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Differential addressing of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in epithelial cells and neurons

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SUMMARY

The 5-HT$_{1A}$ and 5-HT$_{1B}$ serotonin receptors are expressed in a variety of neurons in the central nervous system. While the 5-HT$_{1A}$ receptor is found on somas and dendrites, the 5-HT$_{1B}$ receptor has been suggested to be localized predominantly on axon terminals. To study the intracellular addressing of these receptors, we have used in vitro systems including Madin-Darby canine kidney (MDCK II) epithelial cells and primary neuronal cultures. Furthermore, we have extended these studies to examine addressing in vivo in transgenic mice. In epithelial cells, 5-HT$_{1A}$ receptors are found on both apical and basolateral membranes while 5-HT$_{1B}$ receptors are found exclusively in intracellular vesicles. In hippocampal neuronal cultures, 5-HT$_{1A}$ receptors are expressed on somatodendritic membranes but are absent from axons. In contrast, 5-HT$_{1B}$ receptors are found on both dendritic and axonal membranes, including growth cones where they accumulate. Using 5-HT$_{1A}$ and 5-HT$_{1B}$ knockout mice and the binary tTA/tetO system, we generated mice expressing these receptors in striatal neurons. These in vivo experiments demonstrate that, in striatal medium spiny neurons, the 5-HT$_{1A}$ receptor is restricted to the somatodendritic level, while 5-HT$_{1B}$ receptors are shipped exclusively toward axon terminals. Therefore, in all systems we have examined, there is a differential sorting of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors. Furthermore, we conclude that our in vivo transgenic system is the only model that reconstitutes proper sorting of these receptors.

Key words: Intracellular addressing, MDCK II cell, Hippocampal neuron, Striatum, Transgenic mouse, Axonal transport

INTRODUCTION

The 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors have been suggested to be differentially distributed in neurons. These receptors belong to the 5-HT1 family of serotonin receptors, and like all members of this family, are negatively coupled to adenylyl cyclase (Hoyer et al., 1994; Saudou and Hen, 1994). Both are auto- and hetero-receptors, and their activation modulates the activity of several neuronal systems. A major difference between the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors is their distribution in the central nervous system (Kia et al., 1996; Sari et al., 1997). In addition, even when these two receptors are expressed in the same neurons, their subcellular localization seems to differ as well. In raphe neurons, for instance, the 5-HT$_{1A}$ receptor is localized on somas and dendrites (Sprouse and Aghajanian, 1987; Kia et al., 1996). In contrast, the 5-HT$_{1B}$ receptor has been suggested to be localized on the axon terminals of these neurons (Gothert et al., 1987). This subcellular segregation appears to be respected throughout the central nervous system. In fact, comparisons between expression patterns of mRNA and protein reveal a perfect match for the 5-HT$_{1A}$ receptor (Miquel et al., 1991; Pompeiano et al., 1992), while there is a mismatch for the 5-HT$_{1B}$ receptor (Boschert et al., 1994). For example, a high level of 5-HT$_{1B}$ mRNA is found in striatal neurons, while the 5-HT$_{1B}$ protein is abundant in the substantia nigra and globus pallidus, the main projection areas of the striatum (Boschert et al., 1994). Although these studies, along with lesion studies (Waeber and Palacios, 1990; Waeber et al., 1990), suggest that the 5-HT$_{1B}$ receptor is only localized on axon terminals, it is not clear whether the 5-HT$_{1B}$ receptor is also expressed at the somatodendritic level.

In order to study the addressing of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, we first expressed cDNAs encoding N-terminal hemagglutinin-tagged versions of these receptors in polarized Madin-Darby canine kidney cells (MDCK II) and in differentiated hippocampal cells in culture. We chose MDCKII cells because their plasma membrane is divided into functionally and morphologically distinct domains

(Rodriguez-Boulan and Powell, 1992) and previous studies have suggested that the basolateral and apical domains of epithelial cells correspond to the somatodentritic and axonal domains, respectively, of neurons (Dotti and Simons, 1990). Secondly, we further examined the targeting of the 5-HT_{1A} and 5-HT_{1B} receptors in an in vivo model of 'rescue' mice.

The current study shows that the 5-HT_{1A} and 5-HT_{1B} receptors achieve different localization in both MDCK II cells and hippocampal neurons in culture. Additionally, a comparison between expression patterns of mRNA and protein in 5-HT_{1B} rescue mice revealed that the 5-HT_{1B} receptor is localized exclusively on the projection areas of striatal neurons. In contrast, in 5-HT_{1A} rescue mice, 5-HT_{1A} receptors are localized in the cell bodies of striatal neurons and are not transported to the terminals of these neurons.

MATERIALS AND METHODS

Cell culture

MDCK II cells and MDCK II cells expressing the hemagglutinin tagged alpha 2A adrenergic receptor (α_{2A}-AR) were kindly provided by Dr Lee E. Limbird (Vanderbilt University, Nashville TN). Cells were cultured in minimum essential medium (MEM) with 10% fetal calf serum (Gibco BRL, Gaithersburg, MD), 4 mM L-glutamine, 10 units/ml penicillin, and 10 μg/ml streptomycin (Speciality Media, Lavallette New Jersey). Stably transfected MDCK II cells were maintained in the above medium supplemented with 1 mg/ml Geneticin (Gibco BRL, Gaithersburg, MD). Methods for preparing the hippocampal cell cultures have been described previously (Goslin and Banker, 1992). In brief, hippocampi were dissected from the brains of embryonic day 18 rats and a cell suspension was prepared by trypsin treatment and trituration using a fire-polished Pasteur pipette. Cells were then plated onto acid-washed, poly-L-lysine-treated glass coverslips (Fisher, 18CIR-1 D, German glass, special order) in MEM with 10% horse serum. After the neurons attached to the substrate, the coverslips were inverted and transferred into a dish containing a confluent monolayer of astroglia and were maintained in serum-free medium (MEM containing the N2 supplements of Bottenstein and Sato (1979)), together with 0.1 mM sodium pyruvate and 0.1% ovalbumin. Small dots of paraffin on the coverslips supported them just above the glial monolayer (see Goslin and Banker, 1992).

