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Regional expression and chromosomal localization of the δ opiate receptor gene

(NG108-15/*in situ* hybridization/mouse Chromosome 4/pineal/anterior pituitary)

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ABSTRACT **The δ opiate receptor gene has been cloned from the mouse neuroblastoma–rat glioma hybrid cell NG108-15. The clone that we isolated is apparently identical to that reported by Evans *et al.* [Evans, C. J., Keith, D. E., Jr., Morrison, H., Magendzo, K. & Edwards, R. H. (1992) *Science* 258, 1952–1955] and essentially identical with that of Kieffer *et al.* [Kieffer, B. L., Befort, K., Gaveriaux-Ruff, C. & Hirth, C. G. (1992) *Proc. Natl. Acad. Sci. USA* 89, 12048–12052]. We have found full-length transcripts of the gene in mouse brain but in no other tissues examined. Within the brain the gene is expressed at low levels in many regions but transcripts are found in particularly large amounts in the anterior pituitary and pineal glands. Since these tissues are located outside the blood–brain barrier, opioid peptides easily can reach receptors in these areas from the blood. The gene, which is present as a single copy, has been mapped to the distal region of mouse Chromosome 4.**

The opiates have been used for medicinal and other purposes for at least several thousand years. Morphine, the principal active ingredient of the opium poppy, has long been considered to be one of the few indispensable drugs since it or its relatives are the most effective analgesic agents known. The opiates are also used for treating diarrhea and cough and for general anesthesia in cardiac and other surgery. Accompanying these medically useful attributes of the opiates is a euphoria, which is more or less pronounced depending on the sensibility of the individual taking it, and respiratory, blood pressure, and body temperature depression as well. The fascination of many researchers with the properties of these drugs stems from the hope that real insight into the workings of the mind will emerge from such studies as well as from the expectation that a purer analgesic may be developed. The present report is an account of our isolation of a cDNA clone encoding the δ opiate receptor (1–3) of NG108-15 cells (4, 5). In our study, we used oligonucleotide probes corresponding to conserved regions of mouse inhibitory G protein-coupled receptors to screen an NG108-15 cDNA library for the receptor. Essentially the same clone was isolated by two groups who were independently using a selection system based on expression of a specific opiate binding protein encoded by cDNA from the same NG108-15 cells (6, 7). As previously suspected, based upon pharmacological experiments, there is apparently only a single type of opiate receptor in these cells. We localized the mRNA corresponding to this gene to discrete parts of the rat brain—in particular, the anterior pituitary and pineal glands—and mapped the gene itself to the distal portion of mouse Chromosome 4 (Chr 4). The presence of large amounts of opiate receptor mRNA in the anterior pituitary and pineal is unexpected and, because these tissues are not protected by the blood–brain barrier, suggests that the opiate receptors in these tissues will be particularly susceptible to opiates of exogenous origin.

MATERIALS AND METHODS

Isolation of a δ Opiate Receptor cDNA. Approximately 1×10^6 recombinant plaques from an oligo(dT) and random hexamer primed, size-selected (>1 kb) λZapII NG108-15 cDNA library were screened with the oligonucleotide probes whose sequences were derived from the conserved putative transmembrane domains 2, 5, and 7 of the family of receptors coupled to inhibitory G proteins. Three probes were prepared using an automatic DNA synthesizer (Applied Biosystems model 380B): 2TM, 5′-GGT GGC CAC GAT CAG GTC GGC GCA GGC CAG GCT CAC CAG GAA GTA GTT-3′; 5TM, 5′-TCT CTT GGC GAT TCT GTA GAT TCT CCA GTA CAC CAG GGT CAT GAT-3′; 7TM, 5′-GGC GTA GAT CAC GGG GTT CAG GCT GCT GTT GCA GTA GCC CAG CCA-3′. Plaques were transferred to nitrocellulose filters; the filters were prehybridized and hybridized at 35°C in a solution containing 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.2), 20% formamide, 0.1% SDS, 1× Denhardt's solution (1× Denhardt's solution = 0.2% polyvinylpyrrolidone/0.2% bovine serum albumin/0.2% Ficoll), 20 mM sodium pyrophosphate, and 100 μg of denatured salmon sperm DNA per ml.

