu Asn Arg Thr
35 40 45

Gly Leu Gly Gly Asn Asp Ser Leu Cys Pro Gln Thr Gly Ser Pro Ser
50 55 60

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Met Val Thr Ala Ile Thr Ile Met Ala Leu Tyr Ser Ile Val Cys Val
65 70 75 80

Val Gly Leu Phe Gly Asn Phe Leu Val Met Tyr Val Ile Val Arg Tyr
85 90 95

Thr Lys Met Lys Thr Ala Thr Asn Ile Tyr Ile Phe Asn Leu Ala Leu
100 105 110

Ala Asp Ala Leu Ala Thr Ser Thr Leu Pro Phe Gln Ser Val Asn Tyr
115 120 125

Leu Met Gly Thr Trp Pro Phe Gly Thr Ile Leu Cys Lys Ile Val Ile
130 135 140

Ser Ile Asp Tyr Tyr Asn Met Phe Thr Ser Ile Phe Thr Leu Cys Thr
145 150 155 160

Met Ser Val Asp Arg Tyr Ile Ala Val Cys His Pro Val Lys Ala Leu
165 170 175

Asp Phe Arg Thr Pro Arg Asn Ala Lys Ile Val Asn Val Cys Asn Trp
180 185 190

Ile Leu Ser Ser Ala Ile Gly Leu Pro Val Met Phe Met Ala Thr Thr
195 200 205

Lys Tyr Arg Gln Gly Ser Ile Asp Cys Thr Leu Thr Phe Ser His Pro
210 215 220

Thr Trp Tyr Trp Glu Asn Leu Leu Lys Ile Cys Val Phe Ile Phe Ala
225 230 235 240

Phe Ile Met Pro Val Leu Ile Ile Thr Val Cys Tyr Gly Leu Met Ile
245 250 255

Leu Arg Leu Lys Ser Val Arg Met Leu Ser Gly Ser Lys Glu Lys Asp
260 265 270

Arg Asn Leu Arg Arg Ile Thr Arg Met Val Leu Val Val Val Ala Val
275 280 285

Phe Ile Val Cys Trp Thr Pro Ile His Ile Tyr Val Ile Ile Lys Ala
290 295 300

Leu Ile Thr Ile Pro Glu Thr Thr Phe Gln Thr Val Ser Trp His Phe
305 310 315 320

Cys Ile Ala Leu Gly Tyr Thr Asn Ser Cys Leu Asn Pro Val Leu Tyr
325 330 335

Ala Phe Leu Asp Glu Asn Phe Lys Arg Cys Phe Arg Glu Phe Cys Ile
340 345 350

Pro Thr Ser Ser Thr Ile Glu Gln Gln Asn Ser Thr Arg Val Arg Gln
355 360 365

Asn Thr Arg Glu His Pro Ser Thr Ala Asn Thr Val Asp Arg Thr Asn
370 375 380

His Gln Leu Glu Asn Leu Glu
385 390

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 367 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Pro Val Pro Ser Ala Arg Ala Glu Leu Gln Phe Ser Leu Leu

