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The Dopamine D3 Receptor Interacts with Itself and the Truncated D3 Splice Variant D3nf: D3-D3nf Interaction Causes Mislocalization of D3 Receptors

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ABSTRACT

We have generated a stable cell line expressing FLAG epitope-tagged D3 dopamine receptors and used this cell line to study D3 receptor-protein interactions. To analyze protein interactions, we separately introduced into the stable cell line either D3 receptors carrying an hemagglutinin (HA) epitope tag, or an HA-tagged version of the D3 receptor splice variant D3nf. A combination of confocal laser microscopy and coimmunoprecipitation was used to assay the formation and expression pattern of D3-D3 homodimers or D3-D3nf heterodimers. When coexpressed in HEK 293 cells, FLAG- and HA-tagged D3 receptors exhibited a similar plasma membrane distribution. Using an HA epitope tag-specific antibody, we coimmunoprecipitated HA- and FLAG-tagged D3 receptors, suggesting that D3 receptors are capable of forming homodimers. Epitope-tagged D3nf polypeptides exhibited a markedly different cellular distribution than D3 receptors. When expressed in HEK 293 cells, either alone or in combination with FLAG-tagged D3 receptors, D3nf exhibited a punctate perinuclear distribution. When D3nf was introduced into the stable D3-expressing cell line, D3 receptors were no longer visualized at the plasma membrane. Instead, D3 and D3nf showed a similar, predominantly cytosolic, localization. Using the HA-specific antibody, we were able to coimmunoprecipitate D3 and D3nf polypeptides from transfected cells. These data suggest the existence of physical interaction between D3 and D3nf. This interaction appears to result in the mislocalization of D3 receptors from the plasma membrane to an intracellular compartment, a finding that could be of significance in the etiology of schizophrenia.

Dopamine neurotransmission in mammalian brain is mediated by a cohort of receptors that are members of the superfamily of G protein-coupled receptors (GPCRs). In humans, five dopamine receptor subtypes (D1–D5) have been identified by molecular cloning (reviewed in Missale et al., 1998). The five dopamine receptors have been grouped into two subfamilies based upon sequence homologies and pharmacologic profiles. The D1 class of dopamine receptors is comprised of the D1 and D5 receptor subtypes. These receptors are expressed at high levels in cerebral cortex and are coupled to stimulatory subsets of heterotrimeric G proteins. The D2 class of dopamine receptors, consisting of the D2, D3, and D4 subtypes, is coupled to inhibitory subsets of G proteins and is a major target of antipsychotic drugs.

Among the D2 class of dopamine receptors, the D3 receptor is distinctive in that it is distributed preferentially in limbic areas of the brain (nucleus accumbens, olfactory tubercle, islands of Calleja, and hippocampus) thought to control cognitive and emotional aspects of behavior (Bouthenet et al., 1991). The D3 receptor has also been shown to bind most antipsychotic drugs, both typical and atypical, with high affinity (Levant, 1997). Because of its anatomic distribution and interaction with antipsychotic drugs, the D3 dopamine receptor has been suggested to play a role in the etiology of schizophrenia. Post-mortem examination of patients with chronic schizophrenia has shown a selective loss of D3 mRNA sequences in the parietal and motor cortex (Schmauss et al., 1993). A novel D3 receptor splice variant, termed D3nf, has recently been identified and shown to be present in regions of schizophrenic brains lacking D3 mRNA transcripts (Schmauss et al., 1993). D3nf mRNA encodes a polypeptide with a carboxyl terminus distinct from that of the original human D3 receptor (Schmauss et al., 1993). Immunohistochemical analysis using antibodies raised against the unique carboxyl terminus of D3nf has revealed expression of D3nf polypeptides in rat and monkey brain (Nimchinsky et al., 1997). It is not clear, however, whether the D3nf polypeptide is correctly targeted to, or inserted into, the plasma membrane. The functional significance of D3nf in either normal or

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ABBREVIATIONS: GPCRs, G protein-coupled receptors; HA, hemagglutinin; HEK, human embryonic kidney; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; GABA, γ-aminobutyric acid.

aberrant dopaminergic neurotransmission is also an issue that has not been clearly elucidated.

