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REVIEW

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The D_3 dopamine receptor in cellular and organismal function

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Abstract The D_3 dopamine receptor is a member of the family of D_2-like dopamine receptors. Since the cloning and identification of the D_3 receptor in 1990, considerable progress has been made towards understanding the function of this novel site. Although some avenues of investigation have yielded more definitive results than others, studies to date indicate the D_3 receptor is localized preferentially in limbic brain areas and affects locomotion and perhaps reinforcement and reward. A subpopulation of the receptors appear to be autoreceptors which modulate dopamine synthesis, release, and neuronal activity. These observations have led to the hypothesis that the D_3 receptor may be an appropriate target in the treatment of neuropsychiatric disorders such as schizophrenia and drug addiction. The role of D_3 sites in disease, however, remains to be established. Genetic association of D_3 receptor polymorphisms with neuropsychiatric disorders have been proposed. Alterations in expression of D_3 sites may occur in some diseases. Although the study of this receptor is clearly in the early stages, these findings lay the foundation for future investigation. In this review, dopamine D_3 receptor brain localization, cellular signaling mechanisms, and associated behavior will be discussed. The potential role of the D_3 site in neuropsychiatric disorders and as a therapeutic target is also addressed.

Key words Limbic system · Signal transduction · Autoreceptor · Receptor binding · Behavior · Locomotor activity · Reward · Development · Schizophrenia · Parkinson's disease · Cocaine · Antipsychotic

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Introduction

Dopamine receptors are of considerable interest, as they are the principal target of drugs employed in the treatment of neuropsychiatric disorders such as schizophrenia and Parkinson's disease. Until 1990, the dopamine receptor population in the brain and periphery was believed to consist of two subtypes, D_1 and D_2 (for review, see Seeman and Grigoriadis 1987). Currently, the D_1 and D_2 subtypes represent families of dopamine receptors. Several additional low abundance dopamine receptors were identified following the cloning of the D_1 and D_2 receptors (for review, see Sibley et al. 1993). These novel subtypes include the D_3 and D_4 receptors, which are similar to D_2, and the D_5 receptor, which is similar to D_1. The D_3 receptor was initially cloned from a rat cDNA library by Sokoloff and colleagues (1990) using probes derived from the D_2 dopamine receptor sequence. The cloning of the human D_3 receptor was reported shortly thereafter (Giros et al. 1990), followed by the murine D_3 receptor (Fishburn et al. 1993). Due to the limbic location of this receptor, D_3 receptors have been postulated as a therapeutic target in the treatment of schizophrenia and drug abuse.

Although still in the early stages, considerable progress has been made in the study of the D_3 receptor. This article reviews the progress made to date in assessing the neurobiological role of this novel receptor. Its relevance in disease and as a potential therapeutic target are also discussed.

Molecular biology

Based on amino acid sequence and gene organization, the D_3 receptor has been classified as a member of the family of D_2-like dopamine receptors. Unlike genes for the D_1-like receptors which do not contain introns, the

D_2, D_3, and D_4 receptor genes contain discontinuous gene sequences. Also, in contrast with the D_1-like receptors, which have short third intracellular domains and long carboxy termini, the D_2-like receptors have relatively long third intracellular domains and short carboxy termini. The D_2-like receptors possess moderate sequence homology with the D_1-like receptors. For example, the rat D_1 and D_2 receptors possess only 41% homology in the transmembrane domains (Monsma et al. 1990). In comparison, the overall homology between the D_2 and D_3 receptor is 52%, but increases to 75% when only the transmembrane spanning domains are considered. The D_2 and D_3 receptors exhibit 39% and 41% overall homology with the D_4 receptor, respectively (Van Tol et al. 1991). The rat and human D_3 receptors contain 446 and 400 amino acids, respectively, and exhibit 78% homology (Sokoloff et al. 1990; Giros et al. 1990) (Table 1). Although biochemical evidence for the secondary structure of this receptor has yet to be generated, hydrophobicity analysis indicates that the most probable secondary structure of the D_3 receptor is consistent with those of the seven transmembrane spanning, G-protein coupled receptors.

