Oligomerization of Dopamine and Serotonin Receptors

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Until recently, it has largely been assumed that G protein-coupled receptors (GPCRs) function as monomeric entities. However, over the past few years, we and others have documented that GPCRs can form dimers and oligomers, leading to a re-evaluation of the mechanisms thought to mediate GPCR function. Despite the growing number of investigations into dimerization, little is known about the structural basis of receptor-receptor interactions and the functional consequences of dimer formation. Here, we present a brief review of some insights we have gained into the dimerization of dopamine and serotonin receptors. We have demonstrated that agonist-regulated trafficking is identical for receptor monomers and dimers, however,

agonist treatment appears to stabilise the receptor oligomers. An investigation of the structural assembly between receptors involved in dimerization showed that there are several sites of interaction including hydrophobic transmembrane domain interactions and intermolecular disulphide bonds. We have also examined receptor hetero-oligomerization and demonstrated the potential for novel functions as a result of these associations. Finally, as a result of these observations, we have been able to present evidence that GPCRs function as oligomers in the cell.
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The dopamine receptors are important for a variety of functions in the nervous system and have been identified as having possible roles in drug reward, learning, motor activity, and neuropsychiatric disorders. To date, five different subtypes of dopamine receptors, termed D1 through D5, have been cloned and characterised (Missale et al. 1998). These five subtypes have been grouped into two different classes based on pharmacology and biochemistry: the D1-like and the D2-like receptors. The D1-like receptors, D1 and D5, mediate dopamine stimulation of adenylyl cyclase, whereas D2-like receptors, D2, D3 and D4, mediate dopamine inhibition of adenylyl cyclase.

We have shown that D1 and D2 dopamine receptors expressed in cell lines exist as monomers and dimers (Ng et al. 1994a, 1995, 1997; George et al. 1998) and that the D2 dopamine receptor (D2DR) exists as dimers in human and rat brain tissue (Zawarynski et al. 1998). The D3 dopamine receptor (D3DR) (Nimchinsky et al. 1997), the D4 dopamine receptor, and the D5 dopamine receptor (unpublished observations) have also been shown to form homodimers.

The serotonin (5-hydroxytryptamine; 5-HT) receptors represent a large family of receptors (Uphouse 1997) currently numbering 15. Serotonin receptors, except those in the 5-HT$_3$ subfamily, are G protein-coupled receptors (GPCRs) and serotonin-mediated signal transduction occurs through a variety of effectors.

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Given the number of receptor subtypes, it is not surprising that serotonin receptors have been shown to be important in a diverse range of physiological functions (Barnes and Sharp 1999).

During investigations of the post-translational modifications of the 5-HT$_{1B}$ receptor, we observed that these receptors existed as monomers and homodimers (Ng et al. 1993). This, we believe, was one of the first documentations of GPCR dimers. Recently, we reported that, like the 5-HT$_{1B}$ receptor, the closely related 5-HT$_{1D}$ receptor formed dimers also and that the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors formed heterodimers when co-expressed (Xie et al. 1999).

In this report, we present observations on the agonist-mediated regulation of the monomeric and oligomeric species of serotonin and dopamine receptors and our analyses of the potential sites of interaction between receptors in dimer formation. We also provide evidence of GPCR modulation by heterodimerization to truncated or mutant receptors and, with this evidence, demonstrate that GPCRs exist in large oligomeric complexes in the cell.

HOMODIMERIZATION OF DOPAMINE AND SEROTONIN RECEPTORS

Immunoblot analyses of membranes expressing dopamine receptors have revealed immunoreactive proteins that are approximately equal to and twice the molecular weight of the receptor suggesting that they existed as both dimers and monomers (Ng et al. 1994a, 1995, 1996, 1997; Nimchinsky et al. 1997; George et al. 1998). Autoradiograms showing the phosphorylation and palmitoylation of the D2DR indicated the presence of a higher molecular weight receptor dimer which was post-translationally modified in a similar manner to the receptor monomer (Ng et al. 1994b). In addition, receptor species of higher molecular weight than dimers were observed indicating that higher order oligomers were present. Deglycosylation studies have shown that the higher molecular weight proteins were not glycosylated monomeric receptors. Studies using non-hydrolysable analogues of GTP and using antisera to Gi proteins have shown that the higher molecular weight bands were not complexes of receptor and G proteins (Ng et al. 1996).