Construction of recombinant adenoviruses expressing hemagglutinin-tagged versions of the 5-HT_{1A} and 5-HT_{1B} receptors and infection of neurons in culture

Methods for the construction of adenoviruses expressing the hemagglutinin (HA) tagged version of the 5-HT_{1B} receptor (Adeno-1B) have been described previously (Ghavami et al., 1997). In brief, the hemagglutinin epitope (YPYDVPDYA) which is recognized by the commercially available monoclonal antibody HA12CA5 (Boehringer Mannheim, Indianapolis, IN) was linked to the extracellular amino terminus of the 5-HT_{1A} and 5-HT_{1B} receptors. These constructions were subcloned into the expression vector pAd.CMV (a gift from Dr Falck-Pederson, Cornell University, New York, NY). The resulting plasmids were co-transfected into low passage HEK 293 cells (generously provided by Dr Hamish Young, Columbia University, New York, NY) together with the large *Cla*I DNA fragment of the d1324 mutant of type 5 adenovirus (Rosenfeld et al., 1992). Homologous recombination events between these two DNA molecules generated two replication-deficient adenoviruses, Adeno-1A and Adeno-1B, which were expressing the Tag-5-HT_{1A} and Tag-5-HT_{1B} receptors, respectively, under control of the CMV promoter. These viruses were subsequently plaque purified and then purified by CsCl gradient centrifugation (Becker et al., 1994). The titers of the Adeno-1A and Adeno-1B were determined using a plaque assay and were estimated to be 10^{11} pfu/ml.

Neurons were infected with Adeno-1A or Adeno-1B after 8 days in culture through the addition of an aliquot of viral stock directly to the medium in dishes containing neuronal coverslips. The amount of virus added was titered such that only 1-5% of the neurons were infected.

Stable expression of cDNAs in MDCK II cells

Non-confluent MDCK II cells were cotransfected with 20 μg of circular Tag-1A or Tag-1B plasmids and 1 μg of pcDNA3 (Invitrogen, Carlsband, CA) to provide resistance to Geneticin, in the presence of 0.1 mM chloroquine using the calcium phosphate method as described elsewhere (Sambrook et al., 1989). Stably expressing clones were selected in 1 mg/ml Geneticin, isolated with cloning rings, and screened for expression by immunocytochemistry.

Immunocytochemistry

Parental or transfected MDCK II cells were plated at the density of 10^6 cells on 24 mm polycarbonate membrane filters (Transwell chambers, 0.4 μm pore size, Costar, Cambridge, MA) and were grown for 5-7 days. Cells were rinsed 3 times in phosphate-buffered saline (PBS) and then were fixed in 4% paraformaldehyde in PBS for 15 minutes at 37°C. Then the cells were rinsed three times in PBS and permeabilized with 0.3% Triton X-100 in PBS for 5 minutes at room temperature. Nonspecific sites were saturated with 10% goat serum (Sigma, St Louis, MO) in PBS supplemented with 0.1% Triton for 1 hour at 37°C. The cells were then incubated with anti-HA antibody (1:100 dilution in PBS supplemented with 10% goat serum) overnight at 4°C. After three 10 minute washes with PBS, they were carried through standard avidin-biotin immunohistochemical protocols using an Elite Vectastain Kit (Vector Laboratories Inc, Burlingame, CA). The chromogen reaction was performed with diaminobenzidine (Sigma, St Louis, MO) and 0.003% H_2O_2 for five minutes. After three 2 minute washes in PBS, cells were post-fixed 5 minutes in 2% glutaraldehyde and then rinsed three times, 2 minutes each, in phosphate buffer. Filters were cut out and incubated for 30 minutes with 1% osmium tetroxide (EMS, Washington, PA) in 0.2 M sodium phosphate buffer, pH 7.4. After rinsing four times, 5 minutes each, filters were dehydrated in graded ethanols and embedded gradually in Epon 812. Sections of 1 μm thickness perpendicular to the plane of the monolayer were cut and observed with a light microscope. For the electron microscopy experiments, ultrathin sections (80 nm) of Epon embedded with an Ultracut E microtome (Reichert, Austria) were made. Sections were then slightly stained with uranyle acetate (2 minutes, 5% water solution), and viewed in an electron microscope (Philips 201).

For immunofluorescence staining of cells grown in Transwell cultures, cells were fixed, permeabilized, and the non-specific epitopes were blocked via the same protocol as described above. The cells were then incubated with both a 1:100 dilution of primary mouse HA antibody and 1:500 dilution of rat uvomorulin antibody (Sigma, St Louis, MO). This was followed by incubation in a 1:100 dilution of the secondary Cy3-conjugated goat anti-mouse and FITC-conjugated goat anti-rat antibodies.

To detect proteins present on the cell surface, living MDCK II cells grown in transwell culture were incubated with 1:100 primary HA antibody at both their apical and basolateral domains for 1 hour at 37°C. Filters were rinsed in PBS, fixed, permeabilized by incubation in 0.3% Triton X-100 in PBS for 5 minutes, and then incubated in 10% goat serum supplemented with 0.1% Triton for 1 hour at 37°C. Cells were then incubated with the secondary Cy3-conjugated goat anti-mouse antibody. Samples were visualized by Zeiss fluorescence microscopy.

To detect virally expressed proteins, hippocampal neurons in culture were fixed in 4% paraformaldehyde/0.1% glutaraldehyde/4% sucrose in PBS. Cells were permeabilized by incubation in 0.25%

Triton X-100 in PBS for 5 minutes and then incubated in 10% bovine serum albumin (overnight at 4°C or for 2 hours at 37°C). Coverslips then were incubated with HA antibody (same conditions as above) and polyclonal anti-microtubule associated protein 2 (MAP2) antibody (Caceres and Dotti, 1985), overnight at 4°C. This was followed by incubation in FITC-conjugated streptavidin and the appropriate rhodamine or Texas Red-conjugated secondary antibody to detect MAP2. Processes labeled with MAP2 were considered as dendrites and MAP2-negative processes were considered as axons. Images of immunofluorescently labeled cells were acquired using a Photometrics CH250 chilled CCD camera (12-bit images; 1315×1017 pixels) and a Zeiss Axiophot (×25 Planapo objective; NA 1.2).

Generation of 5-HT$_{1B}$ rescue mice

Plasmid pUHD10-3 containing tet operator sequences upstream of a CMV minimal promoter was kindly provided by Prof. H. Bujard, ZMBH, Heidelberg, Germany. The multiple cloning site of this plasmid was modified by standard cloning procedures and a rabbit β-globin intron II obtained from expression vector p514, a derivative of pSG5 (Green et al., 1988), was cloned into this linker. The previously described hemagglutinin tagged 5-HT$_{1B}$ receptor (Ghavami et al., 1997) was then cloned 3′ of this intron. Transgenic mice were generated by pronuclear injection using standard techniques (Hogan et al., 1994) and analyzed by Southern blot analysis. Positive mice (tetOTag1B mice) were then bred with transgenic mice expressing the tetracycline transactivator (tTA) under the control of the α-calcium calmodulin dependent kinase II (α-CaMKII) promoter. These α-CaMKII-tTA mice have been previously described (Mayford et al., 1996) and were generously provided by Dr Mark Mayford and Dr Eric Kandel (Columbia University, New York, NY). Doubly transgenic mice (α-CaMKII-tTA/tetOTag1B) were then bred with existing 5-HT$_{1B}$ knockout mice (Saudou et al., 1994) until 'rescue' mice were obtained. These final mice were doubly transgenic (α-CaMKII-tTA/tetOTag1B) and homozygous mutant at the 5-HT$_{1B}$ locus (See Fig. 5).