The oligonucleotide probes, labeled with ^{32}P by T4 polynucleotide kinase, were hybridized for 18 hr at 1×10^6 cpm/ml of hybridization solution. Filters were washed with 2× SSC/0.1% SDS at room temperature three times, 10 min each, and finally with 0.2× SSC/0.1% SDS for 5 min at 42°C. Autoradiography was carried out for 48 hr at −80°C with Kodak X-Omat AR film.

The cDNA inserts from 25 positive λZapII clones were excised into pSKII plasmids, following the manufacturer's instructions, and were subjected to restriction mapping and an initial sequence analysis. The δ opiate receptor cDNA, clone TB27, was subcloned into M13mp19 for complete sequence determination. Both strands were sequenced using synthetic oligonucleotide primers by the dideoxynucleotide chain-termination method (8).

Northern Blot Hybridization Analysis. Five-microgram samples of poly(A)$^+$ RNA from adult mouse tissues were fractionated by formaldehyde/agarose gel electrophoresis and transferred to a nylon filter. The blot was hybridized with nick-translated 2.2-kb TB27 cDNA insert at 42°C for 20 hr in 5× SSPE (1× SSPE = 0.15 M NaCl/10 mM phosphate, pH

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Abbreviations: *Nbor*, the neuroblastoma (δ) opiate receptor and the corresponding mouse gene; Chr 4, mouse Chromosome 4.

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7.4/1 mM EDTA)/0.1% SDS/5× Denhardt's solution/50% deionized formamide/100 μg of denatured salmon sperm DNA per ml. The filter was washed with 0.1× SSPE/0.1% SDS four times for 30 min each at 50°C and exposed for 10 days to x-ray film.

cRNA Probe Preparation and *in Situ* Hybridization. The hybridization probes were prepared from pOR plasmid containing 632 bp of the coding region of the opiate receptor (base numbers 559–1191 in Fig. 1). Antisense and sense riboprobes were prepared from the plasmid, which had been linearized with *Bgl* II and *Eco*RI, respectively, by transcribing with appropriate RNA polymerases using a riboprobe system (Promega) in the presence of UTP[α-^{35}S] (1000–1500 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear). A second antisense riboprobe (1210 nucleotides in length, corresponding to base numbers 1014–2224) was prepared from *Not* I-linearized TB27 plasmid DNA by transcribing with T7 RNA polymerase.

In situ hybridization was performed essentially as described (9). Frozen rat brain sections (16 μm thick) were cut, thaw-mounted on gelatin-coated slides, fixed, and dehydrated immediately before hybridization. Brain sections were incubated overnight at 54°C in hybridization buffer containing 50% formamide, 4× saline/sodium citrate (SSC), 10% dextran sulfate, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 250 μg of yeast tRNA per ml,

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  22 CGT GCG GAG CTG CAG TCC TCG CCC CTC GTC AAC CTC TCG GAC GCC TTT CCC AGC GCC TTC   81
 (8) Arg Ala Glu Leu Gln Ser Ser Pro Leu Val Asn Leu Ser Asp Ala Phe Pro Ser Ala Phe  (27)

  82 CCC AGC GCG GGC GCC AAT GCG TCG GGG TCG CCG GGA GCC CGT AGT GCC TCG TCC CTC GCC  141
(28) Pro Ser Ala Gly Ala Asn Ala Ser Gly Ser Pro Gly Ala Arg Ser Ala Ser Ser Leu Ala  (47)

 142 CTA GCC ATC GCC ATC ACC GCG CTC TAC TCG GCT GTG TGC GCA GTG GGG CTT CTG GGC AAC  201
(48) Leu Ala Ile Ala Ile Thr Ala Leu Tyr Ser Ala Val Cys Ala Val Gly Leu Leu Gly Asn  (67)  A

 202 GTG CTC GTC ATG TTT GGC ATC GTC CGG TAC ACC AAA TTG AAG ACC GCC ACC AAC ATC TAC  261
(68) Val Leu Val Met Phe Gly Ile Val Arg Tyr Thr Lys Leu Lys Thr Ala Thr Asn Ile Tyr  (87)