In addition to aiding in the detection of novel receptor subtypes, the use of molecular methods has enabled the elucidation of receptor splice-variants. For example, the D_2 receptor exists in two functional alternate splice variants D_{2L} and D_{2S}, which vary in the length of the third intracellular loop (Dal Toso et al. 1989; Giros et al. 1989). The murine D_3 dopamine receptor undergoes alternative RNA splicing to produce two mRNA species (D_{3L} and D_{3S}) which differ by 63 bp in the putative third intracellular loop (Fishburn et al. 1993). An internal acceptor site is used for the D_3 dopamine receptor splicing mechanism; this is different from the existence of a separate exon, as occurs for D_2 receptor splicing. Alternate splice variants of the third intracellular loop have yet to be detected in the rat or human; however, several truncated isoforms of the D_3 receptor have been reported (Giros et al. 1991; Snyder et al. 1991; Nagai et al. 1993; Pagliusi et al. 1993; Schmauss et al. 1993; Griffon et al. 1996). Although the functional properties of only some of the truncated D_3 receptor variants have been assessed, all of those tested were found to lack binding activity in transfected cell lines (Giros et al. 1991; Schmauss et al. 1993). It is likely that the other truncated forms are also nonfunctional. The physiological role of these receptor variants is also unclear. It is speculated that the truncated receptors could be expressed under certain circumstances as a mechanism for controlling the density of functional D_3 sites or might occur in certain disease states (Giros et al. 1991).

Table 1 Characteristics of the D_3 dopamine receptor

Amino acids	
Human	400
Rat	446
Homology with D_2 receptor (%)	52
D_4 receptor (%)	41
Rat D_3 with human D_3 receptor (%)	78
Brain distribution	Olfactory tubercle Islands of Calleja Nucleus accumbens
Putative selective agonists	7-OH-DPAT PD 128907
Putative selective antagonists	Nafadotride (+)-S-14297 U-99194A

Localization of D_3 receptors in brain

Distribution in rat brain

Although present in significantly lower levels than D_1 or D_2 receptor mRNAs, in situ hybridization studies in the rat brain demonstrate that mRNA for the D_3 receptor appears to be expressed preferentially in limbic brain regions. The highest density is reported in the islands of Calleja where D_3 mRNA is expressed by granule cells (Diaz et al. 1995). High levels of D_3 mRNA are also observed in the nucleus accumbens and olfactory tubercles (Sokoloff et al. 1990; Bouthenet et al. 1991; Mengod et al. 1992; Landwehrmeyer et al. 1993a; Curran and Watson 1995; Diaz et al. 1995). Additional brain regions reported to exhibit dense expression of D_3 mRNA include the medial division of the bed nucleus of the stria terminalis, the nucleus of the vertical limb of the diagonal band of Broca, the paracentral thalamic nucleus, the medial and ventral lateral geniculate nuclei, the magnocellular preoptic nucleus, mammallary nucleus, the lateral part of the substantia nigra pars compacta, the dorsal cochear nucleus, and the Purkinje cell layer of the vestibulocerebellum (Bouthenet et al. 1991; Mengod et al. 1992; Diaz et al. 1995).

Moderately dense levels of D_3 mRNA expression have been observed in areas such as the ventral pallidum, the amygdala, the nucleus of the horizontal limb of the diagonal band of Broca, certain thalamic and hypothalamic nuclei, the superior colliculus, and the inferior olivary nucleus (Bouthenet et al. 1991). Moderate to dense expression of D_3 mRNA has also been reported in the dentate gyrus, olfactory bulb and the anterior and intermediate lobes of the pituitary in in situ hybridization studies (Bouthenet et al. 1991); however, no D_3 mRNA was detected in these brain regions in other studies (Sokoloff et al. 1990; Mengod et al. 1992).

Low densities of D_3 mRNA have been reported in the cerebral cortex, caudate/putamen, ventral pallidum, substantia nigra pars reticulata, ventral tegmental area, and cerebellar cortex (Bouthenet et al. 1991; Mengod et al. 1992).

The distribution of D_3 receptors in rat brain has not been mapped in detail. As the pharmacological specificity of radioligands remains to be extensively validated, data obtained to date must be considered to indicate "putative" D_3 sites. With these limitations in mind, the localization of "putative" D_3 binding sites appears to parallel the distribution of D_3 mRNA. D_3 receptors appear to be expressed in highest density in brain regions such as the islands of Calleja, olfactory bulb, and the pituitary intermediate lobe. Moderately dense D_3 binding is observed in the nucleus accumbens, the molecular layer of the vestibulocerebellum, and substantia nigra pars compacta. Relatively little D_3 binding is observed in the caudate/putamen (Gehlert et al. 1992; Lévesque et al. 1992; Landwehrmeyer et al. 1993a; Levant et al. 1993; Parsons et al. 1993; Booze and Wallace 1995). D_3-like immunoreactivity was associated with neuronal-type cells and was concentrated at the cell body perimeter (Ariano and Sibley 1994; Larson and Ariano 1995).