Generation of 5-HT$_{1A}$ rescue mice

5-HT$_{1A}$ rescue mice were produced by breeding the above described α-CaMKII-tTA transgenic mice with 5-HT$_{1A}$ knockout mice (Ramboz et al., 1998) containing a cassette where the 5-HT$_{1A}$ gene was under the control of tet operator sequences and a CMV minimal promoter.

Animals and tissue collection

Adult mice were decapitated. Brains were rapidly removed and frozen by placing them on dry ice. Coronal sections (20 μm) were obtained with a cryostat at –20°C and thaw-mounted on either poly-L-lysine-coated slides for in situ hybridization experiments or gelatin-coated slides for autoradiography experiments.

In situ hybridization

100 ng of antisense synthetic oligonucleotide corresponding to either the hemagglutinin epitope or tTA (5′ GCTCTACACCT-AGCTTCTGGGCGAGTTTACGGGTTGTAAAC 3′) was labeled with 3 μl of [α-^{33}P]dATP (1825 Ci/mmol, 10 mCi/ml, DuPont NEN, Boston, MA) in the presence of 50 units of terminal transferase (Boehringer-Mannheim, Indianapolis, IN). The protocol used for in situ hybridization using synthetic oligonucleotides has been described previously (Wisden and Morris, 1994).

5-HT$_{1B}$ receptor autoradiography

Sections were preincubated for 1 hour at 4°C in Krebs solution (mM: 118 NaCl, 4.8 KCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 15 Tris-HCl, pH 7.4) as described by Boulenguez et al. (1992). To determine the total binding to 5-HT$_{1B}$ sites, sections were incubated for 1 hour at room temperature in Krebs solution to which pargyline (10 μM), ascorbic acid (0.01%), 10 μM 8-OH-DPAT (RBI, Natick, MA), and 30 pM [^{125}I]GTI (2000 Ci/mmol Immunotech S.A) were added. The [^{125}I]-GTI is a ligand specific for 5-HT$_{1B}$ and 5-HT1Dα receptors, but under the conditions used in our autoradiographic studies, most of the binding sites corresponding to 5-HT1Dα receptors were displaced by 10 μM 8-OH-DPAT. The total binding to 5-HT$_{1B}$ sites was also determined by using 3-[^{125}I] iodocyanopindolol (DuPont NEN, Boston, MA) as the radioligand (not shown). In these experiments 3-[^{125}I] iodocyanopindolol ([^{125}I]-CYP) was used at 30 pM in the presence of 30 μM isoproterenol (Sigma, St Louis, MO) and 100 nM 8-OH-DPAT. In both cases, the nonspecific binding was determined with 10 μM 5-HT (Sigma, St Louis, MO). After incubation, sections were rinsed (2× 1 minute) in cold distilled water and then dried. To measure bound radioactivity, sections were exposed to a film (Kodak Biomax MR) for three days.

5-HT$_{1A}$ receptor autoradiography

Sections were preincubated in 50 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.4, and then in the same buffer supplemented with 0.14 nM of [^{125}I]-4-(2′-methoxy-phenyl)-1-[2′-(n-2′′-pyridinyl)-*p*-iodobenzamido]-ethyl-piperazine (2200 Ci/mmol, [^{125}I]-*p*-MPPI) for 2 hours at room temperature as described by Kung et al. (1995). Non-specific binding was determined in the presence of 10 μM 5-HT.

RESULTS

Differential localization of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in MDCK II cells

To study the sorting properties of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in MDCK II cells, we compared the distribution of these receptors to that of the alpha 2A adrenergic receptors (α_{2A}-AR). We used α_{2A}-AR as a control because this receptor is also tagged with a hemagglutinin epitope on its amino terminus and because the presence of this epitope did not interfere with the proper sorting of this receptor in MDCK II cells (Keefer and Limbird, 1993). The stable expression of the 5-HT$_{1A}$, 5-HT$_{1B}$, and α_{2A}-AR receptors in MDCK II cells did not alter the polarization of these cells as judged by the pattern of distribution of uvomorulin (McNeill et al., 1990), a marker for the lateral membrane (data not shown). Staining of α_{2A}-AR expressing cells with the HA antibody revealed that this receptor was localized at the lateral edges of these cells (Fig. 1A), as had been previously reported (Keefer and Limbird, 1993). In order to more precisely localize these receptors, filters embedded in Epon were cut perpendicular to the plane of the monolayer and observed with either a light (Fig. 1) or electron microscope (Fig. 2). These sections revealed that the majority of the α_{2A}-AR receptor was confined to the lateral microdomain of the basolateral surface (Fig. 1D, 2B3). A lower level of expression was also found on the basal surface (Fig. 1D, Fig. 2B2).

In contrast, sections from 5-HT$_{1A}$ expressing cells examined by light microscopy revealed that this receptor was localized on both basolateral and apical domains (Fig. 1E). Moreover, at the basolateral surface, the 5-HT$_{1A}$ receptor was distributed equally on both lateral and basal microdomains. In a small fraction of cells displaying a low level of immunoreactivity (about 5%), the 5-HT$_{1A}$ receptor could only be detected on the basolateral surface. However, electron microscopy showed that the apical membrane and microvilli were labeled in all 5-HT$_{1A}$ receptor expressing cells (Fig. 2C1).