Like the D2DR, studies examining the phosphorylation and palmitoylation of the 5-HT$_{1B}$ receptor indicated the presence of dimeric receptor that was post-translationally modified in a manner similar to the monomeric receptor (Ng et al. 1993). Immunoblot analyses confirmed that the 5-HT$_{1B}$ existed as monomers and dimers. Subsequent studies on the 5-HT$_{1D}$ receptor demonstrated that it also existed as homo-oligomers.

The initial observations of dopamine receptor dimers and oligomers were documented in heterologous expression systems such as COS7 or Sf9 cells. However, since heterologous expression systems can be used to express receptors at densities above physiological levels, the possibility that dimer formation was related to the density of receptor expression was investigated by examining membranes with varying levels of receptor expression. In D2DR-expressing Sf9 cells, the earliest time point at which receptors were detectable by immunoblotting techniques was approximately 20–24 hours post-infection when the receptor density detected by [^{3}H]spiperone binding was 1.0 ± 0.3 pmol/mg. Both monomers and dimers were observed at this earliest point at which any receptor was detectable. The proportion of dimers to monomers at 24 hours post-infection (Figure 1, Lane 1) was approximately the same as when receptor expression increased to 2.1 ± 0.4 pmol/mg at 48 hours (Figure 1, Lane 2), and to 2.6 ± 0.4 pmol/mg at 72 hours post-infection (Figure 1, Lane 3) as estimated by [^{3}H]spiperone binding.

The identification of D2DR dimers in brain tissue was made through the use of photoaffinity labelling ligands derived from D2DR antagonists (Zawarynski et al. 1998). Photoaffinity labelling with [^{125}I]azidonemonapride indicated the presence of more than one D2DR species in crude homogenates from human caudate nucleus (Figure 2, lanes 1 and 2). The autoradiogram showed the presence of receptor proteins of approximately 100 and 200 kDa molecular weight that specifically bound nemonapride with high affinity. As D3 and D4 receptor are of relatively low abundance in caudate nucleus (Bouthenet et al. 1991; Van Tol et al. 1991), the 100 and
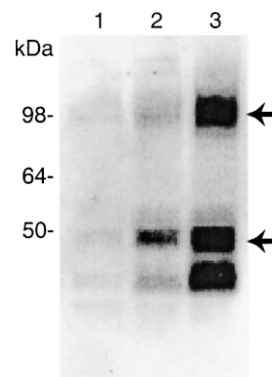

Figure 1. Identification of immunoreactive D2DR in Sf9 cell membranes using AL-26 antibody, a rabbit polyclonal antibody against a 120 amino acid sequence (aa 221–340) in the third intracellular loop of the human D2DR. Membranes were prepared at 24 h (lane 1), 48 h (lane 2), and 72 h (lane 3) post-infection from cells expressing the D2DR. Arrows indicate the monomer and dimer species of the receptors. 25 μg of protein was used for each lane.

200 kDa species correspond to glycosylated forms of the monomeric and dimeric D2DR. D2DR found in mammalian tissue is subject to heavy glycosylation and 100 kDa is close to the molecular weight determined for the glycosylated, monomeric D2DR in brain (Grigoriadis et al. 1988). Immunoblot detection of D2DR in mammalian cells also revealed bands of approximately 100 and 200 kDa (Figure 2, lane 3).

AGONIST-INDUCED TRANSLOCATION OF DOPAMINE RECEPTORS

From confocal microscopy analyses, we have observed that prolonged exposure of D1DR-expressing cells to dopamine results in an internalization of receptors from the cell surface to intracellular regions (Ng et al. 1997). A reverse translocation of receptors from intracellular regions to the plasma membrane is seen when D2DR-expressing cells are treated with dopamine (Ng et al. 1997). We were interested to determine if the agonist-induced trafficking of the D1 and D2 dopamine receptor dimers was different from that of receptor monomers.

D1DR-expressing Sf9 cells were treated with dopamine and membranes were isolated from the cell lysate. These extracts were fractionated using sucrose gradient centrifugation to separate heavy membranes from the light fraction and subjected to immunoblotting (Figure 3).

