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The Serotonin 5-HT_{2A} and 5-HT_{2C} Receptors Interact with Specific Sets of PDZ Proteins*

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The 5-hydroxytryptamine type 2A (5-HT_{2A}) receptor and the 5-HT_{2C} receptor are closely related members of the G-protein-coupled receptors activated by serotonin that share very similar pharmacological profiles and cellular signaling pathways. These receptors express a canonical class I PDZ ligand (S*X*V) at their C-terminal extremity. Here, we have identified proteins that interact with the PDZ ligand of the 5-HT_{2A} and 5-HT_{2C} receptors by a proteomic approach associating affinity chromatography using immobilized synthetic peptides encompassing the PDZ ligand and mass spectrometry. We report that both receptor C termini interact with specific sets of PDZ proteins *in vitro*. The 5-HT_{2C} receptor but not the 5-HT_{2A} receptor binds to the Veli-3·CASK·Mint1 ternary complex and to SAP102. In addition, the 5-HT_{2C} receptor binds more strongly to PSD-95 and MPP-3 than the 5-HT_{2A} receptor. In contrast, a robust interaction between the 5-HT_{2A} receptor and the channel-interacting PDZ protein CIPP was found, whereas CIPP did not significantly associate with the 5-HT_{2C} receptor. We also show that residues located at the −1 position and upstream the PDZ ligand in the C terminus of the 5-HT_{2A} and 5-HT_{2C} receptors are major determinants in their interaction with specific PDZ proteins. Immunofluorescence and electron microscopy studies strongly suggested that these specific interactions also take place in living cells and that the 5-HT_2 receptor-PDZ protein complexes occur in intracellular compartments. The interaction of the 5-HT_{2A} and the 5-HT_{2C} receptor with specific sets of PDZ proteins may contribute to their different signal transduction properties.

Serotonin (5-hydroxytryptamine (5-HT)[1]) is a major neurotransmitter that is involved in numerous functions of the mammalian central nervous system. These functions are mediated by a large number of receptors. Except for the 5-HT_3 receptor, which is a ligand-gated channel, all 5-HT receptors belong to the G-protein-coupled receptor (GPCR) superfamily. Among the GPCRs activated by 5-HT, the 5-HT_2 receptor family, namely the 5-HT_{2A}, the 5-HT_{2B}, and the 5-HT_{2C} receptors, continues to raise particular interest. Indeed, they are involved in multiple physiological functions such as the control of endocrine secretion, motor behavior, mood, pain, sleep, thermoregulation, and appetite (1). Moreover, a large number of psychoactive drugs, including non-classical antipsychotic drugs, hallucinogens, anxiolytics, and anti-depressants, mediate their action at least in part through activation of 5-HT_2 receptors (1–4).

Among the 5-HT_2 receptor family, the 5-HT_{2A} and the 5-HT_{2C} receptor are widely distributed in the central nervous system, whereas the 5-HT_{2B} receptor is sparse. The 5-HT_{2A} and the 5-HT_{2C} receptors share the highest degree of sequence homology (about 50% overall sequence identity). Thus, it is not surprising that these receptors have very similar pharmacological profiles and that only a few selective ligands are available. Initial studies of 5-HT_{2A} and 5-HT_{2C} receptor signaling showed that both receptors activate phosphatidyl inositol hydrolysis. However, some differences in signal transduction characteristics of these receptors have been reported (5, 6). In NIH3T3 cells expressing the 5-HT_{2C} receptor, agonist-independent activity was much more elevated than that measured in cells expressing the same density of 5-HT_{2A} receptors (7). This indicates that the 5-HT_{2A} receptor has lower intrinsic ability to adopt an active conformation than does the 5-HT_{2C} receptor. Different mechanisms of desensitization for the 5-HT_{2A} and 5-HT_{2C} receptor systems have also been described. In Chinese hamster ovary cells, agonist-induced desensitization of the 5-HT_{2A} receptor-mediated phospholipase C activation is inhibited by inhibitors of protein kinase C and Ca^{2+}-calmodulin-dependent protein kinase II (8). In contrast, the 5-HT_{2C} receptor-mediated response is insensitive to these inhibitors. Moreover, the desensitization of the 5-HT_{2C} receptor system but not that of the 5-HT_{2A} receptor is dependent on G-protein receptor kinase activity (8).

A large set of recent studies demonstrate that many GPCR functions, such as G-protein-independent signaling, desensitization, internalization, and resensitization, implicate proteins that bind to their intracellular domains (9–11). To date, many proteins identified as binding partners of GPCRs are PSD-95/Disc-large/Zonula occludens-1 (PDZ) domain proteins, which recognize a PDZ recognition motif (PDZ ligand) located at their extreme C-terminal extremity (11–13). The 5-HT_{2A} and 5-HT_{2C} receptors express a canonical Type 1 PDZ ligand ((S/T)*X*ϕ, where ϕ is a hydrophobic residue, SCV and SSV, respectively). The similitude of these PDZ ligands, which share residues crucially required for the interaction with a PDZ domain (*i.e.*

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[1] The abbreviations used are: 5-HT, 5-hydroxytryptamine; h-, human; CIPP, channel-interacting PDZ protein; CRIPT, cysteine-rich interactor of PDZ three; GPCR, G-protein-coupled receptor; MAGUK, membrane-associated guanylate kinase; PDZ, PSD-95/Disc-large/Zonula occludens-1; SAP, synapse-associated protein; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; HA, hemagglutinin; DTT, dithiothreitol; PBS, phosphate-buffered saline; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.

valine at the 0 position and serine at the −2 position) but differ in the −1 position and in residues upstream the minimal PDZ ligand, raises the question of whether these receptors can recruit specific or common PDZ domain proteins. The multiple PDZ protein 1 (MUPP1) was the first protein identified as a binding partner of the C-terminal domain of the 5-HT$_{2C}$ receptor by a two-hybrid screen (14). Further experiments indicated that this protein is capable of interacting with both the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors *in vitro* (15, 16). Using a proteomic approach associating glutathione *S*-transferase pull-down experiments and MALDI-TOF mass spectrometry, we have recently identified 15 proteins of a complex interacting with the entire C terminus of the 5-HT$_{2C}$ receptor (17). These proteins include scaffolding proteins that contain PDZ domains. A direct interaction of one of them, PSD-95, with the 5-HT$_{2A}$ receptor has also recently been reported (18).

The present study was carried out to provide an exhaustive identification and comparison of the proteins that interact with the PDZ ligand of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors by means of a proteomic approach based on affinity chromatography using synthetic peptides encompassing the C-terminal PDZ ligand of the receptors as baits. This study showed that both receptors interact with specific sets of PDZ proteins *in vitro*. Additional experiments were performed to 1) identify the molecular determinants located on the C terminus of the receptors involved in these specific interactions and 2) determine whether the specificity of these interactions takes place *in vivo*.

EXPERIMENTAL PROCEDURES

Expression Plasmids and Antibodies—The constructs encoding Veli-3 (Veli-3/PRK7) and the c-Myc-tagged h5-HT$_{2C}$ receptor (5-HT$_{2C}$/pRK5) have been previously described. The expression vector coding for CIPP fused to an N-terminal FLAG tag (FLAG-CIPPpCI) was a generous gift from Prof. Michel Lazdunski. QuikChange site-directed mutagenesis (Stratagene) was used to engineer a XbaI restriction site upstream the ATG initiation codon of the pBluescript/h5-HT$_{2A}$ construct (provided by Dr. Christoph Ullmer). h5-HT$_{2A}$ was then subcloned into the XbaI site of the PRK5 plasmid. pRK5/h5-HT$_{2A}$ was hemagglutinin (HA) epitope-tagged on the N-terminal domain by polymerase chain reaction amplification with the forward primer 5′-GCTTGATGCGGATCCATGTACCCATACGACGTCCCCGACTATGCTGATATTCTTTGTGAAAATACTTCTTTGA-3′ (the HA sequence is underlined) and the reverse primer 5′-CATGGATCCGCATCAAGCTTCTAGAGGATC-3′. The amplified products were cut BsaB1/AgeI and ligated into the pRK5/h5-HT$_{2A}$, yielding pRK5/HA-h5-HT$_{2A}$. The construct was verified by sequencing.

