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Cloning and Differentiation-Induced Expression of a Murine Serotonin$_{1A}$ Receptor in a Septal Cell Line

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A neuronal cell model endogenously expressing the 5-HT$_{1A}$ receptor, in which to study the function and regulation of this gene, has yet to be identified. We examined murine SN-48 cells, a septum × neuroblastoma fusion cell line that proliferates in a nondifferentiated state but can be induced to differentiate into neurofilament-positive cells following 24–96 hr treatment with 10 μM retinoic acid in low serum. Northern blot analysis demonstrated the presence of a single 10.9 kilobase (kb) 5-HT$_{1A}$ receptor RNA species in differentiated SN-48 cells, which was not detected in undifferentiated SN-48 cells. The presence of receptor RNA in differentiated SN-48 cells correlated with the appearance of functional responses (i.e., pertussis toxin–sensitive inhibition of cAMP accumulation) to 5-HT$_{1A}$ agonists in differentiated but not in undifferentiated cells. In order to verify that the large 10.9 kb RNA species in SN-48 cells truly corresponded to the mouse 5-HT$_{1A}$ receptor RNA, a cDNA fragment from differentiated SN-48 cells was used to clone the corresponding mouse brain cDNA. The 2.4 kb cDNA contained a single open reading frame that displayed high (>85% predicted amino acid identity) homology to the human and rat 5-HT$_{1A}$ receptor genes. When transfected into receptor-negative Ltk$^-$ cells, this cDNA was found to direct expression of a murine 5-HT$_{1A}$ receptor. Thus, we conclude that upon differentiation SN-48 cells express RNA encoding functional 5-HT$_{1A}$ receptors. The SN-48 septal cells will provide a useful cellular model system for investigating aspects of neuronal differentiation leading to the development of sensitivity to serotonergic input.

[Key words: 5-HT, differentiation, septum, cDNA, 5-HT$_{1A}$ receptor, cAMP]

5-Hydroxytryptamine (serotonin, 5-HT) is a neurotransmitter believed to play a role in various cognitive functions such as feeding, sleep, pain, depression, learning, and anxiety (Blier et al., 1987; Charney et al., 1990; Curzon, 1992). This diversity of actions can be related to the fact that serotonergic neurons send efferents to almost every structure of the brain and especially to the limbic regions (Törk, 1990). 5-HT actions are mediated via activation of a growing family of 5-HT receptors. This receptor family has been divided into four classes designated 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ (Julius, 1991), and 5-HT$_4$ (Bockaert et al., 1992) on the basis of pharmacological properties, intracellular signaling systems, structural homology, and tissue distribution. The 5-HT$_1$ group is the most heterogeneous and is divided into at least four subtypes—5-HT$_{1A}$ (Kobilka et al., 1987; Fargin et al., 1988; Albert et al., 1990), 5-HT$_{1B}$ (Voigt et al., 1991; Jin et al., 1992; Maroteaux et al., 1992), 5-HT$_{1D}$ (Libert et al., 1989; Hamblin and Metcalf, 1991; Demchyshyn et al., 1992), and 5-HT$_{1E}$ (McAllister et al., 1992; Zgombick et al., 1992)—of which the 5-HT$_{1A}$ subtype has been the most studied for two reasons: first, the availability of a highly selective radioligand (8-OH-DPAT) that allows an extensive biochemical, physiological, and pharmacological characterization of the receptors (Hamon et al., 1987); second, the observation that certain 5-HT$_{1A}$ agonists exert anxiolytic and antidepressant effects in clinical paradigms (Blier et al., 1987, 1990).

5-HT$_{1A}$ receptors are expressed in discrete neuronal cell types within the mammalian CNS (e.g., Sotelo et al., 1990). In fact, radioligand binding studies correlated with *in situ* hybridization analysis (Chalmers and Watson, 1991; Pompeiano et al., 1992) indicate that the highest level of expression of the receptor is found in structures such as the dorsal raphe nucleus, septum, hippocampal formation (especially the pyramidal neurons of the CA1 region and the dentate gyrus), and the entorhinal cortex. The 5-HT$_{1A}$ receptor has been shown to couple negatively to adenylyl cyclase through pertussis toxin (PTx)–sensitive G-proteins (Zgombick et al., 1989; Albert et al., 1990) and positively to potassium channels through a different PTx-sensitive G-protein (Innis and Aghajanian, 1987; Innis et al., 1988; Williams et al., 1988; Blier et al., 1993); the latter process inducing membrane hyperpolarization and causing a decrease in neuronal firing rate.

To avoid problems of heterogeneity and complexity associated with most brain preparations, neuronal cell lines provide an abundant and homogeneous source of neurons that facilitates the biochemical analysis of various neuron-specific proteins in their natural environment (Blusztajn et al., 1992; Choi et al., 1992). However, none of these cell lines has been reported to express functional 5-HT$_{1A}$ receptors. Recent progress has led to the construction of cell lines derived from different regions and developmental stages of the CNS (Frederiksen et al., 1988; Lee et al., 1990a,b; Morimoto and Koshland, 1990; Renfranz et al., 1991; Hales et al., 1992). We took advantage of hybrid cell lines derived from pre- and postnatal hippocampus and septum, areas richest in 5-HT$_{1A}$ receptors, to screen for cell lines that express the 5-HT$_{1A}$ receptor. We report the characterization of a 5-HT$_{1A}$ receptor from a hybrid cell line (SN-48) derived from 21 d postnatal (P21) mouse septal neurons fused with a murine neu-

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roblastoma (N18TG2). We also describe the cloning, molecular and pharmacological characterization of the mouse 5-HT_{1A} receptor cDNA.