In Sf9 cells, D1DR monomers and dimers are represented by immunoreactive bands corresponding to ~47 kDa and ~95 kDa, respectively, since recombinant proteins expressed in Sf9 cells are not glycosylated in the same manner as in mammalian cells (Jarvis 1997). Untreated cells showed an approximately equal distribution of D1DR in the light membrane fraction (Figure 3, Lane 1) compared to the heavy plasma membrane fraction (Figure 3, Lane 2). Following 15 minutes exposure to 10 μM dopamine, as expected, there was a reduced amount of total receptor in the heavy fraction (Figure 3, lane 3) and an increase in receptor was detected in the light fraction (Figure 3, lane 4). After prolonged exposure (4 h), the ratio of total D1DR in the light fraction compared to that in the heavy fraction (Figure 3, lanes 7 and 8) returned to the level seen in untreated cells. Interestingly, there was no significant change in the proportion of D1DR dimer to monomer in any of the cell fractions (Figure 4A) indicating that D1DR monomers and dimers were trafficked in a similar manner and that both species may be down-regulated and resensitized by the same mechanism.

We have shown previously using immunoblot and ligand binding studies of the plasma membrane from D2DR-expressing Sf9 cells that there was an increase in the receptor density following agonist exposure, presumably from translocation of receptors from the intracellular regions of the cell (Ng et al. 1997). Densitometric analysis of the immunoblot data revealed that the proportion of D2DR dimer to monomer was not altered (Figure 4B).
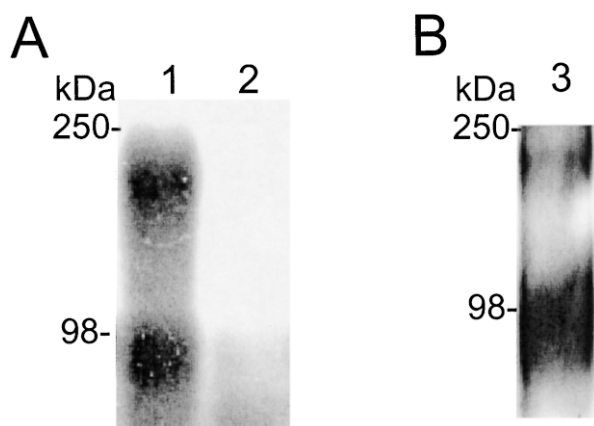

Figure 2. (**A**) Identification of photolabelled D2DR in human caudate nucleus. Tissue homogenate from human caudate nucleus was photolabelled with [^{125}I]iodoazidonemonapride and visualized by autoradiography. Photoincorporation of [^{125}I]iodoazidonemonapride upon photolysis in the absence (lane 1) and the presence (lane 2) of 1 μM (+)-butaclamol is shown. 100 μg of protein was used for each labelling condition. The autoradiogram shown is from a 5 day exposure. The tissue used in this experiment was from CBTB donor #1271. (**B**) Immunoblot analysis using AL-26 antibody of membranes from COS7 cells transiently expressing D2DR.
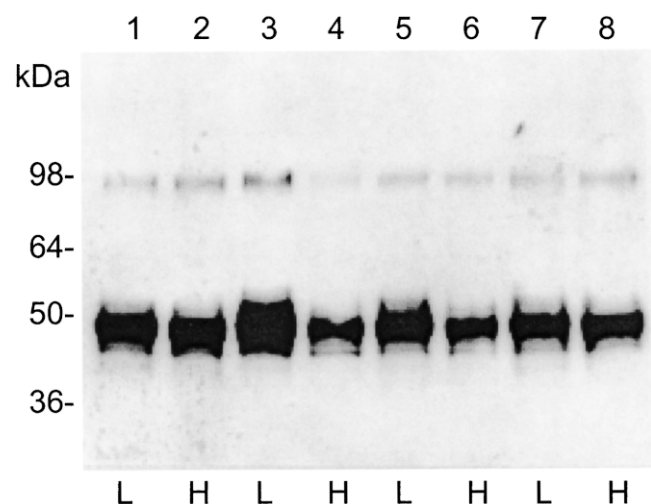
Figure 3. Immunoblot analyses of heavy and light membrane fractions of Sf9 cells expressing the *c-myc* D1DR using 9E10 antibody. Lanes 1, 3, 5, and 7 show membranes from the light fraction and lanes 2, 4, 6, and 8 show membranes from the heavy plasma membrane fraction. The cells were treated with vehicle (lanes 1 and 2) or dopamine for 15 minutes (lanes 3 and 4), 1 hour (lanes 5 and 6) and 4 hours (lanes 7 and 8) immediately prior to membrane preparation.
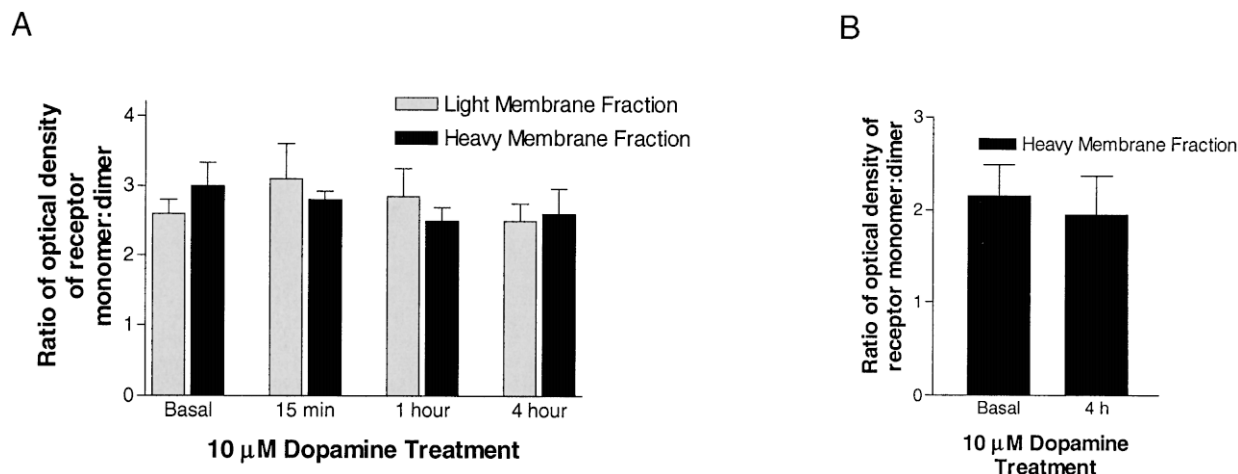
A