The rabbit polyclonal anti-Veli-3 antibody was purchased from Zymed Laboratories Inc. (San Francisco, CA), the mouse monoclonal anti-CASK, anti-Mint1, and anti-5-HT$_{2A}$ receptor antibodies were from Pharmingen, the mouse monoclonal anti-PSD-95 (clone K28/43) antibody was from Upstate Biotechnology (Lake Placid, NY), the mouse monoclonal anti-synapse-associated protein (SAP) 97 was from Stress-Gen Biotechnologies Corp. (Victoria, Canada), the rabbit polyclonal anti-SAP102 antibody was from Oncogene Research Products (Cambridge, MA), the rabbit polyclonal anti-FLAG antibody was from Sigma (Saint Quentin Fallavier, France), and the monoclonal mouse anti-HA antibody (clone 12CA5) was from Roche Applied Science. The rabbit polyclonal anti-5-HT$_{2C}$ receptor antibody was provided by Dr. Abramowski and has been described elsewhere (19). The mouse monoclonal anti-c-Myc antibody was a gift from Dr. Bernard Mouillac (INSERM U469 Montpellier, France).

Peptide Affinity Chromatography—Synthetic peptides (>95% purity, Eurogentec, Seraing, Belgium) encompassing the 14 C-terminal amino acids of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were coupled via their N-terminal extremity to activated CH-Sepharose 4B (Amersham Biosciences) according to the manufacturer's instructions. MALDI-TOF mass spectrometry analysis indicated the coupling efficacy was higher than 90% for each peptide. Immobilized peptides were stored at 4 °C in Tris-HCl (50 mM, pH 7.4) and dithiothreitol (DTT, 10 mM) to prevent cysteine oxidation at the −1 position in the 5-HT$_{2A}$ sequence.

Brains of Swiss mice (obtained from Janvier, Le Genest-St. Isle, France) were thoroughly washed in phosphate-buffered saline (PBS), homogenized with a Polytron homogenizer, and centrifuged at 200 × *g* for 3 min. Pellets were resuspended in ice-cold lysis buffer containing Tris-HCl (50 mM, pH 7.4), EDTA (1 mM), and a mixture of protease inhibitors (Roche Applied Science), homogenized 20 times on ice with a glass Teflon homogenizer, and centrifuged at 10,000 × *g* for 30 min. The membrane pellets were resuspended in CHAPS extraction buffer (50 mM Tris-HCl, pH 7.4, 0.05 mM EDTA, 10 mM CHAPS, and protease inhibitors) for 3 h in rotation at 4 °C. Then samples were centrifuged for 1 h at 10,000 × *g*. Solubilized proteins (2 mg/condition) were incubated overnight at 4 °C with 2 μg of immobilized peptide. Samples were washed twice with 1 ml of extraction buffer and then 4 times with PBS. Proteins were eluted with either 350 μl of isoelectrofocusing medium containing urea (7 M), thiourea (2 M), CHAPS (4%), ampholines (preblended, pI 3.5–9.5, 8 mg/ml, Amersham Biosciences), DTT (100 mM), tergitol NP7 (0.2%, Sigma), and traces of bromphenol blue for two-dimensional electrophoresis analysis or 50 μl of SDS sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, and bromphenol blue) for immunoblotting.

Two-dimensional Electrophoresis and Two-dimensional Gel Protein Pattern Analysis—Proteins were first separated according to their isoelectric point along linear immobilized pH gradient strips (pH 3–10, 18 cm long, Amersham Biosciences). Sample loading for the first dimension was performed by passive in-gel re-swelling. After the first dimension the immobilized pH gradient strips were equilibrated for 10 min in a buffer containing urea (6 M), Tris-HCl (50 mM, pH 6.8), glycerol (30%), SDS (2%), DTT (10 mg/ml), and bromphenol blue and then for 15 min in the same buffer containing 15 mg/ml iodoacetamide instead of DTT. For the second dimension, the strips were loaded onto vertical 12.5% SDS-polyacrylamide gels. The gels were silver-stained according to the procedure of Shevchenko *et al.* (20). Gels to be compared were always processed and stained in parallel. Gels were scanned using a computer-assisted densitometer. Spot detection, gel alignment, and spot quantification were performed using the Image Master 2-D Elite software (Amersham Biosciences). Quantification of proteins was expressed as volumes of spots. To correct for variability resulting from silver staining, results were expressed as relative volumes of total spots in each gel.

MALDI-TOF Mass Spectrometry and Protein Identification—Proteins of interest were excised and digested in gel using trypsin (Gold, Promega, Charbonnières, France), and tryptic peptides were extracted from the gels as previously described (20, 21). Digest products were completely dehydrated in a vacuum centrifuge and resuspended in 10 μl of formic acid (2%), desalted using Zip Tips C18 (Millipore, Bedford, MA), eluted with 10 μl of acetonitrile-trifluoroacetic acid (60−0.1%), and concentrated to a volume of 2 μl. Aliquots (0.5 μl) were loaded onto the target of an Ultraflex MALDI-TOF mass spectrometer (Bruker-Franzen Analytik, Bremen, Germany) and mixed with the same volume of α-cyano-4-hydroxy-*trans*-cinnamic acid (Sigma, 10 mg/ml in acetonitrile-trifluoroacetic acid, 50−0.1%). Analysis was performed in reflectron mode with an accelerating voltage of 20 kV and a delayed extraction of 400 ns. Spectra were analyzed using the XTOF software (Bruker-Franzen Analytik), and auto-proteolysis products of trypsin (M_r, 842.51, 1045.56, and 2211.10) were used as internal calibrates. Identification of proteins was performed using both Mascot and PeptIdent software (available on line at www.matrixscience.com and www.expasy.org/tools/peptident.html, respectively), as previously described (21).

Immunoblotting—Proteins, resolved on 12.5% gels, were transferred electrophoretically onto nitrocellulose membranes (Hybond-C, Amersham Biosciences). Membranes were blocked in blocking buffer (Tris-HCl, 50 mM, pH 7.5, 200 mM NaCl, Tween 20, 0.1 and 5% skimmed dried milk) for 1 h at room temperature and incubated overnight with primary antibodies (anti-Veli-3, 1:500; anti-CASK, 1:500; anti-Mint1, 1:250; anti-PSD-95, 1:5000; anti-SAP102, 1:350, anti-SAP97, 1:500; anti-5-HT$_{2A}$ receptor, 1:250) in blocking buffer. Blots were washed three times with blocking buffer and incubated with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse antibody (1:2,000 in blocking buffer) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (Renaissance Plus, PerkinElmer Life Sciences).

Co-immunoprecipitation—CHAPS-soluble proteins from brain extracts (500 μg per experiment) were incubated overnight at 4 °C either with the anti-5-HT$_{2A}$ receptor antibody, the anti-PSD-95 antibody, or the anti-Veli-3 antibody (1 μg each). Samples were incubated for 1 h at 4 °C with 50 μl of protein A-Sepharose beads (Amersham Biosciences). After 5 washes with extraction buffer, immunoprecipitated proteins were eluted in SDS sample buffer, resolved on 12.5% polyacrylamide gels, and detected by immunoblotting.

Immunocytochemistry and Confocal Microscopy—Subconfluent COS-7 cells, plated onto poly-L-ornithine (15 μg/ml, M_r = 40,000, Sigma)-coated glass coverslips (12-mm diameter) in Dulbecco's modified Eagle's medium

(Invitrogen) containing 10% dialyzed fetal calf serum, were transfected with LipofectAMINE™ 2000 (Invitrogen) according to the manufacturer's instructions using 0.5 μg of each cDNA per coverslip. Twenty-four hours after transfection, cells were washed in PBS and fixed in paraformaldehyde (4% (w/v) in PBS) for 15 min at room temperature. They were washed 3 times with glycine (0.1 M) and permeabilized with 0.1% (w/v) Triton X-100 for 5 min. Cells were then washed 3 times with blocking buffer (gelatin 0.2% in PBS) and incubated overnight at 4 °C with the primary antibody (anti-Myc, 1:500; anti-HA, 1:400; anti-FLAG, 1:250; and anti-Veli-3, 1:500) in blocking buffer. Cells were washed 3 times with blocking buffer and incubated for 1 h at room temperature with either an Alexa green-labeled anti-mouse or a Cy3-labeled anti-rabbit antibody (1:1000 dilution in blocking buffer). After three washes, the cells were mounted on glass slides using gel mount (Biomeda Corp., Foster City, CA). Confocal laser-scanning microscopy was performed using a DMIRB Leica confocal inverted microscope. A series of optical sections were collected with a step of 0.30 μm. Images were collected sequentially to avoid cross-contamination between the fluorochromes and scanned at 1024 × 1024-pixel resolution.