 262 ATC TTC AAT CTG GCT TTG GCT GAT GCG CTG GCC ACC AGC ACG CTG CCC TTC CAG AGC GCC  321
(88) Ile Phe Asn Leu Ala Leu Ala Asp Ala Leu Ala Thr Ser Thr Leu Pro Phe Gln Ser Ala  (107)  B

 322 AAG TAC TTG ATG GAA ACG TGG CCG TTT GGC GAG CTG CTG TGC AAG GCT GTG CTC TCC ATT  381
(108) Lys Tyr Leu Met Glu Thr Trp Pro Phe Gly Glu Leu Leu Cys Lys Ala Val Leu Ser Ile  (127)

 382 GAC TAC TAC AAC ATG TTC ACT AGC ATC TTC ACC CTC ACC ATG ATG AGC GTG GAC CGC TAC  441
(128) Asp Tyr Tyr Asn Met Phe Thr Ser Ile Phe Thr Leu Thr Met Met Ser Val Asp Arg Tyr  (147)  C

 442 ATT GCT GTC TGC CAT CCT GTC AAA GCC CTG GAC TTC CGG ACA CCA GCC AAG GCC AAG CTG  501
(148) Ile Ala Val Cys His Pro Val Lys Ala Leu Asp Phe Arg Thr Pro Ala Lys Ala Lys Leu  (167)

 502 ATC AAT ATA TGC ATC TGG GTC TTG GCT TCA GGT GTC GGG GTC CCC ATC ATG GTC ATG GCA  561
(168) Ile Asn Ile Cys Ile Trp Val Leu Ala Ser Gly Val Gly Val Pro Ile Met Val Met Ala  (187)  D

 562 GTG ACC CAA CCC CGG GAT GGT GCA GTG GTA TGC ATG CTC CAG TTC CCC AGT CCC AGC TGG  621
(188) Val Thr Gln Pro Arg Asp Gly Ala Val Val Cys Met Leu Gln Phe Pro Ser Pro Ser Trp  (207)

 622 TAC TGG GAC ACT GTG ACC AAG ATC TGC GTG TTC CTC TTT GCC TTC GTG GTG CCG ATC CTC  681
(208) Tyr Trp Asp Thr Val Thr Lys Ile Cys Val Phe Leu Phe Ala Phe Val Val Pro Ile Leu  (227)  E

 682 ATC ATC ACG GTG TGC TAT GGC CTC ATG CTA CTG CGC CTG CGC AGC GTG CGT CTG CTG TCC  741
(228) Ile Ile Thr Val Cys Tyr Gly Leu Met Leu Leu Arg Leu Arg Ser Val Arg Leu Leu Ser  (247)

 742 GGT TCC AAG GAG AAG GAC CGC AGC CTG CGG CGC ATC ACG CGC ATG GTG CTG GTG GTG GTG  801
(248) Gly Ser Lys Glu Lys Asp Arg Ser Leu Arg Arg Ile Thr Arg Met Val Leu Val Val Val  (267)  F

 802 GGC GCC TTC GTG GTG TGC TGG GCG CCC ATC CAC ATC TTC GTC ATC GTC TGG ACG CTG GTG  861
(268) Gly Ala Phe Val Val Cys Trp Ala Pro Ile His Ile Phe Val Ile Val Trp Thr Leu Val  (287)

 862 GAC ATC AAT CGG CGC GAC CCA CTT GTG GTG GCC GCA CTG CAC CTG TGC ATT GCG CTG GGC  921
(288) Asp Ile Asn Arg Arg Asp Pro Leu Val Val Ala Ala Leu His Leu Cys Ile Ala Leu Gly  (307)  G

 922 TAC GCC AAC AGC AGC CTC AAC CCG GTT CTC TAC GCC TTC CTG GAC GAG AAC TTC AAG CGC  981
(308) Tyr Ala Asn Ser Ser Leu Asn Pro Val Leu Tyr Ala Phe Leu Asp Glu Asn Phe Lys Arg  (327)

 982 TGC TTC CGC CAG CTC TGT CGC ACG CCC TGC GGC CGC CAA GAA CCC GGC AGT CTC CGT CGT  1041
(328) Cys Phe Arg Gln Leu Cys Arg Thr Pro Cys Gly Arg Gln Glu Pro Gly Ser Leu Arg Arg  (347)

