Synthesis and Processing of D_2 Dopamine Receptors†

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ABSTRACT: Dopamine receptors belong to a superfamily of neurotransmitter receptors that are functionally coupled to guanine nucleotide binding proteins. In this study, we have used Chinese hamster ovary (CHO) cells stably transfected with the rat D_{2L} receptor, in conjunction with specific anti-peptide antibodies that we have developed, in order to visualize this protein and the course of its synthesis. The newly synthesized receptor exists as a 45-kDa protein which undergoes further processing to a 75-kDa glycosylated receptor in the CHO cells. In pulse–chase experiments it was noticed that a 35-kDa precursor was present which disappeared after 30 min. In order to determine whether this 35-kDa protein represents an unprocessed form of the receptor, we have employed an *in vitro* translation system with cDNA constructs coding for both the murine D_2 and D_3 dopamine receptor isoforms. In the absence of processing, the D_2 and D_3 receptors have an apparent molecular mass of 35 kDa. The translated proteins were shown to be the full length receptors by immunoprecipitation with various anti-peptide antibodies and by the demonstration that they can undergo glycosylation to apparent molecular masses of approximately 45 kDa in an *in vitro* system.

Dopamine receptors have been recognized for many years to play a key role in CNS[1] neurotransmission. At least two classes of receptors have been described biochemically and designated D_1 and D_2 by their ability to stimulate or inhibit adenylate cyclase respectively, and by pharmacological differences (Niznik & Jarvie, 1989; Anderson et al., 1990; Civelli et al., 1991; Sibley & Monsma, 1992). Until the cloning of the D_2 dopamine receptor (Bunzow et al., 1988), little was known about the molecular nature of this receptor, which has been implicated in control of movement, behavior, and endocrine and cardiovascular function (Strange, 1990).

The rat D_2 dopamine receptor (Bunzow et al., 1988) was predicted from its hydropathy plot to have seven transmembrane regions, characteristic of a member of a family of guanine nucleotide binding protein (G-protein) coupled receptors (Dohlman et al., 1987). Its subsequent cloning in rat, human, and bovine tissues (Monsma et al., 1989; Dal Toso et al., 1989; Chio et al., 1990) demonstrated that there are two isoforms of the receptor which differ by the presence of an 87 base pair "insert" in the putative third cytoplasmic loop of the originally published sequence. These two isoforms, D_2 "long" (D_{2L}) and D_2 "short" (D_{2S}), were shown to be expressed in the same regions of both rat and human brain and pituitary (Bunzow et al., 1988; Dal Toso et al., 1989; Chio et al., 1990). It is still not clear what functional differences, if any, exist between them. In addition, two other D_2-type receptors (as judged by their pharmacology, structure, and tissue distribution) have been cloned and are termed D_3 (Sokoloff et al., 1990) and D_4 (Van Tol et al., 1991). We have recently cloned the murine D_3 receptor in our laboratory and illustrated that it too undergoes alternative splicing in the putative third cytoplasmic loop (Fishburn et al., 1993), yielding D_3 "long" (D_{3L}) and D_3 "short" (D_{3S}) receptor isoforms. In contrast to other D_3 receptor RNA splice variants which could not be shown to bind dopaminergic ligands (Giros et al., 1991; Snyder et al., 1991), the D_{3L} and D_{3S} receptors isolated in our laboratory do bind ligand in a transfected cell system (Fishburn et al., 1993). With the plethora of sequence data on dopamine receptors, there are many questions that remain to be answered as to the function of each receptor subtype. We have previously developed anti-peptide antibodies against sequences in D_2 dopamine receptors and have used them to localize a region of ligand binding (David & Fuchs, 1991) and for immunocytochemical staining (David et al., 1991). In this study we take advantage of cells stably transfected with the rat D_{2L} dopamine receptor and the anti-peptide antibodies in hand in order to study the synthesis and processing of D_2 dopamine receptors.