Immunohistochemistry and Electron Microscopy—Swiss mice were deeply anesthetized with sodium pentobarbital (50 mg/kg). Animals were perfused through the ascending aorta with PBS, pH 7.4, followed by 300 ml of fixative composed of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were then dissected out and fixed by immersion in the same fixative without glutaraldehyde for 12 h at 4 °C. The forebrain was then sliced frontally with a vibratome (VT 1000S, Leica) into 40–50-μm thick sections. After washing in PBS, sections were successively incubated 1) for 48 h at 4 °C with the primary antibodies against the 5-HT$_{2A}$ receptor, the 5-HT$_{2C}$ receptor, or PSD-95 (1:500 dilution each), 2) for 12 h at 4 °C with a peroxidase-labeled Fab fragment of goat anti-mouse or anti-rabbit IgG (Biosys, Compiègne, France, 1:1000 dilution), and 3) with 0.1% 3,3′ diaminobenzidine diluted in 50 mM Tris-HCl, pH 7.3, in the presence of 0.2% H_2O_2. The primary and secondary antibodies were diluted in PBS containing 1% BSA, 1% normal goat serum, and 0.1% saponin. Immunostained sections were either mounted in Permount (Biomeda, Foster City, CA) and observed under a light microscope or further treated for electron microscopy. The sections were carefully rinsed in 0.1 M cacodylate buffer, pH 7.3, and postfixed in 1% OsO_4 in the same buffer. The sections were then dehydrated in graded concentrations of ethanol and embedded in araldite. Punches of 1.5-mm diameter were cut through the hippocampus or the frontal cortex and mounted on araldite blocks. After slicing into ultrathin sections, they were observed in an electron microscope (Hitachi H 7110) without counterstaining.

RESULTS

Recruitment of Distinct Sets of PDZ Proteins by the PDZ Ligands of the 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors—Proteins interacting with the PDZ ligand of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were isolated by affinity chromatography using synthetic peptides encompassing the 14 C-terminal residues of the receptors as bait. Proteins from whole brain extracts were incubated with this bait because both receptors are widely distributed in the mammalian CNS. Two-dimensional gel analysis of proteins retained by the affinity chromatography showed a similar protein pattern recruited by both peptides, with some differences (Fig. 1). To specifically identify the proteins that were recruited through a PDZ-based mechanism, differential analyses of gels obtained with wild type peptides and peptides in which the C-terminal valine was mutated into alanine were conducted. This mutation is known to drastically reduce the binding to PDZ proteins (13). Seven spots (or groups of spots) that were apparent in the gels obtained with C-terminal peptide of the 5-HT$_{2C}$ receptor were undetectable in the gels obtained with the mutant peptide (indicated by *arrows*, Fig. 1*B*). These proteins were identified by MALDI-TOF mass spectrometry. They include activin receptor-interacting protein 1 (*spot 1*), a membrane-associated guanylate kinase (MAGUK) with inverted domain structure (this protein is also called MAGI2), several proteins associated to the post-synaptic density, SAP97 (*spot 2*), SAP102 *(spot 3*), and PSD-95 (*spots 4* and *5*), MPP-3 (*spot 6*), a member of the P55 MAGUK subfamily, and Veli-3, one of the vertebrate homologues of LIN7 (*spot 7*) (22–26). These proteins possess one or several PDZ domains (Table I), validating our approach to identify PDZ domain partners of membrane-bound receptors. Only three of the binding partners of the 5-HT$_{2C}$ receptor PDZ ligand (PSD-95, MPP-3, and Veli-3) were identified in our previous proteomic study based on a glutathione *S*-transferase pull-down assay using the entire C terminus of the 5-HT$_{2C}$ receptor as bait. This indicates that the approach using short synthetic peptides is more sensitive than that using the entire C-terminal domain fused to glutathione *S*-transferase to detect PDZ-based interactions.

Surprisingly, trypsin digestion of the two major forms of PSD-95 (*spots 4* and *5*, Fig. 1*B*) detected on silver-stained two-dimensional gels, which showed large differences in isoelectric points (~0.6) and molecular masses (~ 15 kDa), generated identical peptide mass fingerprints (34 peptides representing 45% overall sequence coverage). Two alternatively spliced isoforms of PSD-95 have recently been identified in rodents and human, a short isoform containing a pair of N-terminal cysteines that can be palmitoylated and designated PSD-95α and a form containing a longer N terminus, designated PSD-95β (27). The N-terminal peptide of PSD-95α (MDCLCIVTTKK, M_r = 1254.62) was identified in the tryptic digest of all isoforms of PSD-95 detected in the two-dimensional gels, indicating that they do not result from the N-terminal alternative splicing but differ in other regions that were not covered by the peptides identified in our mass spectrometry analyses. We failed to detect any palmitoylation of the cysteines located on the N-terminal peptide because the palmitoylated form of PSD-95 is insoluble in non-ionic detergents such as CHAPS, which was used in our experiments (28).

Five proteins that interacted with the PDZ ligand of the 5-HT$_{2A}$ receptor were also identified (Fig. 1*A*). They include activin receptor-interacting protein 1, SAP97, PSD-95, MPP-3, and CIPP (*spot 8*), a protein containing four PDZ domains that was recently identified as a binding partner of Kir4.0 potassium channel family members, *N*-methyl-D-aspartate receptor NR2 subunits, neurexins, neuroligins, and acid-sensing ionic channel 3 (29, 30). Differential analysis of the proteins recruited by the C-terminal peptides of the 5-HT$_{2A}$ and the 5-HT$_{2C}$ receptors showed a specific recruitment of an additional protein spot by the C terminus of the 5-HT$_{2A}$ receptor through a PDZ-independent mechanism (*spot 9*, Fig. 1*A*). Indeed, mutating the C-terminal valine into alanine did not inhibit the binding of this protein (data not shown). This protein was identified as antioxidant protein-2. Antioxidant protein-2 belongs to the thioredoxin peroxidases, a family of thiol-dependent antioxidant proteins, and was recently proposed to be a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities (31, 32). These bifunctional catalytic properties suggest that antioxidant protein-2 may participate to the regulation of phospholipid turnover and protect against oxidative injury. This result indicates that our approach using short C-terminal peptides also allows the identification of binding partners of 5-HT$_2$ receptors recruited through a PDZ-independent mechanism.

The relative ability of the 5-HT$_2$ receptor PDZ ligands to bind to the identified PDZ partners was next examined by immunoblotting when specific antibodies were available. Indeed, the resolution provided by silver-stained two-dimensional gels does not allow accurate quantitative comparison of protein binding, especially for high molecular weight proteins. These experiments confirmed that Veli-3 and SAP102 are robust interactors of the 5-HT$_{2C}$ receptor, whereas they bind to a much lesser extent to the 5-HT$_{2A}$ receptor PDZ ligand (Fig. 2*A*). Veli-3 belongs to a family of three closely related proteins, Veli-1, Veli-2, and Veli-3. These proteins form a stable ternary com-