Distribution in human brain

Although not as extensively characterized, the distribution of D_3 mRNA in the human brain appears to be generally similar to that observed in the rat. Enrichment of D_3 mRNA was observed in the nucleus accumbens and islands of Calleja with relatively low levels of expression in the anterior caudate and putamen (Landwehrmeyer et al. 1993b). D_3 mRNA has also been observed in the granular cell layer of the dentate gyrus (Meador-Woodruff et al. 1994).

The distribution of "putative" D_3 receptors in human brain is generally similar to that observed in the rat; however, the overall pattern of distribution appears to be somewhat less restricted (Herroelen et al. 1994). Highest densities of putative D_3 binding sites are reported in the nucleus accumbens and islands of Calleja (Murray et al. 1994; Hall et al. 1996). Moderate amounts of D_3 binding were observed in the basal ganglia, parietal, temportal and occipital cortex, and cerebellar cortex, followed by substantia nigra, hippocampus, and the basolateral, lateral and basomedial amygdaloid nuclei (Herroelen et al. 1994; Murray et al. 1994; Lahti et al. 1995). D_3 receptors were also detected in moderate density in the pituitary, with somewhat greater labeling in the posterior lobe than the anterior (Herroelen et al. 1994).

Cellular signaling mechanisms

Signal transduction in expression systems

Determining the signal transduction pathways associated with the dopamine receptor in vivo was initially problematic because of the lack of selective pharmacological tools. The expression of the D_3 receptor in transfected cell lines was the primary means for studying the functional properties of this receptor. However, host cells come from a variety of sources and may not express the appropriate G proteins or effectors that are physiologically relevant for the receptor. Thus, data obtained from the same receptor in different cell lines may be different depending on the endogenous signaling molecules expressed by the cells (for review, see Kenakin 1996). Accordingly, it is not surprising that reports on the coupling of the D_3 receptor to signal transduction systems have varied considerably. The initial cloning report indicated that the D_3 receptor expressed in Chinese hamster ovary (CHO) cells did not exhibit a decrease in affinity for agonists in the presence of guanyl nucleotides, or G-shift, as would be

Table 2 Effect of D_3 dopamine receptor stimulation on signal transduction pathways

Cell line	Effector system	Reference
CCL1.3	$\Leftrightarrow$ Adenylate cyclase	MacKenzie et al. (1994)
	$\Leftrightarrow$ Adenylate cyclase	Tang et al. (1994)
CHO 10001	$\Downarrow$ Adenylate cyclase	Chio et al. (1994)
	$\Uparrow$ Na^+/H^+ exchange	Chio et al. (1994)
CHO-K1	$\Leftrightarrow$ Adenylate cyclase	MacKenzie et al (1994)
CHO	$\Leftrightarrow$ arachadonic acid release	Freedman et al. (1994)
	$\Uparrow$ Na^+/H^+ exchange	Boyfield et al. (1996)
GH_4C_1	$\Leftrightarrow$ K^+ current	Seabrook et al. (1992)
	$\Leftrightarrow$ Adenylate cyclase	Seabrook et al. (1992)
	$\Leftrightarrow$ arachadonic acid release	Seabrook et al. (1992)
	$\Leftrightarrow$ Ca^{2+} current	Seabrook et al. (1992)
MN9D	$\Leftrightarrow$ Adenylate cyclase	Tang et al. (1994)
NG108-15	$\Downarrow$ Adenylate cyclase	Freedman et al. (1994)
	$\Downarrow$ Ca^{2+} current	Freedman et al. (1994)
	$\Leftrightarrow$ Adenylate cyclase	Pilon et al. (1994)
	$\Uparrow$ Fos expression	Pilon et al. (1994)
SK-N-MC	$\Leftrightarrow$ Adenylate cyclase	Mackenzie et al. (1994)
Xenopus melanophores	$\Downarrow$ Adenylate cyclase	Potenza et al. (1994)
293	$\Downarrow$ Adenylate cyclase	McAllister et al. (1995)
C6 glioma	$\Uparrow$ Na^+/H^+ exchange	Cox et al. (1995)

expected for a G-protein-coupled receptor (Sokoloff et al. 1990). This suggested that the D_3 receptor might not be functionally coupled to G-proteins. Other groups studying the receptor expressed in other cell lines, including neuronal mesencephalic MN9D cells, neuroblastoma NG108-15 cells, and insect Sf21 cells, observed a similar lack of G-shift in D_3 binding (Freedman et al. 1994; Tang et al. 1994a; Woodcock et al. 1995). G-shifts in D_3 receptor binding, however, were observed in a number of studies using a variety of other cell lines. Interestingly, the magnitude of the decrease in agonist affinity observed in the presence of guanyl nucleotides ranged from 5- to 10-fold (Seabrook et al. 1992; Sokoloff et al. 1992; Chio et al. 1994; MacKenzie et al. 1994) to 50- to 100-fold, similar to the roughly 100-fold shifts observed for the D_2 receptor (Grigoriadis and Seeman 1985; Castro and Strange 1993; Pilon et al. 1994).