The distribution of the 5-HT$_{1B}$ receptor was completely

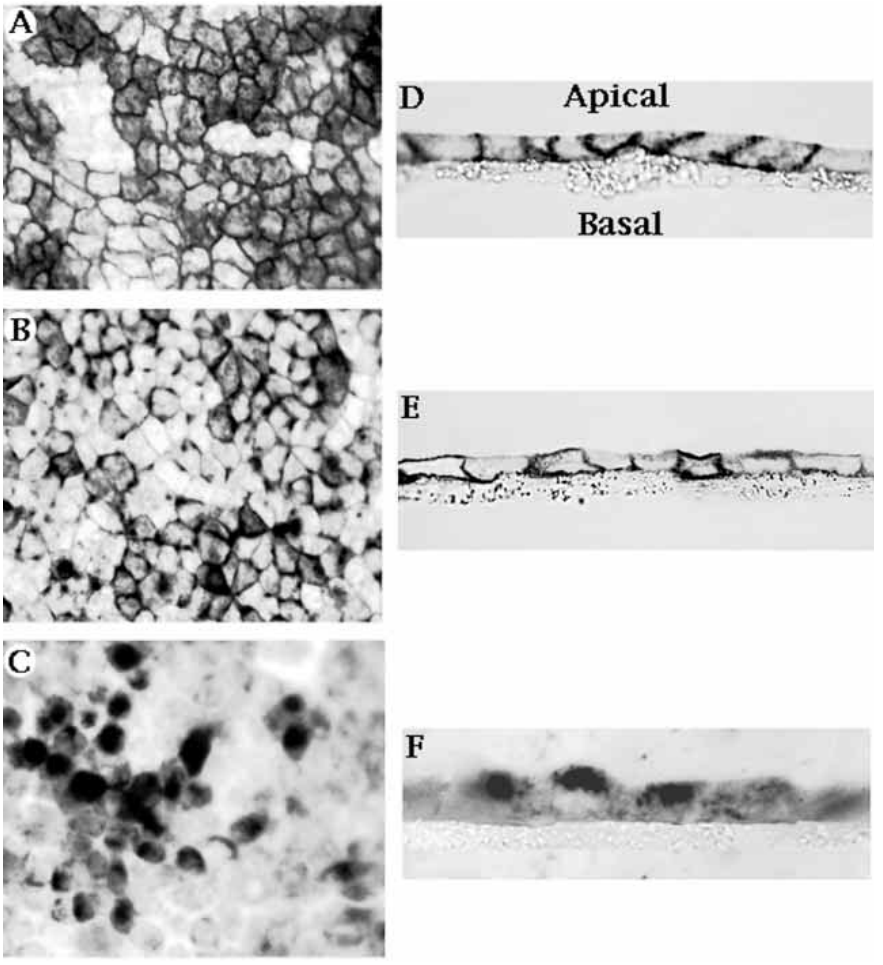

Fig. 1. Differential localization of α_{2A}-AR, 5-HT_{1A}, and 5-HT_{1B} receptors in transfected MDCK II cells. Confluent monolayers of MDCK II clonal cell lines stably expressing either α_{2A}-AR (A and D) or the 5-HT_{1A} receptor (B and E) or the 5-HT_{1B} receptor (C and F) were grown on 0.4 μm filters, fixed, permeabilized, and labeled with an anti-HA antibody. Filters were then carried through standard avidin-biotin immunohistochemical protocols, embedded in Epon 812, and observed on face with a light microscope (A,B,C). Sections of 1 μm thickness perpendicular to the plane of monolayer were cut and observed with a light microscope (D,E,F). The α_{2A}-AR labeling was restricted to the basolateral membrane (A and D). The 5-HT_{1A} receptor labeling was associated with both apical and basolateral surfaces (B and E) while 5-HT_{1B} receptor labeling was found between the apical surface and the nucleus (C and F).

different from that of the α_{2A}-AR and 5-HT_{1A} receptors (Fig. 1C). All the 5-HT_{1B} expressing cells displayed an intense labeling beneath the apical surface, as seen by light microscopy (Fig. 1F). Even cells expressing high levels of 5-HT_{1B} receptors did not appear to display any surface staining. Closer examination of the 5-HT_{1B} receptors by electron microscopy revealed that the plasma membrane of these cells was not labelled (Fig. 2D1-D3). Only vesicular structures found in the apical cytoplasm of the 5-HT_{1B} expressing cells were stained (Fig. 2E). The intracellular localization of the 5-HT_{1B} receptor was further confirmed by immunofluorescence labeling of live cells. When the HA antibody was added to live cells, no immunoreactivity could be detected. In contrast, when the reaction was performed on fixed and permeablilized cells, a vesicular-like staining was observed (data not shown). As a control, 5-HT_{1A} receptor expressing cells displayed the same surface staining under both live and permeabilized conditions (data not shown).

Differential distribution of 5-HT_{1A} and 5-HT_{1B} receptors in infected hippocampal neurons

The distribution of the 5-HT_{1A} receptor in an isolated hippocampal neuron is illustrated in Fig. 3. All the processes of this neuron which were labeled with the anti-HA antibody were also positive for MAP2. The axonal processes visible by phase contrast microscopy did not express MAP2 or the 5-HT_{1A} receptor (10 cells chosen from 3 separate culture preparations). Only very proximal axonal staining was observed in a few 5-HT_{1A} expressing cells (<200 μm). These results show that the expression of the 5-HT_{1A} receptor in cultured hippocampal neurons is highly restricted to the somatodendritic domain of these neurons.

The distribution of the 5-HT_{1B} receptor in hippocampal neurons is illustrated in Fig. 4. Virtually all neuronal processes of the infected neuron were labelled. The 5-HT_{1B} receptor was therefore associated with both MAP2-positive and MAP2-negative processes. The labeling of MAP2-negative processes clearly demonstrated the presence of the 5-HT_{1B} receptor in axons. Furthermore, the axonal expression of the 5-HT_{1B} receptor extended throughout the entire axonal arbor. In particular, axonal growth cones and distal tips were highly labeled (Fig. 4, arrowheads). In contrast to the proximal axonal labeling observed with some 5-HT_{1A} receptor expressing cells, axonal staining of the 5-HT_{1B} receptor, in all cases, extended more than 1 mm from the cell body (10 cells chosen from 3 separate culture preparations).

Differential transport of 5-HT_{1A} and 5-HT_{1B} receptors in striatal neurons in 'rescue mice'

To determine whether the localization of the 5-HT_{1A} and 5-HT_{1B} receptors in cultured neurons was identical to that found in vivo in the striatum, we created two lines of rescue mice by using the binary tTA/tet-O system (Gossen and Bujard, 1992), the α-CaMKII promoter, and 5-HT_{1A} (Ramboz et al., 1998)