1042 CCC CGC CAG GCC ACC ACG CGT GAG CGT GTC ACT GCC TGC ACC CCC TCC GAC GGC CCG GGC  1101
(348) Pro Arg Gln Ala Thr Thr Arg Glu Arg Val Thr Ala Cys Thr Pro Ser Asp Gly Pro Gly  (367)

1102 GGT GGC GCT GCC GCC TGACCTACCC GACCTTCCCC TTAAACGCCC CTCCCAAGTG AAGTGATCCA GAGG  1170
(368) Gly Gly Ala Ala Ala                                                            (372)

1171 CCACACCGAG CTCCCTGGGA GGCTGTGGCC ACCACCAGGA CAGCTAGAAT TGGGCCTGCA CAGAGGGGAG  1240
1241 GCCTCCTGTG GGGACGGGGC CTGAGGGATC AAAGGCTCCA GGTTGGAACG GTGGGGGTGA GGAAGCAGAG  1310
1311 CTGGTGATTC CTAAACTGTA TCCATTAGTA AGGCCTCTCC AATGGGACAG AGCCTCCGCC TTGAGATAAC  1380
1381 ATCGGGTTCT GGCCTTTTTG AACACCCAGC TCCAGTCCAA GACCCAAGGA TTCCAGCTCC AGGAACCAGG  1460
1461 AGGGGCAGTG ATGGGGTCGA TGATTTGGTT TGGCTGAGAG TCCCAGCATT TGTGTTATGG GGAGGATCTC  1530
1531 TCATCTTAGA GAAGATAAGG GGACAGGGCA TTCAGGCAAG GCAGCTTGGG GTTTGGTCAG GAGATAAGCG  1600
1601 CCCCCTTCCC TTGGGGGGAG GATAAGTGGG GGATGGTCAA CGTTGGAGAA GAGTCAAAGT TCTCACCACC  1670
1671 TTTCTAACTA CTCAGCTAAA CTCGTTGAGG CTAGGGCCAA CGTGACTTCT CTGTAGAGAG GATACAAGCC  1740
1741 GGGCCTGATG GGGCAGGCCT GTGTAATCCC AGTCATAGTG GAGGCTGAGG CTGGAAAATT AAGGACCAAC  1810
1811 AGCCTGGGCA ATTTAGTGTC TCAAAATAAA ATGTAAAGAG GGCTGGGAAT GTAGCTCAGT GGTAGGGTGT  1880
1881 TTGTGTGAGG CTCTGGGATC AATAAGACAA AACAACCAAC CAACCAAAAA CCTTCCAAAC AACAAAACCA  1950
1951 ACCCTCAAAC CAAAAAACTA TGTGGGTGTC TCTGAGTCTG GTTTGAAGAG AACCCGCAGC CCTGTATCCC  2020
2021 TGTGGGGCTG TGGACAGTGG GCAGAAGCAG AGGCTCCCTG GATCCTGAAC AAGGGCCCCA AAAGCAAGTT  2090
2091 CTAAAGGGAC CCCTGAAACC GAGTAAGCCT TTGTGTCAAG AAGTGGGAGT AGAACCAGAA AGGTGGCTGA  2160
2161 GTGATTAAGG GCACGTGACT CTCTTGCAGA GGACATAGGT TCGATTCCCA GCACCCACAT AGTGGCTCAC  2220
2221 AGCC                                                                          2224
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FIG. 1. Nucleotide sequence and the derived amino acid sequence of clone TB27. The sequence extends well into the 3′ untranslated region but is incomplete at the 5′ end starting only at amino acid 8 as shown. The putative transmembrane helices were deduced from a combination of hydrophobicity plots and homology with a group of 30 aligned sequences of G protein-coupled, inhibitory, receptors, and are underlined. The shaded amino acids are those that are conserved in at least 29 of those structures. The helices are also designated by the letters A–G.