Signaling through the D3 receptor plays an important role in regulating a variety of neural processes including brain development (Fishburn et al., 1996; Levant, 1997), modulation of locomotor activity (Accili et al., 1996), and motivational aspects of behavior (Caine and Koob, 1993). However, the mechanisms that control signaling through the D3 and other neurotransmitter receptors are complex and, at present, not well understood. Recent work has suggested protein-protein interactions may play a crucial role in receptor-mediated signaling events. For example, specialized proteins such as rapsyn and gephyrin, as well as a large class of proteins containing PDZ domains, have been shown to cluster and localize signaling molecules at neuronal synapses (Craven and Bredt, 1998). To begin to investigate potential mechanisms of D3 receptor regulation, a stable cell line expressing an epitope-tagged D3 receptor was generated and used to analyze D3 receptor-protein interactions. In this study, we provide evidence for the existence of D3-D3 and D3-D3nf interactions. When coexpressed in transfected cells, D3nf appears to function in a dominant-negative fashion to prevent D3 receptors from localizing to the plasma membrane. These observations are an important first step in understanding the functional significance of D3nf in D3 receptor-mediated neurotransmission.

Materials and Methods

DNA Constructs and Transfections. FLAG or hemagglutinin (HA) epitope tags were inserted at the amino termini of the D3 and D3nf polypeptides by polymerase chain reaction mutagenesis as described by Nelson and Long (1989). Each tagged polypeptide was verified by DNA sequencing and then subcloned into the eukaryotic expression vector pCB6 (Brewer and Roth, 1991). Human embryonic kidney (HEK) 293 cells were used as recipients for transfection. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transfections were performed by the calcium phosphate coprecipitation method as previously described (Canfield et al., 1996). For stable cell lines, cells were transfected with a FLAG-tagged D3 receptor cDNA construct and maintained under selection using 800 mg/ml G418 (LifeTechnologies Inc., Rockville, MD). Individual clones were selected approximately 3 weeks following transfection and expanded into cell lines that were maintained in medium containing 800 mg/ml G418. For transient transfections, HEK 293 cells were plated either in 100-mm tissue culture dishes or on glass coverslips and transfected under conditions described above. Neuro-2a cells were obtained from American Type Culture Collection (Manassas, VA) and grown in minimum essential Eagle's medium supplemented with 10% fetal calf serum. Transfection of Neuro-2a cells was performed using the LipofectAMINE 2000 transfection reagent (Life Technologies Inc.) according to instructions supplied by the manufacturer.

Immunofluorescence and Confocal Microscopy. Transiently transfected HEK 293 cells grown on glass coverslips were examined 72 h after transfection. Cells were fixed in 1:1 methanol/acetone (v/v) solution, blocked with PBS containing 2% bovine serum albumin and 10% goat serum at room temperature for 1 h, and then incubated in the same medium with a 1:1000 dilution of the HA-specific monoclonal antibody (mAb) 16B12 (Babco, Berkeley, CA) or a 1:4000 dilution of the anti-FLAG mAb M2 (Kodak, Rochester, NY). Secondary antibody (Cy-3-conjugated goat anti-mouse IgG; Jackson ImmunoResearch, West Grove, PA) was diluted 1:800 and applied in the same buffer. For double labeling, cells were coincubated with a 1:200 dilution of a polyclonal HA antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and a 1:4000 dilution of the FLAG-specific M2 mAb antibody. Labeling was detected by incubation with rhodamine red-conjugated goat anti-mouse (diluted 1:200) and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit (diluted 1:200) secondary antibodies (Jackson ImmunoResearch). Immunofluorescence was visualized by confocal laser scanning microscopy using a Zeiss LSM 210 confocal microscope.

Membrane Preparation, Immunoblotting, and Immunoprecipitation. Crude membrane fractions from either stably or transiently transfected HEK 293 cells were prepared as described previously (Shyjan and Levenson, 1989), and protein concentrations were determined by the method of Bradford (1976). Solubilized membrane fractions were separated on an SDS-containing 12% polyacrylamide gel (70 μg of protein/lane) and transferred to a polyvinylidene fluoride membrane (ICN Biomedicals, Aurora, OH) as described (Karpa et al., 1999). The filter was blocked for 2 h in PBS containing 10% dry milk and 5% goat serum, and then incubated with a 1:1000 dilution of the anti-FLAG M2 mAb. The filter was rinsed with PBS and then incubated with either horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (Jackson ImmunoResearch) for 1 h. Immunoreactivity was detected by enhanced chemiluminescence using an ECL Plus kit (Amersham, Piscataway, NJ).