B

Figure 4. The ratio of receptor dimers to receptor monomers following agonist-mediated redistribution of dopamine receptors. Densitometric measurements on immunoblots were made using the Gel Doc 1000 Video Documentation System and Molecular Analyst software (BioRad, Hercules, CA). **(A)** The ratio of D1DR monomers to dimers in the light and heavy membrane fractions prepared from cells treated with dopamine. **(B)** The ratio of D2DR monomers to dimers in the heavy membrane fraction prepared from cells treated with dopamine.

These studies suggest that dopamine receptor monomers and dimers are trafficked in an identical manner. However, we have recently demonstrated that oligomerization is required for receptor trafficking and that monomers visualized following electrophoresis may reflect dissociation of receptor oligomers (Lee et al. 2000). Our observations from immunoblot analyses that both receptor monomers and dimers are trafficked without disturbing the ratio of monomer to dimer support this theory. In contrast, it has been suggested for the delta opioid receptor that interconversion between the receptor dimers and monomers plays a role in agonist-induced receptor internalization (Cvejic and Devi 1997) and, for the β2-adrenergic receptor, agonist treatment has been reported to increase the amount of dimeric receptor (Hebert et al. 1996).

AGONIST STABILISATION OF HOMO-OLIGOMERS

When cells expressing the serotonin 5-HT$_{1B}$ receptor or the 5-HT$_{1D}$ receptor were treated with agonist and subjected to immunoblot analysis, no change in the ratio of receptor monomer and dimer was observed. However, interestingly, when solubilized 5-HT$_{1B}$ (Figure 5A) and 5-HT$_{1D}$ (Figure 5B) receptors were incubated with the agonists serotonin and 5-carboxamidotryptamine (5-CT), there was an increase in the amount of receptor oligomers. Of particular note, higher order immunoreactive bands, possibly corresponding to higher order oligomers which were poorly detected or not-detected in untreated membranes, became more pronounced. These results were not observed when the solubilized

receptors were treated with antagonist. Since an increase in receptor oligomers was not observed when cells were treated with agonist prior to immunoblot analyses and the membranes processed subsequently, it is possible that agonist binding to the receptor in the membrane preparations does not induce oligomerization, but rather it stabilises the oligomeric receptor complexes.
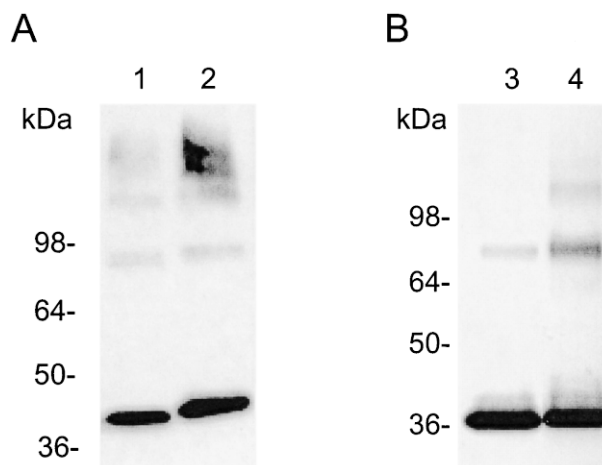