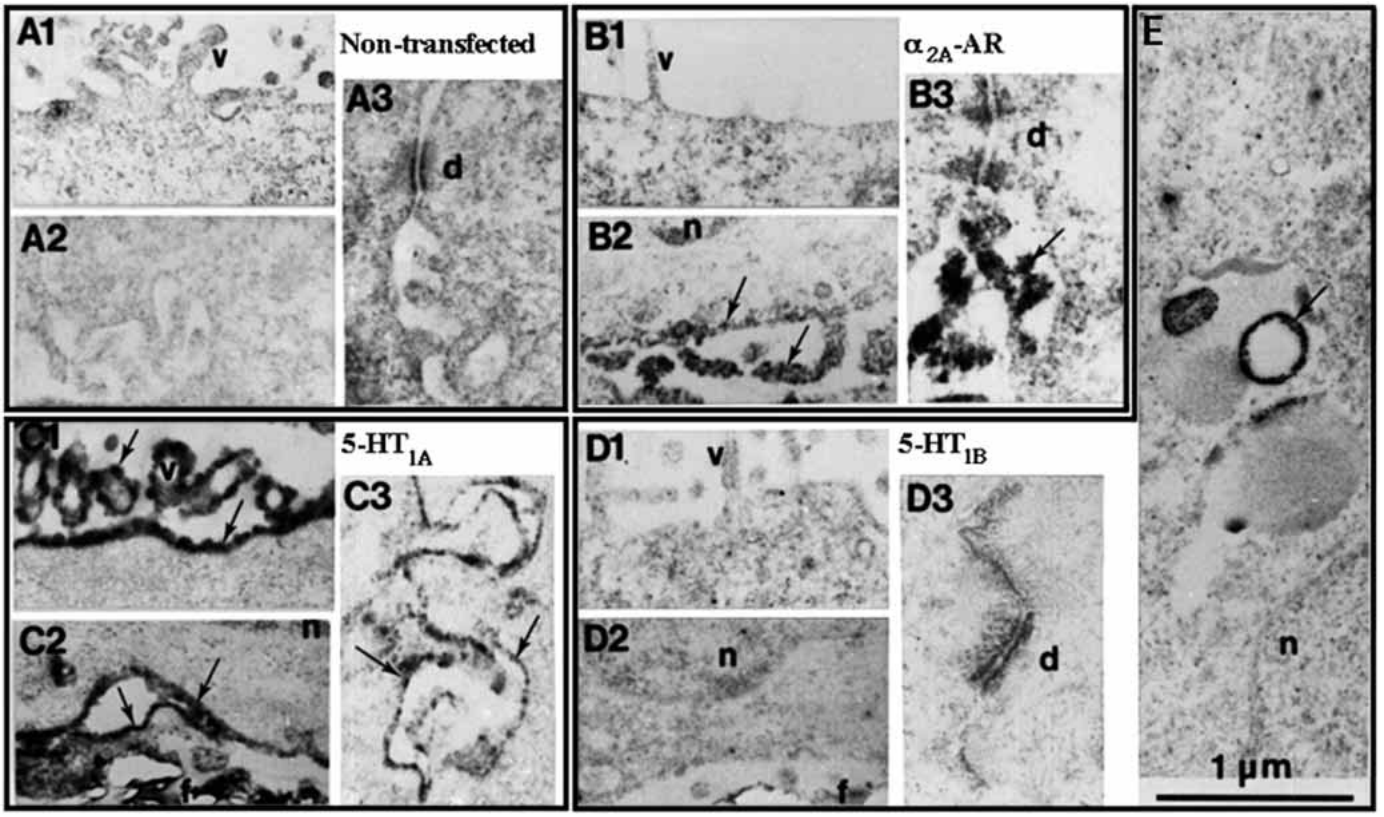

Fig. 2. Electronic microscopy immunolocalization of α_{2A}-AR, 5-HT$_{1A}$, and 5-HT$_{1B}$ receptors in transfected MDCK II cells. 80 nm sections of Epon embedded filters of non-transfected MDCK II cells (A1, A2, A3), α_{2A}-AR expressing cells (B1, B2, B3), 5-HT$_{1A}$ expressing cells (C1, C2, C3), and 5-HT$_{1B}$ expressing cells (D1, D2, D3, E) were cut and observed with an electron microscope. A1, B1, C1, D1 correspond to apical surfaces, A2, B2, C2, D2 correspond to basal surfaces, A3, B3, C3, D3 correspond to lateral surfaces. Specific immunostaining is indicated by arrows. No labeling was detected on the cell surface of non-transfected (A1, A2, A3) or 5-HT$_{1B}$ expressing cells (D1, D2, D3). Basal and lateral membranes of the α_{2A}-AR and 5-HT$_{1A}$ receptor expressing cells were stained (B2, B3; C2, C3). Only 5-HT$_{1A}$ expressing cells were labeled on the apical membrane (C1). A section of the apical cytoplasm of 5-HT$_{1B}$ expressing cells shows labeling in vesicular structure (E). All images have the same scale bar as in E. Abbreviations: f, filter; d, desmosome; n, nucleus; v, microvilli.

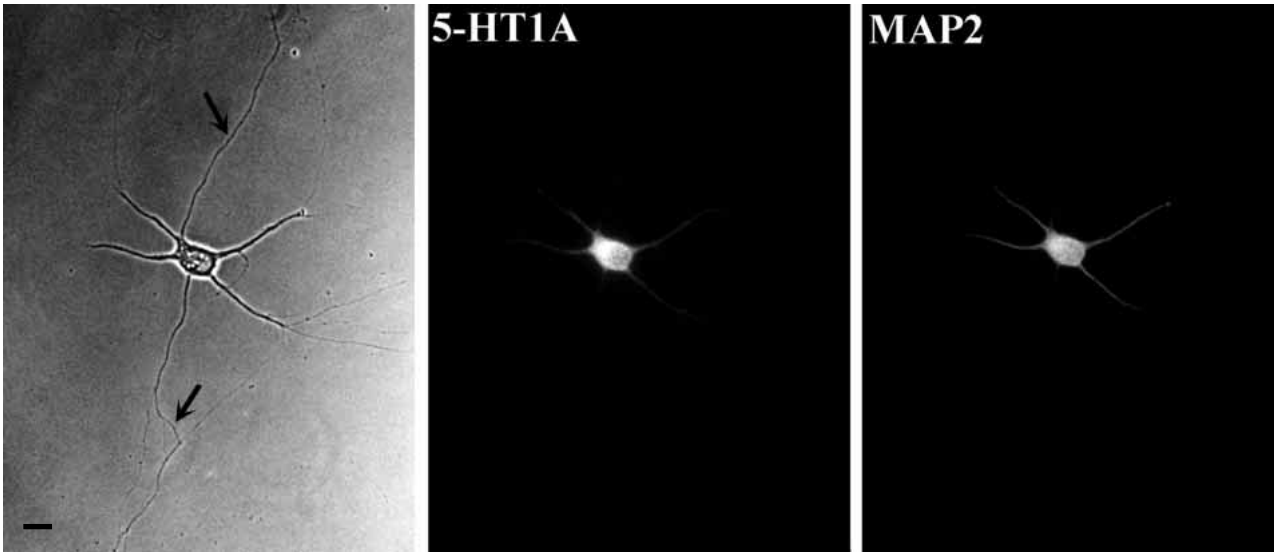

Fig. 3. Colocalization of the 5-HT1A receptor and MAP2 in the soma and dendrites of hippocampal neurons. Neurons cultured for 8 days were infected with Adeno-1A, fixed 36 hours later, and then double-labeled with anti-HA and anti-MAP2 antibodies. The 5-HT$_{1A}$ receptor was expressed in the same processes as the dendritic marker MAP2. The arrows point to axons which were visible in the phase-contrast micrograph but did not stain with either antibody. Bar, 25 µm.