500 μg of sheared salmon sperm DNA per ml, 100 μM dithiothreitol, and the ^{35}S-labeled RNA probe at a density of 1×10^7 cpm/ml of hybridization solution. After hybridization, slides were rinsed four times for 5 min each in 4× SSC, treated with RNase A (20 μg/ml, Boehringer Mannheim) for 30 min at 37°C, and rinsed at room temperature for 5 min two times in 2× SSC, 10 min in 1× SSC, 10 min in 0.5× SSC, and 30 min in 0.1× SSC at 60°C. After dehydration in ethanol and drying, the slides were exposed to film (β-max Hyperfilm, Amersham) for 10 days and developed in Kodak D-19.

Chromosomal Localization of a δ Opiate Receptor Gene. The gene for the opiate receptor of NG108-15 cells, termed *Nbor*, was mapped by Southern blot analysis of DNAs from the progeny of two mouse genetic crosses described previously (10, 11). In addition to *Nbor*, progeny of these crosses were also typed for markers on Chr 4. *Gpd-1* (glucose phosphate dehydrogenase) and *Gnb-1* (G-protein β1) were typed in the *Mus mus musculus* cross as described (12). *Gnb-1* was typed following *Pst* I digestion in the *Mus spretus* cross using the same hybridization probe. *Ccnb1-rs4* (cyclin B1-related sequence 4) was typed as a 5.0-kb *Bam*HI fragment in both crosses using probes described by Hanley-Hyde *et al.* (13). *Lmyc-1* (L-*myc* oncogene) was typed following *Eco*RI digestion in the *musculus* cross using the L-*myc* probe obtained from Oncor. *Lck* (lymphocyte tyrosine kinase) was typed following *Bgl* II digestion in the *musculus* cross and *Apa* I digestion in the *spretus* cross using a cDNA probe obtained from B. Kwon (Indiana University). *Dsi-1* (David Steffen integration site 1) was typed following *Sst* I digestion in the *musculus* cross and *Eco*RI digestion in the *spretus* cross following hybridization with a probe described in ref. 14.

RESULTS

A λZapII NG108-15 cDNA library was screened with the oligonucleotide probes 2TM, 5TM, and 7TM, whose sequences had been derived from the conserved transmembrane domains 2, 5, and 7 of the family of receptors coupled to inhibitory G proteins. The restriction patterns of 25 of the positive λ clones were characterized and some were partially sequenced. Both strands of the cDNA of clone TB27 (2.2 kb) were sequenced completely since it did not correspond to a previously known receptor. While this work was in progress, two groups reported the cloning of the δ opiate receptor by expression of mRNA from NG108-15 cells. The amino acid sequence deduced from TB27 cDNA (Fig. 1) was identical to that of reported by Evans *et al.* (6) and almost identical to that reported by Kieffer *et al.* (7).

Northern blot analysis showed the clear presence of transcripts corresponding to opiate receptor in mouse brain and no evidence for the presence of hybridizing transcripts of size greater than 2 kb in any other tissue examined (Fig. 2). The fact that transcripts of 8- and 12-kb sizes are found in brain suggests the presence of alternatively spliced variants in this tissue since the chromosomal localization data indicate the presence of only a single gene (Table 1). The band seen in the lung in this blot was absent when the experiment was repeated and is not considered significant.

The distribution of neuroblastoma opiate receptor (*Nbor*) mRNA in rat brain was examined by *in situ* hybridization using radiolabeled riboprobes prepared from TB27 cDNA. The transcript distribution in adult rat brain is shown in Fig. 3. The use of second antisense probe complementary to a different region of *Nbor* mRNA gave identical patterns of hybridization (not shown). No signal could be detected with the sense probe when used under identical conditions and at the same specific activity (data not shown).

In general, δ opioid receptor mRNA was not highly expressed throughout brain regions but exhibited restricted

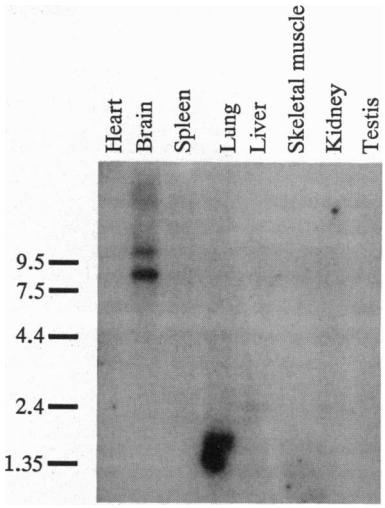

FIG. 2. Northern blot hybridization analysis of *Nbor* expression in various mouse tissues. Shown is an autoradiograph of a blot in which the 2.2-kb TB27 cDNA probe was hybridized to 5-μg samples of poly(A)$^+$ RNA isolated from various mouse tissues. The sizes of the *Nbor* transcripts were determined by comparison with the mobility of RNA standards (BRL). Fluoroautoradiography was for 10 days with an intensifying screen.