Figure 5. Agonist treatment of membrane preparations from cells expressing the **(A)** 5-HT$_{1B}$ or **(B)** 5-HT$_{1D}$ receptor. Lanes 1 and 3 show membranes treated with vehicle. Lane 2 shows membranes treated with 26.3 mM 5-CT for 30 minutes. Lane 4 shows membranes treated with 5.5 mM 5-CT for 30 minutes. Both receptors were c-myc epitope-tagged and the 9E10 antibody was used for immunodetection.

HETERODIMERIZATION OF TWO SEROTONIN RECEPTOR SUBTYPES

The serotonin 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors share a very high degree of homology (Jin et al. 1992) and there are several regions in the brain where the two receptor subtypes are co-expressed (Bonaventure et al. 1998). Therefore, we speculated that the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors formed heterodimers. When membranes from cells co-expressing the receptors were subjected to immunoblot analyses, we observed that the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors formed heterodimers but homodimers of either receptor subtype were not detected (Figure 6). When membranes expressing one receptor subtype were solubilized and mixed with membranes expressing only the other, homodimers of the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors but no heterodimers were seen (Figure 6). This indicated that receptor heterodimerization did not result from non-specific aggregation of the receptors and may require a co-translational or specific cellular mechanism. Furthermore, the absence of homodimers in cells co-expressing the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors suggests that the receptors may favour the heterodimeric conformation.

The preliminary radioligand binding assays of cells co-expressing the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors did not suggest a novel pharmacology for these heterodimeric receptors (Xie et al. 1999). Therefore, the functional significance of heterodimeric 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors remains to be determined. However, data from the

Figure 6. Immunoblot analysis of membranes from Sf9 cells expressing the 5-HT$_{1B}$ receptor (lane 1) and the 5-HT$_{1D}$ receptor (lane 2) and from cells co-expressing both receptors (lane 3). A mixture of membranes expressing only the 5-HT$_{1B}$ receptor with membranes from cells only expressing the 5-HT$_{1D}$ receptor is shown in lane 4. Both receptors were c-myc epitope-tagged and the 9E10 antibody was used for immunodetection. For each lane, 25 μg of protein was used.

GABA$_B$ receptors (Jones et al. 1998; Kaupmann et al. 1998; White et al. 1998; Kuner et al. 1999; Ng et al. 1999) and the opioid receptors (Jordan and Devi 1999) indicates that novel functions may arise from heterodimerization of GPCRs. The formation of "hybrid" receptors is well characterized among insulin receptors and growth factor receptors as a mechanism for increasing the diversity of cellular responses to extracellular signals (Jacobs and Moxham 1991).

ANTAGONISM OF THE D2 RECEPTOR BY A RECEPTOR FRAGMENT: EVIDENCE FOR OLIGOMERIZATION

As a result of naturally occurring mutations or gene manipulation, there are numerous mutant GPCRs with diminished or no function. It has generally been assumed that the co-expression of these mutants with the wild-type receptor does not result in any effect on receptor function. However, we have demonstrated that co-expression of a non-functional mutant D2DR with the wild-type D2DR resulted in diminished receptor function (Lee et al. 2000). In our studies, ligand binding to the D2DR was impaired when co-expressed with a fragment mutant of the D2DR, termed D2trunc$_{1-373}$, truncated in the third intracellular loop and consisting of only the first 373 amino acids.

It has been demonstrated that naturally occurring variant forms of the EP1 prostanoid receptor subtype suppresses prostaglandin E receptor signalling (Okuda-Ashitaka et al. 1996). Similar phenomena with clinical relevance have also been shown for the luteinizing hormone receptor (Osuga et al. 1997), the gonadotropin releasing hormone receptor (Grosse et al. 1997), and the CCR5 chemokine receptor (Benkirane et al. 1997). However, it is only with the CCR5 receptor where the mechanism has been shown to be heterodimerization.