Observations on the coupling of the D_3 receptor in expression systems to specific signal transduction cascades have also varied (Table 2). In some systems, a G-shift in D_3 binding was observed, but alterations in second messengers such as cAMP, phosphoinositides, or arachidonic acid were not detected (Seabrook et al. 1992; MacKenzie et al. 1994). Other groups observed a variety of D_3-initiated signaling events, including stimulation or inhibition of adenylyl cyclase, increased extracellular acidification, alterations in Ca^{2+} and K^+ currents, and induction of c-*fos* expression (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Seabrook et al. 1994; Cox et al. 1995; Griffon et al. 1996, 1997; Liu et al. 1996; Werner et al. 1996). D_3 receptors have also been shown to induce aggregation in melanocytes (Potenza et al. 1994) and to initiate mitogenesis in CHO cells (Chio et al. 1994; Pilon et al. 1994; Pugsley et al. 1995) and NG108-15 glioma cell lines (Griffon et al. 1997). Several of the D_3-mediated signaling events, including stimulation of adenylyl cyclase, mitogenesis, alterations in Ca^{2+} and K^+ currents, and increased rate of extracellular acidification were blocked by pertussis toxin, suggesting coupling to a Gi or Go isoform (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Seabrook et al. 1994; Liu et al. 1996; Werner 1996). In another study, however, D_3-induced increases in the rate of extracellular acidification were not blocked by pertussis toxin (Cox et al. 1995). Thus, these studies demonstrate functional coupling of the D_3 receptor to a variety of signaling cascades in some expression systems. The cellular signaling pathways affected, however, vary depending on the host cell and may not necessarily reflect the signaling pathways associated with the receptor in brain.

Coupling in brain

While coupling of the D_3 receptor has been shown in some expression systems, signaling pathways associated with the receptor in brain have been more difficult to determine. Although all of the D_3-selective radioligands identified to date, such as [^{3}H]7-OH-DPAT (7-hydroxy-diphenylaminotetralin), [^{3}H]PD 128907, and [^{125}I]7-OH-PIPAT ((R)-*trans*-7-hydroxy-2-[*N*-propyl-*N*-(3′-iodo-2′-propenyl)amino]tetralin), have been agonists, which presumably label the high affinity state of a G-protein coupled receptor, most studies indicate that the binding of these ligands at putative D_3 sites is insensitive to guanyl nucleotides (Lévesque et al. 1992; Burris et al. 1994; Kung et al. 1994; Bancroft et al. 1997). In fact, the binding of several non-selective D_2-like receptor agonists remaining in the presence of guanyl nucleotides has been suggested to represent labeling of D_3 sites in autoradiographic studies (Gehlert 1992; Levant et al. 1993; Kung et al. 1994). One study, however, has reported the inhibition of [^{3}H]7-OH-DPAT by guanyl nucleotides and the sulfhydryl alkylating agent N-ethylmaleimide, indicating G-protein coupling (Liu et al. 1994c). This observation, however, is most likely the result of the non-selective labeling of both D_2 and D_3 sites due to the presence of Mg^{2+} in the assay buffer (see below).

While these findings may suggest that the D_3 receptor in brain may lack functional G-protein coupling, there are other possible explanations. Whereas the high affinity state of the D_2 receptor exhibits about 100-fold higher affinity for agonists than the low affinity state, the high affinity conformation of the cloned D_3 receptor in expression systems has been most often reported to exhibit only about 5- to 10-fold higher affinity for agonists than the low affinity state. Thus, while agonist radioligands are presumed preferentially to label the high affinity conformation of G-protein coupled receptors, the putative D_3 binding observed in brain, albeit of nanomolar affinity, may be to receptors in the low affinity state. As such, the binding of either agonist or antagonist ligands to these sites would be unaltered in the presence of guanyl nucleotides.