Materials and Methods

Cell culture. SN-48 (Lee et al., 1990b) fusion cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (GIBCO), 2 mM glutamine (GIBCO), and antibiotics (GIBCO) at 37°C in 5% CO_2. Differentiation was achieved by plating the cells at a density of 10^4 cells/cm^2 in DMEM supplemented with 1% (v/v) heat-inactivated FCS, 10 μM retinoic acid (RA) (all *trans*-) (Sigma, St. Louis, MO), and 2 mM glutamine. Ltk$^-$ mouse fibroblast cells were maintained in α-modified Eagle's medium (α-MEM) supplemented with 10% (v/v) heat-inactivated FCS and antibiotics at 37°C in 5% CO_2.

Polymerase chain reaction and cDNA cloning. The 918 base pair (bp) fragment from reverse-transcription polymerase chain reaction (RT-PCR) of differentiated SN-48 5-HT_{1A} mRNA was obtained as follows: total cytoplasmic RNA was isolated from 48 hr differentiated SN-48 as described (Sambrook et al., 1989). RNA (0.3 μg) was reverse-transcribed with Superscript (GIBCO/Bethesda Research Labs) using random hexamers (Pharmacia). An aliquot of the resulting cDNA was used as template during the subsequent PCR amplification with sense oligonucleotide (#45, 5′-GTGCTGGTGCTGCCCATGG-3′) and antisense oligonucleotide (#44, 5′-GGGAGTTGGAGTAGCCTAGCC-3′) derived from sequences of the second and seventh predicted transmembrane domains of the rat and human 5-HT_{1A} receptor genes. The 918 bp product of the amplification was isolated, and cloned into the EcoRV site of pBluescript KS+ (Stratagene). This 918 bp fragment was then radiolabeled using a random-primed labeling kit (Boehringer Mannheim). An 18-d-old NIH Swiss mouse brain λgt11 cDNA library (constructed by J. Kamholz, C. Puckett, and R. A. Lazzarini, NINCDS) was probed at high stringency with the radiolabeled 918 bp fragment as described elsewhere (Sambrook et al., 1989) and a single clone (clone A) containing a 2.4 kilobase (kb) cDNA insert was isolated and subcloned into the EcoRI site of pBluescript KS+. Both strands of the cDNA insert were sequenced by the Sanger method using a T7 polymerase-based DNA sequencing kit (Pharmacia).

Stable eukaryotic expression. The 2.4 kb EcoRI fragment of clone A was subcloned into EcoRI-cut pcDNA eukaryotic expression vector (InVitrogen), and 20 μg was cotransfected by the calcium phosphate method (Sambrook et al., 1989) with 2 μg of pRSV-*neo* plasmid. Clones were selected in medium containing 500 μg/ml of G418 (Geneticin, GIBCO) and screened for expression of the transfected 5-HT_{1A} receptor by RT-PCR (see above). Positive clones were further screened for level of functional receptors by measurement of calcium release from intracellular pools upon activation of the receptors as described previously (Liu and Albert, 1991). One clone (LM1A-5) was selected for further characterization.

Radioligand binding studies. Membranes from the positive LM1A-5 clone were harvested and assayed for binding as described previously (Albert et al., 1990) using ^{3}H-8-OH-2-(di-*n*-propylamino)1,2,3,4-tetrahydronaphthalene (^{3}H-8-OH-DPAT) (Amersham) as ligand, and 10 μM 5-HT to determine nonspecific binding. The results were analyzed by the LIGAND computer software (Biosoft, Cambridge, UK). Specific ^{3}H-8-OH-DPAT binding was not detected in nontransfected Ltk$^-$ cells or nondifferentiated SN-48 cells (data not shown). Attempts to demonstrate specific ^{3}H-8-OH-DPAT binding in differentiated SN-48 cells were unsuccessful, partly because of low cell number and low proportions of differentiated cells in large preparations.

cAMP assay. Measurement of cAMP was performed as described elsewhere (Albert et al., 1990). In brief, SN-48 cells were differentiated in six-well 35 mm dishes; medium was changed 24 hr prior to experimentation. After removal of the medium, cells were incubated in 1 ml of serum-free medium containing 100 μM 1-methyl-3-isobutylxanthine (IBMX) (Sigma, St. Louis, MO) with the various test compounds for 20 min at room temperature. The medium was discarded and the cells were lysed by addition of 1 ml of serum-free medium containing 0.05% of Triton X-100 and 100 μM IBMX. The media were collected and centrifuged (2000 × *g*, 5 min), and the supernatant was assayed for cAMP. cAMP was assayed by a specific radioimmunoassay (ICN) as described previously (Albert et al., 1990) with antibody used at 1:500 dilution. After 16 hr of incubation at 4°C, 20 μl of 10% BSA and 1 ml of ice-cold 95% ethanol were added sequentially to precipitate the antibody–antigen complex. Data are presented as mean ± SEM for triplicate wells.

Northern blot analysis. Total RNA (extracted from differentiated and nondifferentiated SN-48 cells, and from LM1A-5 cells), polyA$^+$ selection of mRNA species, and Northern blot analysis were performed as described (Sambrook et al., 1989). Molecular weight was extrapolated from the semilogarithmic relation to migration using molecular weight standards.