Interestingly, photoaffinity labeling of membranes where the D2DR was co-expressed with D2trunc$_{1-373}$, revealed that ligand binding to the receptor monomer was markedly attenuated (Figure 7, lanes 3 and 4) compared to membranes only expressing the wild-type receptor (Figure 7, lanes 1 and 2). It would be expected that, if a proportion of the D2DR pre-existed in the cell in a monomeric state, ligand binding to the D2DR monomers would be unaffected by the presence of the receptor fragment. However, since binding to the receptor monomer appeared to be decreased, it can be inferred that the receptor fragment, mimicking part of the full-length receptor, was associated with the D2DR in the cell. This evidence suggested that D2DR visualized following SDS PAGE existed as oligomers in the cell membrane and that dissociation of receptor oligomers may occur during electrophoresis.
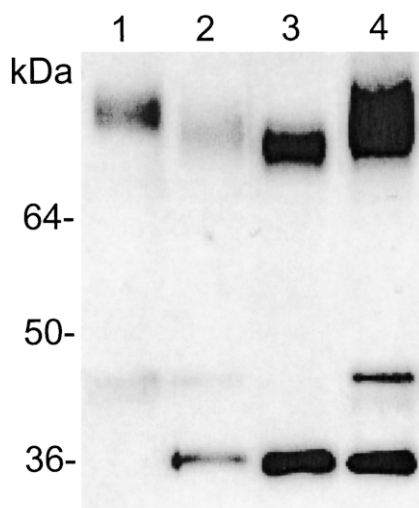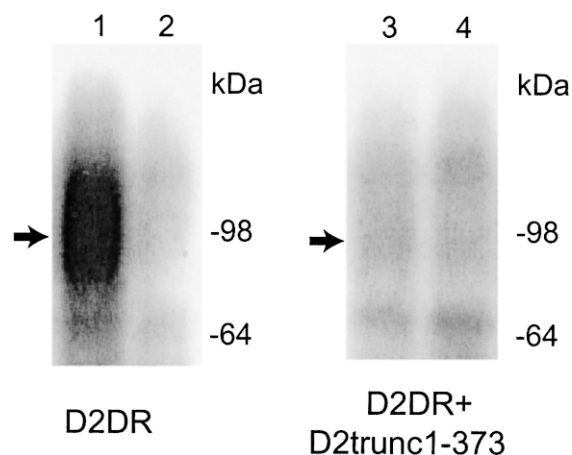
Figure 7. Autoradiogram showing photoaffinity labelling by [^{125}I]azido-YM of membranes from COS7 cells expressing D2DR (lanes 1 and 2) or expressing D2trunc$_{1-373}$ (lanes 3 and 4). Lanes 1 and 3 show total labelling. Lanes 2 and 4 show non-specific labelling as defined by 1 μM (+)-butaclamol. For each labelling condition, 50 μg of protein was used.

INTERACTION OF THE D3 RECEPTOR WITH A D3 RECEPTOR SPLICE VARIANT: EVIDENCE FOR MODULATION OF D3DR BY A SPLICE VARIANT

The mRNA encoding D3DR is subject to alternative splicing and, as a result, there are several variants of the receptor (Liu et al. 1994). One of the best characterised of these splice variants, the D3nf, is essentially a truncated form of the D3DR that prematurely terminates in the third intracellular loop and has been shown to have expression levels similar to those of the D3DR (Liu et al. 1994). Not unexpectedly, the D3nf does not bind dopaminergic ligands and is incapable of signal transduction—the name D3nf was given to the splice variant to represent "D3 non-functional."

Hetero-oligomeric complexes of the D3DR and the D3nf have been shown to be present in mammalian cell-lines and in brain tissue (Nimchinsky et al. 1997). We hypothesised that interactions between the D3DR and the D3nf could alter the function of the D3DR. Using Sf9 cells, we examined membranes co-expressing the receptor and the splice variant by immunoblot analyses. Homo-oligomers of both proteins were observed, but an immunoreactive band corresponding to hetero-oligomers was not observed (Elmhurst et al., in press). However, an association of the D3DR and D3nf was demonstrated by co-immunoprecipitation studies (Figure 8) suggesting that D3DR/D3nf hetero-oligomers, while present in the cell, dissociate when subjected to electrophoresis. Ligand binding to the D3DR in membranes in which the receptor was co-expressed with the D3nf was diminished compared to membranes only expressing the D3DR suggesting that D3nf may have a physiological role as a natural antagonist.
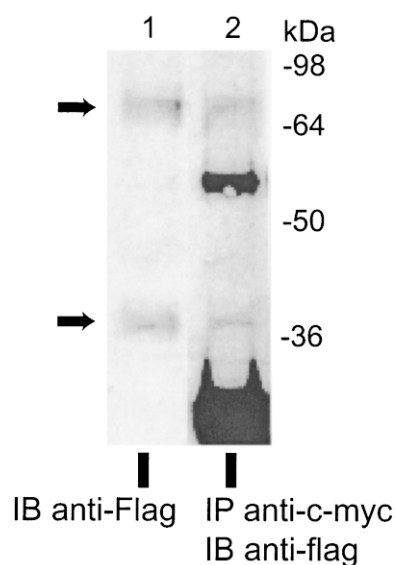