EXPERIMENTAL PROCEDURES

Materials. *N*-(4-Azido-3-[^{125}I]iodophenethyl)spiperone ([^{125}I]-NAPS) (2200 Ci/mmol) was purchased from New England Nuclear (Boston, MA). ^{35}S-Methionine (>3000 Ci/mmol) and ^{3}H-spiperone (116 Ci/mmol) were purchased from Amersham (Aylesbury, England). Phenylmethanesulfonyl fluoride (PMSF), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), Triton X-100, soybean trypsin inhibitor, benzamidine, leupeptin, trypsin, and bovine serum albumin (BSA) were all purchased from Sigma Chemical Co. (St. Louis, MO). Protein A–Sepharose was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). All cell culture media were from Gibco Laboratories (Grand Island, NY). Rabbit reticulocyte lysate, canine microsomal membranes, and RNasin were from Promega (Madison, WI).

Antibody Preparation. The following four synthetic peptides derived from the predicted amino acid sequence of the rat D_2 dopamine receptor (Bunzow et al., 1988; Monsma et al., 1989) were synthesized in the laboratory of peptide synthesis of the Weizmann Institute of Science by the solid-phase method of Merrifield (1965): SREKALQTTNY (pep-

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[1] Abbreviations: CHO, Chinese hamster ovary; CNS, central nervous system; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; G protein, guanine nucleotide binding protein; kDa, kilodalton; MEM, modified Eagle's medium; PBS, phosphate-buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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tide 60–71); KSNGSFPVNRRR (peptide 258–269); SPPERTRYSPIPPS (peptide 288–301); and NIEFRKAFMKILHC (peptide 431–444). Specific anti-peptide antibodies were elicited as described previously (David & Fuchs, 1991). Peptide $D_3$333–346 (SPTMAPKLSLEVRK) was taken from the sequence of the murine D_3 dopamine receptor (Fishburn et al., 1993) and antibodies were elicited as described above.

Preparation of Cell Membranes and Immunoprecipitation of Photolabeled Receptors. Cell pellets were homogenized in 10–20 volumes of ice-cold 50 mM Hepes, pH 7.4, containing 0.25 M sucrose and the following protease inhibitors: 20 mM EDTA, 15 μg/mL benzamidine, 5 μg/mL soybean trypsin inhibitor, 5 μg/mL leupeptin and 1 mM PMSF (homogenization buffer). The homogenate was centrifuged at 1000*g* for 10 min and the supernatant was recentrifuged at 40000*g* for 50 min. The resulting pellet was resuspended in 50 mM Hepes buffer, pH 7.4, containing 100 mM NaCl and protease inhibitors (assay buffer). Protein concentration was determined using the BCA protein assay (Pierce) with BSA as standard and membranes were resuspended to yield a final concentration of approximately 0.5 mg/mL, representing 0.5–1.0 nM D_2 receptor, as determined by ^{3}H-spiperone binding. Labeling of membranes and immunoprecipitation was carried out as described previously (David & Fuchs, 1991).

^{35}S-Met Labeling. Permanently transfected Chinese hamster ovary (CHO) cells (Monsma et al., 1989) (~5×10^6/10 mL) were washed twice with PBS and then placed in methionine-free MEM (5% dialyzed FCS) for 1 h. Cells were labelled for the time indicated in the presence of 200 μCi of ^{35}S-methionine (15 mCi/mL of stock) in methionine-free medium. All incubations were performed at 37 °C in a humidified incubator (5% CO_2). At the end of labeling, cell plates were placed on ice, washed twice with cold PBS, and solubilized with 0.7 mL of 50 mM Hepes, 150 mM NaCl, 10% glycerol, and 1% Triton X-100 (solubilization buffer) with 2 mM PMSF, 15 μg/mL benzamidine, 5 μg/mL soybean trypsin inhibitor, and 5 μg/mL leupeptin (protease inhibitors) for 15 min on ice with intermittent vortexing and spun for 10 min at 12000*g*. The supernatants were preadsorbed to 10 μL of protein-A Sepharose CL-4B (preswollen in solubilization buffer) for 10 min at 4 °C and spun for 2 min at 12000*g*. Equal amounts of radioactivity (as judged by 10% TCA precipitation and counting on a ^{14}C channel) were immunoprecipitated in a given experiment (see below). Where indicated, at the end of labeling, cells were washed twice and then chased with methionine-containing medium. In this case, the beginning of the pulse for the different time points was staggered so that all cells were lysed, solubilized and processed for immunoprecipitation at the same time.