Results and Discussion

A neuroblastoma × septum hybrid cell line expressing the 5-HT_{1A} receptor

The characterization of neuronal hybrid cells derived from the fusion of primary cultures of pre- and postnatal hippocampal and septal neurons with a neuroblastoma cell line (N18TG2) has been described (Lee et al., 1990a,b). An intrinsic feature of these cell lines is the potential inducibility of certain phenotypic neuronal markers (e.g., neurofilaments) upon treatment of the cells with differentiation-inducing agents (e.g., RA or dibutryl-cAMP). Although the mechanisms underlying this phenomenon are unknown and likely to involve complex systems, inducibility of neuronal phenotype by chemical inducers is a well-established property of various systems (Jones-Villeneuve et al., 1982; Hammond et al., 1986; Rudnicki and McBurney, 1987; McBurney et al., 1988). Several murine hybrid cell lines (including SN-56, SN-48, HN-8, HN-9, HN-10, HN-25.2, HN-33.11) and neuroblastoma N18TG2 cells were screened by RT-PCR for the presence of mRNA coding for the 5-HT_{1A} receptor. Of the different hybrid clones screened, only one clone (SN-48) showed increased level of 5-HT_{1A} receptor gene expression in the differentiated state (RA treated) over the nondifferentiated state (data not shown). The SN-48 cell line is derived from primary culture of septum from P21 C57BL/6 mouse fused with the N18TG2 neuroblastoma cell line (Lee et al., 1990b) and has a noncholinergic, GABAergic phenotype (B. H. Wainer, unpublished observations). The SN-48 cell line was chosen for further characterization.

Correlation of neuronal phenotype and 5-HT_{1A} receptor expression

In the nondifferentiated state, the SN-48 cells are rapidly dividing and do not exhibit morphological features of neurons (Fig. 1*a*). However, 48 hr of treatment with 10 μM RA (see Materials and Methods) resulted in neuronal differentiation, as assessed by the appearance of long, axon-like processes (Fig. 1*b*). Since the concentration of RA used was not toxic to the cells, the differentiation observed is induced and not due to selection of a minor population of differentiated cells preexisting within the undifferentiated population. Concomitant with the morphological change, the appearance of mRNA encoding the 5-HT_{1A} receptor was observed by Northern blot analysis of RNA harvested from cells differentiated for 48 hr, but absent from nondifferentiated SN-48 cells (Fig. 2). The temporal association of enhanced levels of receptor RNA with the development of a neuronal phenotype suggested that the increase in 5-HT_{1A} receptor RNA may be due to indirect differentiation-inducing actions of RA that result in neurons capable of expressing the receptor, although a direct action of RA on receptor gene transcription was not ruled out. The size of the 5-HT_{1A} receptor RNA present in the differentiated SN-48 cells (10.9 kb) is larger than species observed for the rat and human receptor RNAs. Northern blot analysis of rat brain tissues detects three message sizes (3.9, 3.6, and 3.3 kb) encoding the 5-HT_{1A} receptor and