There are several possible reasons why the observed D_3 binding in brain tissue may represent receptors in the low affinity state. The first and simplest explanation is that the affinity state of these sites is a function of the in vitro assay conditions used to obtain putatively selective labeling of D_3 sites. Specifically, obtaining selective labeling of the D_3 site with the radioligands currently available appears to be dependent on the use of assay conditions which disfavor agonist binding at the D_2 site. The greatest D_3/D_2 selectivity for these ligands has been obtained in the absence of Mg^{2+} and the presence of EDTA (Lévesque et al. 1992; Akunne et al. 1995) in concordance with previous studies indicating that the high affinity agonist state of D_2-like receptors is not favored in the absence of Mg^{2+} (Sibley and Creese 1983). Although these conditions may also affect the affinity state of the D_3 receptor, the low affinity conformation of the D_3 site exhibits much

higher affinity for agonists than the low affinity state of the D_2 receptor. As such, selective labeling of D_3 sites occurs.

Alternatively, D_3 sites in rat brain may exist predominantly in the low affinity state under basal conditions as has been suggested for the D_1 receptor (Richfield et al. 1989). Although "D_3-selective" radioligands, such as [^{3}H]7-OH-DPAT, label a single putative D_3 site in rat brain (Lévesque et al. 1992; Akunne et al. 1995; Levant 1995), depletion of endogenous catecholamines resulted in the detection of an additional [^{3}H]7-OH-DPAT binding site ex vivo. This additional binding site exhibited roughly 10-fold higher affinity than the single binding site detected in control animals without a significant increase in the total number of sites (Levant 1995). Although the higher affinity sites may have been occupied, and thus masked, by endogenous dopamine in control animals (Schotte et al. 1992, 1996), preincubation and extensive washing of membranes from control animals to remove any residual dopamine failed to alter binding of [^{3}H]7-OH-DPAT (Levant 1995). These observations suggest that in the absence of dopamine, some D_3 sites, which under normal conditions are predominantly in the low affinity state, assume a high affinity conformation. This hypothesis is supported by the observation that the high affinity component of [^{3}H]7-OH-DPAT binding in catecholamine-depleted rats is inactivated by the alkylating agent 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), while the low affinity component is not, a difference that might well be conferred by a conformational change. In fact, when catecholamine-depleted rats are treated with EEDQ, only low affinity sites remain and the density of these sites is reduced by roughly the same amount as the density of high affinity sites present in the catecholamine-depleted animals (Levant 1995).

Although coupling to classical second messengers has yet to be demonstrated for the D_3 receptor in the brain, modulation of expression of the transcription factor *c-fos* has been observed following stimulation or blockade of the receptor. 7-OH-DPAT and quinpirole attenuated clozapine-induced Fos expression in the nucleus accumbens, suggesting the possible involvement of dopamine D_3 receptors (Vahid-Ansari and Robertson 1996). U-99194A, a D_3-preferring antagonist, produced an increase in *c-fos* mRNA expression in the medial prefrontal cortex (Merchant et al. 1996). Similarly, another D_3-preferring antagonist, *l*-nafadotride, induced Fos protein expression in the nucleus accumbens and olfactory tubercles (Shafer and Levant 1997). However, no induction of c-*fos* mRNA was observed after treatment with the selective D_3 receptor antagonist GR 103691 (Hurley et al. 1996b). Further study is required to establish the exact role D_3 receptors play in the induction of Fos protein expression.

The D_3 receptor as an autoreceptor

Anatomical studies

One of the issues of interest regarding the D_3 receptor is whether, like the D_2 site, this receptor is localized pre- or postsynaptically. The detection of D_3 mRNA in the substantia nigra and ventral tegmental areas and putative binding sites in dopaminergic terminal fields suggests that a subset of D_3 receptors may be presynaptic. In keeping with this hypothesis, unilateral dopaminergic lesions produced a marked decrease in D_3 receptor density in the nucleus accumbens, suggesting the loss of presynaptic sites (Lévesque et al. 1995). While this observation could also result from a decrease in postsynaptic sites, Tepper et al. (1997) also report a marked decrease in D_3 receptor binding in the substantia nigra in rats treated with D_3 receptor antisense oligonucleotides.