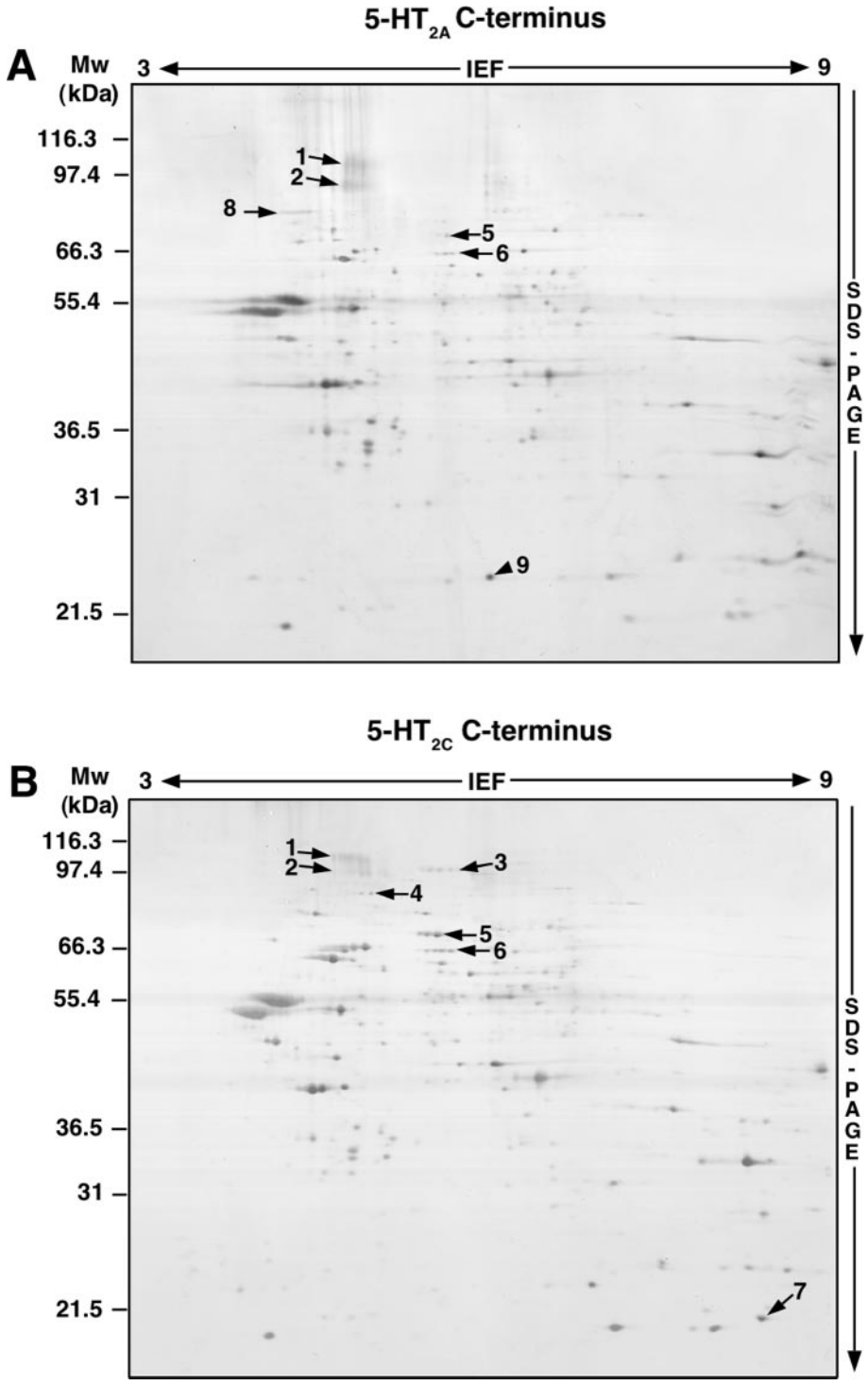

FIG. 1. **Two-dimensional analysis of proteins interacting with the PDZ ligands of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.** Proteins from mice brains that bind to the C terminus of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were purified by affinity chromatography using synthetic peptides encompassing the 14 C-terminal residues of the receptors immobilized on CH-Sepharose beads as bait, separated by two-dimensional electrophoresis, and stained with silver. Typical two-dimensional gels representative of four experiments performed independently are illustrated. To identify proteins that interact with the peptides through a PDZ-based mechanism, differential analyses between gels obtained with wild type peptides and peptides in which the C-terminal valine was replaced by an alanine (not illustrated) were performed. *Arrows* indicate the position of protein spots that are not detectable in the corresponding gels obtained with the mutant peptide. The *arrowhead* indicates the position of an additional protein spot that is specifically recruited by the C terminus of the 5-HT$_{2A}$ receptor but through a PDZ-independent mechanism (this protein interacts equally with the wild type and mutated peptides). IEF, isoelectrofocusing.

plex with two other modular proteins, CASK, a MAGUK that contains one PDZ domain and a N-terminal calmodulin kinase II domain, and Mint1, a protein that contains two PDZ domains. Although CASK and Mint1 were not detectable in the silver-stained two-dimensional gels, immunoblotting indicated that both proteins associated with the 5-HT$_{2C}$ receptor but not the 5-HT$_{2A}$ receptor PDZ ligand (Fig. 2*A*). These experiments also indicate that PSD-95 preferentially interacts with the 5-HT$_{2C}$ receptor when compared with the 5-HT$_{2A}$ receptor, whereas SAP97 binds each receptor in similar amounts. Moreover, quantitative analysis of two-dimensional gels showed that the 5-HT$_{2C}$ receptor interacted more strongly with MPP-3 than did the 5-HT$_{2A}$ receptor (Fig. 3). Altogether, these results indicate that although the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors share a similar canonical PDZ ligand, they each recruit a specific set of PDZ proteins.

Molecular Determinants Located on the C Termini of the 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors Are Involved in Their interaction with Specific PDZ Proteins—To determine the amino acids involved in the interaction of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors with specific PDZ proteins, experiments using mutant peptides were performed. We focused on residues located around the canonical PDZ ligand, which diverge between both 5-HT$_2$ receptor subtypes. The peptide corresponding to the 5-HT$_{2A}$ receptor C terminus in which the cysteine residue at −1 position was replaced with a serine (the residue at −1 position in the

TABLE I

Proteomic analysis of proteins interacting with the C terminus of the 5-HT$_{2A}$and the 5-HT$_{2C}$ receptor

SWISS-PROT and TrEMBL accession numbers are listed. Proteins that were recruited by both receptor subtypes were identified by MALDI-TOF mass spectrometry from both the gels obtained with the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor C-terminal peptides. For these proteins, the results of MALDI-TOF analyses that yielded the larger sequence coverage are indicated.

Position in gels in Fig. 1	Protein name	Accession number	MALDI-TOF MS		PDZ domains	5-HT$_{2A}$	5-HT$_{2C}$
			Peptides	Coverage			
				%			
1	ARIP-1 (activin receptor-interacting protein 1)	Q9WVQ1	41	63.8	6	++	++
2	SAP97 (synapse-associated protein 97)	Q62402	14	28.0	3	++	++
3	SAP102 (synapse-associated protein 102)	P70175	18	23.4	3	−	++
4	PSD-95 (post-synaptic density protein-95)	Q62108	34	44.6	3	+	++
5	PSD-95 (post-synaptic density protein-95)	Q62108	34	44.6	3	+	++
6	MPP-3 (MAGUK p55 subfamily member-3)	O88910	20	43.0	1	+	++
7	Veli-3 (vertebrate homolog of LIN 7)	O88952	8	49.7	1	+/−	++
8	CIPP (channel-interacting PDZ domain protein)	O70471	16	52.6	4	++	+/−
9	AOP-2 (antixoxidant protein 2)	O08709	11	48.4	0	++	−

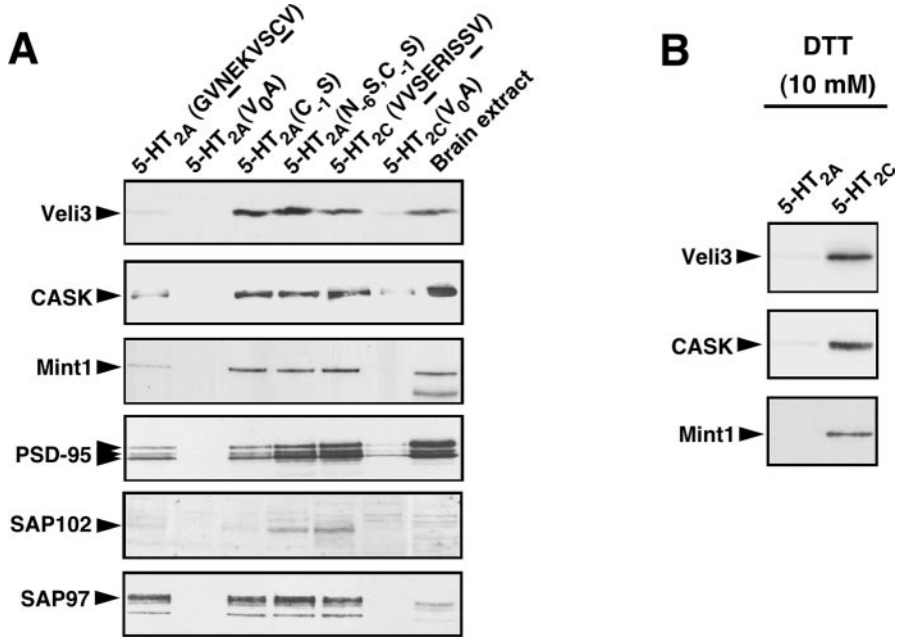

FIG. 2. **Analysis of the interaction of PDZ proteins with the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors by Western blotting.** *A*, proteins from brain extracts were incubated in the presence of either immobilized peptides encompassing the 14 C-terminal residues of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (the C-terminal residues that are implicated in the specificity of interactions with PDZ proteins are indicated in *parentheses*), mutant peptides in which the C-terminal valine was replaced by an alanine (5-HT$_{2A}$(V0A) and 5-HT$_{2C}$(V0A), respectively), or mutant peptides in which one or two residues of the C terminus of the 5-HT$_{2A}$ receptor were substituted to the corresponding residues of the 5-HT$_{2C}$ receptor (5-HT$_{2A}$(C−1S) and 5-HT$_{2A}$(N−6S,C−1S), respectively). *B*, brain extracts were incubated with immobilized peptides in the presence of DTT (10 mM). Proteins were eluted with SDS sample buffer, resolved onto 12.5% polyacrylamide gels, and transferred electrophoretically onto nitrocellulose membranes. Input (brain extract) represents 10% of the protein total used for affinity chromatography. The data representative of three experiments are illustrated.