To deglycosylate D3 receptors, microsomes from transfected cells were digested with *N*-glycosidase F (Boehringer Mannheim, Indianapolis, IN). Protein samples (70 μg) were solubilized by denaturation in 125 mM $NaPO_4$ (pH 7.4), 10 mM EDTA, 1% SDS for 1 h at 37°C, and then treated in the same buffer with 1.0 unit of *N*-glycosidase F, 1.0% Triton X-100 (Smith et al., 1987). Samples were digested for 1 h at 37°C.

For immunoprecipitation, cell lysates were prepared from transfected cells using protocols described in the ImmunoCatcher immunoprecipitation kit (CytoSignal, Irvine, CA). Cell lysates were incubated with a 1:100 dilution of the anti-HA mAb 12CA5 (Boehringer Mannheim) for 2 h at room temperature. Protein A/G resin (Cytosignal) was used to capture antigen, and bound proteins were eluted with SDS-polyacrylamide gel electrophoresis gel loading buffer. Proteins were analyzed by electrophoretic separation on an SDS-containing 12% polyacrylamide gel.

Results

Generation and Characterization of D3 Dopamine Receptor Expressing Cell Lines. To study D3 dopamine receptor-protein interactions, we generated an HEK 293 cell line stably expressing FLAG epitope-tagged D3 dopamine receptors. Confocal laser microscopy was used to analyze the cellular distribution of the epitope-tagged D3 receptors. As shown in Fig. 1A, cells transfected with the FLAG-tagged D3 receptor were reactive with the FLAG-specific M2 mAb. Strong staining was visualized at cell margins, indicating a predominantly plasma membrane localization of the D3 receptor polypeptides. Untransfected cells, or cells treated with secondary antibody alone, produced no visible staining (data not shown).

To further evaluate D3 receptor expression, we analyzed immunoblots containing microsomes prepared from stably transfected HEK 293 cells. Figure 1B (− lane) shows that the FLAG-specific mAb reacted most intensely with a broad band ~40 to ~45 kDa in size. A series of less intense bands ~58, ~60, and ~80 kDa in size were also reactive with the M2 mAb. Each of these immunoreactive bands appear to represent differentially glycosylated forms of the D3 receptor. Treatment of the microsomes with *N*-glycosidase F, an enzyme that removes *N*-linked sugars, produced a predominant immunoreactive band ~40 kDa in size (Fig. 1B, + lane). This

band corresponds well with the size of the D3 dopamine receptor predicted from cDNA cloning (Sokoloff et al., 1990). These results demonstrate that transfected HEK 293 cells stably express epitope-tagged D3 receptors. These receptors are post-translationally modified by the addition of *N*-linked sugars and appear to be properly targeted to the plasma membrane.

Cellular localization of epitope-tagged D3 and D3nf polypeptides was examined in transiently transfected HEK 293 cells. Cells transiently transfected with either FLAG- (Fig. 2B) or HA-tagged (Fig. 2D) D3 constructs showed predominantly plasma membrane localization of D3 receptors. Untransfected cells produced no visible staining with anti-FLAG (Fig. 2A) or anti-HA (Fig. 2C) antibodies. These results indicate that transiently expressed D3 receptors exhibit the same plasma membrane distribution as stably expressed D3 receptors. Transient transfection of epitope-tagged D3nf constructs produced a strikingly different staining pattern. Cells transiently transfected with either FLAG- (Fig. 3B) or HA-tagged (Fig. 3D) D3nf constructs showed a predominantly punctate, cytosolic staining pattern. Little, if any, staining was observed at the margins of cells transiently transfected with D3nf constructs. Untransfected cells showed virtually no antibody reactivity with either the anti-FLAG (Fig. 3A) or anti-HA (Fig. 3C) antibodies. These results provide the first evidence that the D3 splice variant, D3nf, does not appear to traffic to the plasma membrane. Instead, D3nf polypeptides appear to be retained within some intracellular compartment in transfected HEK 293 cells.

D3-D3nf and D3-D3 Interactions. The distinct intracellular distributions exhibited by D3 and D3nf prompted us to examine the effect of coexpression of D3 and D3nf constructs on the subsequent localization of the two polypeptides. To address this issue, we used confocal laser microscopy to localize epitope-tagged D3 and D3nf polypeptides after cells stably expressing FLAG-tagged D3 receptors were transiently transfected with an HA-tagged D3nf construct. As shown in Fig. 4A, cells reactive with anti-HA antibodies showed a punctate staining pattern (arrow), suggesting cytosolic localization of D3nf polypeptides. To localize D3 receptors, the same field of cells was stained with anti-FLAG antibodies (Fig. 4B). In cells not expressing D3nf, anti-FLAG D3 receptor staining was observed at cell margins (arrowhead). In cells expressing D3nf (arrow), anti-FLAG antibodies gave a predominantly cytosolic staining pattern. Superimposition of anti-HA and anti-FLAG images (Fig. 4C) showed overlap in the cytosolic distribution of anti-FLAG and anti-HA immunoreactivity in cells (arrow) exhibiting coexpression of D3 and D3nf polypeptides. These results suggest that when D3 and D3nf polypeptides are coexpressed in the same cell, D3 receptors no longer traffic to the plasma membrane.