patterns of distribution. By far the greatest amount of hybridization was observed in the anterior pituitary and the pineal glands (Fig. 3 *D* and *E*). Relatively high levels of *Nbor* mRNA were also found in the internal granular layer of the olfactory bulb (Fig. 3*A*), dorsomedial, ventromedial, and arcuate nuclei of the hypothalamus, amygdala, and hippocampal area (Fig. 3*C*), pontine nuclei (Fig. 3*E*), and inferior olive (Fig. 3*F*). Moderate levels of *Nbor* mRNA were also detected in the cerebral cortex (Fig. 3 *C* and *D*) and granular layers of the cerebellum (Fig. 3*F*).

Analysis of two previously described genetic crosses (10, 11) was done to position *Nbor* in the mouse genome. Digestion with *Hin*dIII produced a 22-kb fragment in *M. m. musculus* and a 14-kb fragment in NFS/N parental mouse DNAs. Seventy-six of 146 mice of the backcross (NFS/N or C58/J × *M. m. musculus*)F_1 × *M. m. musculus* contained the 14-kb fragment, consistent with single gene segregation. For

Table 1. Segregation of *Nbor* with *Lck* and *Dsi-1* in progeny of the intersubspecies backcrosses (NFS/N or C58/J × *M. m. musculus*)F_1 × *M. m. musculus* or (NFS/N or C58/J × *M. spretus*)F_1 × *M. spretus*

Mice	Inheritance of parental alleles			Number of mice	
	Lck	*Nbor*	*Dsi-1*	*musculus* cross	*spretus* cross
Parental	+	+	+	18	30
	−	−	−	18	31
Single	+	+	−	0	0
Recombinant	−	−	+	1	0
	+	−	−	1	1
	−	+	+	1	3

Locus pair	Recombination* r/*n*	% recombination
Lck, Nbor	6/104	5.8 ± 2.3
	16/225†	7.1 ± 1.7
Nbor, Dsi-1	1/104	0.96 ± 0.96

*Mice were typed as heterozygous (+) or homozygous (−) for inheritance of allelic variants at each locus. Percent recombination ± SE between restriction fragments was calculated according to Green (15) from the number of recombinants (r) in a sample size of *n*.
†121 mice were typed for *Lck* and *Nbor* but not *Dsi-1*.