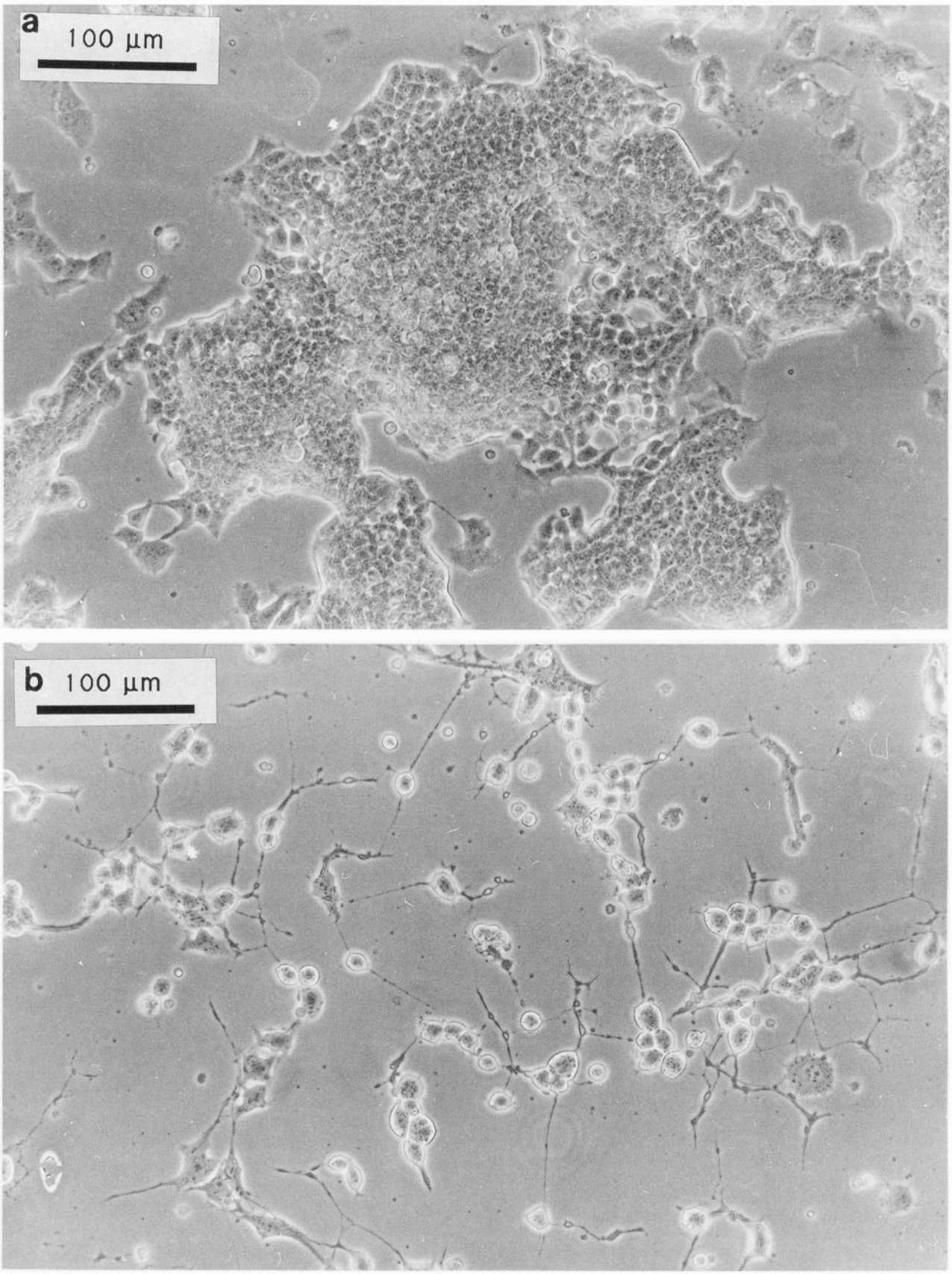


Figure 1. Morphology of nondifferentiated and differentiated SN-48 cells: nondifferentiated SN-48 cells (*a*) and 48 hr RA-differentiated SN-48 cells (*b*). The differentiation procedure is described in Materials and Methods. Note the adherent fibroblast-like morphology of the nondifferentiated cells contrasts with the rounded neuronal morphology of cells in the differentiated sample, most of which extend long processes.

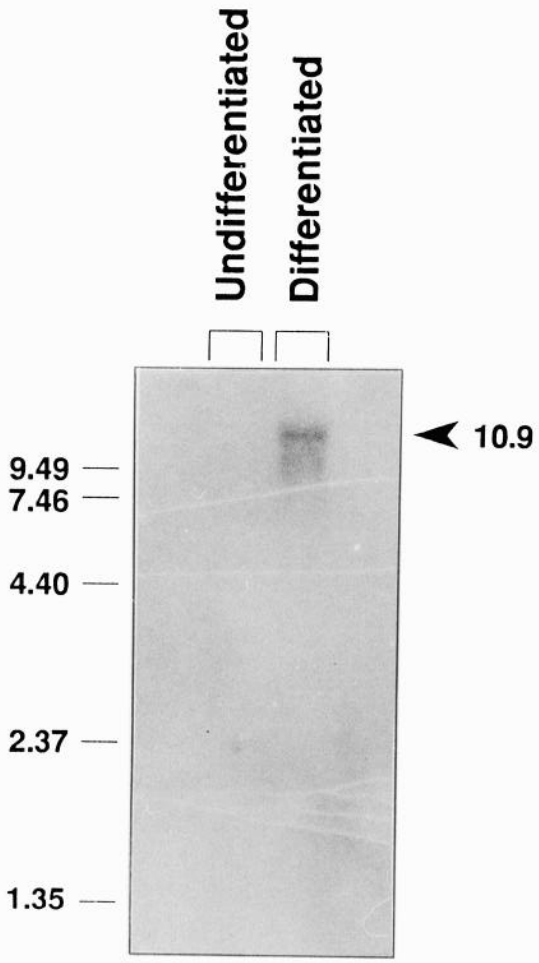


Figure 2. Northern blot analysis of the mouse 5-HT_{1A} receptor from SN-48 cells. Ten micrograms of polyA$^+$ selected RNA isolated from differentiated and nondifferentiated SN-48 cells were loaded in the designated lanes. The blot was probed with ^{32}P-labeled cloned 2.4 kb mouse 5-HT_{1A} receptor cDNA (clone A; see below). The migration of RNA MW standards (GIBCO/Bethesda Research Labs) is indicated at the *left*.

analysis of fetal human peripheral tissues demonstrate a receptor mRNA species of ~6.0 kb (Kobilka et al., 1987; Albert et al., 1990). These differences in size between the three species appear to result from variation in the lengths of untranslated regions of the RNA.

Functional characterization of the 5-HT_{1A} receptors

In order to determine whether the increase in 5-HT_{1A} receptor mRNA that precedes morphologic neuronal differentiation was associated with expression of function responses to 5-HT_{1A} receptor activation in SN-48 cells, actions of receptor activation on intracellular cAMP accumulation were tested (see Materials and Methods). In the nondifferentiated SN-48 cells, and to a lesser extent in differentiated cells, both vasoactive intestinal peptide (VIP) and prostaglandin E_2 (PGE_2) markedly elevated basal cAMP levels (Fig. 3*A*). The 5-HT_{1A} receptor agonist 8-OH-DPAT had no effect on basal or on VIP- or PGE_2-stimulated cAMP accumulation in the nondifferentiated cells, even at high (10 μM) concentration. This suggests that 5-HT_{1A} receptors are not expressed by undifferentiated SN-48 cells, consistent with the lack of 5-HT_{1A} receptor mRNA in Northern blot analysis