It is possible that the failure of the D3 dopamine receptor to correctly traffic to the plasma membrane, when coexpressed in the same cell as D3nf, may represent an artifact of the transient expression assay system. As a control, we an-

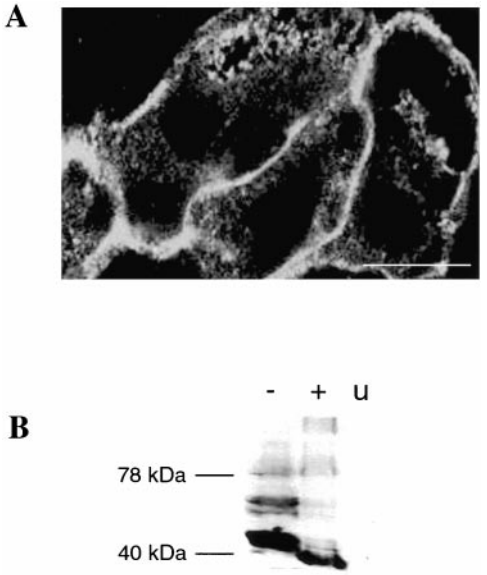

Fig. 1. Stable expression of dopamine D3 receptors in HEK 293 cells. A, FLAG-tagged D3 dopamine receptors were detected using the FLAG-specific M2 mAb and visualized by confocal laser scanning microscopy. Bar = 10 μm. B, immunoblots containing crude membrane fractions prepared from HEK 293 cells stably expressing FLAG-tagged D3 receptors. Proteins were separated on SDS gels containing 12% polyacrylamide gels, electroblotted to polyvinylidene fluoride membranes and probed with the anti-FLAG M2 mAb. −, untreated microsomes; +, treatment of microsomes with *N*-glycosidase F; U, untransfected HEK 293 cells. Positions of molecular mass markers are shown at left.

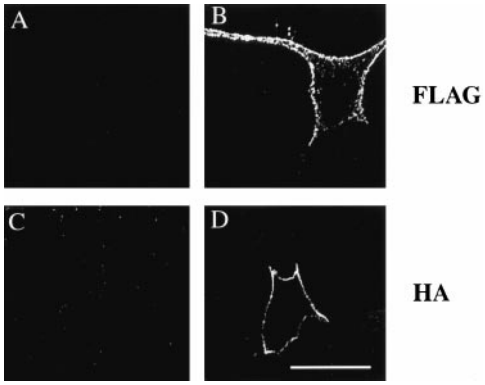

Fig. 2. Subcellular localization of epitope-tagged dopamine D3 receptors visualized by confocal laser microscopy. A and C, untransfected HEK 293 cells stained with anti-FLAG and anti-HA antibodies, respectively. HEK 293 cells transiently expressing FLAG-tagged (B) or HA-tagged (D) D3 receptors. Reactivity was detected using anti-FLAG (B) or anti-HA (D) antibodies and visualized with Cy3-conjugated anti-mouse secondary antibodies. Bar = 10 μm.

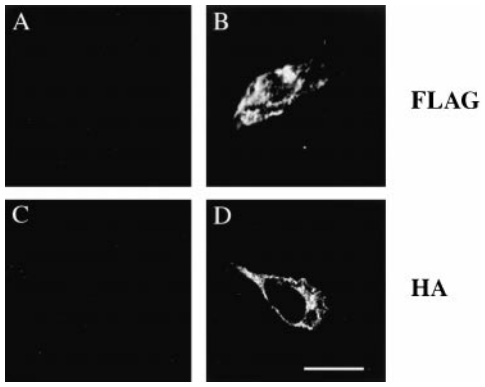

Fig. 3. Cytosolic localization of the D3nf splice variant. A and C, untransfected HEK 293 cells stained with anti-FLAG and anti-HA antibodies, respectively. HEK 293 cells transiently expressing FLAG-tagged (B) or HA-tagged (D) D3nf polypeptides. Reactivity was detected using anti-FLAG (B) or anti-HA (D) antibodies and visualized by confocal laser scanning microscopy. Bar = 10 μm.