DNA Constructs. Cloned cDNAs of the murine D_{2L}, D_{2S} (Fishburn et al., 1991) and D_{3L} and D_{3S} receptors (Fishburn et al., 1993) were subcloned into pBluescript (Stratagene). DNA was prepared by conventional methods (Sambrook et al., 1989).

In Vitro Transcription and Translation. Linearized DNA was transcribed essentially according to the manufacturer's instructions (Boehringer Mannheim), and RNA was stored at −70 °C until use. *In vitro* translation was carried out using the Promega rabbit reticulocyte kit, according to the manufacturer's instructions. Following translation, samples were placed on ice and 100 μL of RIPA (1% Triton X-100, 0.1% SDS, and 0.5% sodium deoxycholate in 10 mM sodium phosphate buffer, pH 7.5) buffer (plus protease inhibitors) was added to each before immunoprecipitation. Samples were prebound to 10 μL of protein A–Sepharose for 10 min at 4 °C and then spun for 10 min at 12000*g*. The supernatants were then used for immunoprecipitation as described below.

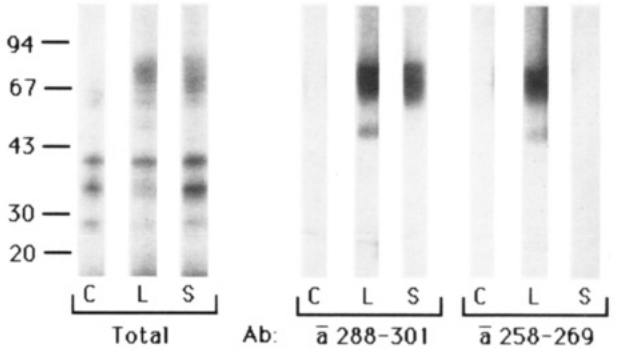

FIGURE 1: Photolabeling of membranes from D_{2L}- and D_{2S}-transfected cells and immunoprecipitation by specific antibodies. Membranes from cells stably transfected with either rat D_{2L} (L) or D_{2S} (S), or non-transfected cells (C) were labelled with [^{125}I]-NAPS as outlined in Experimental Procedures. Immunoprecipitation of 250 μL of RIPA-solubilized membranes was carried out with the antisera indicated (prebound to Sepharose) for 3.5 h at 4 °C. Samples were washed, eluted, and run on a 7–15% polyacrylamide gel as described in Experimental Procedures. Total: 20 μL of RIPA solubilized membranes prior to immunoprecipitation.

Immunoprecipitation of ^{35}S-Met-Labeled Products. Antibodies were prebound to protein A–Sepharose as follows. For each immunoprecipitation sample, 10 μL of protein A–Sepharose was incubated with 1 μL of serum in 50 μL of PBS for at least 1 h at 4 °C. When the same antibody was to be used for more than one sample, the antibodies were prebound to Sepharose in one pool and then split to each sample for immunoprecipitation. The solubilized cell lysates, containing equal amounts of total labelled proteins, were incubated batchwise with the antisera for 1–2 h of immunoprecipitation at 4 °C. For *in vitro* translation products, each *in vitro* translation reaction (50 μL + 100 μL of RIPA buffer) was immunoprecipitated with one antibody. In all experiments, beads were washed in 50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.1% SDS, 0.1% Triton X-100, and 5 mM EDTA; in 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.1% Triton X-100, and 5 mM EDTA; and finally in 10 mM Tris-HCl pH 8.0, 0.1% Triton X-100, and 5 mM EDTA, all supplemented with protease inhibitors as mentioned above and carried out at 4 °C. On the last wash, the samples were transferred to a new tube. The immunoprecipitates were eluted and run on polyacrylamide gels as in David and Fuchs (1991).