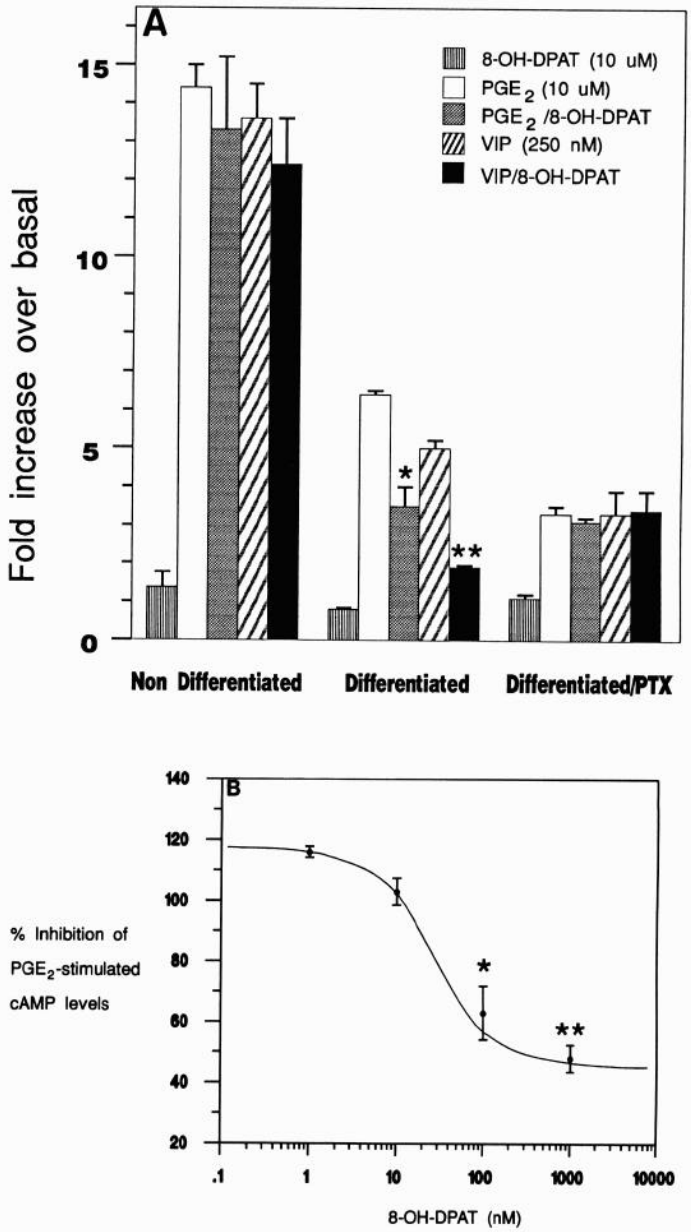
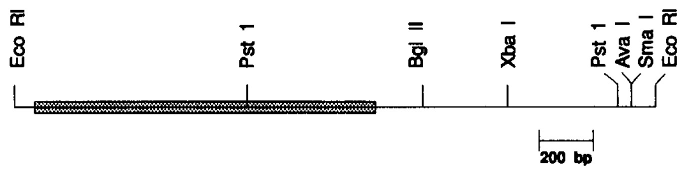


Figure 3. 5-HT_{1A}–mediated inhibition of stimulated cAMP accumulation. Each experiment is representative of three independent assays. *A*, Inhibitory action of 8-OH-DPAT (10 μM) on stimulation of cAMP levels by PGE_2 or VIP in nondifferentiated, differentiated, and PTx-pretreated/differentiated SN-48 cells. PTx (250 ng/ml) was added to differentiated cells 16 hr prior to assay, and the other compounds were present at the indicated concentrations during the assay. The data are presented as the mean ± SEM of three determinations from individual wells of *n*-fold increase over basal cAMP level. The basal cAMP levels (pmol/well) for nondifferentiated, differentiated, and differentiated/PTx were 3.0 ± 0.7, 0.73 ± 0.032, and 0.94 ± 0.10, respectively. Student's *t* test comparing PGE_2- or VIP-stimulated samples in the absence and presence of 8-OH-DPAT are designated *, $p < 0.02$; **, $p < 0.01$; the significance of other data points is readily apparent. *B*, Dose dependency of 8-OH-DPAT–induced inhibition of PGE_2-stimulated cAMP levels. Data are presented as percentage of the cAMP level in the presence of 10 μM PGE_2 alone. The basal and PGE_2-treated cAMP levels were 1.8 ± 0.14 and 7.5 ± 0.9 pmol/well. The data represent mean ± SEM of three determinations from individual wells. Statistically significant differences from PGE_2-stimulated samples using Student's *t* test are indicated as *, $p < 0.05$; **, $p < 0.01$.