5-HT$_{2C}$ receptor) strongly interacted with Veli-3 (Fig. 2*A*). As expected, this mutant peptide also recruited CASK and Mint1 (Fig. 2*A*). This suggests that the serine residue at the −1 position in the C terminus of the 5-HT$_{2C}$ receptor plays a critical role in the specific interaction of this receptor with the Veli-3·CASK·Mint1 complex. The lack of binding of Veli-3, CASK, and Mint1 to the 5-HT$_{2A}$ receptor C terminus was probably not due to oxidation of cysteine at the −1 position during peptide storage or the chromatography step; respective selectivities in binding were observed under reducing conditions (Fig. 2*B*). Contrasting to what was observed for the Veli-3·CASK·Mint1 complex, the single substitution of the −1 position in the 5-HT$_{2A}$ sequence did not modify the binding of PSD-95, SAP102, and MPP-3 when compared with the wild type C-terminal peptide (Figs. 2*A* and 3). Only a peptide carrying a double substitution at the −1 and −6 positions (5-HT$_{2A}$(N−6S,C−1S) generated a robust interaction with all three proteins, which was similar to that observed with the wild type 5-HT$_{2C}$ receptor C-terminal peptide (Figs. 2*A* and 3). We next examined the molecular determinants involved in the specificity of the interaction between the 5-HT$_{2A}$ receptor and CIPP. Mutant peptides with a single substitution at the −1 position (5-HT$_{2A}$C−1S) or a double substitution at the −1 and −6 positions (5-HT$_{2A}$N−6S,C−1S) bound CIPP at levels similar to wild type 5-HT$_{2A}$ receptor C-terminal peptide (Fig. 4). In contrast, a mutant peptide with a single substitution at the −8 position (5-HT$_{2A}$G−8V) showed little interaction with CIPP, indicating that this residue is critical for the interaction of the 5-HT$_{2A}$ receptor with CIPP. In agreement with this finding, the reciprocal substitution on the 5-HT$_{2C}$ receptor C-terminal peptide (5-HT$_{2C}$V−8G) generated a robust binding to CIPP, comparable with that found with the 5-HT$_{2A}$ receptor (Fig. 4). Altogether, these results show that amino acids at the −1 position or located upstream the canonical PDZ ligand play a critical role in the interaction between the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and specific sets of PDZ proteins *in vitro*.

Interaction of the 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors with Specific PDZ Proteins in COS7 Cells—We next examined whether the specificity of interaction between the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and PDZ proteins demonstrated in the *in vitro* binding assay also occurs in intact cells. We evaluated this by immunocytochemistry and confocal microscopy in transfected COS7 cells. We focused on Veli-3 and CIPP because both proteins specifically interact with one of the 5-HT$_2$ receptor subtypes *in vitro*. In single-transfected COS7 cells, the HA-tagged 5-HT$_{2A}$ receptor and the c-Myc-tagged 5-HT$_{2C}$ receptor showed a wide distribution at intracellular membrane structures with a prominent immunostaining around the nucleus (Fig. 5*A*). In contrast, Veli-3 and FLAG-tagged CIPP showed a diffuse distribution throughout the cytosol, with some perinuclear accumulation of

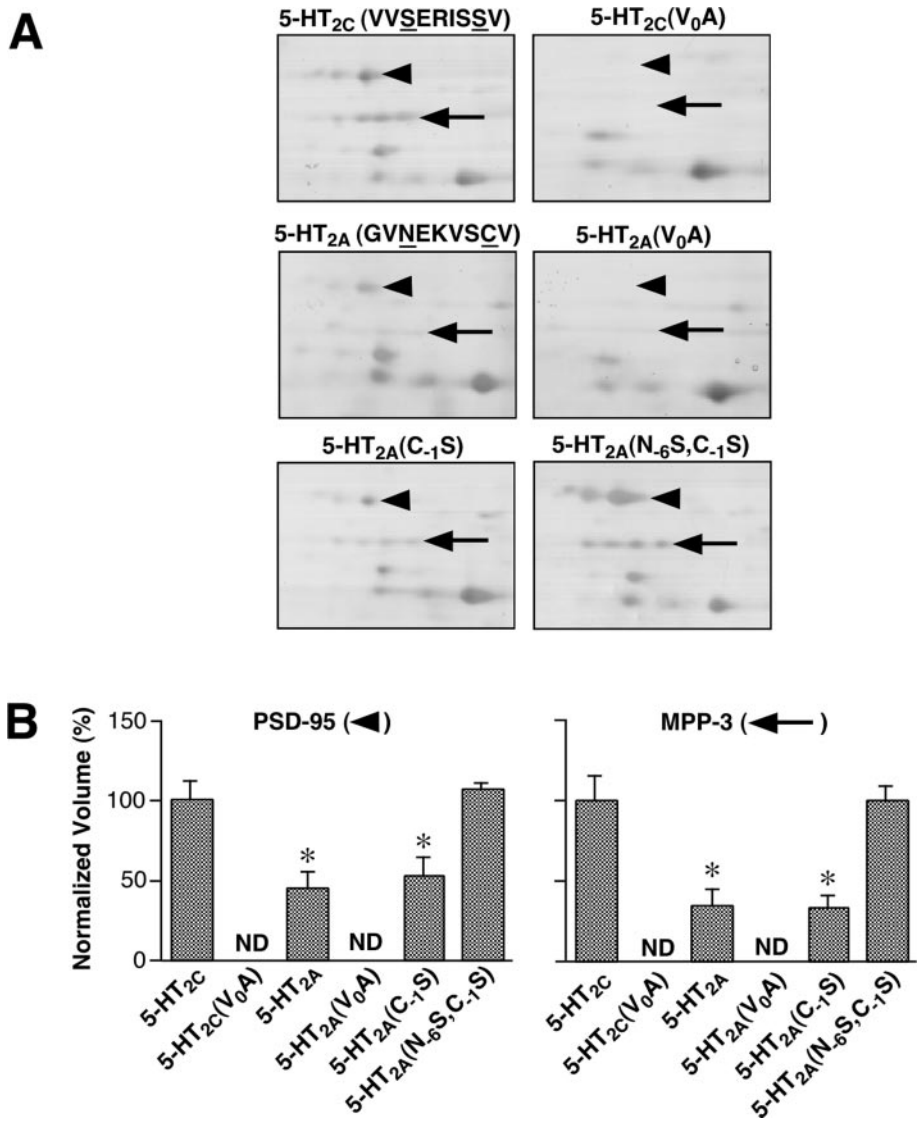

FIG. 3. **Molecular determinants in the C termini of 5-HT$_2$ receptors involved in the preferential interaction of MPP-3 and PSD-95 with the 5-HT$_{2C}$ receptor.** Proteins from brain extracts were incubated with the same peptides as those described in the legend of Fig. 2. Proteins were eluted in isoelectrofocusing medium, separated by two-dimensional electrophoresis, and stained with silver. *A*, areas of gels including MPP-3 (*arrows*) and the low molecular weight forms of PSD-95 (*arrowheads*) are illustrated. The data are representative of four experiments. *B*, the quantification of proteins (spot volume relative to the volume of total spots) was performed with the Image Master software. For each protein the volumes of all spots identified as a single protein were added. Data, expressed in % of the spot volume measured in the gels obtained with the peptide corresponding to the C terminus of the 5-HT$_{2C}$ receptor, are the means ± S.E. of values obtained from four gels. *, $p < 0.01$ *versus* 5-HT$_{2C}$, $n = 4$, analysis of variance followed by Dunnett's test. *ND*, not detectable with Image Master.