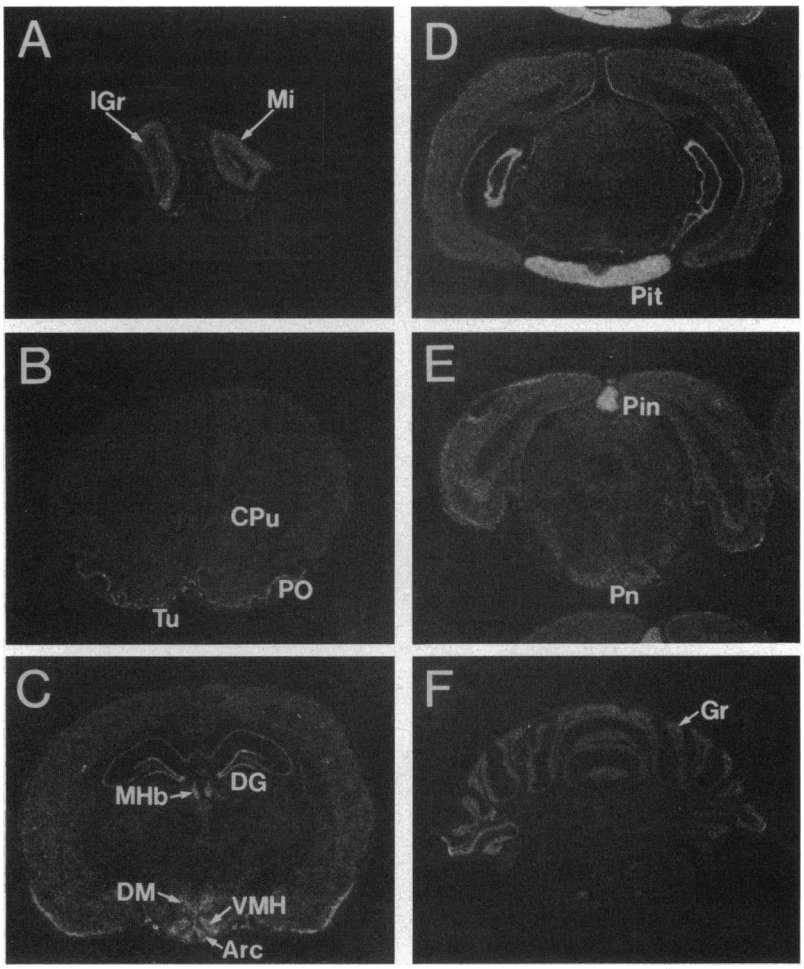

FIG. 3. Localization of the δ opiate receptor mRNA in adult rat brain by *in situ* hybridization. A negative film image of *in situ* hybridization of coronal sections is shown. Arc, arcuate nucleus; CPu, caudate-putamen; DG, dentate gyrus; DM, dorsomedial hypothalamic nucleus; Gr, granule cell layer of cerebellum; IGr, internal granular layer of olfactory bulb; MHb, medial habenula; Mi, mitral cell layer of olfactory bulb; Pin, pineal gland; Pit, pituitary gland; Pn, pontine nuclei; PO, piriform cortex; Tu, olfactory tubercle; VMH, ventromedial hypothalamic nucleus. (×2.47.)

analysis of the second cross, digestion with *Pst* I produced a *M. spretus* fragment of 9.6 kb and NFS/N or C58/J fragments of 7.2 kb and 5.7 kb. Forty-five of 110 mice in the cross (NFS/N or C58/J × *M. spretus*)F_1 × *M. spretus* were heterozygous, as would also be expected with single gene segregation.

Inheritance of *Nbor* in these crosses was compared with >400 markers typed on all 19 autosomes and the X chromosome. Results of this comparison indicated linkage to the Chr 4 markers *Lck* and *Dsi-1* (Table 1). Additional markers in this same region of Chr 4 were typed in both crosses. Gene order and distances (centimorgans ± standard error, in parentheses) for this region in the *musculus* cross are as follows: *Ccnb1-rs4* (4.7 ± 3.2) *Lmyc-1* (6.8 ± 3.3) *Lck* (5.8 ± 2.1) *Nbor* (2.0 ± 2.0) *Dsi-1* (1.9 ± 1.8) *Gpd-1* (2.7 ± 1.9) *Gnb-1*. Gene order and distances (centimorgans ± standard error, in parentheses) for the *spretus* cross are as follows: *Ccnb1-rs4* (4.7 ± 3.2) *Lmyc-1* (6.8 ± 3.3) *Lck* (5.8 ± 2.1) *Nbor* (2.0 ± 2.0) *Dsi-1* (1.9 ± 1.8) *Gpd-1* (2.7 ± 1.9) *Gnb-1*. These results place the *Nbor* gene in the distal region of mouse Chr 4, between *Lck* and *Dsi-1*.

DISCUSSION

Clone TB27 is an almost full-length version of the coding sequence of the NG108-15 opiate receptor. We isolated the clone by virtue of its hybridization with oligonucleotide probes taken from highly conserved regions of the family of inhibitory G protein-coupled receptors. The clone extends well into the 3′ untranslated region of the gene and is missing just a short segment at its 5′ end, accounting for 7 amino acids. The amino acid sequence of the opiate receptor is characteristic of the family of seven transmembrane helical G protein-coupled receptors. Among its most highly conserved features are some aspects of the sequence of the transmembrane helices, all of which are found in almost every member of the family of G_i-coupled receptors. Included in these are the Gly-Asn sequence in transmembrane helix A, the Asp residue in helix B, the Ser residue in helix C, and the Pro residues in each of helices D, E, F, and G (Fig. 1). These conserved features are likely to play important roles in the determination of the structure or function of these receptors. Even though the ligand binding sites are probably located within the transmembrane helices, the fact that these features are so well conserved among so many different receptors suggests that they play a more general role in governing structure or activity than simply binding. The size of the receptor, as deduced from its amino acid sequence, is 40,600, a value consistent with the mobility value of 58,000 measured by SDS gel electrophoresis for the natural, highly glycosylated, δ opiate receptor isolated from NG108-15 cells (16).