A



B

-1
gaattccgcgagggagatccccttgcatttctttttccctcccttccgcccaccccagcgggagtgcaggcaggc
1 90
ATG GAT ATG TTC AGT CTT GGC CAG GGC AAC AAC ACC ACA ACG TCC CTG GAG CCC TTC GGG ACA GGC GGC AAC GAT ACT GGC CTC TCC AAC
MET Asp Met Phe Ser Leu Gly Gln Gly Asn Asn Thr Thr Thr Ser Leu Glu Pro Phe Gly Thr Gly Gly Asn Asp Thr Gly Leu Ser Asn
1 10 20 30
180
GTG ACC TTC AGC TAC CAA GTG ATC ACC TCT CTT TTG CTG GGG ACG CTC ATT TTC TGC GCG GTG CTC GGC AAT GCC TGC GTG GTT GCT GCC
Val Thr Phe Ser Tyr Gln Val Ile Thr Ser Leu Leu Leu Gly Thr Leu Ile Phe Cys Ala Val Leu Gly Asn Ala Cys Val Val Ala Ala
40 50 60
270
ATC GCC CTG GAG CGC TCC CTT CAG AAT GTT GCC AAC TAT CTC ATC GGC TCC TTG GCG GTC ACC GAT CTC ATG GTG TCA GTG CTG GTG CTG
Ile Ala Leu Glu Arg Ser Leu Gln Asn Val Ala Asn Tyr Leu Ile Gly Ser Leu Ala Val Thr Asp Leu Met Val Ser Val Leu Val Leu
70 80 90
360
CCC ATG GCT GCT CTG TAC CAG GTG CTC AAC AAG TGG ACT CTG GGT CAG GTC ACC TGT GAC CTG TTT ATC GCC CTG GAT GTG CTG TGC TGC
Pro Met Ala Ala Leu Tyr Gln Val Leu Asn Lys Trp Thr Leu Gly Gln Val Thr Cys Asp Leu Phe Ile Ala Leu Asp Val Leu Cys Cys
100 110 120
450
ACT TCG TCC ATC CTG CAC CTG TGC GCC ATC GCG CTA GAC AGG TAC TGG GCA ATC ACC GAC CCT ATA GAC TAC GTG AAC AAG AGG ACG CCC
Thr Ser Ser Ile Leu His Leu Cys Ala Ile Ala Leu Asp Arg Tyr Trp Ala Ile Thr Asp Pro Ile Asp Tyr Val Asn Lys Arg Thr Pro
130 140 150
540
CGG CGC GCC GCT GCG CTG ATC TCG CTC ACT TGG CTC ATT GGC TTT CTC ATC TCC ATC CCG CCT ATG CTG GGC TGG CGC GCC CCG GAA GAC
Arg Arg Ala Ala Ala Leu Ile Ser Leu Thr Trp Leu Ile Gly Phe Leu Ile Ser Ile Pro Pro Met Leu Gly Trp Arg Ala Pro Glu Asp
160 170 180
630
CGC TCG AAC CCC AAC GAG TGC ACC ATC AGC AAG GAC CAC GGG TAC ACC ATC TAC TCC ACT TTC GGC GCT TTC TAT ATT CCG CTG CTG CTC
Arg Ser Asn Pro Asn Glu Cys Thr Ile Ser Lys Asp His Gly Tyr Thr Ile Tyr Ser Thr Phe Gly Ala Phe Tyr Ile Pro Leu Leu Leu
190 200 210
720
ATG CTG GTC CTC TAT GGG CGC ATC TTC AGA GCC GCG CGC TTC CGA ATC CGC AAG ACG GTC AAG AAG GTG GAA AAG AAG GGA GCG GGC ACC
Met Leu Val Leu Tyr Gly Arg Ile Phe Arg Ala Ala Arg Phe Arg Ile Arg Lys Thr Val Lys Lys Val Glu Lys Lys Gly Ala Gly Thr
220 230 240
810
AGC TTC GGA ACA TCG TCG GCC CCG CCC CCC AAG AAG AGC CTG AAT GGT CAG CCA GGT AGT GGG GAC TGC AGG CGC AGT GCT GAG AAC AGG
Ser Phe Gly Thr Ser Ser Ala Pro Pro Pro Lys Lys Ser Leu Asn Gly Gln Pro Gly Ser Gly Asp Cys Arg Arg Ser Ala Glu Asn Arg
250 260 270
900
GCG GTG GGG ACT CCG TGC GCT AAT GGG GCG GTG AGA CAG GGT GAG GAC GAC GCC ACC CTG GAG GTG ATC GAG GTG CAT CGA GTG GGC AAC
Ala Val Gly Thr Pro Cys Ala Asn Gly Ala Val Arg Gln Gly Glu Asp Asp Ala Thr Leu Glu Val Ile Glu Val His Arg Val Gly Asn
280 290 300
990
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Ser Lys Gly Asp Leu Pro Leu Pro Ser Glu Ser Gly Ala Thr Ser Tyr Val Pro Ala Cys Leu Glu Arg Lys Asn Glu Arg Thr Ala Glu
310 320 330
1080
GCA AAG CGC AAG ATG GCC TTG GCC CGT GAG AGG AAG ACA GTG AAG ACA CTG GGC ATC ATC ATG GGC ACC TTC ATC CTC TGC TGG CTG CCC
Ala Lys Arg Lys Met Ala Leu Ala Arg Glu Arg Lys Thr Val Lys Thr Leu Gly Ile Ile Met Gly Thr Phe Ile Leu Cys Trp Leu Pro
340 350 360
1170
TTT TTC ATT GTG GCG CTG GTC CTA CCT TTC TGT GAG AGC AGT TGC CAC ATG CCT GAG TTG TTG GGT GCC ATA ATT AAC TGG CTA GGC TAC
Phe Phe Ile Val Ala Leu Val Leu Pro Phe Cys Glu Ser Ser Cys His Met Pro Glu Leu Leu Gly Ala Ile Ile Asn Trp Leu Gly Tyr
370 380 390
1260
TCC AAC TCC CTG CTC AAC CCA GTT ATT TAT GCT TAT TTC AAC AAA GAC TTT CAA AAC GCT TTT AAG AAG ATC ATC AAG TGC AAG TTC TGC
Ser Asn Ser Leu Leu Asn Pro Val Ile Tyr Ala Tyr Phe Asn Lys Asp Phe Gln Asn Ala Phe Lys Lys Ile Ile Lys Cys Lys Phe Cys
400 410 420
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CGC TGA TGA TGA TGA tggtggtggtagtggtggtaatagtggtggtgatggagaaggaagaagtggaggaagagtgtagggcttacaggacccttcctgttcactcaatatcccc
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gccctggaagcaacacctaagaaaacttgcttttcctagaacacgcagcttcttagcccatggctttcagatcttctctctctatctcattttaaggggacagtgcttttttgcagaaatc
ttagagaacccctagcatctgtttgtaaaattattggctcggtctttggccctaggatcagttttgatagttgcaataacctcctctctattcgaaatcccaattcttcacgatggaagt
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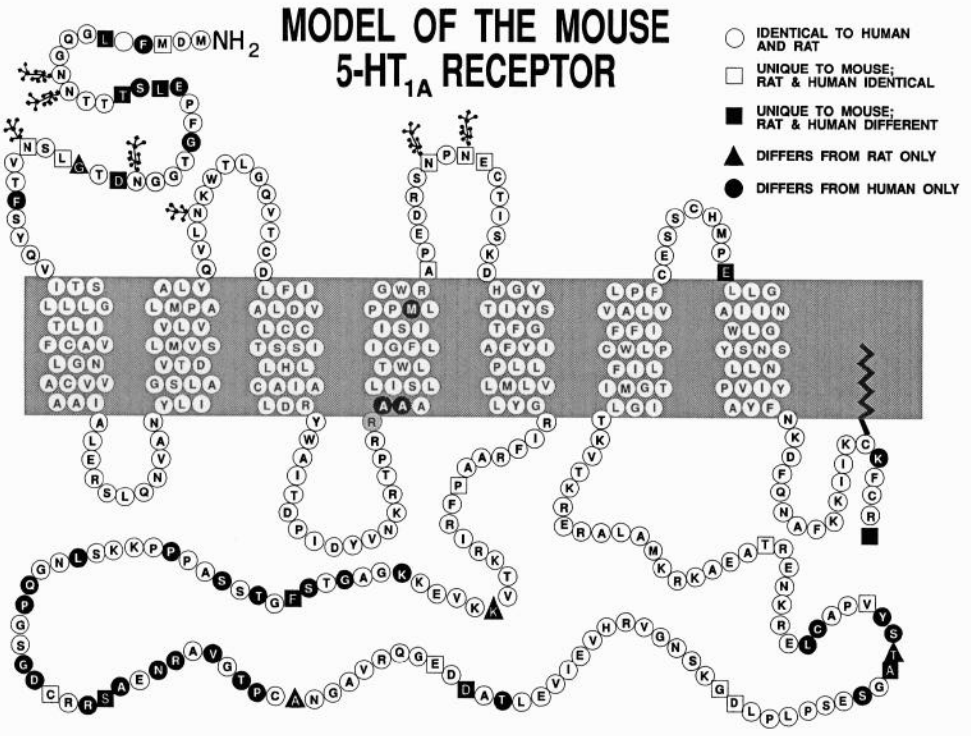