-continued

1 5 10 15

Ala Asn Val Ser Asp Thr Phe Pro Ser Ala Phe Pro Ser Ala Ser Ala
20 25 30

Asn Ala Ser Gly Ser Pro Gly Ala Arg Ser Ala Ser Ser Leu Ala Leu
35 40 45

Ala Ile Ala Ile Thr Ala Leu Tyr Ser Ala Val Cys Ala Val Gly Leu
50 55 60

Leu Gly Asn Val Leu Val Met Phe Gly Ile Val Arg Tyr Thr Lys Leu
65 70 75 80

Lys Thr Ala Thr Asn Ile Tyr Ile Phe Asn Leu Ala Leu Ala Asp Ala
85 90 95

Leu Ala Thr Ser Thr Leu Pro Phe Gln Ser Ala Lys Tyr Leu Met Glu
100 105 110

Thr Trp Pro Phe Gly Glu Leu Leu Cys Lys Ala Val Leu Ser Ile Asp
115 120 125

Tyr Tyr Asn Met Phe Thr Ser Ile Phe Thr Leu Thr Met Met Ser Val
130 135 140

Asp Arg Tyr Ile Ala Val Cys His Pro Val Lys Ala Leu Asp Phe Arg
145 150 155 160

Thr Pro Ala Lys Ala Lys Leu Ile Asn Ile Cys Ile Trp Val Leu Ala
165 170 175

Ser Gly Val Gly Val Pro Ile Met Val Met Ala Val Thr Gln Pro Arg
180 185 190

Asp Gly Ala Val Val Cys Thr Leu Gln Phe Pro Ser Pro Ser Trp Tyr
195 200 205

Trp Asp Thr Val Thr Lys Ile Cys Val Phe Leu Phe Ala Phe Val Val
210 215 220

Pro Ile Leu Ile Ile Thr Val Cys Tyr Gly Leu Met Leu Leu Arg Leu
225 230 235 240

Arg Ser Val Arg Leu Leu Ser Gly Ser Lys Glu Lys Asp Arg Ser Leu
245 250 255

Arg Arg Ile Thr Arg Met Val Leu Val Val Val Gly Ala Phe Val Val
260 265 270

Cys Trp Ala Pro Ile His Ile Phe Val Ile Val Trp Thr Leu Val Asp
275 280 285

Ile Asn Arg Arg Asp Pro Leu Val Val Ala Ala Leu His Leu Cys Ile
290 295 300

Ala Leu Gly Tyr Ala Asn Ser Ser Leu Asn Pro Val Leu Tyr Ala Phe
305 310 315 320

Leu Asp Glu Asn Phe Lys Arg Cys Phe Arg Gln Leu Cys Arg Ala Pro
325 330 335

Cys Gly Gly Gln Glu Pro Gly Ser Leu Arg Arg Pro Arg Gln Ala Thr
340 345 350

Ala Arg Glu Arg Val Thr Ala Cys Thr Pro Ser Asp Gly Pro Gly
355 360 365

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 330 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(i i) MOLECULE TYPE: protein

(v i) ORIGINAL SOURCE:

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(A) ORGANISM: Rat

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Glu Ser Pro Ile Gln Ile Phe Arg Gly Glu Pro Gly Pro Thr Cys
1 5 10 15

Ala Pro Ser Ala Cys Leu Leu Pro Asn Ser Ser Ser Trp Phe Pro Asn
20 25 30

Trp Ala Glu Ser Asp Ser Asn Gly Ser Val Gly Ser Glu Asp Gln Gln
35 40 45

Leu Glu Pro Ala His Ile Ser Pro Ala Ile Pro Val Ile Ile Thr Ala
50 55 60

Val Tyr Ser Val Val Phe Val Val Gly Leu Val Gly Asn Ser Leu Val
65 70 75 80

Met Phe Val Ile Ile Arg Tyr Thr Lys Met Lys Thr Ala Thr Asn Ile
85 90 95

Tyr Ile Phe Asn Leu Ala Leu Ala Asp Ala Leu Val Thr Thr Thr Met
100 105 110

Pro Phe Gln Ser Ala Val Tyr Leu Met Asn Ser Trp Pro Phe Gly Asp
115 120 125

Val Leu Cys Lys Ile Val Ile Ser Ser Ser Val Gly Ile Ser Ala Ile
130 135 140

Val Leu Gly Gly Thr Lys Val Arg Glu Asp Val Asp Val Ile Glu Cys
145 150 155 160

Ser Leu Gln Phe Pro Asp Asp Glu Tyr Ser Trp Trp Asp Leu Phe Met
165 170 175

Lys Ile Cys Val Phe Val Phe Ala Phe Val Ile Pro Val Leu Ile Ile
180 185 190

Ile Val Cys Tyr Thr Leu Met Ile Leu Arg Leu Lys Ser Val Arg Leu
195 200 205

Leu Ser Gly Ser Arg Glu Lys Asp Arg Asn Leu Arg Arg Ile Thr Lys
210 215 220

Leu Val Leu Val Val Val Ala Val Phe Ile Ile Cys Trp Thr Pro Ile
225 230 235 240

His Ile Phe Ile Leu Val Glu Ala Leu Gly Ser Thr Ser His Ser Thr
245 250 255

Ala Val Leu Ser Ser Tyr Tyr Phe Cys Ile Ala Leu Gly Tyr Thr Asn
260 265 270

Ser Ser Leu Asn Pro Val Leu Tyr Ala Phe Leu Asp Glu Asn Phe Lys
275 280 285

Arg Cys Phe Arg Asp Phe Cys Phe Pro Ile Lys Met Arg Met Glu Arg
290 295 300

Gln Ser Thr Asn Arg Val Arg Asn Thr Val Gln Asp Pro Ala Ser Met
305 310 315 320

Arg Asp Val Gly Gly Met Asn Lys Pro Val
325 330

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: Not Relevant

(i i) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

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(v i) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly Gln Trp Val Val Leu Leu Pro Asp Ser Leu Val Ser His Gly Phe
1 5 10 15

Leu Leu Val Pro Leu Pro Pro Asn Pro Ser Pro Ala
20 25

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACGATGAAGC ACTGAGCCAC CACCA 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:
(F) TISSUE TYPE: rat brain

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Leu Val Val Val Ala Val Phe Ile Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTTCAATCCT GGCTCTTGCC TGAT 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER corresponding to nucleotides 51 to 71 of SEQ ID NO:1"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGGGCACAGA CATCCTACTG G 21

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER corresponding to nucleotides 546-566 of SEQ ID NO:1"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCCTGAAAG CAGACGGACA C 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACCCTGGTCT TGCTAACA 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR PRIMER"

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CAGCACCAGT CGAGTGAT 18

We claim:

1. A method for detecting the expression, in a tissue, of MRNA encoding a polypeptide having an amino acid sequence comprising amino acid residues 88–269 of the amino acid sequence of SEQ ID NO:2, said method comprising:

(a) selecting at least one oligonucleotide sequence unique to said polypeptide, wherein said sequence comprises from about 15 to about 30 nucleotides;

(b) synthesizing said oligonucleotides;

(c) hybridizing said oligonucleotide to total mRNA isolated from said tissue under stringent conditions; and

(d) detecting said hybridization.

2. A method as defined in claim **1**, wherein said detecting step comprises polymerase chain reaction.

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