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The dopamine D_4 receptor: one decade of research

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Abstract

Dopamine is an important neurotransmitter involved in motor control, endocrine function, reward, cognition and emotion. Dopamine receptors belong to the superfamily of G protein-coupled receptors and play a crucial role in mediating the diverse effects of dopamine in the central nervous system (CNS). The dopaminergic system is implicated in disorders such as Parkinson's disease and addiction, and is the major target for antipsychotic medication in the treatment of schizophrenia. Molecular cloning studies a decade ago revealed the existence of five different dopamine receptor subtypes in mammalian species. While the presence of the abundantly expressed dopamine D_1 and D_2 receptors was predicted from biochemical and pharmacological work, the cloning of the less abundant dopamine D_3, D_4 and D_5 receptors was not anticipated. The identification of these novel dopamine receptor family members posed a challenge with respect to determining their precise physiological roles and identifying their potential as therapeutic targets for dopamine-related disorders. This review is focused on the accomplishments of one decade of research on the dopamine D_4 receptor. New insights into the biochemistry of the dopamine D_4 receptor include the discovery that this G protein-coupled receptor can directly interact with SH3 domains. At the physiological level, converging evidence from transgenic mouse work and human genetic studies suggests that this receptor has a role in exploratory behavior and as a genetic susceptibility factor for attention deficit hyperactivity disorder.

Keywords: Dopamine D_4 receptor; G protein-coupled receptor; Structure; Pharmacology; Signaling; Physiology; Genetics

1. Introduction

The dopaminergic system has received a significant amount of attention due to the important role it plays in the central nervous system (CNS) in motor control, cognition, reward and endocrine regulation. The importance of the dopaminergic system in these processes is best exemplified by (1) disorders with deficits in dopaminergic signaling, such as Parkinson's disease and L-DOPA (L-3,4-dihydroxyphenylalanine) responsive dystonia, (2) the addictive properties of drugs that enhance dopaminergic signaling such as cocaine and amphetamine and (3) the therapeutic efficacy of neuroleptic medication in controlling the symptoms of Gilles de la Tourette syndrome and the psychoses seen in schizophrenia, Huntington's disease and Alzheimer's disease. In addition, psychostimulant drugs which enhance dopamine release, such as methylphenidate, are effective in the treatment of attention deficit hyperactivity disorder (ADHD). Even though the dopaminergic system may not be essential for normal development, dopamine-deficient transgenic mice will not survive post-weaning due to motor impairment and abnormalities in feeding behavior, but can be rescued by life-long L-DOPA treatment (Zhou and Palmiter, 1995).

The presence of receptors for dopamine in the brain that could mediate intracellular signaling through the activation of adenylyl cyclase were recognized almost three decades ago (Kebabian and Greengard, 1971; Kebabian et al., 1972). This notion was soon followed by the direct demonstration of the existence of binding sites for dopamine in

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brain and the identification of these sites as the target for neuroleptic medications (Burt et al., 1975; Seeman et al., 1975; Seeman and Lee, 1975; Creese et al., 1976; Seeman et al., 1976). Soon thereafter it was realized that two dopamine receptor subtypes existed, termed dopamine D_1 and D_2, which coupled to the stimulation and blockade of adenylyl cyclase, respectively (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). The functional coupling of these receptors is mediated by GTP-binding proteins (Maeno, 1982; Kilpatrick and Caron, 1983; Niznik et al., 1986; Senogles et al., 1987), hence dopamine receptors belong to the superfamily of G protein-coupled receptors.

After the initial cloning of several G protein-coupled receptors through expression cloning or protein purification strategies, it became clear that this class of receptors shared a relatively high homology. Structurally, this is characterized by seven conserved hydrophobic domains that were proposed to span the plasma membrane (Hanley and Jackson, 1987). Based on this observation, homology cloning strategies using the cloned β_2-adrenoceptor sequence as a probe were employed to identify novel G protein-coupled receptors. This led to the cloning of the dopamine D_2 receptor (Bunzow et al., 1988), and it was soon found that two dopamine D_2 receptor subtypes are generated through alternative splicing (Dal Toso et al., 1989; Giros et al., 1989; Grandy et al., 1989; Monsma et al., 1989; Selbie et al., 1989). Subsequently, the other major dopamine binding site in the brain, the dopamine D_1 receptor, was cloned (Dearry et al., 1990; Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990). The homology cloning approach rapidly resulted in the identification of three novel dopamine receptor subtypes, called D_3 (Sokoloff et al., 1990), D_4 (Van Tol et al., 1991) and D_5 (Grandy et al., 1991; Sunahara et al., 1991; Tiberi et al., 1991; Weinshank et al., 1991), the existence of which were unanticipated. Due to the similarity of these new dopamine receptor subtypes with either the dopamine D_1 (for D_5) or D_2 (for D_3 and D_4) receptor and their relative low abundance, these novel receptors had evaded previous detection by classic pharmacological and biochemical approaches. No other functional dopamine receptors have been found in mammalian species to date, although two pseudogenes for the dopamine D_5 receptor subtype are found in humans (Grandy et al., 1991; Nguyen et al., 1991; Weinshank et al., 1991). Additional dopamine D_1-like receptor subtypes have been identified in non-mammalian species (Sugamori et al., 1994; Demchyshyn et al., 1995; Lamers et al., 1996; Cardinaud et al., 1997).

With the identification of five dopamine receptor subtypes, it was apparent that the distinct physiological and behavioral roles attributed to the dopamine D_1 and D_2 receptors were now less clear. The anatomical localization and pharmacological properties of the dopamine D_4 receptor led to intense interest in this receptor as a possible target of neuroleptic drugs. Unfortunately, many of the pharmacological tools available at that time did not provide the necessary specificity to discriminate the dopamine D_4 receptor from the other D_2-like receptor subtypes. In the last decade, significant advances have been made in this respect, and a plethora of specific receptor agonists

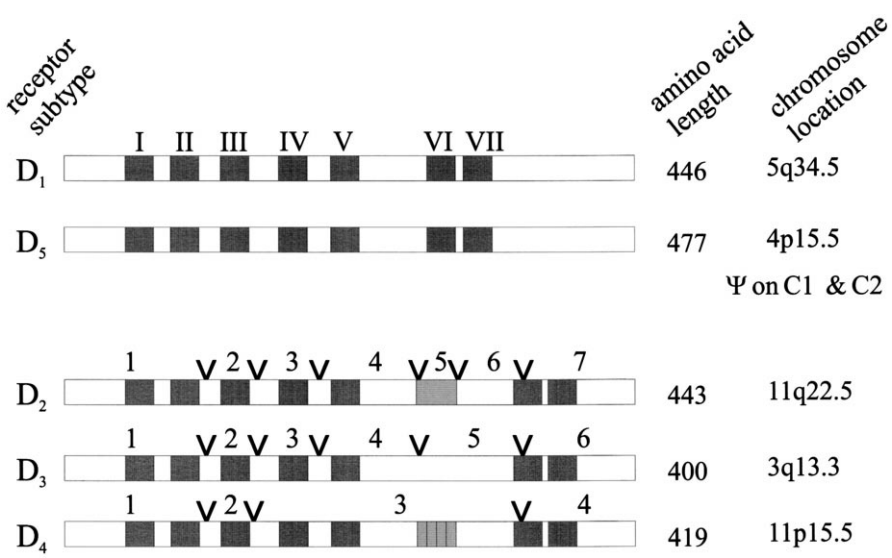

Fig. 1. Diagrammatic representation of the structural organization of dopamine receptor subtypes. The seven hydrophobic stretches characteristic for G protein-coupled receptors are indicated in dark grey blocks and numbered on the top of the figure with Roman numerals. The amino acid length and chromosomal location is indicated beside the diagram of the receptor. The position of introns in the coding portion of the genomic sequence is indicated with V and the exons are numbered in Arabic numerals. The light grey portion in the dopamine D_2 receptor indicates the alternatively spliced 29 amino acid sequence of exon 5. The light grey tandem repeated boxes in exon 3 of the dopamine D_4 receptor denote the position of the repeat polymorphism that is present in the human receptor.