alyzed the distribution of D3 receptors following transient expression of HA-tagged D3 receptors in HEK 293 cells stably expressing FLAG-tagged D3 receptors. Strong anti-HA antibody staining was observed at cell margins (Fig. 4D), indicating targeting of transiently expressed HA-tagged D3 receptors to the plasma membrane. A similar staining pattern was obtained when the same field of cells was reacted with anti-FLAG antibodies (Fig. 4E). Merging the HA and FLAG images (Fig. 4F) produced almost complete overlap of the anti-HA and anti-FLAG staining patterns, indicating colocalization of FLAG- and HA-tagged D3 receptors in the plasma membrane of transfected cells. These results suggest that transient expression of HA-tagged D3 receptors did not adversely affect the trafficking of either HA- or FLAG-tagged D3 receptors to the plasma membrane. Taken together, these experiments provide initial evidence suggesting that coexpression of D3 and D3nf polypeptides causes mislocalization of D3 receptors to an intracellular compartment.

The trafficking of D3 and D3nf was also examined in transiently transfected murine neuroblastoma Neuro-2a cells. Cells transiently transfected with an HA-tagged D3 construct (Fig. 5A) showed predominantly plasma membrane localization of D3 receptors, whereas cells transiently transfected with a FLAG-tagged D3nf construct (Fig. 5B) showed a predominantly cytosolic staining pattern. In Neuro-2a cells coexpressing HA-tagged D3 and FLAG-tagged D3nf constructs, D3 (Fig. 5C) and D3nf (Fig. 5D) polypeptides appeared to localize within the cytosol. Superimposing the HA and FLAG images (Fig. 5E) showed almost complete overlap in the cytosolic distribution pattern of D3 and D3nf polypeptides. These results further support the view that D3nf causes redistribution of D3 receptors from the plasma membrane to the cytosol.

Immunoprecipitation experiments were performed to investigate potential interactions between D3-D3 and D3-D3nf polypeptides. The results are shown in Fig. 6. To analyze D3-D3 interactions, lysates were prepared from HEK 293 cells stably expressing FLAG-tagged D3 receptors and transiently expressing HA-tagged D3 receptors. These cells are the same cells as depicted previously in Fig. 4, D–F. D3 receptors were immunoprecipitated with anti-HA antibodies, and the immunoprecipitate was analyzed by Western blotting using anti-FLAG antibodies. As shown in Fig. 6 (lane A), the anti-FLAG antibody detected a band of ~44 kDa, a size consistent with that of the D3 dopamine receptor. These results suggest that anti-HA antibodies can coimmunoprecipitate HA- and FLAG-tagged D3 receptors. A similar approach was used to immunoprecipitate HA-tagged D3nf polypeptides from transfectants stably expressing FLAG-tagged D3 receptors and transiently expressing D3nf polypeptides. These are the same cells shown previously in Fig. 4, A–C. Western blot analysis of the immunoprecipitate using anti-FLAG antibodies produced an immunoreactive band of ~44 kDa (Fig. 6, lane B), suggesting that anti-HA antibodies can coimmunoprecipitate HA-tagged D3nf and FLAG-tagged D3 polypeptides. No immunoreactive bands were visible (Fig. 6, lane C) in lysates prepared from cells expressing FLAG-tagged D3 receptors alone. These results provide strong evidence for physical interaction between D3-D3 and D3-D3nf polypeptides. It is not known, however, whether the proteins interact directly or indirectly via an additional partner or partners.

Discussion

In this study, we describe several novel features of D3 dopamine receptor structure and function. The ability to coimmunoprecipitate HA- and FLAG-tagged D3 receptors from doubly transfected cells provides biochemical evidence that D3 receptors can form homodimers. Immunoprecipita-