Figure 8. Co-immunoprecipitation of Flag-D3nf with c-myc-D3DR. Immunoblot analysis was performed using monoclonal M5 anti-Flag antibody on membranes from Sf9 cells expressing Flag-D3nf and c-myc-D3DR (lane 1) and on solubilized protein immunoprecipitated using 9E10 antibody from membranes expressing Flag-D3nf and c-myc-D3DR (lane 2).

MECHANISMS OF RECEPTOR INTERACTION

Despite the numerous reports of GPCR dimerization, little is known about the structure of receptor dimers and the types of interactions involved in receptor-receptor association. However, several studies have yielded some insight into the mechanisms of interaction between GPCRs. It has been proposed that a portion of the carboxy terminus is involved in the dimerization of the delta opioid receptor (Cvejic and Devi 1997). Recently, it has been demonstrated that a coiled-coil interaction between carboxy termini forms the basis of heterodimerization in the GABA$_B$ receptors (Marshall et al. 1999). Several reports have also indicated that intermolecular disulphide linkages between cysteine residues in the extracellular domains occur in GPCR dimerization (Romano et al. 1996; Fan et al. 1998; Ward et al. 1998; Zhu and Wess 1998; Jordan and Devi 1999; Pace et al. 1999; Zeng and Wess 1999). Finally, it has been proposed that hydrophobic interactions between the transmembrane (TM) domains are also involved (Gouldson et al. 1997).

During our examination of the serotonin receptors, we observed that homo-oligomers visualised by immunoblot analysis could be partially dissociated into receptor monomers upon treatment with the reducing agent, dithiothreitol, followed by the alkylating agent, iodoacetamide (Figure 9, lanes 1 and 2). This indicated that the serotonin receptors formed intermolecular dis-
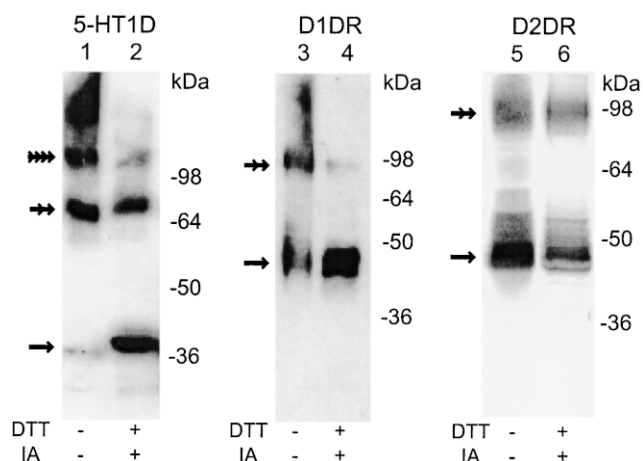
Figure 9. Dithiothreitol and iodoacetamide treatment of membranes from Sf9 cells expressing serotonin and dopamine receptors. The 5-HT$_{1D}$ receptor (lanes 1 and 2), the D1DR (lanes 3 and 4), or the D2DR (lanes 5 and 6) are shown. Membranes in lanes 1, 3, and 5 were incubated with DTT vehicle for 30 minutes followed by treatment with iodoacetamide vehicle for 30 minutes. Membranes in lanes 2, 4, and 6 were incubated with DTT for 30 minutes followed by treatment with iodoacetamide for 30 minutes. All treatments were performed at 37°C. Single-headed, double-headed, and four-headed arrows indicate receptor monomers, dimers, and tetramers, respectively. The 5-HT$_{1D}$ receptor and the D1DR were both c-myc epitope-tagged and the 9E10 antibody was used for immunodetection. The D2DR was detected using AL-26 antibody.

ulphide bridges, possibly involving the cysteine residues in extracellular loop 1 and extracellular loop 2. However, a complete disruption of all oligomers to monomers could not be achieved suggesting that additional SDS-resistant intermolecular interactions were also involved in GPCR oligomerization.