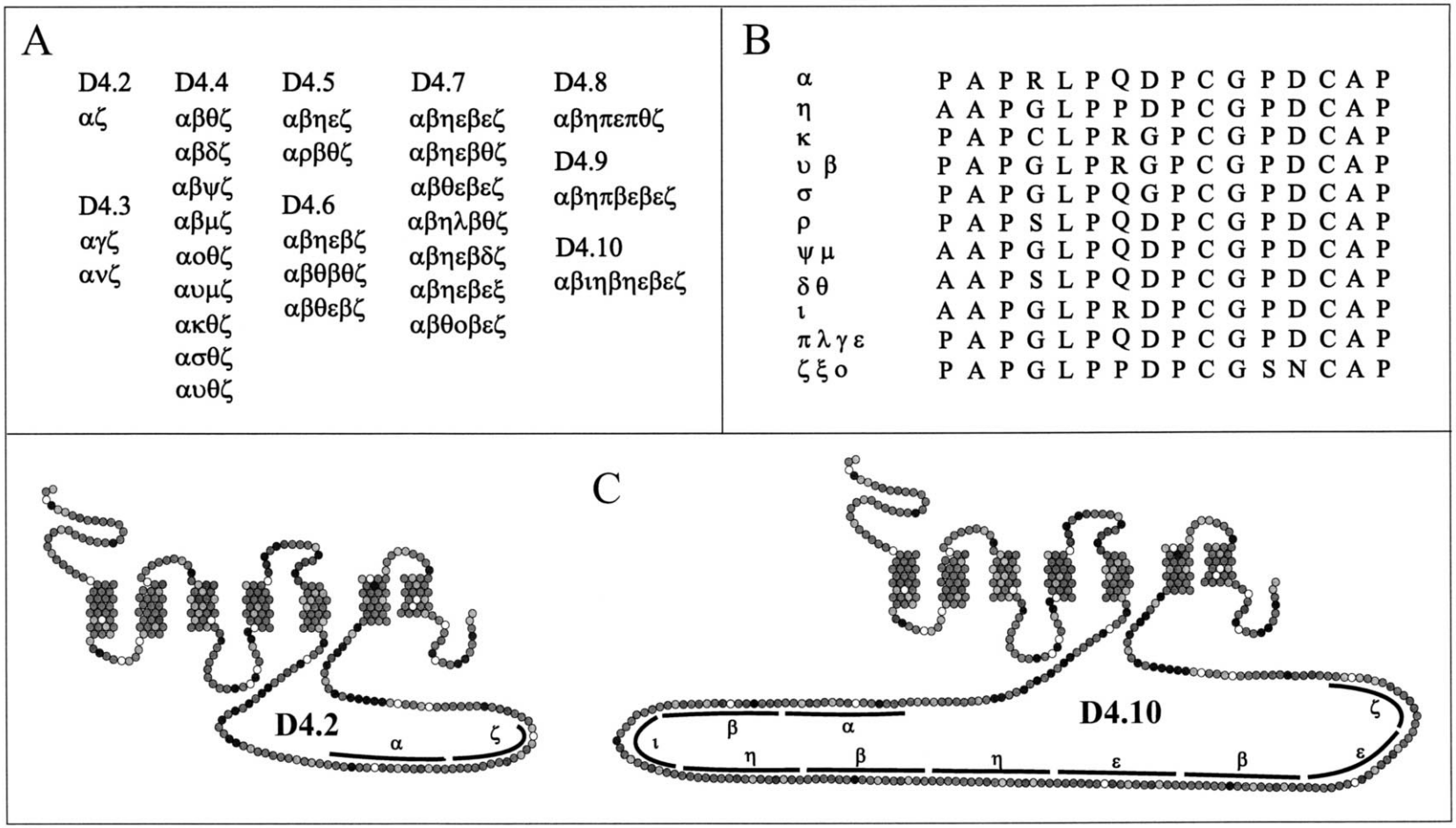

Fig. 2. Schematic overview of the polymorphic variants of the exon 3 VNTR that have been identified, to date, for the human dopamine D_4 receptor gene (DRD4). (A) The various haplotypes according to length (i.e. number of 48 bp repeats). The Greek letters identify variants of the 48-bp repeat sequences found within the DRD4 VNTR. (B) The 16 amino acid sequences encoded for by the different 48 bp repeat sequences. (C) Snake plots of the dopamine D_4 receptor variants with the shortest and longest exon 3 VNTR identified to date (dopamine $D_{4.2}$ and $D_{4.10}$, respectively). This figure is reproduced with permission from: Jovanovic et al. (1999). Comparative pharmacological and functional analysis of the human dopamine $D_{4.2}$ and $D_{4.10}$ receptor variants. Pharmacogenetics 9(5): 561–568.

and antagonists have been developed. In addition, the development of transgenic mouse models deficient for the individual receptor subtypes have significantly contributed to the understanding of the functional roles of the different receptors. This review summarizes a decade of research into the molecular biology, biochemistry, human genetics and physiology of the dopamine D_4 receptor to clarify what we have learned and to identify what questions remain unanswered.

2. Gene structure

The human dopamine D_4 receptor gene contains four exons (Van Tol et al., 1991; Van Tol et al., 1992), and this genomic organization is conserved within the mouse and rat homologues (O'Malley et al., 1992; Asghari et al., 1994; Fishburn et al., 1995; Matsumoto et al., 1995a; Suzuki et al., 1995). This organization is also partially found in the dopamine D_2 (Grandy et al., 1989) and D_3 receptors (Giros et al., 1990, 1991; Fishburn et al., 1993; Fu et al., 1995; Park et al., 1995; Griffon et al., 1996) (Fig. 1). The human dopamine D_4 receptor gene (DRD4) contains a transcription initiation site approximately 400–500 basepair (bp) upstream from the translation initiation codon. Promoter sequences that can confer expression of DRD4 are located within the first kilobase upstream from the initiation codon (Kamakura et al., 1997). Whether these sequences are sufficient to mediate tissue-specific expression is still unknown and will require direct testing using transgenic approaches.

The human dopamine D_4 receptor gene contains a number of polymorphisms in its coding sequence. The most extensive polymorphism is found in the third exon of the gene in a region that encodes the putative third cytoplasmic loop of the receptor (Van Tol et al., 1992). This polymorphism consists of a variable number of tandem repeats (VNTR) in which a 48 bp sequence exists as a 2- to 10-fold repeat (indicated as dopamine $D_{4.2}$ to $D_{4.10}$ receptors). Several of the repeat units vary in sequence from each other. To date, 18 different repeat units have been recognized, and are identified by Greek letters (see Fig. 2). The different repeat units have been found in various positions, although the first and last repeat unit always encode the amino acid sequence of α and ζ, respectively. At least 27 different haplotypes have been found for this polymorphism, encoding 20 different proteins variants of the receptor (Van Tol et al., 1992; Lichter et al., 1993; Asghari et al., 1994). The frequency of occurrence of different polymorphic variants differs significantly between several population groups, but at a global level the dopamine $D_{4.4}$ receptor variant occurs most frequently at about 64%, followed by the dopamine $D_{4.7}$ receptor at about 20% (Lichter et al., 1993; Chang et al., 1996). A similar polymorphism has been found in various primate species (Livak et al., 1995; Matsumoto et al., 1995b), but not in rodents (Lichter et al., 1992; Asghari et al., 1994; Suzuki et al., 1995). In an apparent parallel evolutionary process, a polymorphic repeat sequence has emerged in the canine dopamine D_4 receptor; however, this repeat consists of 12- and 39-bp repeat units (Niimi et al., 1999).

Other coding region polymorphisms are found in exon 1. These include a 12-bp sequence that commonly exists as a tandem direct repeat, but less often as a single 12-bp sequence (Catalano et al., 1993), a 21 bp deletion of amino acids 36–42 and a transversion changing Gly^{11} into Arg^{11} (Cichon et al., 1995). A 13-bp frame shift deletion mutant of the human dopamine D_4 receptor has been reported that is predicted to give rise to a truncated gene product of 89 amino acids (Nothen et al., 1994). It was found to occur at a 2% frequency in the general population, and one individual homozygous for this mutation was found. Although this human null mutant displays some psychiatric and somatic abnormalities, it is unclear whether this is related to a dopamine D_4 receptor deficiency. A $Val^{194} \rightarrow Gly^{194}$ mutation in exon 3 results in the formation of a receptor that cannot be activated by dopamine. A homozygous individual has been found for this mutation, but no overt psychiatric or somatic abnormalities were observed (Seeman et al., 1994; Liu et al., 1996). Additional mutations and polymorphisms have been found in intron 1 and in the 5′ flanking sequence of the gene (Petronis et al., 1994a,b; Cichon et al., 1995; Paterson et al., 1996; Mitsuyasu et al., 1999; Okuyama et al., 1999b; Seaman et al., 1999). Interestingly, a $C \rightarrow T$ mutation at -521 bp from the initiation codon reduces activity of a 5′ flanking promoter fragment in heterologous expression assays (Okuyama et al., 1999b; Okuyama et al., 2000).

The human dopamine D_4 receptor gene, DRD4, is located at the distal tip of the short arm of chromosome 11 at position 11p15.5. The gene is located proximal to the Harvey-RAS oncogene locus and distal to the tyrosine hydroxylase locus (Kennedy et al., 1991; Gelernter et al., 1992; Petronis et al., 1993, 1994a). This region of the human genome contains the imprinted genes H19 and IGF2, however DRD4 is likely not imprinted since bi-allelic expression has been observed (Cichon et al., 1996).

3. Protein structure

The primary sequence of the dopamine D_4 receptor displays highest homology to the dopamine D_2-like receptor and α_2-adrenoceptor families. This similarity is particularly evident in the postulated transmembrane domains of the receptor. The existence of a seven transmembrane topology is predicted by hydrophobicity analysis of the primary structure and the observed sequence similarities with other G protein-coupled receptors (Van Tol et al.,

1991). The dopamine D_4 receptor does not contain a signal sequence and is classified as a type IIIb integral membrane receptor. Recombinant human dopamine D_4 receptors on which we have added a hemagglutinin signal sequence and epitope-tag to the amino-terminal tail show normal functional pharmacology and functional activation (Oldenhof et al., 1998). Immunofluorescence experiments with heterologously expressed dopamine D_4 receptors show that the receptor is located in the membrane, with the amino-terminal epitope-tag located on the extracellular surface of the plasma membrane (Oldenhof et al., 1998). Western blotting of wildtype and epitope-tagged dopamine $D_{4.2}$, $D_{4.4}$ and $D_{4.7}$ receptors has confirmed that the VNTR polymorphism is present in the translated product (Fig. 3) (see also Lanau et al., 1997a; Kazmi et al., 2000).

The translation initiation methionine of the receptor was originally predicted by its position as the most upstream initiation codon in the reading frame of the transcription initiation site. However, we have found evidence that in heterologous expression systems, up to 20% of the receptor population may use an alternative non-AUG initiation codon at 105 to 117 nucleotides downstream of the normal initiator-methionine (Schoots et al., 1996). The most likely initiation codon is CUG at position 112. This amino-terminal truncated receptor form displays normal pharmacology but has a reduced ability to maintain the receptor in the active state.

The seven hydrophobic domains of the dopamine D_4 receptor are proposed to have a conformation similar to the membrane-spanning α-helices of rhodopsin (Baldwin et al., 1997; Unger et al., 1997). Low-resolution projection-density mapping of rhodopsin has established that the transmembrane domains form a circular bundle oriented counterclockwise when viewed from the extracellular surface. The α-helices are arranged such that the hydrophobic residues form a boundary with the plasma membrane and the less hydrophobic residues face the interior, where 11-*cis* retinal is located in rhodopsin.

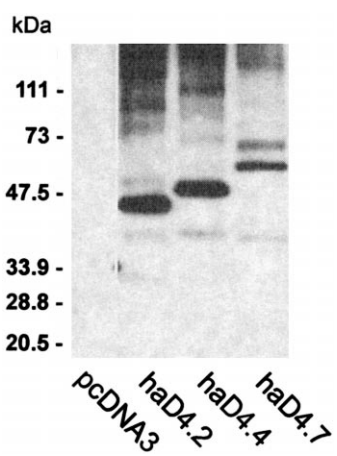

Fig. 3. Immunoblot of HA epitope-tagged dopamine $D_{4.2}$, $D_{4.4}$, and $D_{4.7}$ receptors. Amino-terminal HA-tagged dopamine D_4 receptors transiently expressed in HEK 293 cells by the calcium phosphate method (Chen and Okayama, 1987) were immunoprecipitated from a detergent-solublized membrane preparation with monoclonal rat anti-HA IgG 3F10 (Roche)/Protein G-agarose. Western blotting was carried out on immunoprecipitated receptors with polyclonal rabbit anti-HA IgG Y11 (Santa Cruz Biotechnology)/anti-rabbit-HRP.