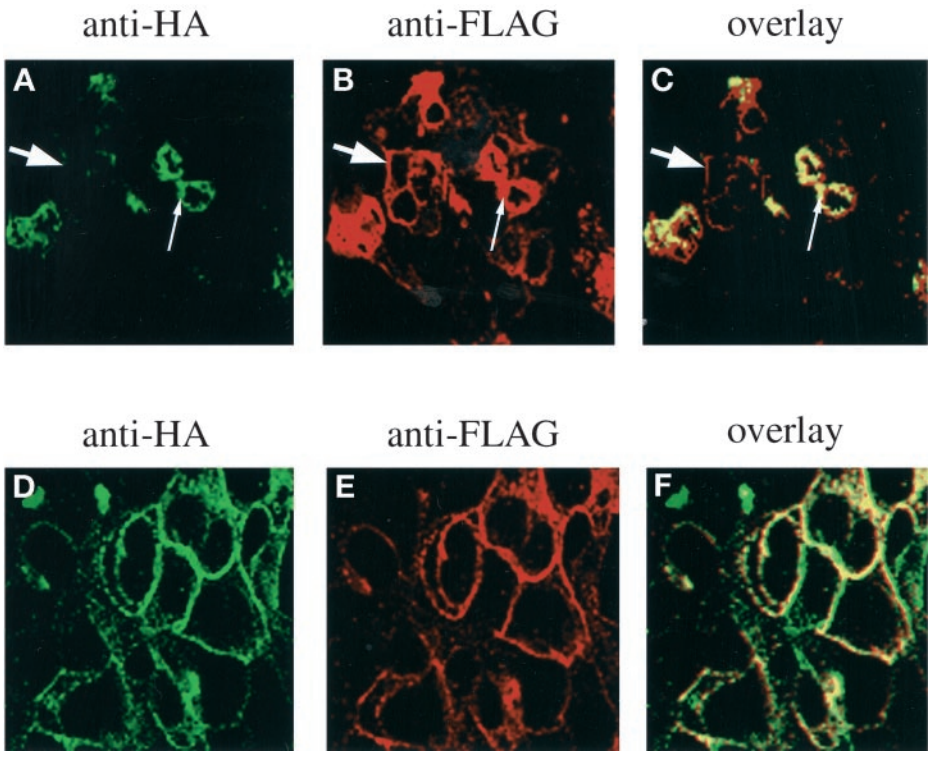

Fig. 4. Immunolocalization of D3 and D3nf polypeptides in doubly transfected cells visualized by confocal laser microscopy. HEK 293 cells stably expressing FLAG-tagged D3 receptors were transiently transfected with either HA-tagged D3nf (A–C) or HA-tagged D3 receptor (D–F) constructs. Cells expressing HA-tagged D3nf (A) or HA-tagged D3 (D) polypeptides were stained with rabbit polyclonal anti-HA antibodies and visualized by FITC-conjugated goat anti-rabbit IgG. Stably expressed FLAG-tagged D3 receptors (B and E) were detected using the M2 anti-FLAG mAb and visualized by Cy3-conjugated anti-mouse secondary antibodies. Confocal detection of anti-HA and anti-FLAG antibody double labeling (C and F). Magnification: (A–C) 1000×; (D–F) 2000×.

tion analysis indicates that physical interaction between D3 and D3nf polypeptides can occur as well. As a result of this interaction, D3 receptors no longer appear to be capable of trafficking to the plasma membrane. D3nf, whose normal cellular function is unknown, may act in transfected cells as a dominant-negative regulator of D3 receptor activity.

A number of GPCRs have been shown to be capable of forming homodimeric (Hebert et al., 1996; Fukushima et al., 1997; Xie et al., 1999) or heterodimeric (Jordan and Devi, 1999; Marshall et al., 1999; Xie et al., 1999) structures. It has recently been suggested that the dopamine D3 receptor may also form higher order (dimeric and tetrameric) structures (Nimchinsky et al., 1997). However, this conclusion is based primarily on the identification of D3 antibody-reactive polypeptides that migrate on gels at the positions expected for multimers of the D3 receptor core protein (Nimchinsky et al., 1997). Here we provide direct biochemical data supporting the view that the D3 receptor is capable of forming homodimers. This conclusion is based on coexpression of D3 receptors containing HA or FLAG epitope tags in transfected HEK 293 cells. The ability of an antibody directed against one of the epitope tags to immunoprecipitate D3 receptors carrying the heterologous tag provides very strong evidence for D3-D3 receptor interaction. It is possible that D3 receptor polypeptides may interact directly with one another, as has been described for muscarinic (Maggio et al., 1996) and $GABA_B$ (White et al., 1998) receptors. Alternatively, D3 receptors could interact indirectly via interaction with receptor accessory proteins, as has been described for $GABA_A$ receptors (Essrich et al., 1998; Wang et al., 1999). At present, the functional significance of D3 receptor homodimerization remains unknown. It will clearly be of interest to define the domains through which D3 receptors interact, and to ascertain whether dimerized D3 receptors exhibit different pharmacological properties than do monomeric D3 receptors. Recently, dopamine D5 receptors have been shown to interact directly with $GABA_A$ receptors (Liu et al., 2000). Our expression system could therefore be used to study interaction between D3 and other dopamine receptor subtypes, as well as additional GPCR family members.