RESULTS

D_2 Dopamine Receptor Exists as a 75-kDa Protein in Stably Transfected Cells. In order to learn more about the synthesis and processing of the D_2 dopamine receptor we used stably transfected CHO cells as a model system. Even though the stably transfected cells express approximately 10 times more receptor than striatal brain tissue, the receptor cannot be visualized by conventional methods such as protein staining or radiolabeling without further concentrating the receptor by immunoprecipitation. In order to visualize the fully glycosylated receptor, we used the photoaffinity label, *N*-(4-azido-3-[^{125}I]iodophenethyl)spiperone ([^{125}I]-NAPS), to label the receptor in membranes prepared from cells stably transfected with the rat D_{2L} or D_{2S} dopamine receptors or nontransfected cells (Figure 1). As can be clearly seen, the labelled receptor runs as a heavily glycosylated protein of approximately 75 kDa, as was previously observed by McVittie

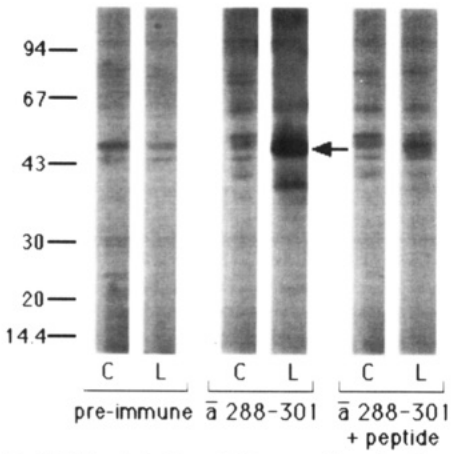

FIGURE 2: ^{35}S-Met labeling of D_{2L}-transfected cells and specific immunoprecipitation. CHO cells stably transfected with rat D_{2L} (L) or nontransfected cells (C) were labelled for 15 min in the presence of ^{35}S-methionine and immunoprecipitated, as described in Experimental Procedures, with either preimmune serum, anti-peptide 288–301, or anti-peptide 288–301 preincubated with 20 μM of peptide 288–301 for 1 h at 22 °C prior to use. The samples were run on a 7–15% polyacrylamide gel and autoradiographed. Arrow indicates the 45-kDa D_{2L} receptor.

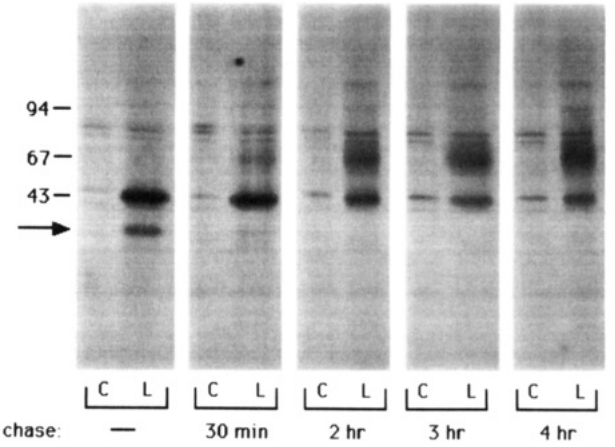

FIGURE 3: ^{35}S-Met pulse–chase of D_{2L}-transfected cells. CHO cells stably transfected with rat D_{2L} (L) or nontransfected cells (C) were labelled with ^{35}S-Met for 15 min, and then chased for the time periods indicated, as outlined in Experimental Procedures. Labelled cell lysates for each time point (each representing $\sim 5 \times 10^6$ cells) were prepared and immunoprecipitated with anti-peptide 288–301 as described in Experimental Procedures. The samples were run on a 7–15% polyacrylamide gel and autoradiographed. The arrow indicates the D_{2L} receptor precursor (see text for details).

et al. (1991). This apparent molecular mass is lower than that previously shown for D_2 receptors in labelled striatal membranes (David & Fuchs, 1991; Amlaiky & Caron, 1986). It is possible that the D_2 dopamine receptor is not processed (e.g., glycosylated) in exactly the same way in the stably transfected cells as it is in striatal brain tissue. Anti-peptide 288–301 antibodies recognize a region common to both D_{2L} and D_{2S} receptors and therefore immunoprecipitate the receptor from both these cell lines. The anti-peptide 258–269 antibodies, however, were generated against a peptide specific for the D_{2L} receptor (David et al., 1991) and indeed only immunoprecipitate the receptor from the D_{2L} transfected cells. This gives further evidence that this band represents the D_2 dopamine receptor. Furthermore, prior to immunoprecipitation, this band is absent from the nontransfected cells Figure 1, total).