Our understanding of the structure and function of bioamine receptors has been guided by studies of the β-adrenoceptors. In the β_2-adrenoceptor, an aspartate in transmembrane domain 3 acts as a counterion to the protonated amine group of epinephrine, while conserved serines in helix 5 can act as hydrogen bond acceptor sites for the hydroxyl groups of the catechol ring (Strader et al., 1988, 1989). A phenylalanine in helix 6 appears to interact with the aromatic catechol ring of the agonist to mediate activation (Strader et al., 1994). Another aspartate in helix 2 is highly conserved in G protein-coupled receptors and is important in producing the conformation change required for activation (Strader et al., 1988). Site-directed mutagenesis of the dopamine D_2 receptor has confirmed the importance of serines 193, 194 and 197 and aspartates 80 and 114 in dopamine binding and activation (Neve et al., 1991; Cox et al., 1992; Mansour et al., 1992; Woodward et al., 1996). The combination of biophysical data from rhodopsin, mutagenesis studies and sequence conservation within bioamine receptors all support a model for the transmembrane arrangement of the dopamine D_4 receptor consisting of seven α-helices of approximately 25 amino acids traversing the membrane. Although site-directed mutagenesis studies have not been carried out on these transmembrane residues of the dopamine D_4 receptor, their conservation (Asp^{80}, Asp^{115}, Ser^{196}, Ser^{197}, Ser^{200} and Phe^{362} in the dopamine $D_{4.4}$ receptor) suggests that they play a similar role.

The human dopamine D_4 receptor contains consensus sequences for various post-translational modifications, including a single *N*-linked glycosylation site at the extracellular amino-terminal tail and several putative phosphorylation sites for protein kinase A, protein kinase C and casein kinase II (Van Tol et al., 1991). It has been shown that the receptor is *N*-glycosylated when expressed in Chinese hamster ovary (CHO) cells (Lanau et al., 1997a). Mrzljak et al. (1996) and Khan et al. (1998) carried out Western analysis of the dopamine D_4 receptor from brain and found that the predominant form had a molecular size close to that of the unglycosylated protein. In contrast, another study detected the presence of higher molecular size forms that may represent glycosylated dopamine D_4 receptors (Defagot et al., 1997). To date, there are no published experimental data on other post-translational modifications of the dopamine D_4 receptor, and this area of research requires further attention. Two highly conserved extracellular cysteine residues between transmembrane domains 2 and 3 are present in the dopamine D_4 receptor and are thought to from a disulfide bond in other G protein-coupled receptors (Fraser, 1989; Hwa et al., 1997).

The dopamine D_4 receptor contains intracellular sequences that are conserved among G protein-coupled receptors and are thought to be involved in G protein activation (for a review see Helmreich and Hofmann, 1996). An Asp–Arg–Tyr (Asp–Arg–Phe in dopamine D_4 receptor) sequence in the second intracellular loop has been shown to be a key determinant in receptor activation. Within the third cytoplasmic loop, basic regions at the amino and carboxyl ends of the loop appear to be important, but there is a general lack of conservation within this region (Hedin et al., 1993). Previous work in our lab found that deletion of all but six residues of the first repeat ($D_{4.4}$ Δ254–315) resulted in a functional receptor (Asghari et al., 1995). We have also found that deletion of the repeat region and the arginine-rich region amino-terminal to it ($D_{4.4}$ Δ221–315) results in a receptor that is partially impaired at inhibiting adenylyl cyclase, while a larger deletion mutant that also lacks the carboxyl-terminal basic domain of the proposed third intracellular loop ($D_{4.4}$ Δ221–337) does not couple to G_i but has normal [^{3}H]spiperone binding (unpublished, Hubert H.M. Van Tol; Oldenhof et al., 1998). These results are supported by Kazmi et al. (2000), who found that the deletion of amino acids 237–326 of the dopamine $D_{4.4}$ receptor abolished functional coupling. Studies comparing the pharmacological and functional characteristics of the dopamine $D_{4.2}$ and $D_{4.7}$ receptors (Asghari et al., 1994, 1995; Kazmi et al., 2000) or dopamine $D_{4.2}$ and $D_{4.10}$ receptors (Jovanovic et al., 1999) found only 2- to 3-fold differences.

The putative third cytoplasmic loop is highly proline-rich, particularly the VNTR region and the sequence immediately surrounding it. This proline-rich section contains multiple copies of the PXXP motif, which is considered to be the core consensus sequence for SH3 binding domains (ie. the SH3 ligand). Indeed, we have recently shown that this section of the receptor can interact with a variety of SH3 domain-containing proteins such as Grb2 (Oldenhof et al., 1998). The functional significance of this interaction is presently unknown and continues to be an active area of research. Interactions between G protein-coupled receptors and PDZ, WW, SH2 and SH3 domain-containing proteins have recently been discovered with the β_1-, β_2- and α_2-adrenoceptors (Cao et al., 1999; Prezeau et al., 1999; Tang et al., 1999), the angiotensin II AT_1 receptor (Ali et al., 1997) and the metabotropic glutamate receptors mGluR1 and mGluR5 (Kato et al., 1998; for a review see Hall et al., 1999). Given that the polymorphic VNTR region affects the number of putative SH3 binding domains present in dopamine D_4 receptor, it is tempting to speculate that a protein–protein interaction with this region may underlie potential functional differences between dopamine $D_{4.2}$ and $D_{4.10}$ receptors. However, this hypothesis remains unproven.

Whether the dopamine D_4 receptor exists in monomeric form or multimeric complex, as seen for other G protein-coupled receptors, is unknown (Hebert et al., 1996; for a review see Hebert and Bouvier, 1998). Our own experimental data indicate that we can co-immunoprecipitate two differently epitope-tagged dopamine D_4 receptors when co-expressed, suggesting that the receptor can exist as a multimeric complex (unpublished, Hubert H.M. Van Tol). The functional significance of this is unknown, but may be related to signal transduction or receptor regulation (Hebert and Bouvier, 1998; Jones et al., 1998; Jordan and Devi, 1999; Kuner et al., 1999; Ng et al., 1999).

4. Expression

Northern blot and RT-PCR (reverse transcriptase-polymerase chain reaction) analyses have demonstrated that the dopamine D_4 receptor is expressed in various brain areas, albeit at relatively low levels compared to dopamine D_2 receptor levels found in striatum (Van Tol et al., 1991; O'Malley et al., 1992; Matsumoto et al., 1995a, 1996). Expression of the dopamine D_4 receptors is most abundant in retina (Cohen et al., 1992), cerebral cortex, amygdala, hypothalamus and pituitary (Valerio et al., 1994; Asghari et al., 1995), but sparse in the basal ganglia. This was confirmed in more refined analyses by using in situ hybridization, establishing that expression was highest in retina followed by prefrontal cortex, hippocampus, amygdala and hypothalamus (Cohen et al., 1992; O'Malley et al., 1992; Meador-Woodruff et al., 1994, 1996; Meador-Woodruff, 1995; Ariano et al., 1997; Lidow et al., 1998). While it is difficult to make accurate direct comparisons, it appears that dopamine D_4 mRNA levels in the prefrontal cortex are comparable to the levels of dopamine D_1 and D_2 mRNA (Meador-Woodruff, 1995; Meador-Woodruff et al., 1996).

Immunohistochemistry and Western analyses using dopamine D_4 receptor-specific antisera essentially confirmed these observations (Mrzljak et al., 1996; Ariano et al., 1997; Defagot et al., 1997; Rubinstein et al., 1997; Khan et al., 1998; Mauger et al., 1998). Immunohistochemical studies in primate brain indicate that the dopamine D_4 receptor is present in both pyramidal and non-pyramidal neurons of the cerebral cortex, particularly layer V, and in the hippocampus (Mrzljak et al., 1996; Lidow et al., 1998). Most of the non-pyramidal dopamine D_4 receptor-positive cortical and hippocampal neurons are γ-aminobutyric acid (GABA)-producing interneurons. Similarly, dopamine D_4 receptor-positive neurons in thalamic nuclei, globus pallidus and substantia nigra pars reticulata are also GABAergic (Mrzljak et al., 1996).

The identification of dopamine D_4 receptors by ligand binding has been more difficult due to the absence of adequate radioligands. It was initially attempted to demonstrate the presence of these receptors with subtractive ligand binding techniques, based on the observation that dopamine D_4 receptors have low affinity for the dopamine

D_2/D_3 receptor antagonist raclopride but can effectively bind to the dopamine $D_2/D_3/D_4$ receptor antagonists [^{3}H]spiperone and [^{3}H]nemonapride (Seeman et al., 1993b). While this approach indicated the presence of dopamine D_4-like binding sites in the prefrontal cortex and hippocampus (Lahti et al., 1995; Defagot and Antonelli, 1997; Tarazi et al., 1997b; Defagot et al., 2000), it also detected a significant number of such sites in the basal ganglia (Seeman et al., 1993a; Lahti et al., 1995; Murray et al., 1995; Schoots et al., 1995; Sumiyoshi et al., 1995; Defagot and Antonelli, 1997; Florijn et al., 1997; Marzella et al., 1997; Tarazi et al., 1997a,b,c; Tarazi et al., 1998a,b,c; Defagot et al., 2000).

The presence of dopamine D_4 binding sites in striatum appears to be in conflict with several mRNA and immunohistochemical data, and may be attributed in part to the presence of a raclopride-insensitive dopamine D_2-like receptor isoform in this region (Seeman et al., 1997b; Wilson et al., 1998). However, there are also several observations that support the presence of dopamine D_4 mRNA and receptor protein in striatum, albeit in low levels (Van Tol et al., 1991; Schoots et al., 1995; Surmeier et al., 1996; Ariano et al., 1997; Defagot et al., 1997; Lidow and Goldman-Rakic, 1997; Khan et al., 1998; Mauger et al., 1998; Stefanis et al., 1998). The dopamine D_4 receptor-selective radioligand [^{3}H]NGD 94-1 (2-[4-[(2-phenyl-1*H*-imidazol-5-yl)methyl]-1-piperazinyl]pyrimidine) detected the presence of dopamine D_4 binding sites predominantly in areas of the cerebral cortex, septum, hippocampus, amygdala and hypothalamus, generally reflecting the distribution profiles seen by in situ hybridization and immunohistochemistry (Primus et al., 1997; Lahti et al., 1998). The density of the dopamine D_4 binding sites in the cerebral cortex is low, but about equivalent to the level of dopamine D_2/D_3 receptors. These data are supported by a subtractive approach using the non-selective dopamine D_2-like receptor radioligand [^{3}H]nemonapride and the unlabeled dopamine D_4 receptor-specific ligand L-745,870 (3-[4-(4-chlorophenyl)piperazin-1-yl]methyl-1*H*-pyrrolo[2,3-b]pyridine) and by comparing dopamine D_2-like binding sites between dopamine D_4 receptor-deficient and wild-type mice (Defagot et al., 2000).