Our genetic mapping results place the NG108-15 opiate receptor gene (*Nbor*) in the distal region of mouse Chr 4, between *Lck* and *Dsi-1* (Table 1). The human homologues of

two of the flanking genes, *LCK* and *GNB1*, have been mapped to human chromosome 1p (17), and it is therefore likely that the human gene for the δ opiate receptor will also map to chromosome 1p. The genes and cDNAs corresponding to the μ and κ opiate receptors remain to be identified but it seems likely that all three major subtypes are very closely related members of a family within the G protein-coupled receptor superfamily. Genes specifying another member of the superfamily, the dopamine receptors, are found to be widely dispersed throughout the human genome (18). Determination of the gene structure and chromosomal localization of the other members of the opiate receptor family should provide insight into its evolution.

It is reasonable to ask why it has taken so long to isolate clones of an opiate receptor when it has been under intense study for the past 20 years (19–21). There is no good answer to this question, especially in light of the fact that the δ opiate receptor turns out to be a typical member of the class of G protein-coupled receptors, many of which have been cloned in the past decade. The putative κ opiate receptor, which has been recently cloned by expression (22), is not very similar to clone TB27 and, though it is clearly a receptor, seems not to have the specificity expected of an opiate receptor. The interesting opiate-binding protein cloned by Schofield *et al.* (23) is clearly not a transmembrane G protein-coupled receptor. A clue to the solution of this question may be our observation during Northern blot analysis that the amount of δ opiate receptor mRNA present in brain is surprisingly small. Our studies of distribution within brain regions confirm the fact that, with the exception of the pineal and anterior pituitary glands, the δ opiate receptor transcript is expressed at a low level in other brain regions.

The results of our studies of the regional distribution of mRNA have been both surprising and provocative. We indeed found the δ opiate receptor gene transcript to be located in expected brain regions like the olfactory bulb, hippocampus, hypothalamic nuclei, cortex, and basal ganglia, but the distribution was not identical to the regional distribution of enkephalin binding to presumed δ receptors (24, 25). Perhaps the most surprising feature of the distribution is the relative absence of labeling in the caudate. We point out, however, that we are measuring the distribution of mRNA and not of binding sites. The interpretation of the differences will be clarified when an antibody becomes available that can measure the amounts of δ opiate receptor protein directly. The most striking concentrations of δ opiate receptor message were, however, found in the anterior pituitary and pineal glands, neither of which is famous as a center of opiate activity. There is, however, compelling evidence that even opiates that do not cross the blood–brain barrier affect the anterior pituitary so as to inhibit the release of luteinizing hormone and stimulate prolactin secretion (26, 27). Therefore there are indeed functionally significant opiate receptors in the anterior pituitary. The pineal gland is also outside of the blood–brain barrier (28) and contains opiate receptors (29) that may be largely δ (30). In view of this distribution, and of its neuroblastoma origin, the possibility should be considered that *Nbor* specifies a peripheral rather than a central nervous system opiate receptor. Most hydrophilic substances, such as peptides, are hindered in their ability to reach the brain by this barrier and the natural peptide ligands for the δ opiate receptor cross into the brain from the circulation only with difficulty (31). But blood-borne peptides should have no difficulty reaching the receptors found in the pituitary and pineal glands. These structures can then be considered to be target tissues for opioid peptides circulating as classic hormones. These receptors can also be targets for a class of opioid peptide generated from food proteins, the exorphins (32). Some of these exogenously derived peptides are known to activate δ receptors *in vitro* (33). The finding that there are many δ opiate receptors encoded by mRNA transcripts located outside the blood–brain barrier removes a conceptual obstacle to the consideration of the role of opioid peptides generated from food proteins to physiology and to pathology as well.

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