Flag-CIPP (Fig. 5*A*). In cells coexpressing the HA-tagged 5-HT$_{2A}$ receptor and CIPP, CIPP concentrated around the nucleus, where the protein was co-localized with the 5-HT$_{2A}$ receptor (Fig. 5*B*). In contrast, Veli-3 still showed a generally diffuse distribution and did not co-localize with the 5-HT$_{2A}$ receptor in cells co-transfected with the HA-tagged 5-HT$_{2A}$ receptor and Veli-3. Opposite observations were made in cells coexpressing the c-Myc-tagged 5-HT$_{2C}$ receptor and either CIPP or Veli-3; the intracellular localization of CIPP remained unchanged, whereas Veli-3 concentrated in perinuclear structures, similar to the distribution of the 5-HT$_{2C}$ receptor (Fig. 5*C*). Altogether, these observations indicate that in COS cells, the 5-HT$_{2A}$ receptor interacts with CIPP but not Veli-3, whereas the 5-HT$_{2C}$ receptor specifically interacts with Veli-3, consistent with the specificity of interactions detected *in vitro*.

Interaction of PDZ Proteins with Native 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors from Mice Brain—To examine whether some of the PDZ proteins identified as binding partners of both 5-HT$_2$ receptor subtypes interact with native receptors from mice brain, we performed co-immunoprecipitation experiments. In agreement with our previous findings, we found that both PSD-95 and Veli-3 were immunoprecipitated with the 5-HT$_{2C}$ receptor antibody (Fig. 6*A*). In contrast, only PSD-95 but not Veli-3 coimmunoprecipitated with the anti-5-HT$_{2A}$ receptor antibody (Fig. 6*A*). In the same manner, we were able to immunoprecipitate the 5-HT$_{2A}$ receptor with the PSD-95 antibody but not the Veli-3 antibody, which is very efficient in immunoprecipitation (Fig. 6*B*). These results suggest that the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors interact with PDS-95, whereas only the 5-HT$_{2C}$ receptor associates with the Veli-3·CASK·Mint1 complex *in vivo*, consistent with the specificity of interaction detected in our *in vitro* binding assay.

Comparison of the Subcellular Distribution of the 5-HT$_{2A}$ and the 5-HT$_{2C}$ Receptors with That of PSD-95 within the Brain—Electron microscope immunocytochemistry was performed to compare the subcellular distribution of the 5-HT$_{2A}$ and the 5-HT$_{2C}$ receptor with that of PSD-95, one of the common binding partners of both receptor subtypes, within the mouse brain. Observations were made in two regions exhibiting intense immunostaining for either the 5-HT$_{2A}$ receptor or the 5-HT$_{2C}$ receptor, *i.e.* the frontal cortex (33) and the anterior olfactory nucleus (17), respectively. Throughout the frontal cortex, 5-HT$_{2A}$ receptor and PSD-95 immunostaining showed very similar subcellular localizations. Immunostaining was associated with both the cytoplasm of dendritic profiles of various sizes and post-synaptic thickenings located along dendritic profiles or dendritic spines (Figs. 7, *A* and *B*). Moreover, no labeling was found with both antibodies in axonal terminals present in this region. In the anterior olfactory nucleus, 5-HT$_{2C}$ receptor immunostaining was found to be associated with both post-synaptic and presynaptic thickenings of axo-dendritic synapses (Fig. 7*C*), whereas PSD-95 immunostaining was essentially associated with the cytoplasm and post-synaptic thickenings of

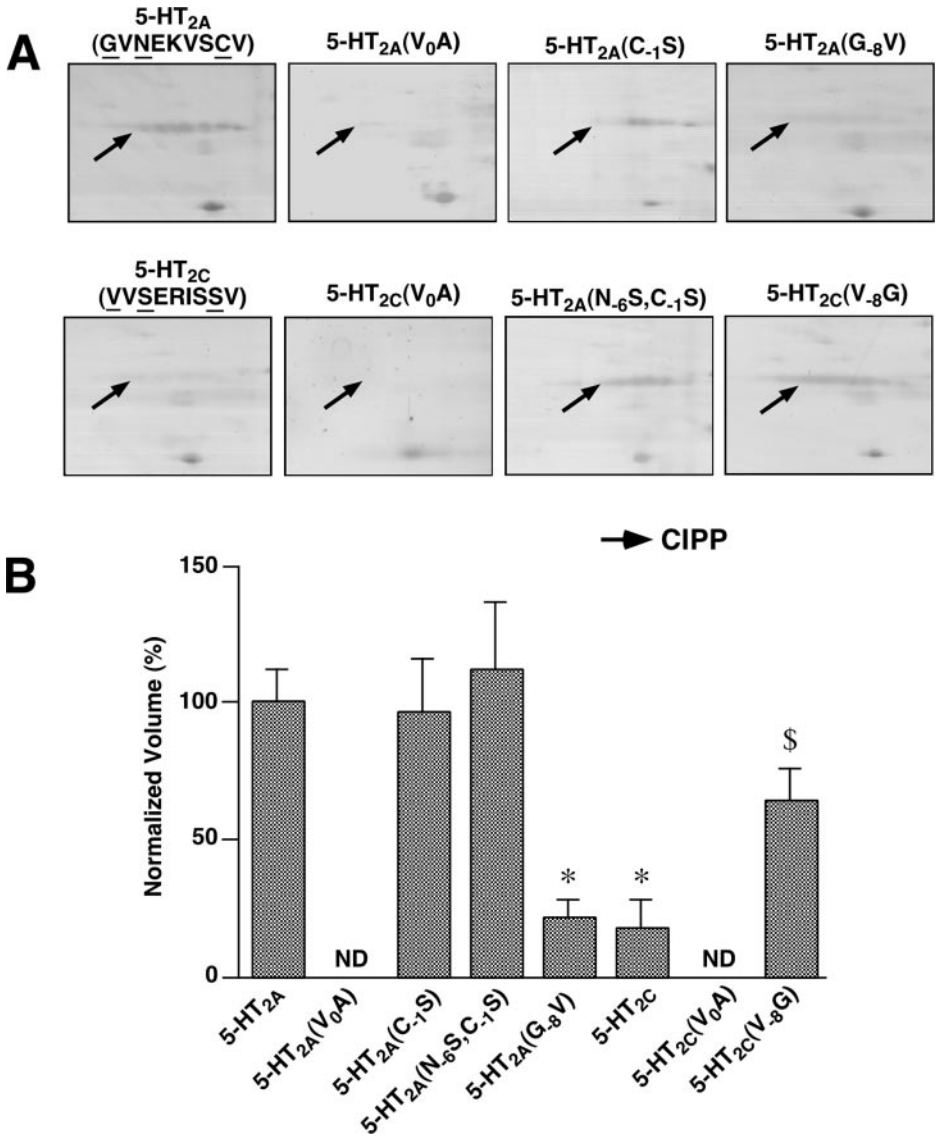

FIG. 4. **Molecular determinants in the C terminus of the 5-HT$_{2A}$ receptor involved in its preferential interaction with CIPP.** Proteins from brain extracts were incubated with the same peptides as those described in the legend of Fig. 2 plus two peptides in which the residue located at the −8 position in each 5-HT$_2$ receptor subtype was replaced by the corresponding residue of the other receptor (5-HT$_{2A}$(G−8V) and 5-HT$_{2C}$(V−8G), respectively). *A*, areas of gels including CIPP (*arrows*) are depicted. The data are representative of four experiments. *B*, the quantification of proteins was performed as indicated in the legend to Fig. 3. Data, expressed in % of the spot volume measured in the gels obtained with the peptide corresponding to the C terminus of the 5-HT$_{2A}$ receptor, are the means ± S.E. of values obtained from four gels. $p < 0.01$ *versus* 5-HT$_{2A}$ (*) and $p < 0.05$ *versus* 5-HT$_{2C}$ ($), $n = 4$, analysis of variance followed by Student-Newman-Keul's test. *ND*, not detectable with Image Master.

dendritic processes (Fig. 7*D*). Altogether, these electron microscopy observations indicate that in the two brain regions examined, the 5-HT$_{2A}$ receptor and the 5-HT$_{2C}$ receptor show ultrastructural distribution that coincide with that of PSD-95, *i.e.* the post-synaptic thickenings of axo-dendritic synapses.