The expression of dopamine D_4 receptors is not confined to the CNS. A significant level expression has been observed in the cardiac atrium (O'Malley et al., 1992; Ricci et al., 1998). Dopamine D_4 receptor expression has also been reported in lymphocytes (Bondy et al., 1996; Amenta et al., 1999) and the cortical and medullary collecting ducts of the kidney (Sun et al., 1998).

In vivo regulation of dopamine D_4 receptor expression has not been extensively examined, and existing studies were carried out in the context of schizophrenia and antipsychotic drug response. Analysis of dopamine D_4-like receptor expression in schizophrenic brain by subtractive pharmacological methods based on the low affinity of dopamine D_4 receptors for raclopride suggests that dopamine D_4-like receptors are up-regulated in schizophrenic striatum (Seeman et al., 1993a; Murray et al., 1995; Sumiyoshi et al., 1995; Marzella et al., 1997) (see also Reynolds and Mason, 1994, 1995; Seeman et al., 1995; Seeman and Van Tol, 1995; Helmeste et al., 1996a). However, as pointed out above, it can be debated whether this increased number of raclopride-insensitive dopamine D_2-like binding sites is genuine dopamine D_4 sites. First of all, dopamine D_4 receptor-selective radioligands and specific antibodies fail to detect significant amounts of this receptor in striatum. Secondly, since these sites are not detected in dopamine D_2 receptor-deficient mice, contrary to wild-type mice, one could argue for the existence of a raclopride-insensitive dopamine D_2 receptor isoform derived from the dopamine D_2 gene, DRD2 (Seeman et al., 1997; Wilson et al., 1998). This latter observation from DRD2 knock-out mice also makes it highly unlikely that the detection of raclopride-insensitive dopamine D_2 binding sites is due to non-selective binding conditions that allow non-dopaminergic sites to be labeled, as has been suggested by Helmeste et al. (1996b) (also see Wilson et al., 1998).

There are conflicting data with respect to dopamine D_4 mRNA levels in the frontal cortex of schizophrenics. Lack of change (Mulcrone and Kerwin, 1996; Roberts et al., 1996) as well as decreases (focal abnormalities) (Meador-Woodruff et al., 1997) and increases in dopamine D_4 mRNA have been reported (Stefanis et al., 1998). An increase in hippocampal dopamine D_4 binding sites has also been observed using [^{3}H]NGD 94-1 (Lahti et al., 1998). It is unclear how and to what extent antipsychotic medication alters dopamine D_4 receptor expression. While it has been argued that some of the observed changes in dopamine D_4 receptor levels in schizophrenics are drug-independent (Lahti et al., 1998), it has also been shown that antipsychotic medication can affect dopamine D_4 mRNA expression. These changes appear to be region specific, since it was found that haloperidol downregulates dopamine D_4 mRNA levels in the hippocampus and entorhinal cortex (Ritter and Meador-Woodruff, 1997), but increases levels in the striatum and temporal and prefrontal cortex (Schoots et al., 1995; Lidow and Goldman-Rakic, 1997). Like antipsychotics, low doses of the non-competitive NMDA receptor antagonist (+)-MK-801 ((5*R*, 10*S*)-(+)-5-methyl-10, 11-dihydro-5*H*-dibenzo(a,d)cyclohepten-5,10-imine) can down-regulate dopamine D_4 mRNA in the hippocampus, although at high doses this effect is reversed (Healy and Meador-Woodruff, 1996). To what extent these changes in mRNA levels translate into actual changes in functional dopamine D_4 receptors is not known.

The mechanism by which dopamine D_4 receptor expression is regulated is not yet understood. Promoter analysis of the human receptor indicates the presence of specific domains that mediate or silence expression (Kamakura et al., 1997). It is unknown whether these domains are involved in dynamic regulation of receptor expression or

whether they solely mediate tissue specific expression. A naturally occurring point mutation in the human dopamine D_4 promoter can significantly reduce expression potential in heterologous expression systems (Okuyama et al., 1999b; Okuyama et al., 2000). The in vivo changes in dopamine D_4 mRNA expression due to antipsychotic and MK-801 treatment suggests that receptor density may be regulated at the level of transcription or RNA stability. However, heterologous expression experiments with dopamine D_4 receptors in a variety of different cell lines suggest that post-transcriptional mechanisms can also strongly contribute to expression (Knapp et al., 1998). There is little known about how dopamine D_4 receptor density is regulated through desensitization and downregulation in vivo, if at all. Experimental data derived from heterologous expression experiments in human embryonic kidney (HEK) 293 cells suggests that this receptor type is not strongly regulated through desensitization or internalization mechanisms compared with β_2-adrenoceptors (unpublished, Hubert H.M. Van Tol; Watts et al., 1999).

5. Pharmacology

The pharmacological profile of the dopamine D_4 receptor has been the topic of several extensive reviews by us (Seeman and Van Tol, 1994; Seeman et al., 1996, 1997; Wilson et al., 1998). In general, the dopamine D_4 receptor displays a pharmacological profile that is very comparable to that of the dopamine D_2 and D_3 receptors. The most striking observations are that the dopamine D_2/D_3 receptor-specific ligands raclopride and S-sulpiride fail to recognize the dopamine D_4 receptor with good affinity. This observation formed the basis for the experimental approach to measure dopamine D_4 receptors using subtractive pharmacological methods (see above). The classic atypical antipsychotic clozapine binds the dopamine D_4 receptor with an affinity that is 5- to 10-fold higher than its affinity for the dopamine D_2 receptor (Van Tol et al., 1991, 1992; Asghari et al., 1994). This observation suggested that the dopamine D_4 receptor may mediate atypical features of antipsychotics; however, this still remains to be established (Wilson et al., 1998). Although dopamine is the most potent endogenous ligand known to activate the dopamine D_4 receptor, the receptor can also be activated by (nor)epinephrine at submicromolar concentrations (Lanau et al., 1997b; Newman-Tancredi et al., 1997; Jovanovic et al., 1999). While the affinity of the dopamine D_4 receptor for (nor)epinephrine is at least 5- to 10-fold lower than its affinity for dopamine, these concentrations may still be relevant to receptor activation under certain physiological conditions. Other endogenous ligands like serotonin and histamine do not have a significant affinity for this receptor.

The extensive repeat polymorphism found in the third cytoplasmic loop of the human dopamine D_4 receptor does not affect the pharmacological profile in a major way (Asghari et al., 1994). In fact, the repeat sequence can be deleted from the receptor without consequence to its pharmacological profile (Asghari et al., 1994; Kazmi et al., 2000). Although an accurate side by side comparison of the rat dopamine D_4 receptor, which does not contain a repeat sequence in the third cytoplasmic loop, with the human dopamine D_4 receptor has not been reported, it appears that there may be some subtle pharmacological differences between receptors derived from different species (Asghari et al., 1994). Small 2- to 3-fold differences in affinity for some dopamine D_2 receptor antagonists, such as (+)butaclamol, fluphenazine, epidepride and *cis*-flupentixol, has been observed between the dopamine $D_{4.2}$ and $D_{4.10}$ receptor variants (Jovanovic et al., 1999). We also observed that different repeat variants of the dopamine D_4 receptor display differences in the sodium-sensitivity of clozapine binding. However, such small pharmacological differences are unlikely to be the cause for non-responsiveness in neuroleptic treatment, considering the large dose-range at which neuroleptics are being used (Marder et al., 1991).

The repeat polymorphism of the dopamine D_4 receptor is located in a region of the receptor that has previously been implicated in specificity and efficacy of G protein coupling. The two alternative splice forms of the dopamine D_2 receptor, which differ by the presence or absence of a 29 amino acid insertion in the third cytoplasmic loop at the same position where the dopamine D_4 receptor repeat polymorphism is found, show differences in specificity for G protein coupling and functional efficacy (Dal Toso et al., 1989; Hayes et al., 1992; Liu et al., 1992; Montmayeur et al., 1993; Guiramand et al., 1995). A direct comparison between dopamine $D_{4.2}$, $D_{4.4}$ and $D_{4.7}$ receptor isoforms indicated that the dopamine $D_{4.7}$ receptor has a 2- to 3-fold lower potency for dopamine-mediated coupling to adenylyl cyclase as compared to dopamine $D_{4.4}$ and $D_{4.2}$ receptors (Asghari et al., 1995). However, a careful analysis of dopamine $D_{4.2}$ vs. $D_{4.10}$ receptors indicated that the dopamine $D_{4.10}$ receptor is 2- to 3-fold more potent in coupling to adenylyl cyclase than the dopamine $D_{4.2}$ receptor variant. Parallel executed binding analysis indicated that the differences in potency are also reflected in the high affinity binding state of the receptor (Jovanovic et al., 1999). These results strongly suggest that there is no direct linear relationship between the length of the polymorphism and functional receptor pharmacology. This is an important observation since various genetic studies have employed strategies that pool the short and long alleles of the repeat polymorphism based on the assumption that there is a linear relationship between length of this polymorphism and biological activity. Thus, the pooling strategy may contribute to the loss of significance in studies searching for a genetic association between the dopamine D_4 recep-