Neurochemical studies

In vivo and in vitro neurochemical studies also suggest a role for the D_3 site as an autoreceptor that modulate dopaminergic activity. Stimulation of D_3 receptors expressed in neuronal mesencephalic MN9D cells resulted in a dose-dependent inhibition of dopamine release (Tang et al. 1994b). Likewise, the D_3-preferring agonist 7-OH-DPAT produced a decrease in dopamine release in vivo as assessed by microdialysis or voltametry, as well as in accumbal slice preparations (Damsma et al. 1993; Rivet et al. 1994; Yamada et al. 1994; Devoto et al. 1995; Gilbert et al. 1995; Patel et al. 1995; Gainetdinov et al. 1996). Similar effects were also reported for PD 129807, another D_3 receptor-preferring agonist (Pugsley et al. 1995; Gobert et al. 1996). Both 7-OH-DPAT and PD 128907 have also been shown to decrease extracellular DOPAC concentrations as assessed by in vivo microdialysis, consistent with a decrease in dopamine release (Pugsley et al. 1995; Gainetdinov et al. 1996). In addition, mice deficient in D_3 sites resulting from a targeted mutation of the D_3 receptor gene, or "knockout" animals, exhibited higher basal levels of extracellular dopamine (Cooper et al. 1996). It is difficult, however, to ascertain that the inhibition of dopamine release observed in heterogeneous tissues results from selective actions at the D_3 receptor; as stimulation of D_2 receptors expressed in MN9D cells also inhibits dopamine release (Tang et al. 1994b). Likewise, care must be taken in the interpretation of data obtained with "knockout" animals because of the considerable plasticity in the developing nervous system, compensation changes may occur such as the expression of other receptors in place of the original D_3 receptor. It must also be noted that similar inhibitory responses to PD 128907 were observed for both D_3 "knockout" and wild-type mice (Cooper et al. 1996).

The D_3 receptor has also been implicated in the modulation of dopamine synthesis. In D_3-expressing MN9D cells, the application of agonist produced a decrease in K^+-stimulated tyrosine hydroxylase activity, the enzyme responsible for dopamine synthesis (O'Hara et al. 1996). In vivo, the D_3-selective agonists 7-OH-DPAT and PD 128907 have been reported to decrease dopamine synthesis (Aretha et al. 1995; Gobert et al. 1995; Pugsley et al. 1995; Gainetdinov et al. 1996). This effect appears to be presynaptic, as it is observed in both normal rats and in rats treated with γ-butyrolactone, which blocks impulse flow in nigrostriatal and mesolimbic dopamine neurons (Aretha et al. 1995; Pugsley et al. 1995). The involvement of the D_3 receptor in this effect is supported by the observation that 7-OH-DPAT produced a greater decrease in dopamine synthesis in the nucleus accumbens, where D_3 sites are relatively abundant, than in the caudate nucleus where D_3 sites are sparse (Aretha et al. 1995). A preliminary report by Nissbrandt et al. (1995) also suggests that reduction in the density of D_3 sites by intracerebroventricular infusion of antisense oligonucleotides for the D_3 receptor may result in increased DOPA accumulation, indicating a possible increase in dopamine synthesis. However, a preliminary report on D_3 receptor deficient mice indicated no alteration in dopamine synthesis compared to wild-type animals (Cooper et al. 1996). Taken together, these observations suggest a potential role for the D_3 receptor as an autoreceptor modulating dopamine release and/or synthesis. Further study, however, must confirm the role of specific dopamine receptor subtypes in these observations.

Electrophysiological studies

The D_3-preferring agonist 7-OH-DPAT has been shown to inhibit firing of neurons in both the substantia nigra and ventral tegmental areas as well as in brain slice preparations by activation of an 85 pS K^+ channel (Bowery et al. 1994; Liu et al. 1994a; Devoto et al. 1995; Kreiss et al. 1995; Lejeune and Millan 1995). Although selective action of 7-OH-DPAT at D_3 receptors cannot be assumed in these studies, Kreiss et al. (1995) have shown that the potencies of ten dopamine agonists in inhibiting firing of neurons in the substantia nigra pars compacta correlated with their affinities at D_3, but not D_2 receptors. Caution, of course, must be exercised in the interpretation of such findings in view of the significant variability in the in vitro pharmacological profile of the D_2 and D_3 sites in various assay systems (see below). Recent electrophysiological data demonstrates, by D_3 receptor antisense knockout, that dopaminergic neurons possess functional D_3 autoreceptors at their axonal terminal and somatodentritic regions (Tepper et al. 1997). D_3 antisense knockouts produced an attenuation of the inhibitory response to apomorphine, decreased [^{3}H]7-OH-DPAT binding in the substantia nigra, and exhibited spontaneous contralateral rotations which suggest an increased dopamine release. D_3 autoreceptors may modulate terminal excitability and presumably the synthesis/release of dopamine from nerve terminals. On the somatodendrites, D_3 autoreceptors may play a role in modulating the excitability of local dendritic regions.