A similar partial disruption of receptor oligomers under reducing conditions was seen in immunoblots of the D1DR (Figure 9, lanes 3 and 4). However, when the D2DR (Figure 9, lanes 5 and 6), the D3DR, and the D3nf (data not shown) were subjected to dithiothreitol and iodoacetamide treatment, there was no reduction in the oligomeric receptor species detected. Based on these observations, we hypothesised that, in the D2-like receptor subfamily, the intermolecular interactions not based on disulphide bridges were more robust than in the D1DR and in the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors.

A study using truncated mutants of the D2DR revealed evidence that this reducing agent-resistant interaction in GPCR oligomerization may be hydrophobic interactions between TM domains. It was observed that D2trunc$_{1-373}$ which contained TM domains 1 through 5 formed oligomers similar to the full-length receptor under reducing conditions, but that a counterpart D2DR fragment containing TM domains 6 and 7 did not form

oligomers under reducing conditions (Figure 10). A more thorough study using additional receptor fragments (Lee et al., in preparation) indicated that intermolecular interactions among specific TM domains are responsible for D2DR oligomers, as well as disulphide linkages. Similar results have been reported for the V2 vasopressin receptor (Zhu and Wess 1998) which also suggested that specific TM domain interactions together with disulphide bonds were responsible for receptor dimerization.

CONCLUSIONS

Dopamine and serotonin receptors are both members of the rhodopsin-like family of GPCRs, often referred to as family A. Increasing evidence for oligomerization in many other members of the family A GPCRs and in all the GPCR families is emerging. Although all of the hypotheses and theories being formed from the growing number of studies of GPCR oligomerization are not always consistent, it appears clear that dimerization and oligomerization are universal aspects of GPCR struc-
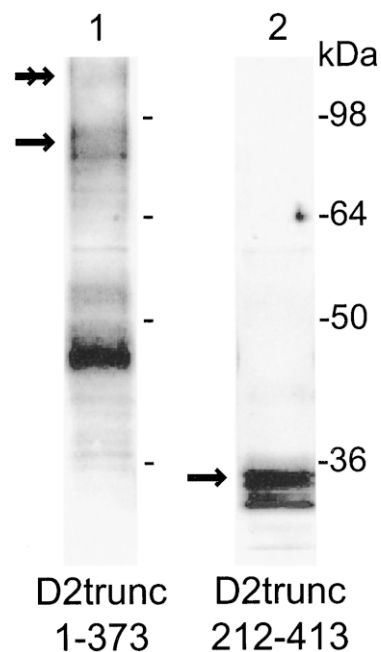

Figure 10. Immunoblot analysis using AL-26 antibody of membranes from COS7 cells transiently transfected with cDNA encoding D2trunc$_{1-373}$ (lane 1) and D2trunc$_{212-413}$ (lane 2). In lane 1, the single headed arrow labels glycosylated monomers of D2trunc$_{1-373}$ and the double-headed arrow indicates glycosylated dimers of D2trunc$_{1-373}$. Other immunoreactive bands likely represent degraded protein or D2trunc$_{1-373}$ that is unglycosylated or partially glycosylated. In lane 2, the arrow indicates monomeric D2trunc$_{212-413}$. There are no putative N-glycosylation sites with D2trunc$_{212-413}$. For each lane, 25 μg of protein was used.

ture. Furthermore, while the functional role of oligomer formation in GPCRs still needs to be better understood, it is also apparent that traditional models of GPCR function based on the idea that the receptor is a monomeric protein on the cell surface need to be revised.

Our studies of the dopamine and serotonin receptors have yielded several interesting insights into the oligomerization of these GPCRs. We have demonstrated that the oligomeric state may be stabilised by agonist binding. We have provided evidence that GPCRs are in oligomeric complexes in the cell and that these oligomers may dissociate upon gel electrophoresis. Oligomer formation appears to be mediated via several interactions including hydrophobic TM domain interactions and intermolecular disulphide bonding. Finally, we have shown that hetero-oligomerization among GPCRs may result in a greater diversity of GPCR function than predicted by the number of cloned receptor genes due to inter-receptor interactions that result in potential modulation by other receptor subtypes, alternatively spliced variants, and receptor mutants.

ACKNOWLEDGMENTS

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