The dopamine D3 receptor splice variant D3nf was originally identified in post-mortem brains from patients with schizophrenia (Schmauss et al., 1993). The polypeptide encoded by D3nf mRNA lacks the sixth and seventh transmembrane segments found in the full-length D3 receptor. Although D3nf polypeptides have been detected in human, monkey, and rodent brain (Liu et al., 1994; Nimchinsky et al., 1997), it is not clear whether D3nf functions as an authentic GPCR (Schmauss et al., 1993). Here we show that in transiently transfected HEK 293 and Neuro-2a cells, epitope-tagged D3nf polypeptides do not traffic to the plasma membrane. Instead, D3nf polypeptides localize to an as yet undefined intracellular compartment. It is possible that transient D3nf overexpression could lead to the cytoplasmic accumulation of D3nf polypeptides. We view this possibility as unlikely, however, since transiently expressed wild-type D3 receptors are correctly targeted to the plasma membrane in both HEK 293 and Neuro-2a cells. Our data thus lends support to the idea that D3nf is not likely to function as a typical GPCR.

In cells stably expressing FLAG-tagged D3 receptors, anti-FLAG staining was detected almost exclusively at the plasma membrane. Introduction of D3nf into these cells produced a dramatic shift in anti-FLAG staining from the plasma membrane to the cytoplasm. In contrast, the plasma membrane distribution of anti-FLAG staining was not af-