Table 1
Selective ligands for the dopamine D_4 receptor

Compound	Code name	Activity	D_4 affinity
1-(3-Cyanobenzylpiperidin-4-yl)-5-methyl-4-phenyl-1, 3-dihydroimidazol-2-one		antagonist	0.96 nM (Carling et al., 1999)
[1-[4-Iodobenzyl]-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine	RBI-257	ND	0.3 nM (Kula et al., 1997)
1-Benzyl-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine	PNU-101,958	antagonist[a]	2.0 nM (Kula et al., 1997; Schlachter et al., 1997)
2-(4-(1-fluorenylmethyl)-1-piperazinyl)-pyrimidine		ND	20 nM (Arlt et al., 1998)
[2-[4-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]	S 18126	antagonist	2.4 nM (Millan et al., 1998)
2-[4-[(2-Phenyl-1*H*-imidazol-5-yl)methyl]-1-piperazinyl]pyrimidine	NGD 94-1	antagonist[a]	3.6 nM (Tallman et al., 1997)
3-[4-(3-Chloro-4-hydroxymethyl-phenyl)piperazin-1-yl]-1-(4-chloro-phenyl)-propan-1-ol	PD 108635	ND	11 nM (Belliotti et al., 1998)
3-[4-(4-Chlorophenyl)piperazin-1-yl]methyl-1*H*-pyrrolo[2,3-b]pyridine	L-745,870	antagonist[a]	0.43 nM (Patel et al., 1997)
3-[4-(4-Iodophenyl)piperazin-1-yl]methyl-1*H*-pyrrolo(2,3-b)pyridine	IPMPP	antagonist	0.39 nM (Kung et al., 1997)
3-Benzyl-8-methyl-1,2,3,4-tetrahydrochromeno[3,4-c]pyridin-5-one		antagonist	3.6 nM (Unangst et al., 1997)
5-[2-(4-(3-Fluorobenzylidene)piperidin-1-yl) ethyl]-4-(4-fluorophenyl)thiazole-2-carboxamide	NRA0160	ND[b]	1.4 nM (D_3, 39 nM) (Okuyama et al., 1999a)
5-(4-Chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole		antagonist	3.5 nM (Rowley et al., 1996, 1997)
5-Fluoro-2-[4-[(2-phenyl-1*H*-imidazol-5-yl)methyl]-1-piperazinyl]pyrimidine	SCH 66712	antagonist	6.6 nM (Kim et al., 1999)
7-[(2-Phenylaminoethylamino)methyl]chromen-2-one		antagonist	5.8 nM (Kesten et al., 1999)
7-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-4*H*-benz[1,4]oxazin-3-one		antagonist	4.3 nM (Belliotti et al., 1999)
7-(4-Fluoro-phenoxymethyl)-2-(5-fluoro-pyrimidin-2-yl)-octahydro-pyrido[1,2a]pyrazine;	CP-293,019	antagonist	3.4 nM (Sanner et al., 1998)
N-[2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl]-3-methoxybenzamide		ND	0.06 nM (Perrone et al., 1998)
N-[4-(2-Cyanophenyl)piperazin-1-ylmethyl]-3-methoxybenzamide;	PD 168077	agonist (partial)	8.7 nM (Glase et al., 1997)
N-4-Chlorobenzyl-4-[2-(3-(2-thienyl)-1,2, 4-oxadiazolyl)]piperidine		ND	5 nM (Williams and Lavrador, 2000)
(*R*)-(+)-2-Amino-4-(4-fluorophenyl)-5-[1-[4-(4-fluorophenyl)-4-oxobutyl]pyrrolidin-3-yl] thiazole	NRA0045	ND[b]	1.2 nM ($5HT_{2a}$ 1.9 nM; α_1 1.4 nM) (Okuyama et al., 1997b)
(*S*)-(−)-4-[4-[2-(Isochroman-1-yl)ethyl]piperazin-1-yl]benzenesulfonamide	PNU-101,387G	antagonist	7.2 nM (Merchant et al., 1996; TenBrink et al., 1996)
5-Fluoro-2-(4-pyridin-2-yl-piperazin-1-methyl)-1*H*-indole	CP-226,269	agonist	6.0 nM (Zorn et al., 1997)
2-Amino-benzoic acid 1-benzyl-piperidin-4-yl ester	RO-61-6270	antagonist	5.0 nM (Hartman et al., 1996)

In this table are listed a selection of the D_4-selective ligands; however, not all reported derivatives are included. Compounds that are not more than 100-fold selective over the D_2 receptor are excluded. Other receptors for which the listed ligand has significant affinity are given after the D_4 affinity value. Listed affinities for the dopamine D_4 receptor are the average value from those given in the listed references. The classifi cation of compounds as an antagonist or agonist is based on in vitro functional assays using cloned receptors which measured coupling to adenylyl cyclase and/or mitogesis as measured by [^{3}H]thymidine incorporation and/or dopamine mediated GTPγ^{35}S binding.

[a] These ligands have also been reported to possess agonist activity (Gazi et al., 1998; Gazi et al., 1999a,b). ND stands for not determined.

[b] Indicates ligands that are thought to act as antagonists based on their in vivo biological activities and behavioural profile.

tor and certain disorders or traits. In this respect, it should also be considered that several sequence variants exist for alleles of the same length.

The potential of the novel dopamine receptors as an antipsychotic target resulted in the development of various dopamine D_4 receptor-selective ligands (see Table 1). Possibly the best characterized selective compound in the literature to date is L-745,870, which has been analyzed extensively for its (biological) pharmacology (Patel et al., 1997; Gazi et al., 1998, 1999a; Ceci et al., 1999), in various behavioral paradigms (Bristow et al., 1997a; Patel et al., 1997; Mansbach et al., 1998) and in clinical trials for schizophrenia (Bristow et al., 1997b; Kramer et al., 1997) (see below). While this compound was reported to be an antagonist for the dopamine D_4 receptor (K_i 0.43 nM) (Patel et al., 1997), it could also serve as a partial agonist (Gazi et al., 1998, 1999a). Other reported selective dopamine D_4 receptor antagonists are PNU-101,387G ((*S*)-(−)-4-[4-[2-(isochroman-1-yl)ethyl]piperazin-1-yl]benzenesulfonamide) (K_i 10 nM) (Merchant et al., 1996), PNU-101,958 (1-benzyl-4-[*N*-(3-isopropoxy-2-pyridinyl)-*N*-methyl]-aminopiperidine) (K_i 1.4 nM) (Schlachter et al., 1997) (note that this compound was reported to be a partial agonist (Gazi et al., 1998, 1999a)), CP-293,019 (7-(4-fluoro-phenoxymethyl)-2-(5-fluoro-pyrimidin-2-yl)-octahydro-pyrido[1,2a]pyrazine) (K_i 3.4 nM) (Sanner et al., 1998) and NGD 94-1 (K_i 3.6 nM) (Tallman et al., 1997) (this compound was also reported to possess agonist activity (Gazi et al., 1999b)).

Over the last few years, considerable progress has been made in the identification of subtype-specific determinants that contribute to ligand selectivity for dopamine dopamine D_2-like receptors. Using a substituted-cysteine accessibility method, Javitch et al. (1995,1998,1999) have mapped water-accessible residues of transmembrane domains 2, 3, 5, 6 and 7 in the dopamine D_2 receptor ligand binding pocket. Simpson et al. (1999) and Schetz et al. (2000) furthered this work by examining the molecular basis for dopamine D_2/D_4 receptor ligand specificity. By mutating 14 water-accessible residues of the dopamine D_2 receptor binding pocket that are not conserved in the dopamine D_4 receptor, residues that confer the selectivity of ligands such as CPPMA (chlorophenylpiperazinyl methylazaindole; 3-[4-(4-chlorophenyl)piperazin-1-yl]methyl-1*H*-pyrrolo[2,3-b]pyridine) and Ro 62-4599 (2-methyl-6-(6-phenyl-3,4-dihydro-1*H*-isoquinolin-2-ylmethyl)-pyrimidin-4-ylamine) for the dopamine D_4 receptor were identified near the extracellular surface of transmembrane domains 2, 3 and 7 (Simpson et al., 1999). They conclude that selectivity is primarily determined by a bundle of aromatic residues, which is located adjacent to the amine of the bound dopamine molecule. The binding of dopamine D_4 receptor-selective antagonists containing an amine substituted with two large aromatic groups is facilitated by the smaller aliphatic sidechains of these critical transmembrane domains that are present in the dopamine D_4 receptor. Schetz et al. (2000) also found that the residues close to the extracellular surface of transmembrane domains 2 and 3 affect the binding of the dopamine D_4 receptor-selective ligand L-750,667 (3-{[4-(4-iodophenyl) piperazin-1-yl]methyl}-1*H*-pyrrolo[2,3b]pyridine).

Chimeric dopamine $D_{2L}/D_{4.2}$ and $D_{4.2}/D_{2L}$ receptors have been constructed with the junction located near the amino-terminal portion of transmembrane domain 3 (Shih et al., 1997). The dopamine $D_{2L}/D_{4.2}$ receptor chimera was not detected with a [^{3}H]spiperone probe, while the dopamine $D_{4.2}/D_{2L}$ receptor retained high-affinity binding. However, perturbations that were observed in the binding affinity of dopamine agonists suggest that the transmembrane domains of dopamine D_4 and D_{2L} receptors may be incompatible, with gross conformational changes resulting in the chimeric receptor. Our own work using a large number of single chimeric (D_2/D_4, D_4/D_2) and double chimeric ($D_2/D_4/D_2$, $D_4/D_2/D_4$) dopamine receptors indicates that [^{3}H]nemonapride binding is maintained in these chimeras (unpublished, Hubert H.M. Van Tol). This work suggests that transmembrane domain 4 plays an important role for the dopamine D_4 receptor-selectivity of CP-293,019.

6. Cellular signaling

The D_2-like family of receptors couple to multiple intracellular effectors (reviewed by Huff, 1996). Inhibition of adenylyl cyclase by the dopamine D_2-like receptor was first identified in the pituitary prior to its cloning (De Camilli et al., 1979; Onalli et al., 1981; Stoof and Kebabian, 1981). In the mouse retina, the dopamine D_4 receptor has been shown to reduce dark-adapted cAMP levels, indicating that this subtype is active in vivo (Cohen et al., 1992). Dopamine D_2-like receptors also couple to the inhibition of inositol phosphate hydrolysis, inhibition of arachidonic acid release, opening of K^+ channels, and inhibition of Ca^{2+} channels in native tissue (Huff, 1996). Due to the historical lack of selective agonists and antagonists, the contribution of dopamine D_4 receptors has not been determined by in vivo studies. Experiments with cultured cells expressing this receptor, however, have provided insight into its signaling properties (see Table 2).