Figure 5. Schematic representation of the murine 5-HT_{1A} receptor. A hypothetical model of the 5-HT_{1A} receptor based on hydropathicity analysis and structural evidence from other G-protein–coupled receptors is shown. The amino acid residues that differ from the rat and/or human receptor sequences are indicated. Putative N-linked glycosylation sites are indicated by *small arboreous symbols.*

(Fig. 2). In contrast, the nondifferentiated cells apparently express PGE_2 and VIP receptors. However, in the differentiated state, 8-OH-DPAT significantly decreased both the VIP- and the PGE_2-stimulated levels of cAMP by 54% and 37%, respectively (Fig. 3*A*). This inhibition is mediated through PTx-sensitive G-proteins since 16 hr of pretreatment of the differentiated SN-48 cells with 250 ng/ml of PTx completely abolished the 8-OH-DPAT–mediated inhibition of stimulated cAMP accumulation (Fig. 3*A*). PTx pretreatment did not, however, exert any effect in the undifferentiated cells (data not shown).

In order to determine whether the inhibitory actions of 10 μM 8-OH-DPAT are mediated by a 5-HT_{1A}–like receptor, reduced concentrations of 8-OH-DPAT were shown to inhibit PGE_2-stimulated cAMP levels in differentiated SN-48 cells (Fig. 3*B*), with a maximal inhibition identical to that obtained with 10 μM 8-OH-DPAT (Fig. 3*A*). In three independent experiments, 8-OH-DPAT inhibited PGE_2-induced cAMP accumulation with an EC_{50} value of 20 ± 5 nM. At the lowest concentration examined (1 nM), 8-OH-DPAT appeared to enhance slightly PGE_2-induced cAMP accumulation; however, this change was not statistically significant. The estimated EC_{50} value for inhibition of cAMP levels by 8-OH-DPAT is consistent with the values of 10–20 nM obtained for the mouse hippocampal (Dumuis et al., 1988) or the cloned rat 5-HT_{1A} receptors (Liu and Albert, 1991; Fowler et al., 1992), and supports the 5-HT_{1A}–like nature of the receptor in differentiated SN-48 cells.

These results correlate with the increase in 5-HT_{1A} receptor mRNA levels, and strongly suggest that differentiated SN-48 cells express functional 5-HT_{1A} receptors that are coupled to PTx-sensitive G-proteins to inhibit adenylyl cyclase. The GABAergic phenotype of these cells is consistent with the recent observation that 5-HT_{1A} receptors are located on certain GABAergic neurons of the rat septum (Pompeiano et al., 1992), the neurons from which the SN-48 cells may be derived.

Cloning of a murine 5-HT_{1A} receptor cDNA

Because of the large size of the mouse 5-HT_{1A} receptor mRNA, we wished to determine the nature of the coding sequence in SN-48 and mouse brain. A 918 bp fragment was isolated by RT-PCR of RNA from the differentiated SN-48 cells using primers specific for the 5-HT_{1A} receptor. The cloned RT-PCR fragment had striking similarity to corresponding regions of the rat and human 5-HT_{1A} receptor genes, and was identical to an analogously isolated fragment from mouse hippocampus total RNA (data not shown). Using this fragment as a probe, a P18 mouse brain λgt11 cDNA library was screened under high stringency and a single clone containing a 2.4 kb cDNA insert (clone A) was isolated and sequenced (Fig. 4). The sequence of clone A contained a single open reading frame encoding a 421 amino acid residue protein with a predicted molecular weight of 46 kDa. Analysis of clone A revealed a region with identity (100%) to sequences derived from the 918 bp RT-PCR fragments from mouse brain and differentiated SN-48 cells (data not shown).