Newly Synthesized D_{2L} Receptor Exists as a 45-kDa Protein Which Undergoes Further Processing. The newly synthesized D_{2L} receptor was visualized by metabolic labeling with ^{35}S-Met followed by solubilization of cell lysates and immunoprecipitation. It should be stressed again that in the absence of immunoprecipitation it is very difficult to follow the receptor because it represents about 0.005% of the total protein (or about 500 cpm from 10^7 cpm total). Figure 2 shows that a 45-kDa protein is specifically immunoprecipitated from cells labelled with ^{35}S-Met for 15 min (Figure 2, arrow). This band is not present in the nontransfected cells (C) and is not immunoprecipitated by preimmune serum or by preincubation of the serum with its specific peptide. In addition, a lower band of approximately 35 kDa was also specifically immunoprecipitated by the antibodies and was only present in the transfected cells. At this point we were not sure if this lower molecular mass band represented a proteolytic fragment of the receptor or a D_2 receptor precursor.

In order to view the processing of the receptor, we pulsed cells for 15 min with ^{35}S-Met and then chased them with cold methionine for various time points (Figure 3). Between 30 min and 2 h after the pulse, the protein is clearly seen to shift to a higher molecular weight of approximately 70 kDa, most probably due to glycosylation of the receptor. At the same time, the lower molecular mass form of 45 kDa seems to decrease. The apparent molecular mass reached in this experiment seems to be slightly lower than that observed in the fully processed receptor, which runs at about 75 kDa (refer to Figure 1). Attempts at visualization of the 75-kDa protein using longer chase times were unsuccessful, probably due to dilution of the radioactive label. Here again, we visualized a lower specific band of approximately 35 kDa (Figure 3, arrow). This band did not seem to be a proteolytic fragment of the 45-kDa protein, because it disappeared during the course of the chase. During shorter pulse times, as short as 5 min, the 35-kDa protein was also observed, although never in the absence of the 45-kDa protein. Although the predicted molecular mass of the D_{2L} receptor is close to 45 kDa, we suspected that the newly synthesized D_2 receptor precursor displays an apparent molecular mass of 35 kDa, and that the 45-kDa protein is probably already partially processed. This was later supported by evidence from *in vitro* translation of the cloned cDNAs for the different receptor subtypes.

In-Vitro Translation of the Cloned Murine D_2 and D_3 Dopamine Receptors Yields Proteins of 35 kDa. We have utilized the recently cloned murine D_{2S}, D_{2L} (Fishburn et al., 1991) and D_{3S} and D_{3L} (Fishburn et al., 1993) dopamine receptors in *in vitro* transcription and translation systems and performed immunoprecipitation in order to visualize the translated proteins (Figure 4). In this system all receptors displayed apparent molecular masses in the range of 35 kDa, which is approximately 10 kDa less than the molecular mass predicted by their sequence, in accordance with the results obtained for the D_{2L} using metabolic labeling (Figure 3). Even so, a clear difference was observed between the molecular mass of the long and short isoforms of both receptors (about 2 kDa), consistent with a difference of between 20 and 30 amino acids (Figure 4). Antibodies directed against peptides from the first cytoplasmic loop (60–71) and the C-terminal tail (431–444), which have been shown to react with both D_2 and D_3 receptors, were able to immunoprecipitate the 35-kDa *in vitro* translation products, as is shown for the D_{3L} receptor in Figure 4, panels e and f, respectively. This supports the notion that the 35-kDa proteins are not truncated and represent the full-length receptors. *In vitro* translated D_2 receptors yielded smaller products as well, probably representing proteolytic fragments of the receptor. The specificity of these bands was illustrated by their immunoprecipitation using

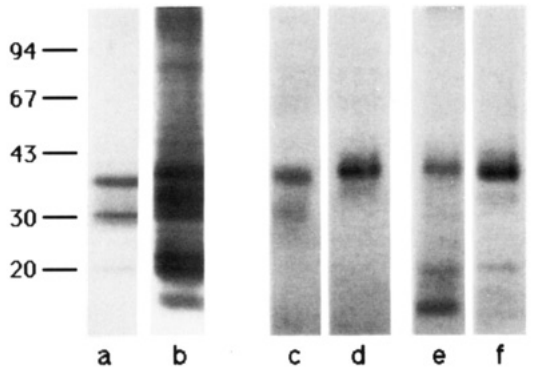

FIGURE 4: *In vitro* translation of murine D_2 and D_3 receptor subtypes. *In vitro* translation products of cloned murine D_{2S} (a), D_{2L} (b), D_{3S} (c), and D_{3L} (d–f) receptors were immunoprecipitated with either anti-peptide 288–301 (a and b), anti-peptide $D_3$333–346 (c and d), anti-peptide 60–71 (e), or anti-peptide 431–444 (f), as described in Experimental Procedures. The samples were run on a 7–15% acrylamide gel and autoradiographed.