Almost all studies with recombinant dopamine D_4 receptors have shown that functional coupling is dependent on a pertussis toxin-sensitive G protein (ie. G_i/G_o). In MN9D mesencephalic cells, the rat dopamine D_4 receptor failed to couple to $G\alpha_{oA}$, $G\alpha_{oB}$, and $G\alpha_{i1/2/3}$ (Tang et al., 1994; O'Hara et al., 1996). However, use of a pertussis toxin-resistant mutant of $G\alpha_{t2}$ (transducin) has revealed that the dopamine D_4 receptor can activate this G protein in MN9D cells (Yamaguchi et al., 1997). G_{t2} was shown to express outside the retina and to inhibit adenylyl cyclase upon activation. Despite identifying the dopamine D_4 receptor as the first non-opsin receptor to couple to G_{t2}, Yamaguchi et al. (1997) did not identify any effects on

Table 2
Functional coupling of the dopamine D_4 receptor

Functional response	Dopamine D_4 receptor	Tissue or cell line	G protein coupling	Refs.
Adenylyl cyclase inhibition	mouse	Retina		Cohen et al., 1992
	human $D_{4.2}$	CHO *lac1*	G_i/G_o	Chio et al., 1994
	rat	MN9D	G_i/G_o	Tang et al., 1994
	human $D_{4.2}$, $D_{4.4}$, $D_{4.7}$	GH_4C_1		Sanyal and Van Tol, 1997
	rat	MN9D	$G\alpha_{t2}$	Yamaguchi et al., 1997
	human $D_{4.2}$, $D_{4.4}$, $D_{4.7}$	CHO-K1		Asghari et al., 1995
	human $D_{4.2}$, $D_{4.4}$, $D_{4.7}$	HEK 293	G_i/G_o	Watts et al., 1999
	human	COS-7	G_i/G_o $G\alpha z$	Obadiah et al., 1999
Potentiation of adenylyl cyclase II stimulation	human $D_{4.4}$	HEK 293	$G\beta\gamma$	Watts and Neve, 1997
Arachidonic acid elease (potentiation)	human $D_{4.2}$	CHO *lac1*	G_i/G_o	Chio et al., 1994
		CHO 10001	G_i/G_o	Lajiness et al., 1995
Na^+/H^+ antiporter stimulation	human	CHO 10001	G_i/G_o	Lajiness et al., 1995
	human $D_{4.4}$	CHO-K1	G_i/G_o	Coldwell et al., 1999
K^+-channel activation GIRK1 (Kir3)	human $D_{4.2}$	*Xenopus* oocytes	G_i/G_o	Werner et al., 1996
	human $D_{4.2}$	*Xenopus* oocytes	$G\beta\gamma$	Pillai et al., 1998
K^+-channel inhibition (voltage-dependent)	rat	Hypophysial nerve terminals		Wilke et al., 1998
	rat	MES-23.5	$G\alpha o$	Liu et al., 1999
Ca^{2+}-channel current inhibition	human $D_{4.2}$	GH_4C_1		Seabrook et al., 1994
	human $D_{4.2}$,$D_{4.4}$,$D_{4.7}$	AtT-20, HEK 293T	$G\alpha i_{1,2,3}$	Kazmi et al., 2000
Cl^-influx	mouse	*Xenopus* oocytes	G_i/G_o	Jensen et al., 1997
Changes to neural morphology	D_4	Fetal cortical neurons		Swarzenski et al., 1994
Mitogenesis (DNA synthesis ↑)		CHO 10001	G_i/G_o	Lajiness et al., 1995
MAPK activation (ERK1/2)	human $D_{4.7}$	CHO-K1		Oldenhof et al., 1998

cGMP phosphodiesterase activity, the effector of rhodopsin-stimulated $G\alpha_t$. Recently, the dopamine D_4 receptor has been shown to potently and preferentially couple to pertussis toxin-resistant $G\alpha_z$, a G protein expressed in the cerebral cortex and retina (Obadiah et al., 1999). In contrast to findings with the rat dopamine D_4 receptor expressed in MN9D cells, in vitro results have implied that the human receptor is not selective with respect to the $G\alpha_i$ subtype (Kazmi et al., 2000). Using dopamine D_4 receptor-containing COS-1 cell membranes that were stripped with urea and reconstituted with $G\beta\gamma_t$ and $G\alpha_{i1}$, $G\alpha_{i2}$ or $G\alpha_{i3}$, robust activation of all three heterotrimeric G proteins (as measured by GTPγ^{35}S binding) was detected.

Besides mediating effects through the α-subunit of G proteins, it has become increasingly evident that receptor signals can be transduced by Gβγ. The cloning of G protein-coupled inward rectifying K^+ channels such as GIRK1 (Kir3.1) has revealed that these proteins are regulated by Gβγ subunits (Reuveny et al., 1994; Wickman et al., 1994). The activation of GIRK1 in *Xenopus* oocytes by dopamine D_4 receptors suggests that they can open K^+ channels via Gβγ as well (Werner et al., 1996; Pillai et al., 1998). Another report showed that potentiation of phorbol ester- or G_s-stimulated type II adenylyl cyclase activity by the dopamine D_4 receptor was most likely due to dopamine-activated Gβγ subunits (Watts and Neve, 1997).

The dopamine D_4 receptor has been shown to attenuate intracellular cAMP levels in a pertussis toxin-sensitive manner (Chio et al., 1994; Tang et al., 1994; Lajiness et al., 1995). Human dopamine D_4 receptor variants contain-

ing 2, 4 or 7 of the 16 amino acid repeats in the third cytoplasmic loop can effectively inhibit adenylyl cyclase activity (Asghari et al., 1995; Watts et al., 1999). Persistent exposure of the dopamine D_4 receptor to agonists does not produce a desensitization of adenylyl cyclase inhibition in HEK 293 cells (Watts et al., 1999). In contrast, this study found that long-term agonist exposure (2–18 h) induced the heterologous sensitization of forskolin-stimulated cAMP accumulation, while exposure to quinpirole for 18 h led to a reduction in cellular $G\alpha_i$ levels.

Several authors have measured a potentiation of ATP- or Ca^{2+} ionophore-stimulated release of arachidonic acid mediated by dopamine D_4 receptors expressed in CHO cells. This pathway appears to require a pertussis toxin-sensitive G protein, with Lajiness et al. (1995) indicating that protein kinase C is required for the potentiation of arachidonic acid release. The dopamine D_4 receptor stimulates pertussis toxin-sensitive extracellular acidification by activation of the amiloride-sensitive Na^+/H^+ antiporter (Lajiness et al., 1995; Coldwell et al., 1999). The inhibition of a voltage-dependent K^+ current by a dopamine D_4 receptor-specific agonist has been reported (Wilke et al., 1998). Liu et al. (1999) expressed dopamine D_2-like receptors in a mesencephalic cell line and found that while D_2 and D_3 increased a voltage-dependent outward K^+ current, the dopamine D_4 receptor reduced this current by a mechanism that involved $G\alpha_o$. As with other dopamine D_2-like receptors, however, the D_4 receptor can also produce a hyperpolarization through the pertussis toxin-sensitive/$G\beta\gamma$-dependent activation of the K^+ channel GIRK1 (Kir3) (Werner et al., 1996; Pillai et al., 1998).

Dopamine D_4 receptor activation leads to the depression of dihydropyridine-sensitive Ca^{2+} current in GH_4C_1 pituitary cells (Seabrook et al., 1994). In GH_4C_1 cells, the dopamine D_4 receptor is able to inhibit adenylyl cyclase but fails to significantly reduce stimulated prolactin release or the activity of the prolactin promoter, in contrast to effective inhibition of prolactin release and prolactin promoter activity by the dopamine D_2 receptor (Sanyal and Van Tol, 1997). The reason for this difference in coupling between dopamine D_4 and D_2 receptors is presently unknown. The inhibition of L-type Ca^{2+} channels by quinpirole in a human dopamine D_4 receptor-expressing AtT-20 cell line was also shown recently (Kazmi et al., 2000). In addition, this study also used a dopamine D_4 receptor-mediated transient increase in cytosolic Ca^{2+} to measure functional coupling in HEK 293T cells. Results indicated that there is no significant difference in coupling between dopamine $D_{4.0}$, $D_{4.4}$, and $D_{4.7}$ receptor variants, while the dopamine $D_{4.2}$ receptor had a marginally lower EC_{50}.

When transfected into MN9D cells, the dopamine D_4 receptor can increase the number, branching, and extension of neurites in a manner distinct from the dopamine D_2 receptor (Swarzenski et al., 1994). In CHO 10001 cells, expression of dopamine D_4 receptor leads to a stimulation of DNA synthesis as measured by increased [^{3}H]thymidine incorporation (Lajiness et al., 1995). Recently, it was shown by Luo et al. (1998) that the mitogenic effect of the dopamine D_2 receptor occurs through a pathway involving G_i/G_o and Ras/MEK (MAPK/ERK kinase). Other reports have indicated that the dopamine D_2 receptor can activate the MAPK (mitogen-activated protein kinase) pathway by a G protein-dependent mechanism (Faure et al., 1994; Welsh et al., 1998). Dopamine D_4 receptors can also activate the MAP kinases ERK1 (extracellular signal-regulated kinase 1) and ERK2 in CHO-K1 cells (Fig. 4) (also see Oldenhof et al., 1998). This pathway may mediate the observed effects of the dopamine D_4 receptor on arachidonic acid release, mitogenesis and neuronal morphology. The recently identified role of the MAPK pathway in the brain, such as in the establishment of long-term potentiation, suggests that these kinases may be important cellular targets of G protein-coupled receptors (Orban et al., 1999).

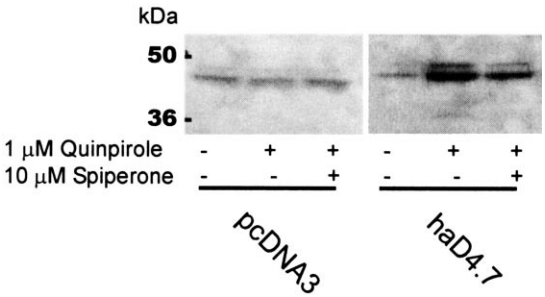

Fig. 4. Activation of MAPK by dopamine $D_{4.7}$ receptors. Serum-starved CHO-K1 cells stably transfected with the plasmids pcDNA3 or pcDNA3-ha$D_{4.7}$ were treated for 5 min with quinpirole (1 μM) alone or quinpirole (1 μM) + the dopamine D_4 receptor antagonist spiperone (10 μM). Stimulation of ERK1/ERK2 phosphorylation was detected by Western blotting with anti-phosphoMAPK(Tyr204) IgG as described in Oldenhof et al. (1998).