DISCUSSION

The major finding of the present study is that the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, which are two closely related members of the GPCRs activated by serotonin, interact with specific sets of PDZ proteins. Although both receptors equally interacted with activin receptor-interacting protein 1 and SAP97, we found that the 5-HT$_{2C}$ receptor preferentially bound to PSD-95, SAP102, MPP-3, and the Veli-3·CASK·Mint1 ternary complex when compared with the 5-HT$_{2A}$ receptor. In contrast, a robust interaction between the 5-HT$_{2A}$ receptor and CIPP was found, whereas CIPP did not significantly associate with the 5-HT$_{2C}$ receptor.

PDZ domains consist of 80–90 amino acids including six β strands (βA to βF) and two α-helices (αA and αB) organized in a globular structure (12, 13). One characteristic of PDZ domains is to bind to specific sequences at the very C-terminal extremity of their interacting proteins. Extensive structural, peptide screening, and biochemical studies performed in the past few years have established general principles of ligand binding specificities for PDZ domains. These analyses indicate that the amino acids located at the 0 and −2 positions in the peptide ligands play a dominant role in the binding to PDZ domains (12, 13). The −2 residue of the ligand constitutes the basis for the classification of PDZ specificity. Class I PDZ domains select for peptides with a serine or threonine at the −2 position. In this case, the hydroxyl group of the −2 residue of the ligand forms a strong hydrogen bond with the histidine at the position 1 in the βB strand. Besides the 0 and −2 residues, the residue at the −1 position and further residues upstream the minimal PDZ ligand seem to play additional roles in fine tuning, binding specificity, and affinity for PDZ domains (12, 13).

Our study shows that the −1 position in the C terminus of the 5-HT$_{2C}$ receptor is a critical determinant in its specific interaction with the Veli-3·CASK·Mint1 complex. Indeed, the single substitution of this residue in the 5-HT$_{2A}$ receptor C-terminal peptide markedly strengthened the interaction with this ternary complex. These results are consistent with previous observations showing a determinant role of this position in specifying interactions with PDZ domains (12, 13). For example, the −1 position in the protein CRIPT (for cysteine-rich interactor of PDZ three) determines its binding preference for PDZ3 over PDZ1/2 of PSD-95, SAP102, and SAP97 (34). In-

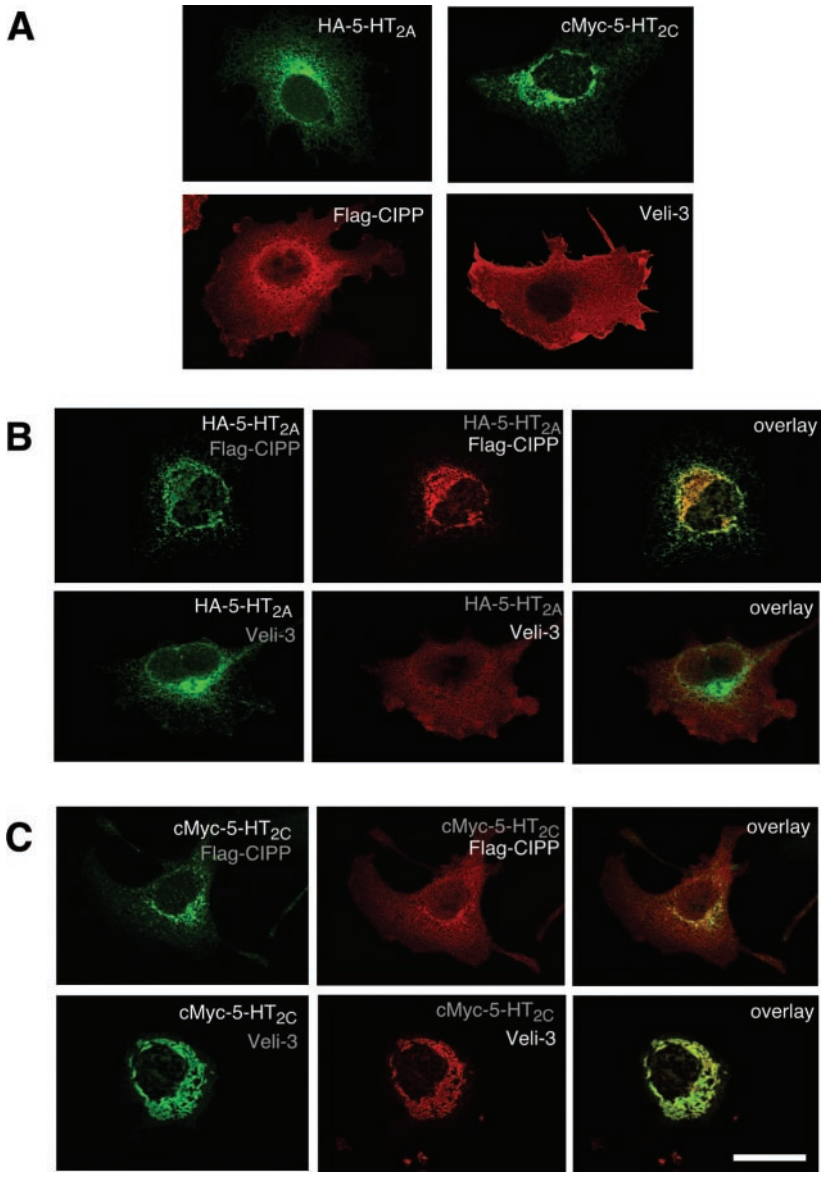

FIG. 5. **Interaction of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors with specific PDZ proteins in transiently transfected COS7 cells.** *A*, COS7 cells were transfected with either the HA-tagged 5-HT$_{2A}$ receptor, the c-Myc-tagged 5-HT$_{2C}$ receptor, FLAG-tagged CIPP, or Veli-3. Cells were examined by confocal laser-scanning microscopy. Representative cells are depicted. *B*, cells were cotransfected with the HA-tagged 5-HT$_{2A}$ receptor and either FLAG-tagged CIPP (*top panels*) or Veli-3 (*bottom panels*). The *left panels* illustrate the HA staining, the *middle panels* illustrate the FLAG or Veli-3 staining, and the *right panels* illustrate merged images. C- COS7 cells were cotransfected with the c-Myc-tagged 5-HT$_{2C}$ receptor and either FLAG-tagged CIPP (*top panels*) or Veli-3 (*bottom panels*). The *left panels* illustrate the c-Myc staining, the *middle panels* illustrate the FLAG or Veli-3 staining, and the *right panels* illustrate merged images. *Scale bar*, 20 μm (for *A–C*).

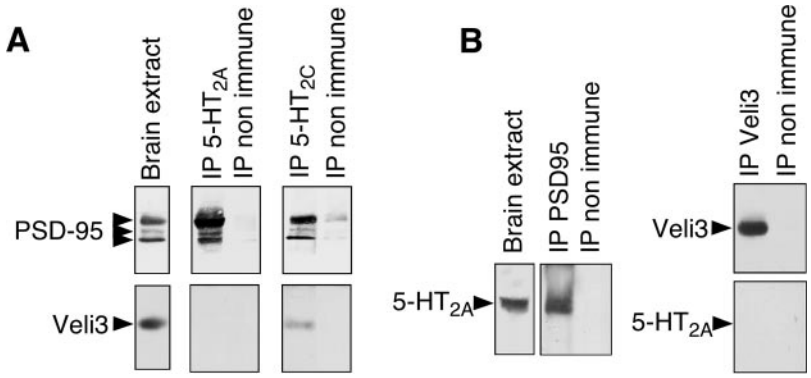

FIG. 6. **Interaction of PDZ proteins with native 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors from mice brain.** Proteins from brain extracts were immunoprecipitated with either the 5-HT$_{2A}$ receptor, the 5-HT$_{2C}$ receptor, the Veli-3, or the PSD-95 antibody. Immunoprecipitated proteins were resolved onto 12.5% polyacrylamide gels and detected by Western blotting. Input (*Brain extracts*) represents 10% of the total protein content used for the immunoprecipitation (*IP*). The data illustrated are representative of three experiments.

deed, changing the −1 residue of CRIPT to that of Kv1.4 potassium channel subunit, which preferentially interacts with PDZ1/2, abolished binding of CRIPT to PDZ3 while strengthening the interaction with PDZ1/2 (34). The structural basis of the role of the −1 position is uncertain because the side chain of the −1 residue usually points away from the binding pocket of the PDZ domain. However, in a few cases, specific interactions have been demonstrated between this residue and the interaction surface (13). Several pieces of evidence suggest that the Veli-3·CASK·Mint1 complex is recruited by the 5-HT$_{2C}$ receptor via the PDZ domain of Veli-3; Veli-3 was detected on silver-stained two-dimensional gels, whereas the binding of