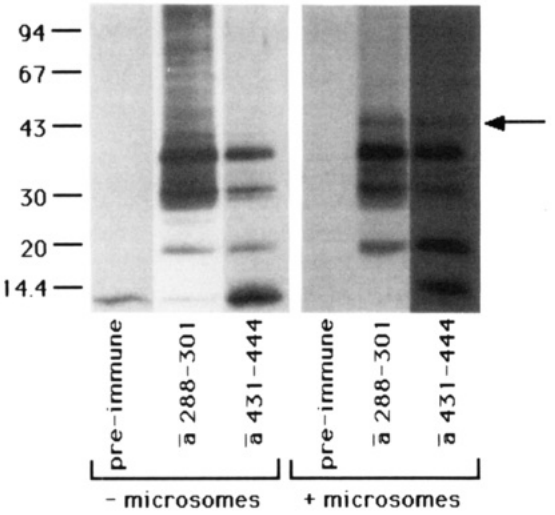

FIGURE 5: Glycosylation of D_{2S} dopamine receptors. *In vitro* translation of cloned murine D_{2S} receptors was carried out in the presence or absence of canine microsomal membranes, as indicated. *In vitro* translation products were immunoprecipitated with the anti-peptide antisera indicated or preimmune serum as described in Experimental Procedures. The samples were run on a 7–15% acrylamide gel and autoradiographed. The first panel (–microsomes) was exposed for less time.

different anti-peptide antibodies (Figure 4 and Figure 5, left) and by inhibition with the respective peptides (data not shown).

In an extension of our studies on the processing of the D_2 dopamine receptor, we have tested whether the *in vitro* translation product could undergo glycosylation. Figure 5 shows the results of immunoprecipitation of the D_{2S} dopamine receptor after translation in the presence or absence of canine microsomal membranes, which provide the basic machinery needed for glycosylation. Indeed, when translation is carried out in the presence of microsomes, a higher molecular mass protein of about 45 kDa is specifically immunoprecipitated by two anti-peptide antisera. Three putative glycosylation sites are present within the first 25 amino acids of the protein (Bunzow et al., 1988) and therefore the demonstration that the 35-kDa protein can undergo glycosylation gives further evidence that it represents the full-length receptor. A similar increase in molecular mass was seen after *in vitro* translation of the D_{2L} receptor in the presence of microsomes (data not shown). Interestingly, the glycosylated protein now runs at 45 kDa, reminiscent of the newly synthesized D_2 dopamine receptor seen in the metabolic labeling experiments (refer to Figure 2). It is quite possible that the glycosylation occurring in the *in vitro* system is only partial and is comparable to the fast processing we see in the transfected cells, which yield a 45-kDa protein as early as 5 min after the pulse of ^{35}S-Met on the cells.

DISCUSSION

The study presented here describes the synthesis and processing of the D_2 dopamine receptors. We have shown that the receptor is first synthesized as a protein with an apparent molecular mass of approximately 35 kDa, is then processed within min to a 45-kDa protein (Figure 2), and over the next hours is further modified (probably glycosylated) to a protein of about 75 kDa (Figure 3).

The first set of experiments carried out to visualize the D_2 receptor in the transfected cells used the photoaffinity label [^{125}I]-NAPS and immunoprecipitation with specific anti-peptide antibodies. We and others have used [^{125}I]-NAPS in the past to visualize D_2 receptors in brain tissue (David & Fuchs, 1991; Amlaiky & Caron, 1986; Jarvie et al., 1988). In the striatum, the D_2 dopamine receptor was seen as a 90 kDa protein, whereas in the present study we show that in transfected cells it runs at an apparent molecular mass of 75 kDa (Figure 1). It should be mentioned that in bovine striatal membranes it is quite possible that [^{125}I]-NAPS is labeling more than one subtype of D_2 dopamine receptors, since it is a very high affinity antagonist. In a stably transfected cell system however, we are sure to be looking at only one subtype of the receptor and this may account for the difference in glycosylation. It is also possible that the glycosylation machinery differs between the transfected cells and the native tissue.