7. Genetic association studies

Despite early optimism that the higher affinity of clozapine for the dopamine D_4 receptor compared with the dopamine D_2/D_3 receptor may form the biochemical basis for its atypical antipsychotic profile, association and linkage studies failed to find evidence identifying DRD4 as a risk factor for schizophrenia (Daniels et al., 1994; Shaikh et al., 1994; Petronis et al., 1995; Kohn et al., 1997; Serretti et al., 1999). Two recent publications report a relationship between the exon 3 polymorphism and the response to neuroleptic drugs in schizophrenia, although the studies involved small sample sizes (Hwu et al., 1998; Cohen et al., 1999). Both found that the shorter VNTR variants were associated with positive response to these drugs. Since the receptor was not found to be a major risk

factor in schizophrenia, attention has been focused on finding a relationship between polymorphisms of dopamine D_4 receptor and other diseases or personality traits.

Reports of an association between the trait of novelty seeking (impulsive, exploratory, sensation-seeking behavior) and long VNTR alleles in Israeli and American populations surfaced in 1996 (Benjamin et al., 1996; Ebstein et al., 1996) (for a critical review, see Paterson et al., 1999). These studies were cited as the first demonstration of a genetic basis to personality and it was claimed that the polymorphism explained 10% of the genetic variation of novelty seeking (Benjamin et al., 1996). Since that time, others have failed to replicate these findings with different populations and/or measures of novelty seeking (Jonsson et al., 1997, 1998; Vandenbergh et al., 1997; Pogue-Geile et al., 1998; Kuhn et al., 1999), although one study found linkage with harm avoidance (Hill et al., 1999a). Ebstein et al. (1997) subsequently published further evidence of an association, and this has been followed by recent papers claiming an association between the exon 3 polymorphism and novelty seeking in German and Japanese populations (Strobel et al., 1999; Tomitaka et al., 1999). In addition, studies examining the effects of both the DRD4 exon 3 and serotonin transporter (5-HTTLPR) polymorphisms in young children found a relationship between long alleles and this personality trait (Ebstein et al., 1998; Auerbach et al., 1999). Benjamin et al. (2000) recently looked at the modulation of DRD4 long allele effects by 5-HTTLPR and catechol *O*-methyltransferase (COMT) polymorphisms and again found that the 7-repeat VNTR variants are associated with novelty seeking. A study examining novelty seeking in a large sampling of the Finnish population found a significant association between the 2- and 5-repeat variants and high novelty seeking scores, which disagrees with the other positive findings (Ekelund et al., 1999). Another polymorphism, 521 bp upstream from the initiation codon of DRD4, has also been associated with this trait and was suggested to have a weak link with schizophrenia (Okuyama et al., 1999b; Okuyama et al., 2000).

Due to the variety of populations, methodologies and statistical tests utilized in the studies of novelty seeking, it is difficult to make direct comparisons between these reports. The variety of positive and negative results suggests a weak association at best, and concern has been raised regarding the statistical validity of some studies (Paterson et al., 1999). Several authors use a strategy of grouping short and long alleles of DRD4 despite a lack of biochemical evidence supporting a relationship between receptor function and the number of VNTR repeats, as discussed earlier (Jovanovic et al., 1999). An interesting footnote to the discussion of novelty seeking is a recent publication that reported a higher proportion of long DRD4 alleles in migratory populations, presumably due to natural selection (Chen et al., 1999). For instance, a striking difference was observed between South American indigenous peoples (69% long alleles) compared with North and East Asians (5%), despite the fact that the former group is thought to have migrated from Asia relatively recently.

Despite the importance of the dopaminergic system in reward and dependence, association studies implicating the dopamine D_4 receptor as a risk factor in substance abuse have also been inconsistent. Studies supporting (George et al., 1993; Muramatsu et al., 1996) and rejecting (Adamson et al., 1995; Chang et al., 1997; Parsian et al., 1997; Sander et al., 1997) an association between the VNTR polymorphism and alcoholism have emerged. Hill et al. (1999b) propose that linkage is detectable with more severe forms of alcoholism. Two studies in 1997 claimed that the 7-repeat VNTR allele is a risk factor in opioid dependence among Chinese and Israeli populations (Kotler et al., 1997; Li et al., 1997). A relationship between the exon 3 polymorphism and substance abuse (alcohol and drugs) was rejected in population studies by Comings et al. (1999) and Gelernter et al. (1997). In a German study using both association and linkage analyses, no difference was found in VNTR frequency between addicts and controls and no preferential transmission of long alleles was observed (Franke et al., 2000).

ADHD is distinguished by inattention and impulsivity and is treated with amphetamines and dopamine agonists. Studies concluding that ADHD has a strong genetic component have been followed by reports linking the dopaminergic genes DAT1 (encoding the dopamine transporter) and DRD4 with this disorder (reviewed in Swanson et al., 2000). Four family-based studies (Rowe et al., 1998; Smalley et al., 1998; Swanson et al., 1998; Faraone et al., 1999) have reported linkage between the 7-repeat VNTR allele and ADHD, while population-based approaches produced evidence for (LaHoste et al., 1996) and against (Castellanos et al., 1998) an association. While DRD4 is not an "ADHD gene", Swanson et al. (2000) conclude that there is converging evidence of a dopaminergic component to this disorder. Tourette syndrome, a disease that is characterized by multiple motor and vocal tics, was also linked to the 7-repeat DRD4 variant in a study of 64 family trios (Grice et al., 1996). Subsequent linkage (Hebebrand et al., 1997) and population studies (Comings et al., 1999) do not support that conclusion, but a study of obsessive–compulsive disorder patients did report an association between subjects exhibiting tics and the 7-repeat DRD4 variant (Cruz et al., 1997).

Mood disorders, including unipolar depression and bipolar disorder, have also been examined to look for involvement of the exon 3 polymorphism of DRD4. Positive association studies have found that the 4-repeat variant was less frequent in mood disorder patients (Manki et al., 1996). A weak association between depression and the VNTR polymorphism in patients compared with controls was found to be insignificant when corrected for multiple testing (Serretti et al., 1999). Others have found the polymorphic variants made no contribution to bipolar disorder in both population studies (Lim et al., 1994; Oruc et al.,

1997; Li et al., 1999) and a family-based study (Bocchetta et al., 1999).

Our understanding of the importance of dopamine in the CNS has provided a rational basis for proposing as association between the polymorphic dopamine D_4 receptor and novelty seeking, substance abuse, ADHD, and depression (Emilien et al., 1999). While a strong relationship between the dopamine D_4 receptor and disease/personality has not been identified, numerous reports of linkage or a weak association between the 7-repeat/long alleles of DRD4 and novelty seeking, drug and alcohol abuse, ADHD and Tourette syndrome may indicate that the dopamine D_4 receptor polymorphism is one of several genetic contributions to these traits or disorders.

8. Physiological role

The importance of dopamine in movement, mood/cognition, and pituitary hormone secretion is well established (reviewed by Emilien et al., 1999). However, insight into the function of the dopamine D_4 receptor has been limited until very recently by the lack of selective antagonists and agonists. The search for these ligands has been driven by speculation that antagonism of this receptor may underlie the activity of atypical antipsychotics such as clozapine. It has been proposed that the ability of clozapine to treat both the positive symptoms (psychoses) and negative symptoms (loss of affect) of schizophrenia while having a lower propensity to induce extrapyramidal side-effects may be partly related to the fact that it has a higher affinity for the dopamine D_4 receptor compared with dopamine D_2/D_3 receptors (Wilson et al., 1998). The anatomical localization of the dopamine D_4 receptor in the prefrontal cortex, thought to be involved in cognition, compared with little or no expression in the basal ganglia also hints that a dopamine D_4 receptor-selective drug may possess antipsychotic actions with a low propensity for extrapyramidal side-effects.

The recent synthesis of compounds which can block the dopamine D_4 receptor but do not bind to dopamine D_2/D_3 receptors has allowed the use of animal models to study receptor-specific behaviors (see Table 3). In rodents, reversal of apomorphine-induced loss of prepulse inhibition, reversal of amphetamine-induced hyperactivity, increased dopamine turnover in the forebrain and effects on neuronal firing in the ventral tegmental area are known to correlate with the potency of antipsychotics. Drugs that reduce locomotor activity, induce catalepsy, increase prolactin secretion or block apomorphine/amphetamine-induced stereotypy are predicted to cause extrapyramidal side-effects. In rats, L-745,870, CP-293,019 and PNU-101,387G were found to block the loss of prepulse inhibition resulting from apomorphine administration (Mansbach et al., 1998). Others have failed to replicate these findings. L-745,870 had no effect on apomorphine-induced loss of prepulse inhibition, amphetamine-induced hyperactivity or dopamine metabolite levels in rodents, predicting that a "pure" dopamine D_4 receptor antagonist is not an effective antipsychotic (Bristow et al., 1997a; Patel et al., 1997). Similarly, Merchant et al. (1996) did not observe antagonism of apomorphine- or amphetamine-induced effects in rats with PNU-101,387G. In another animal model for schizophrenia, behaviors induced by phencylidine (PCP) treatment such as locomotor hyperactivity and social isolation were not affected by L-745,870 (Sams-Dodd, 1998).

Many studies have found that dopamine D_4 receptor-selective antagonists can potently induce c-*fos* expression in limbic and cortical regions, areas where dopamine D_4 receptor expression has been identified. Feldpausch et al. (1998) found that the behavioral and neuroadaptive effects resulting from long-term amphetamine treatment, including behavioral sensitization and the refractoriness of amphetamine-induced c-*fos* mRNA expression in the medial prefrontal cortex, were blocked by PNU-101,387G. In primates, NGD 94-1 was able to reverse PCP-induced impairments in object retrieval/detour performance and also increased the level of the dopamine metabolite homovanillic acid (HVA) found in the cerebrospinal fluid (Jentsch et al., 1999). It should be noted that results using animal models must be interpreted with caution. Positive results may be due to the antagonism of other receptors (e.g. serotonin 5-HT_{2A}, sigma, or dopamine D_2/D_3 receptors), particularly given the range of doses administered in these studies. Also, many of the animal models used to predict antipsychotic potential were designed to identify drugs that act on dopamine D_2 receptors. The absence of activity in these models does not necessarily preclude antipsychotic activity with dopamine D_4 receptor-selective antagonists. However, clinical studies with L-745,870 and the combined dopamine D_4/serotonin 5-HT_{2A} receptor antagonist fananserin have borne out the more pessimistic predictions. These drugs were found to be ineffective as an antipsychotic in patients with acute schizophrenia (Bristow et al., 1997b; Kramer et al., 1997; Truffinet et al., 1999).