Mediation of drug effects

Radioligand binding studies

The D_3 receptor is of interest particularly because of its relatively restricted distribution in brain. Unlike the D_2 receptor, which is abundant in the caudate/putamen and pituitary as well as in limbic brain regions (for review, see Levant 1996), very low levels of expression of the D_3 receptor are detected in either the caudate/putamen or pituitary, brain areas associated with the untoward neurological and endocrine effects produce by most conventional antipsychotics. These observations suggest that the D_3 receptor, alone or in conjunction with other receptors, may be a target for novel antipsychotic drugs which might be free of extrapyramidal and neuroendocrine effects (Sokoloff et al. 1990).

Considering the extensive homology between the D_2 and D_3 sites, it is not unexpected that the pharmacological profile of the D_3 receptor is generally similar to that of the D_2 receptor. As such, the D_3 receptor exhibits high affinity for non-selective and D_2-selective agonists, such as dopamine, quinpirole, and apomorphine, and significantly lower affinity for the D_1-selective agonist SKF 38393 (Sokoloff et al. 1990). The D_3 site also possess significantly higher affinity for D_2-selective antagonists, such as spiperone and haloperidol, than the D_1-selective antagonist SCH 23390 (Freedman et al. 1994). Likewise, the D_3 receptor exhibits stereospecificity with higher affinity for (+)-butaclamol than (–)-butaclamol and (–)-sulpiride than (+)-sulpiride (Freedman et al. 1994; Kula et al. 1994; MacKenzie et al. 1994).

A number of studies have also examined the relative affinities of dopaminergic compounds for D_2 and D_3 receptors in various expression systems and in brain. These studies suggest that some dopaminergic agonists, such as dopamine and quinpirole, possess higher affinity for the D_3 site whereas antagonists, such as haloperidol, spiperone, and domperidone have higher affinity for D_2 (Sokoloff et al. 1990). However, results of these studies vary considerably, depending, at least in part, on the expression system or tissue, the radioligand, and the in vitro assay conditions used (Tang et al. 1994a; Burris et al. 1995; Levant et al. 1995). For example, quinpirole was found to have more than

100-fold higher affinity for the D_3 receptor than the D_2 receptor in some assay systems (Sokoloff et al. 1990; Lèvesque et al. 1992; Burris et al. 1995) but roughly equal affinity for these sites in others (Levant and DeSouza 1993; Tang et al. 1994a; Burris et al. 1995). Similar variation in D_2/D_3 selectivity in vitro has been observed for several other agonists including dopamine, quinelorane, 7-OH-DPAT, pramipexole, PD 128907 and 7-*trans*-OH-PIPAT (Sokoloff et al. 1990, 1992; Levant and DeSouza 1993; Chumpradit et al. 1994; Freedman et al. 1994; Kula et al. 1994; MacKenzie et al. 1994; Tang et al. 1994a; Burris et al. 1995; Levant et al. 1995; Lévesque et al. 1995; Mierau et al. 1995; Pugsley et al. 1995; Sautel et al. 1995a; Flietstra and Levant 1996). In fact, the high affinity state of the D_2 receptor appears to have similar affinity for agonists as the D_3 site (Burris et al. 1995). As such, the observed D_3-selectivity of many agonists may have resulted from the use of in vitro conditions which disfavor the high affinity conformation of the D_2 receptor, such as the inclusion of Na^+ in in vitro assay systems used for benzamide radioligands (Grigoriadis and Seeman 1985; Burris et al. 1995; Levant et al. 1995). In accordance with this hypothesis, the D_2/D_3 selectivity of antagonists has exhibited much less variability between studies. Compounds such as domperidone, haloperidol, spiperone, chlorpromazine, clozapine and risperidone exhibit slight to substantial selectivity for the D_2 receptor over the D_3 while others, such as (–)-sulpiride, amisulpiride, (+)-UH 232 exhibit little selectivity between these sites (Sokoloff et al. 1990, 1992; Levant and DeSouza 1993; Malmberg et al. 1993; Freedman et al. 1994; Kula et al. 1994; MacKenzie et al. 1994; Tang et al. 1994a; Burris et al. 1995; Levant et al. 1995; Mierau et al. 1995; Millan et al. 1995b; Pugsley et al. 1995; Sautel et al. 1995a; Flietstra and Levant 1996). Several antagonist possessing modest selectivity for the D_3 receptor over the D_2 have been identified, including *l*-nafadotride, (+)-S-14297, U-99194A and GR103691 (Waters et al. 1993; Sautel et al. 1995b; Millan et al. 1995; Flietstra and Levant 1996). What is clear from these studies is that under certain conditions, a number of compounds exhibit significant selectivity between the D_2 and D_3 dopamine receptors. This information is likely to be of considerable utility in the design and interpretation of in vitro studies, particularly for the determination of the localization and density of D_3 sites. On the other hand, the attribution of in vivo pharmacological effects of these drugs to specific receptor subtypes based on these data is, in most instances, premature.