In previous reports, D_2 dopamine receptors in striatal membranes were shown to have an apparent molecular mass of 90 kDa (Amlaiky & Caron, 1986). The high molecular mass was explained by glycosylation, as deglycosylation of the receptor yielded primarily a 45-kDa band (Jarvie et al., 1988; Grigoriadis et al., 1988; Clagett-Dame et al., 1989). However, it should be noted that all of these studies, using photoaffinity labeling of D_2 dopamine receptors, consistently reported specific labeling of a 32-kDa protein, in addition to the 90-kDa band. This smaller molecular weight band was believed to be the result of proteolysis, but this was not shown unequivocally. These studies, which used photoaffinity labels to visualize D_2-type receptors, preceded their cloning and were the first indication of their apparent molecular weight. Cloning and sequencing of D_2, D_3 and D_4 receptors have shown them to have between 390 and 450 amino acids and therefore a predicted molecular mass of between 45 and 50 kDa.

The results presented here from *in vitro* translation of D_{2L}, D_{2S}, D_{3L}, and the novel D_{3S} receptors showed that for each receptor subtype the deglycosylated protein has an apparent molecular mass around 35 kDa (Figure 4), which is lower than the molecular masses predicted from the sequences of the cloned receptors. This unglycosylated protein could also be shown to undergo further glycosylation to an apparent molecular mass of 45 kDa in an *in vitro* system (Figure 5). There are several indications suggesting that the 35-kDa band represents a full-length *in vitro* translation product. This band is immunoprecipitated by antibodies against peptides close to the N-terminus and from the C-terminal tail of the receptor (Figures 4 and 5). The peptide close to the N-terminus (60–71) corresponds to residues 55–66 of the D_3 receptor (Fishburn et al., 1993), and is positioned upstream to the second

methionine residue (position 83). In addition, the illustration that the D_2 receptor can undergo glycosylation (Figure 5) suggests that the N-terminus is present as this is the location of putative glycosylation sites. It therefore seems unlikely that the apparent molecular mass of 35 kDa is due to aberrant transcription or translation.

Apparent molecular masses are obtained by polyacrylamide gel electrophoresis in SDS on the assumption that the proteins bind a constant amount of about 1 SDS molecule per 2 amino acid residues and that they assume identical conformations (Jorgensen, 1982). These assumptions are uncertain in the case of proteins with high concentrations of hydrophobic amino acids, as has been demonstrated with the Na^+,K^+-ATPase, which also runs at an apparent molecular mass lower than that calculated from its amino acid sequence (96 kDa as opposed to 113 kDa). It is therefore understandable that dopamine receptors, which have seven putative transmembrane regions and are highly hydrophobic, will have an anomalous migration in SDS–PAGE.

There are several explanations for the discrepancy between the apparent molecular mass reported here for the unmodified D_2 receptor protein (35 kDa) and that previously determined by deglycosylation experiments (45 kDa) (Jarvie et al., 1988; Grigoriadis et al., 1988; Clagett-Dame et al., 1989). It is possible that the deglycosylation carried out in the experiments cited above was not complete. There may be a complex sugar moiety that has not been removed by the enzymes used in these experiments. Another possible explanation is that the shift from 35 to 45 kDa seen in the transfected cells is due to a posttranslational modification other than glycosylation. For example, the C-terminus of the D_2 dopamine receptor is thought to be palmitoylated (Civelli et al., 1991), as are at least two other members of the G-protein linked receptor family: rhodopsin (Ovchinnikov et al., 1988) and the β_2-adrenergic receptor (O'Dowd et al., 1989). Although palmitoylation alone would probably not account for such a difference in apparent molecular weight, there may be other covalent or noncovalent modifications present. Despite the fact that no general function for modification of proteins with lipids has been identified, lipid is, in several cases, clearly required for the binding of proteins to cellular membranes. Palmitoylation has been reported to affect protein recycling, lateral diffusion, and signalling in other proteins (Crise & Rose, 1992). It will be of interest to see if D_2 dopamine receptors are in fact modified by fatty acylation and what may be the presumed function of this modification.

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