Apart from animal models that are believed to be predictive of antipsychotic activity, it is interesting that specific dopamine D_4 receptor ligands show little or no observable effect. In a mouse plus-maze experiment, no significant behavioral alterations were observed in animals administered the dopamine D_4 receptor-selective antagonists L-745,870 and L-741,742 (5-(4-chlorophenyl)-4-methyl-3-(1-(2-phenylethyl)piperidin-4-yl)isoxazole) (Cao and Rodgers, 1997). In an effort to distinguish a clear behavioral drug profile or "ethogram" for dopamine D_4 receptor-selective drugs, Clifford and Waddington (2000) looked at the effects of the antagonists L-745,870, Ro 61-6270 and CP-293,019 and the agonists CP-226,269 (5-fluoro-2-(4-pyridin-2-yl-piperazin-1-methyl)-1*H*-indole) and PD-168077 (*N*-[methyl-4-(2-cyanophenyl)piperazinyl-3-methylbenzamide) on the behavior of rats. They failed to find any consistent "ethogram" for these drugs and were also unable to unequivocally identify any behavioral ef-

Table 3
Behavioral and physiological effects of dopamine D_4 receptor-selective antagonists in rodents

Behavioral or physiological action	Effective	Not effective
Reversal of apomorphine/amphetamine-induced loss of prepulse inhibition	L-745,870 (10 mg/kg s.c.)[a] CP-293,019 (17.8 mg/kg s.c.)[a] LU-111995 (10 mg/kg i.p.)[b] NGD 94-1[c] NRA0045 (3 mg/kg i.p.)[d] NRA0160 (3 mg/kg p.o.)[e] PNU-101,387G (30 mg/kg s.c.)[a]	L-745,870 (1 mg/kg p.o.)[f]
Block of amphetamine-induced hyperactivity	L-745,870 (10 mg/kg p.o.)[f] NRA0045 (0.4 mg/kg i.p.)[d] NRA0160 (29 mg/kg p.o.)[e]	L-745,870 (1 mg/kg p.o.)[f] L-745,870 (10 mg/kg s.c.)[g] S 18126 (10 mg/kg s.c.)[g] PNU-101,387G (28 mg/kg i.p.)[h]
Block of apomorphine/amphetamine-induced stereotypy		L-745,870 (1 mg/kg p.o.)[f] NGD 94-1[c] NRA0045 (39 mg/kg i.p.)[d] NRA0160 (1000 mg/kg p.o.)[e]
Block of phencylidine (PCP)-induced prolonged swimming latency	NRA0045 (0.3 mg/kg i.p.)[d] NRA0160 (3 mg/kg p.o.)[e]	
Block of apomorphine/amphetamine inhibition of neuronal firing in A9 (substantia nigra pars compacta)	NRA0160 (1 mg/kg i.v.)[i]	L-745,870 (3 mg/kg i.v.)[i] PNU-101,387G (6.4 mg/kg i.v.)[h]
Block of apomorphine/amphetamine inhibition of neuronal firing in A10 (ventral tegmental area)	L-745,870 (3 mg/kg i.v.)[i] NRA0045 (0.1 mg/kg i.v.)[d] NRA0160 (1 mg/kg i.v.)[i]	L-745,870 (0.5 mg/kg i.v.)[g] S 18126 (0.5 mg/kg i.v.)[g]
Increase in dopamine or dopamine metabolite levels in the brain	S 18126 (40 mg/kg s.c.) (weak)[g]	L-745,870 (30 mg/kg p.o.)[j] L-745,870 (10 mg/kg s.c.)[g] PNU-101,387G (19 mg/kg i.p.)[h]
Block of amphetamine-induced behavioral sensitization	PNU-101,387G (10 mg/kg i.p.)[k]	
Increase in c-*fos* mRNA or Fos-like immunoreactivity in the medial prefrontal cortex	NRA0045 (10 mg/kg i.p.)[n] NRA0160 (10 mg/kg i.p.)[n] Ro 61-6270 (10 mg/kg i.p.)[l] PNU-101,387G (0.2 mg/kg i.p.)[h]	
Reduction in spontaneous locomotor activity	LY-745,870 (30-300 mg/kg p.o.)[f]	NRA0045 (10 mg/kg i.p.)[d] PNU-101,387G (28 mg/kg i.p.)[h]
Catalepsy	L-745,870 (100 mg/kg p.o.)[f] NRA0045 (30 mg/kg i.p.)[m] NRA0160 (1000 mg/kg p.o.)[e]	L-745,870 (10 mg/kg p.o.)[f] L-754,870 (10 mg/kg s.c.)[g] NGD 94-1[c] S 18126 (40 mg/kg s.c.)[g] PNU-101,387G (28 mg/kg i.p.)[h]
Increase in plasma prolactin levels		L-745,870 (10 mg/kg p.o.)[j] L-754,870 (10 mg/kg s.c.)[g] S 18126 (40 mg/kg s.c.)[g]

[a] Mansbach et al. (1998).
[b] Geyer et al. (1999).
[c] Hoffman et al. (1995).
[d] Okuyama et al. (1997a).
[e] Okuyama et al. (1999a).
[f] Bristow et al. (1997a).
[g] Millan et al. (1998).
[h] Merchant et al. (1996).
[i] Kawashima et al. (1999b).
[j] Patel et al. (1997).
[k] Feldpausch et al. (1998).
[l] Hartman et al. (1996).
[m] Okuyama et al. (1997b).
[n] Kawashima et al. (1999a).

fects that were due to dopamine D_4 receptor occupancy. Ceci et al. (1999) were able to block dopamine-induced facilitation of pyramidal neuron firing in rat prelimbic cortex using L-745,870. However, their conclusion that this

facilitation is mediated by the dopamine D_4 receptor is undermined by the effectiveness of quetiapine, a drug with low affinity for this receptor, in these experiments. Thus, this demonstration of dopamine D_4 receptor-specific action needs to be confirmed with a selective agonist.

Despite disappointments with dopamine D_4 receptor-selective antagonists, the use of transgenic mice has begun to provide an understanding of the role of this receptor in the CNS. Homozygous, dopamine D_4 receptor-deficient (DRD4 $-/-$) mice created by Rubinstein et al. (1997) showed no gross anatomical irregularities. No significant reduction in [^{3}H]nemonapride binding in the brain of the dopamine D_4 receptor knock-outs was found, emphasizing the predominance of dopamine D_2/D_3 receptors in the brain. Despite this, DRD4 $-/-$ mice exhibited several phenotypes that suggest a physiological role for the dopamine D_4 receptor in locomotion and drug sensitivity. DRD4 $-/-$ mice displayed less spontaneous locomotion and rearing than wildtype animals. Conversely, the knock-outs scored higher than DRD4 $+/+$ mice in the rotorod test of coordination, which may be a result of the higher level of dopamine synthesis that was detected in the DRD4 $-/-$ animals. Low-dose clozapine administration in DRD4 $-/-$ animals did not affect apomorphine-induced locomotor activity, while DRD4 $+/+$ animals showed a significant reduction. At higher clozapine doses, both DRD4 $-/-$ and DRD4 $+/+$ animals experienced akinesia, presumably due to antagonism on all dopamine D_2-like receptors. DRD4 knock-out mice were supersensitive to the locomotor-stimulating effects of cocaine, methamphetamine and ethanol. These findings, taken together with mRNA hybridization and immunohistochemical analyses, point to the dopamine D_4 receptor acting as inhibitory postsynaptic receptors that maintains a tonic control on neurons of the frontal cortex and basal ganglia. However, the observation that DRD4 $-/-$ animals have an increased level of dopamine biosynthesis and degradation has been argued to point to a role as an inhibitory autoreceptor (Rubinstein et al., 1997).

The existence of dopamine D_2, D_3 and D_4 receptor knock-out mice has allowed a further examination of the role of these receptors in the blockade of prepulse inhibition. Amphetamine treatment was found to disrupt prepulse inhibition in wildtype, DRD3 $-/-$, and DRD4 $-/-$ mice, while the drug was ineffective with DRD2 $-/-$ mice (Ralph et al., 1999). The previously cited reports of "selective" dopamine D_4 receptor antagonists blocking apomorphine-induced loss of prepulse inhibition are not consistent with these results, and possibly reflect antagonism of the dopamine D_2 receptor instead. Results with DRD4 $-/-$ knock-out mice have provided support for the controversial finding that the dopamine D_4 receptor VNTR polymorphism is associated with novelty-seeking in humans (Benjamin et al., 1996; Ebstein et al., 1996). Using three measures of novelty seeking in mice, the open field, emergence and novel object tests, DRD4 $-/-$ mice were found to be significantly less responsive to novel stimuli compared with DRD4 $+/+$ littermates (Dulawa et al., 1999). While this convergence of data from human genetics and transgenics is exciting, it must be approached with caution since the contribution and effects of compensatory developmental changes induced by disruption of the DRD4 gene are unknown.

9. Conclusions

Almost a decade after it was cloned, the dopamine D_4 receptor continues to be a source of intense interest. The critical function that the dopaminergic system plays in the CNS and the unique polymorphic structure of this receptor ensures that the drive to understand this receptor will continue. While the exon 3 VNTR polymorphism remains the most ubiquitous and structurally divergent polymorphism that has been identified in the dopamine receptor family, its physiological role, if any, remains uncertain. Pharmacological evidence has ruled against a significant role in ligand binding or G protein activation, but the demonstration that the third cytoplasmic loop of the dopamine D_4 receptor can act as a SH3 binding domain suggests that we may be close to finding a role for the VNTR. This new paradigm in G protein-coupled receptor biology opens additional avenues of research to explore.

Many of the human genetic studies that have examined the involvement of the dopamine D_4 receptor gene in personality and disease point to a small but detectable contribution of the VNTR polymorphism, but once again the biological basis is a mystery. In fact, the physiological function of the dopamine D_4 receptor is not yet fully understood. Whereas some drug studies have identified neuroleptic-like effects in animal models, others have failed to observe a dopamine D_4 receptor-dependent phenotype. Yet knock-out mice provide compelling evidence that this receptor may influence exploratory behavior and substance dependence. The growing number of novel pharmacological tools that are specific for the dopamine D_4 receptor may soon refine our preliminary understanding of its physiological role. Discerning what role the dopamine D_4 receptor has in development also needs to be further clarified and may be possible through the use of conditional knock-out and knock-in transgenic mice.

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