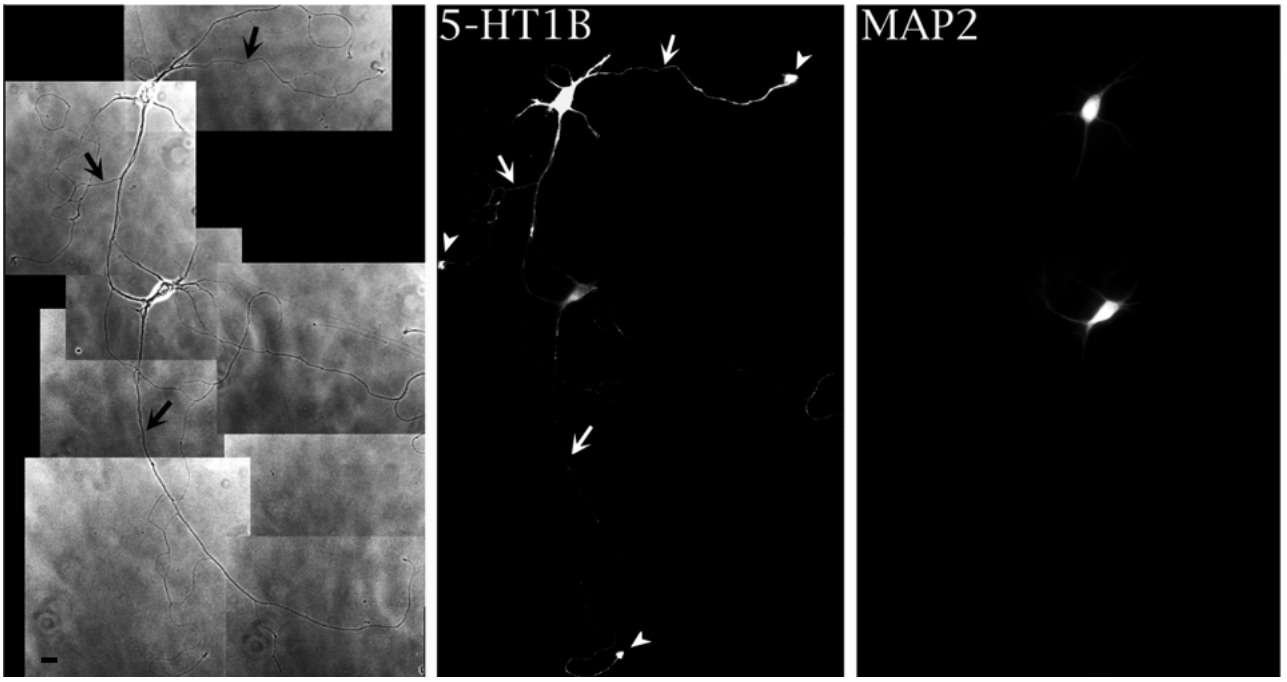

Fig. 4. Distribution of the 5-HT_{1B} receptor in both axons and dendrites of cultured hippocampal neurons. Neurons cultured for 8 days were infected with Adeno-1B. The cells were fixed 36 hours later and double labeled with anti-HA and anti-MAP 2 antibodies. One of the two neurons in this field (upper neuron) expressed the 5-HT_{1B} receptor. The arrows indicate the axons, as defined by non-labeling with the anti-MAP2 antibody. The 5-HT_{1B} receptor was expressed in both MAP2 positive and negative processes. The 5-HT_{1B} staining extended throughout the cell's entire axonal arbor, with axonal staining appearing most intense in growth cones (arrowheads). Bar, 25 μm.

and 5-HT_{1B} knockout mice (Saudou et al., 1994; see Materials and Methods and Fig. 5). Although the same α-CaMKII-tTA transgenic line was used for the creation of both the 5-HT_{1A} and 5-HT_{1B} rescue mice, the final pattern of distribution of these receptors depends not only on the distribution pattern of tTA (Fig. 5) but also on the integration site of the tet-O recombinant (our unpublished observations). The in situ hybridization experiments showed that the mRNA coding for the 5-HT_{1A} receptor in the 5-HT_{1A} rescue mice had a pattern of expression resembling to that of tTA (Fig. 5A and and Fig. 7A and 7C), while the 5-HT_{1B} rescue mice produced a subset of this pattern (Fig. 6A and C).

The regional expression of the 5-HT_{1B} mRNA and the 5-HT_{1B} receptor was examined in the brains of 5-HT_{1B} rescue mice by in situ hybridization and autoradiography with the specific 5-HT_{1B} receptor radioligand [^{125}I]-GTI (Fig. 6). The 5-HT_{1B} mRNA was found predominately in the striatum (Fig. 6A) but not in the globus pallidus and substantia nigra (Fig. 6A and C). In contrast, the 5-HT_{1B} binding sites were highly concentrated in the substantia nigra and globus pallidus (Fig. 6B and D), the main projection areas of striatal neurons. Thus, the 5-HT_{1B} receptor was transported to the terminals of striatal neurons in vivo. No expression of the 5-HT_{1B} protein was detectable in the striatum (Fig. 6B). We also performed autoradiographic studies with another radiolabeled ligand, [^{125}I]-CYP. When used in the presence of appropriate masking agents (30 μM isoproterenol, 100 nM 8-OH-DPAT), this radioligand binds specifically to the 5-HT_{1B} receptor (Hoyer et al., 1985). The distribution of [^{125}I]-CYP binding sites in the rescue mice was similar to that of the [^{125}I]-GTI sites (data not shown).

The 5-HT_{1A} rescue mice had more widespread expression. The 5-HT_{1A} mRNA was found in the striatum, hippocampus, and cortex (Fig. 7A and C). The distribution of the 5-HT_{1A} receptor was analyzed in the brains of 5-HT_{1A} rescue mice autoradiography with the specific 5-HT_{1A} receptor antagonist [^{125}I]-*p*-MPPI (Fig. 7). In contrast to the 5-HT_{1B} receptor, no 5-HT_{1A} receptor was detected in the globus pallidus and substantia nigra (Fig. 7B and D), indicating that the 5-HT_{1A} receptor is not transported toward striatal terminals.

DISCUSSION

In the present study, we have shown that 5-HT_{1A} and 5-HT_{1B} receptors are differentially localized in both stably transfected MDCK II cells and hippocampal neurons in culture. Additionally, we have used in vivo mouse models to demonstrate that the 5-HT_{1B} receptor is transported to the axon terminals of striatal neurons while the 5-HT_{1A} receptor is localized at the somatodendritic level of these neurons.