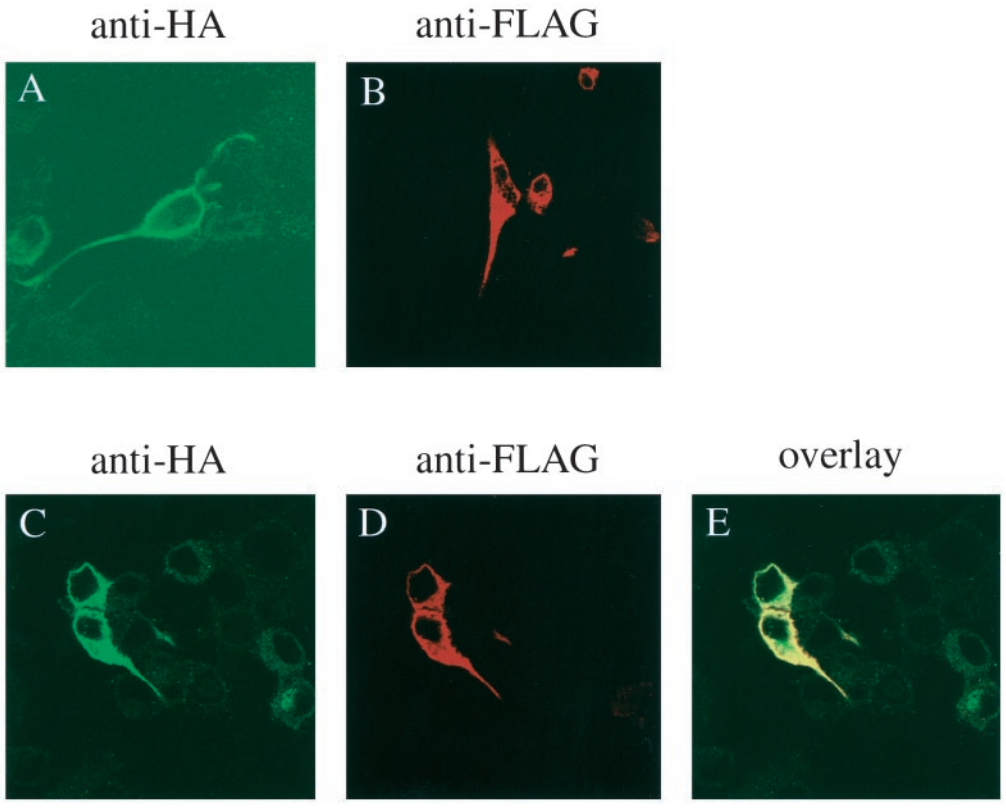

Fig. 5. Coexpression of D3 and D3nf in murine Neuro-2a cells. Neuro-2a cells were transiently transfected with either HA-tagged D3 (A), FLAG-tagged D3nf (B) constructs, or were doubly transfected (C–E). Cells expressing HA-tagged D3 polypeptides (A and C) were stained with a 1:200 dilution of a rabbit polyclonal anti-HA antibody and visualized by FITC-conjugated goat anti-rabbit secondary antibodies (diluted 1:200). Cells expressing FLAG-tagged D3nf (B and D) were stained with a 1:1000 dilution of the anti-FLAG M2 antibody and visualized with Rhodamine Red-X-conjugated goat anti-mouse secondary antibodies (1:200 dilution). Confocal detection of anti-HA and anti-FLAG antibody double labeling (E). Magnification: 100×.

fected by transient expression of HA-tagged D3 receptors in stably transfected HEK 293 cells. These results strongly imply that D3nf causes mislocalization of D3 receptors in doubly transfected cells and that mislocalization of D3 receptors is not an artifact of the transient expression system. The fact that D3 and D3nf can be coimmunoprecipitated from doubly transfected cells suggests that the physical interaction of D3 and D3nf polypeptides is the mechanism that underlies the failure of D3 receptors to traffic to the plasma membrane. A number of truncated receptor variants have recently been described, including truncated forms of the V2 vasopressin (Zhu and Wess, 1998), gonadotropin-releasing hormone (Grosse et al., 1997), and chemokine 5 (Benkirane et al., 1997) receptors. In each case, coexpression of truncated and wild-type receptors results in diminished cell surface expression of the full-length receptors. The immunolocalization studies reported here provide the first evidence that D3nf may play an inhibitory role in dopaminergic signaling through D3 receptors.

The physiological significance of D3-D3nf protein interaction is not yet understood. A truncated version of the D3 receptor has recently been generated in transgenic mice (Accili et al., 1996). Binding of the dopamine antagonist iodosulpride was greatly reduced in mice heterozygous for the D3 receptor mutation, suggesting that the truncated D3 receptor may act in a dominant-negative fashion to inhibit antagonist binding to D3 receptors produced from the wild-type allele (Accili et al., 1996). It will therefore be of considerable interest to determine whether in coexpression studies, D3nf acts in a dominant-negative fashion to alter the pharmacological profile of the D3 receptor for various ligands, or whether D3-D3nf interaction affects activation of appropriate subsets of G proteins in transfected cells. For D3-D3nf interactions to have physiological relevance in vivo, it is necessary that the two polypeptides be coexpressed in the same neurons. Immunostaining with D3- and D3nf-specific antibodies has revealed overlap in the distribution of D3 and D3nf polypeptides within cortical pyramidal neurons of rat brain (Nimchinsky et al., 1997). Experiments designed to determine the subcellular localization of D3 and D3nf polypeptides within specific neuronal cell types could help clarify the role D3nf plays in the intracellular trafficking of D3 receptors.

Finally, D3-D3nf interaction may be of relevance for understanding the etiology of schizophrenia. D3nf has been detected in brains of normal human subjects as well as brains from patients with chronic schizophrenic (Schmauss et al., 1993). In schizophrena, the ratio of D3 to D3nf mRNA sequences is altered in several brain regions including the parietal and motor cortices. It is conceivable that within these brain regions, alterations in the ratio of D3 to D3nf polypeptides could profoundly affect cell surface expression of D3 receptors and thus contribute to the manifestation of schizophrenia. Clearly, investigation of the role of D3 receptor function is just beginning. Further study is required to verify whether alterations in the homo- or heterodimeric structure of this receptor contribute to abnormalities in dopaminergic neurotransmission in various pathological conditions.

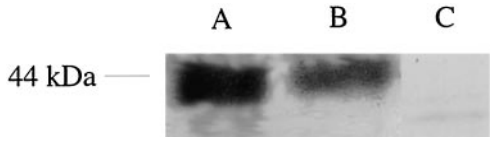

Fig. 6. D3-D3 and D3-D3nf interaction. Immunoblot of proteins immunoprecipitated from lysates of HEK 293 cells stably expressing FLAG-tagged D3 receptors and transiently transfected with HA-tagged D3 (lane A) or HA-tagged D3nf (lane B) constructs, or not transiently transfected (lane C). Lysates were immunoprecipitated using the 12CA5 anti-HA mAb, and the blot was probed with polyclonal anti-FLAG antibodies (Santa Cruz Biotechnology). The 44-kDa band runs at the size expected for the D3 receptor.

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