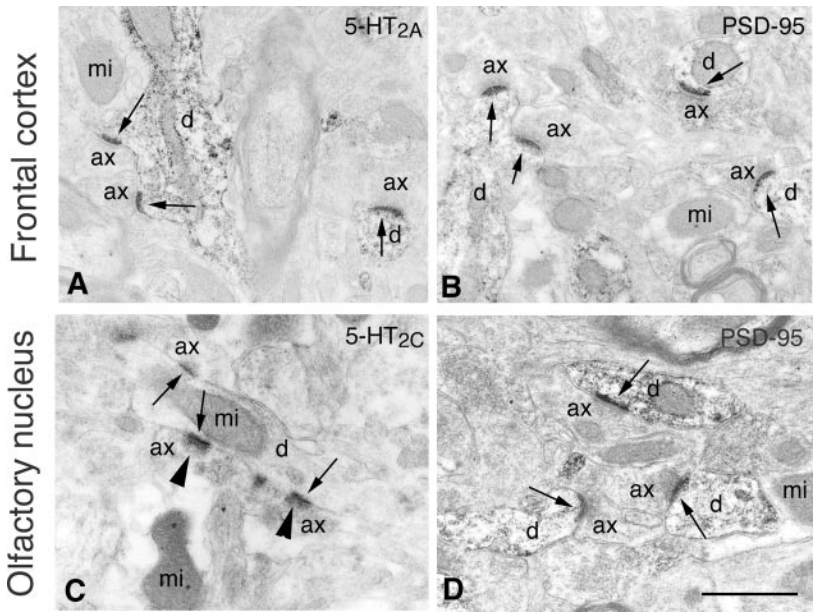

FIG. 7. **Comparison of the subcellular distribution of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors with that of PSD-95 within the brain.** Electron micrographs illustrate immunoperoxidase labeling for the 5-HT$_{2A}$ receptor and PSD-95 within the mouse frontal cortex (*A* and *B*) and for the 5-HT$_{2C}$ receptor and PSD-95 within the anterior olfactory nucleus (*C* and *D*). In both brain regions, electron-dense precipitates corresponding to immunostaining for the 5-HT$_{2A}$ receptor or the 5-HT$_{2C}$ receptor are associated with the postsynaptic thickenings of axo-dendritic synapses (*arrows* in *A* and *C*) that also exhibit intense immunostaining for PSD-95 (*arrows* in *B* and *D*). Note that immunostaining for the 5-HT$_{2A}$ receptor and PSD-95 are also associated with intracytoplasmic structures located within the postsynaptic dendritic profiles (*A*, *B*, and *D*), whereas immunostaining for the 5-HT$_{2C}$ receptor is associated with presynaptic thickenings of some axo-dendritic synapses (*arrowheads* in *C*). No peroxidase staining was observed in sections processed without primary antibodies (not shown). *ax*, axonal profile; *d*, dendritic profile; *mi*, mitochondria. *Scale bar*, 1 μm (applies for *A–D*).

CASK and Mint1 was only shown by immunoblotting, consistent with an indirect recruitment of these proteins; the PDZ domain of CASK is of class II, making unlikely a direct interaction with the PDZ ligand of the 5-HT$_{2C}$ receptor. In addition to a PDZ domain, Veli proteins also express another interaction domain designated L27 domain. This domain is involved in the interaction of Veli proteins with CASK. The L27 domain of Veli proteins is also capable of interacting with other PDZ proteins identified in our study, such as SAP97 and MPP-3 (35, 36). This may suggest that Veli-3 could be indirectly recruited through another modular protein. In this regard, it should be pointed out that our approach cannot discriminate between direct and indirect interactions. However, the distinct binding profile of Veli-3, SAP97, and MPP-3 to the peptides used in our study suggests that these proteins are recruited independently by the PDZ ligands of the receptors and, therefore, that the −1 residue at the 5-HT$_{2C}$ receptor C terminus determines its specific interaction with the PDZ domain of Veli-3.

Our data also indicate that residues upstream from the −2 position of 5-HT$_2$ receptor carboxyl peptides also specify differential recognition between PDZ proteins. Indeed, the double substitution of the −1 and −6 positions of the 5-HT$_{2A}$ receptor C terminus to those of the 5-HT$_{2C}$ receptor strengthened the binding to PSD-95, SAP102, and MPP-3 to a similar level as that measured with the 5-HT$_{2C}$ receptor, whereas the single permutation of the −1 position did not improve the interaction of the 5-HT$_{2A}$ receptor with these PDZ proteins. Similarly, the residue at the −8 position is a key determinant of the preferential interaction of the 5-HT$_{2A}$ receptor *versus* the 5-HT$_{2C}$ receptor, with CIPP. These results are consistent with previous findings indicating that switching the last eight or nine residues of the Kv1.4 C terminus to those of CRIPT is required to convert their binding behavior to PSD-95 PDZ domains (34). Similarly, the tyrosine at −7 position in the tyrosine kinase receptor ErbB2 C terminus is involved in the binding to the Erbin PDZ domain (37). We also must point out that our approach only provides a global overview of the specific interactions of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor with PDZ proteins. Several PDZ domains may participate individually or as dimers (38, 39) in the binding of multi-PDZ proteins such as PSD-95, SAP102, and CIPP to the receptor C terminus, and the observed binding properties probably result from the sum of these interactions. Systematic identification of the PDZ domains contributing to the interactions with the carboxyl peptides of each 5-HT$_2$ receptor would allow the identification of the structural determinants of the specificity observed in our study.

Most of the proteins identified as binding partners of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are recognized for their role in organizing synaptic protein networks. Electron microscopy experiments indicated that in neurons the 5-HT$_{2C}$ receptor is highly concentrated at post-synaptic and presynaptic thickenings of axo-dendritic synapses, consistent with its interaction with both proteins of the post-synaptic density such as PSD-95, SAP97, and SAP102 and the Veli-3·CASK·Mint1 ternary complex, which is known to interact with protein components of presynaptic termini (40–42). In contrast, the 5-HT$_{2A}$ receptor, which does not interact with this ternary complex, was only detected on post-synaptic processes in the frontal cortex, where it showed ultrastructural distribution similar to that of PSD-95, *i.e.* the cytosol of dendritic shafts and post-synaptic thickenings of axo-dendritic synapses. These observations are consistent with several ultrastructural analyses that reported not only a plasma membrane (including synaptic portions) but also a cytoplasmic (within dendrites) localization of the 5-HT$_{2A}$ receptor and PSD-95 or others MAGUKs (33, 43–45).

Our immunofluorescence experiments performed in transfected COS-7 cells indicated that the interaction of recombinant 5-HT$_2$ receptors and PDZ proteins mainly occurred in intracellular compartments, especially within membrane-type structures in the perinuclear region. These experiments are consistent with recent findings indicating that MAGUK-*N*-

methyl-D-aspartate receptor complexes are formed in intracellular compartments, possibly the Golgi apparatus and the endoplasmic reticulum (46). It is difficult to conclude from such experiments performed in heterologous cells whether this intracellular distribution of PDZ protein-receptor complexes reflects early interaction in the biosynthetic pathway because co-overexpression may force an earlier than normal interaction. However, intracellular localization of these proteins is probably not an overexpression artifact. Indeed, it is consistent with electron microscopy observations that revealed a prominent intracellular localization of the 5-HT_{2A} receptor and an equally prevalent plasma membrane and intracytoplasmic distribution of PSD-95 (44, 45).

In conclusion, our study demonstrates that the 5-HT_{2A} and the 5-HT_{2C} receptors, two closely related GPCRs that share an identical canonical PDZ ligand, interact with distinct sets of PDZ proteins. It has been recently reported that the interaction of the 5-HT_{2A} receptor with PSD-95 enhances the 5-HT_{2A} receptor-mediated signal transduction and decreases agonist-induced 5-HT_{2A} receptor internalization (18). Similarly, the interaction of the 5-HT_{2C} receptor with PDZ proteins may regulate receptor signal transduction. In fact, the deletion of its PDZ ligand delays resensitization of the receptor responses (47). Hence, the specific set of PDZ proteins to which each 5-HT_2 receptor subtype binds may contribute to their differences in signal transduction properties.

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