Previous studies comparing the pattern of 5-HT_{1A} and 5-HT_{1B} mRNA and the corresponding proteins demonstrated a mismatch between the mRNA and protein of the 5-HT_{1B} receptor, whereas the mRNA and protein of the 5-HT_{1A} receptor colocalized (Miquel et al., 1991; Pompeiano et al., 1992; Boschert et al., 1994). However, in a number of brain regions such as the striatum, the distribution of the 5-HT_{1B} mRNA and protein had been shown to overlap. It was therefore impossible to assess whether 5-HT_{1B} receptors were localized only on axons or also on somas and dendrites. To address the issue of exact localization of the 5-HT_{1B} receptor in the basal ganglia, we studied the transport of the 5-HT_{1B} receptor in a

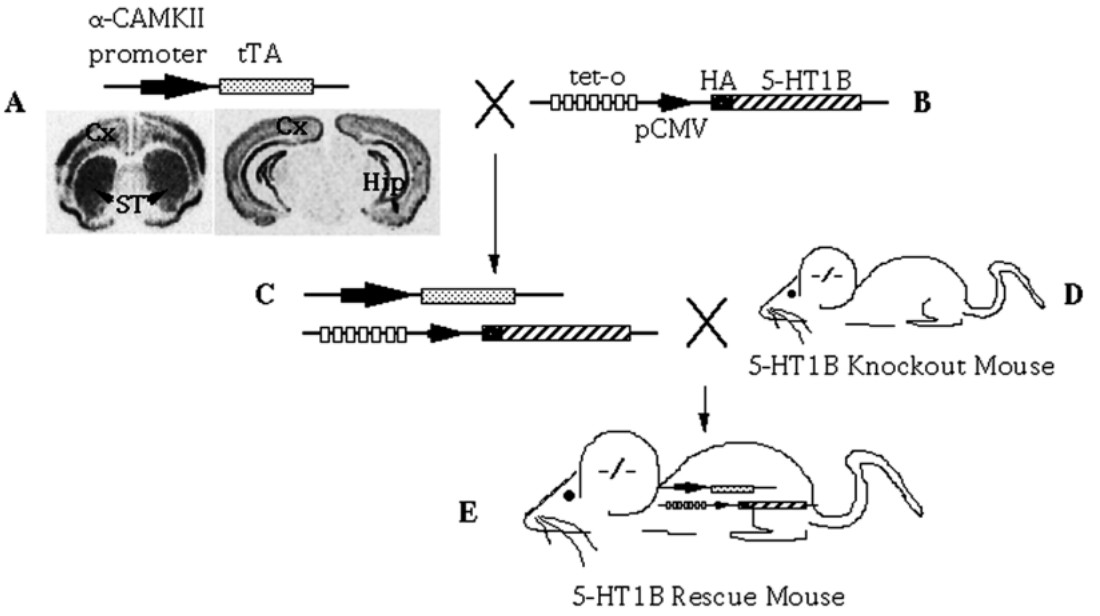

Fig. 5. Creation of rescue mice expressing the tagged 5-HT$_{1B}$ receptor. This cartoon summarizes the creation of a line of transgenic mice (rescue mice) where the hemagglutinin tagged version of the 5-HT$_{1B}$ receptor was expressed predominately in the striatum of otherwise 5-HT$_{1B}$ knockout mice. (A) Transgenic mouse expressing tTA under the control of the α-CaMKII promoter and the distribution pattern of tTA mRNA detected by in situ hybridization in coronal brain sections of this mouse. The tTA mRNA was detected in striatum, hippocampus, and cortex. (B) Transgenic mouse expressing the hemagglutinin tagged version of the 5-HT$_{1B}$ receptor under the control of tet-O sequences and a CMV minimal promoter. (C) Doubly transgenic mouse created by breeding A and B together. (D) The 5-HT$_{1B}$ receptor knockout mouse. (E) Doubly transgenic mouse on the knockout background created by breeding C and D together. HA, hemagglutinin; tTA, tetracycline transactivator; tet-O, tetracycline operator sequences; α-CaMKII, α-calcium calmodulin dependent kinase II; pCMV, cytomegalovirus minimal promoter; Cx, cortex; Hip, hippocampus; ST, striatum.

'rescue mouse'. Additionally, the creation of a 5-HT$_{1A}$ rescue mouse allowed us to compare the distributions of these receptors in this in vivo system. We designed a strategy to express the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in the striatum of mice which are otherwise knockouts for these receptors. Our results demonstrate that the 5-HT$_{1B}$ receptor was transported exclusively to the terminals of projecting striatal neurons in these rescue mice. In contrast, in 5-HT$_{1A}$ rescue mice, the expression of the 5-HT$_{1A}$ receptor was restricted to the somatodendritic domain of striatal neurons. These results show that the coding sequence of both the 5-HT$_{1B}$ and 5-HT$_{1A}$ receptors contain all the necessary addressing information for proper localization in these neurons.

Unlike in wild-type mice, we could not detect any striatal expression of the 5-HT$_{1B}$ receptor, even on long exposures of autoradiographic films. Striatal neurons possess collaterals which project back to the striatum. Therefore, the absence of the 5-HT$_{1B}$ receptor in the striatum of our rescue mice strongly suggests that, not only is the 5-HT$_{1B}$ receptor exclusively transported to axon terminals of these neurons, but also that this receptor is preferentially transported in axons projecting to the globus pallidus and substantia nigra. These results also strongly suggest that the 5-HT$_{1B}$ receptors found in the striatum of wild-type mice correspond to receptors which are localized on the terminals of nonstriatal neurons projecting to the striatum.

Although the 5-HT$_{1B}$ receptor is localized exclusively in the axonal compartments of striatal neurons in rescue mice, this receptor is found in both axonal and somatodendritic compartments in primary hippocampal neurons. There are a number of other studies showing that exogenously expressed axonal proteins have been found in both the dendrites and

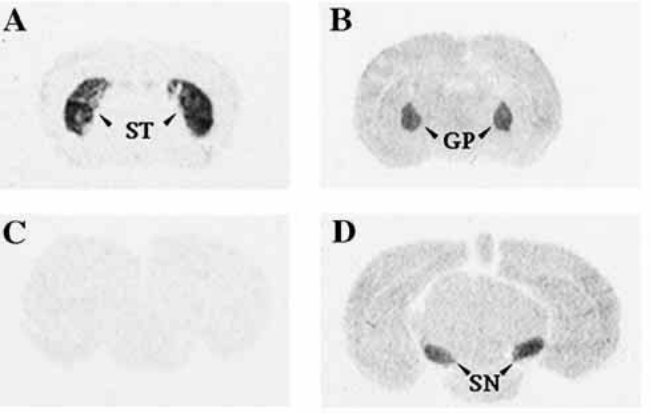

Fig. 6. Localization of the 5-HT$_{1B}$ receptor in 5-HT$_{1B}$ rescue mice. (A and C) 5-HT$_{1B}$ mRNA expression detected by in situ hybridization. Coronal brain sections of 5-HT$_{1B}$ rescue mice were hybridized with an antisense oligonucleotide probe for the HA epitope. (B and D) 5-HT$_{1B}$ receptor distribution detected by autoradiography. The distribution of tagged 5-HT$_{1B}$ receptor binding sites was determined by using the [^{125}I]-GTI radioligand in the presence of 10 μM 8-OH-DPAT. 5-HT$_{1B}$ mRNA was detected in the striatum, whereas 5-HT$_{1B}$ receptor protein was detected in the globus pallidus and substantia nigra. GP, globus pallidus; SN, substantia nigra; ST, striatum.