The overall sequence homology with the rat and human 5-HT_{1A} receptor gene (nucleotide : amino acid) was as follows: mouse–rat: 93:94%; mouse–human: 80:86%. Computer-aided hydrophobicity analysis of the predicted amino acid composition of clone A indicated the presence of seven hydrophobic domains

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Figure 4. Restriction map and sequence of the mouse 5-HT_{1A} receptor cDNA. *A,* Restriction endonuclease map of the 2.4 kb cDNA clone A isolated from a mouse brain λgt11 library. The *shaded box* represents the position of the coding sequences. *B,* Nucleotide sequence of the mouse 5-HT_{1A} receptor cDNA. *Underlined* are the putative seven transmembrane domains and *bolditalic underlined nucleotides* represent a putative polyadenylation signal sequence.

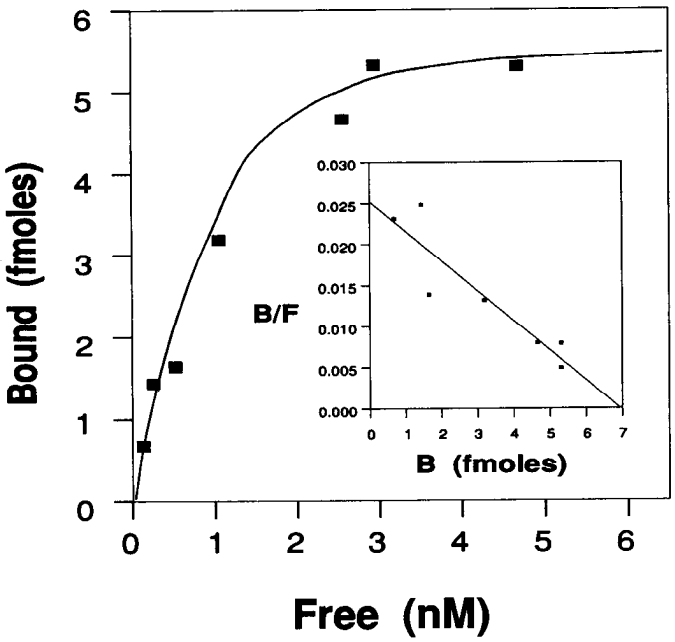

Figure 6. Specific binding of ^{3}H-8-OH-DPAT to membranes from LM1A-5 cells: saturation analysis of the specific binding of ^{3}H-8-OH-DPAT to membranes prepared from Ltk$^-$ cells stably transfected with the murine 5-HT$_{1A}$ receptor. The *abscissa* represents the free concentration of ^{3}H-8-OH-DPAT corrected by substracting the amount bound specifically to membranes. The *ordinate* is the amount of specific bound ^{3}H-8-OH-DPAT (total minus nonspecific). *Inset,* Transformation of the above data for Scatchard analysis. The data were best fit by a one-site model (*plotted line*) with a K_D of 1.68 ± 0.36 nM and a B_{max} of 0.19 ± 0.05 pmol/mg protein.

with the potential of forming transmembrane α-helices (Fig. 5), consistent with the structure predicted for G-protein–coupled receptors (Caron and Lefkowitz, 1991). Sporadic amino acid differences were localized in regions of the receptor that have low conservation between human and rat homologs (e.g., the amino-terminal and the central portion of the third cytoplasmic loop), consistent with species differences observed in other G-protein–coupled receptors. The strong overall sequence similarity between clone A and the rat and human 5-HT$_{1A}$ receptors suggested that clone A encodes the mouse 5-HT$_{1A}$ receptor. In order to substantiate this hypothesis, radioligand binding assays were performed.

Clone A was subcloned into a mammalian expression vector and stably transfected in mouse Ltk$^-$ fibroblast cells as described in Materials and Methods. Membranes prepared from the cloned cells LM1A-5 exhibited binding features characteristic of 5-HT$_{1A}$ receptors. Scatchard analysis of saturation binding experiments using ^{3}H-8-OH-DPAT as ligand indicated a single site with K_D of 1.68 nM ± 0.36 (Fig. 6), similar to the value (0.8 nM) obtained for the cloned rat 5-HT$_{1A}$ receptor (Albert et al., 1990). Moreover, ligand binding competition experiments demonstrated the same rank order of potency as observed for the rat and human 5-HT$_{1A}$ receptors for the following compounds: 8-OH-DPAT > 5-HT > spiroxatrine > spiperone. Thus, clone A encodes a murine 5-HT$_{1A}$ receptor.

Conclusion

Based on the pharmacological profile exhibited by clone A when expressed heterologously in mammalian cultured cells, as well as its strong homology with the human and rat 5-HT$_{1A}$ receptors, we conclude that clone A encodes the mouse 5-HT$_{1A}$ receptor cDNA. In addition, we have identified an inducible neuronal cell line that expresses endogenously the murine 5-HT$_{1A}$ receptor upon morphological differentiation. This cell line represents an excellent model system to investigate the natural environment in which the 5-HT$_{1A}$ receptor exerts its actions. Moreover, the SN-48 cells provide a powerful paradigm for investigating the aspects of neuronal differentiation leading to the development of sensitivity to serotonergic input through 5-HT$_{1A}$ receptors.

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