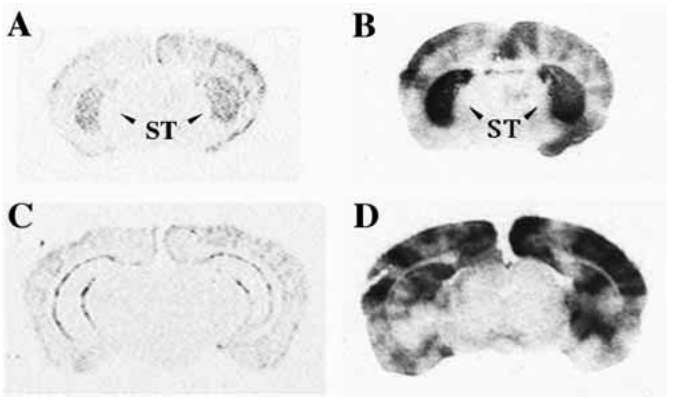

Fig. 7. Localization of the 5-HT_{1A} receptor in 5-HT_{1A} rescue mice. (A and C) 5-HT_{1A} mRNA expression detected by in situ hybridization. (B and D) 5-HT_{1A} receptor distribution detected by autoradiography. 5-HT_{1A} receptor binding sites were determined by using the 5-HT_{1A} specific antagonist [^{125}I]-*p*-MPPI and were found to be highly concentrated at the somatodendritic level of striatal neurons. Both 5-HT_{1A} mRNA and protein were detected in the striatum. ST, striatum.

axons of hippocampal neurons (Ahn et al., 1996; West et al., 1997b). In these studies, the dendritic localization of axonal proteins was attributed either to detection problems due to the close proximity of axons and dendrites in culture, or to mis-sorting to dendrites due to overexpression. In our experiment, since we used a low titer of adenovirus to infect low density neuronal cultures, labeled neurons were widely separated from each other. Therefore, it is unlikely that the dendritic labeling of the 5-HT_{1B} receptor was due to fasciculating axons. Though mis-sorting due to overexpression cannot be ruled out, however, NgCAM was confined to axons even when expressed at high levels in hippocampal neurons under similar conditions (Jareb and Banker, 1998). An alternative explanation for the dendritic localization of the 5-HT_{1B} receptor in our hippocampal cultures may stem from the fact that this receptor is not normally expressed in all types of hippocampal neurons. In vivo, the 5-HT_{1B} receptor is found only in CA1 hippocampal pyramidal neurons, while our cultures contained a mixed population of hippocampal neurons. It has been shown, for example, that the sorting of several glypiated adhesion molecules differs in different types of neurons (Faivre-Sarrailh and Rougon, 1993). Finally, it is also possible that the differentiation state of neurons in culture differs from that of neurons in the adult brain, thereby contributing to the mis-sorting of certain proteins. Numerous membrane proteins are polarized to dendrites or axons in hippocampal neurons after 9 days in culture, although the clustering of glutamate receptors and the maturation of synaptic sites occurs later in culture (Craig et al., 1994; Rao et al., 1998).

It has been suggested that there is a common mechanism of intracellular addressing between neurons and epithelial cells. Specifically, a parallel between basolateral and dendritic sorting and between apical and axonal sorting has been proposed (Dotti and Simons, 1990). Since we have shown that the 5-HT_{1A} and 5-HT_{1B} receptors are expressed in somatodendritic and axonal compartments respectively, we decided to test whether they would also be selectively expressed in the corresponding epithelial compartments. We found that the 5-HT_{1A} receptor was expressed on both basolateral and apical surfaces in MDCK II cells. In contrast, the 5-HT_{1B} receptor was localized intracellulary in vesicles. These vesicles might correspond either to apical recycling endosomes which have previously been reported in the case of polymeric IgA receptors in MDCK cells (Apodaca et al., 1994), or to lysosomes. Double labeling experiments will be necessary to determine the exact nature of the 5-HT_{1B} receptor containing vesicles. The exclusively intracellular localization of the 5-HT_{1B} receptor in MDCK II cells is not due to a general inability of this receptor to be transported to the plasma membrane since it has been found at the surface of both COS7 cells and ventricle myocytes (Ghavami et al., 1997). Such results suggest that the intracellular addressing machinery is different in these different cell types.

Recent studies on the addressing of the 5-HT_{1A} and 5-HT_{1B} receptors in another epithelial cell line, LCC-PK1 cells, have shown that the 5-HT_{1A} receptor was targeted to the basolateral domain while the 5-HT_{1B} receptor was localized in a Golgi-like intracellular compartment (Langlois et al., 1996). The basolateral distribution of the 5-HT_{1A} receptor in LCC-PK1 cells was in contrast to its nonpolarized distribution in MDCK cells. In both cell lines, the 5-HT_{1B} receptor was expressed intracellularly. However, the Golgi-like distribution of the 5-HT_{1B} receptor in LCC-PK1 is in contrast to its localization in vesicles beneath the apical surface in MDCK cells. This differential sorting behavior further supports the hypothesis that distinct sorting mechanisms exist in these two renal epithelial cells (Gu et al., 1996; Caplan, 1997).

Our findings are in disagreement with the hypothesis that axonal and dendritic targeting signals are interpreted as apical and basolateral sorting signals, respectively, in epithelial cells. A lack of correspondence between epithelial and neuronal compartments has also been observed in the case of the Na^+/K^+-ATPase (Pietrini et al., 1992), the β-amyloid precursor protein (Haass et al., 1994; Tienari et al., 1996), and neurotransmitter transporters (Gu et al., 1996). For instance, serotonin and norepinephrine transporters are expressed on axon terminals in vivo, but these proteins are restricted to the basolateral membrane of MDCK cells (Gu et al., 1996). Thus, targeting signals may be interpreted differently in MDCK cells than in neurons. There is increasing evidence for the existence of multiple targeting signals within a particular protein (Tienari et al., 1996; West et al., 1997a,b). It is therefore conceivable that the hierarchy between various sorting signals will be different in different cell types. For example, the dominant basolateral addressing signal of the β-amyloid precursor protein in epithelial cells (Haass et al., 1995) is not a primary sorting signal in neurons. This basolateral signal exerts its effect only after the removal of a dominant axonal signal (Tienari et al., 1996).

In summary, our in vivo rescue models clearly demonstrate that 5-HT_{1B} receptors, unlike 5-HT_{1A} receptors, are expressed on the axon terminals, but not on the dendrites, of striatal neurons. Furthermore, this transgenic system appears to be the only expression system that reconstitutes the normal distribution of the 5-HT_{1B} receptor. Such a system will therefore be necessary to establish the identity of the axonal addressing sequences of 5-HT_{1B} receptors. This inducible rescue model will also allow us to study the kinetics of

transport as well as the half-life of receptor mRNA and protein. Finally, this model will allow us to determine the function of this receptor in the basal ganglia.

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