Functional assays

Several functional assays have established the agonist or antagonist activity of a variety of dopaminergic compounds at the D_3 receptor. D_2 agonists, such as dopamine, quinpirole, and bromocriptine, have been shown to possess agonist activity at the D_3 receptor as assessed by the induction of CHO cell mitogenesis, melanocyte aggregation, or extracellular acidification (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Sautel et al. 1995a; Boyfield et al. 1996). The putatively D_3-selective compounds 7-OH-DPAT and PD 128907 also exhibit agonist activity in the mitogenesis test (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Pugsley et al. 1995; Sautel et al. 1995). In contrast, antagonists, such as spiperone, (±)-sulpiride, (+)-S-14297 and nafadotride, block agonist-induced activity in these tests (Potenza et al. 1994; Sautel et al. 1995b; Gobert et al. 1996). The D_2/D_3 ligand (+)-UH 232 has been shown to be a partial agonist at the D_3 receptor in the mitogenesis assay (Griffon et al. 1995).

In addition to elucidating the agonist or antagonist activity of compounds at the D_3 receptor, the assays described above are also useful in determining the D_2/D_3-selectivity of various compounds. In contrast to the significant D_3-selectivity reported in some binding studies, the agonists tested, including dopamine, quinpirole, and 7-OH-DPAT, exhibited only modest, if any, D_3-selectivity in the mitogenesis, melanocyte aggregation, or extracellular acidification assays (Chio et al. 1994; Pilon et al. 1994; Potenza et al. 1994; Sautel et al. 1995a; Boyfield et al. 1996). In contrast, the antagonists spiperone and (±)-sulpiride were roughly 65-fold more potent in inhibiting agonist-induced melanocyte aggregation at D_2 receptors than D_3 (Potenza et al. 1994). These observations further underscore the need of caution in the use of in vitro binding data in the interpretation of in vivo or in vitro functional studies.

Modulation by antidopaminergic drugs

The up-regulation of D_2 receptors by antidopaminergic drugs is well established (Seeman 1981) and is believed to result from the blockade of those receptors. Because the D_3 receptor has been proposed as a potential antipsychotic site, there has been a great deal of interest in whether antipsychotic drugs produce a similar up-regulation in D_3 sites. To date, several studies have been performed to examine the effects of antipsychotic drugs on the expression of D_3 receptor mRNA with differing results. Chronic treatment with haloperidol, sulpiride, and clozapine has been reported to produce increases in D_3 receptor mRNA in whole brain of 3- to 5-fold as assessed by RNAse protection (Buckland et al. 1992, 1993). More modest increases in D_3 receptor mRNA (40–60%) were observed in olfactory tubercle following treatment with haloperidol and sulpiride, but not clozapine, for 14 days as assessed by polymerase chain reaction (Wang et al. 1996). Hurley et al. (1996c) reported significant increases in D_3 receptor mRNA expression following 21 days of treatment with clozapine in striatal and

limbic brain areas by in situ hybridization. Clozapine also produced a decrease in D_3 mRNA in the islands of Calleja. The density of [^{3}H]7-OH-DPAT-labeled sites were unaltered after treatment with either clozapine or sulpiride. In contrast, haloperidol did not produce significant alterations in D_3 mRNA expression but an increase in D_3 binding was observed in the islands of Calleja. Other studies, using different treatment paradigms, reported no change in D_3 receptor mRNA expression (Fishburn et al. 1994; Fox et al. 1994; Lévesque et al. 1995) or D_3 binding (Lèvesque et al. 1995; Levant 1997). Clearly, the conflicting results observed in these studies may result from the different treatment paradigms used and must be resolved by further study. Moreover, these studies did not assess whether the observed antipsychotic-induced alterations in D_3 receptor mRNA expression resulted in changes in the density or affinity of D_3 receptors.

Role in behavior

One of the primary aims in the study of the novel dopamine receptors is the elucidation of their role in organismal function. To date, pharmacological and molecular methods have been used in attempt to selectively study these novel sites. In the case of the D_3 receptor, numerous pharmacological studies have been performed as well as several studies using targeted mutation and antisense technologies. To date, a large body of data has been generated. Care, however, must be taken in the interpretation of these findings. It must be noted that the D_2/D_3 selectivity of many drugs, particularly agonists, varies considerably in in vitro binding studies. In addition, compensatory adaptations must be considered in the interpretation of studies using "knockout" or antisense approaches. While all of methodologies currently available for the study of the functional role of the D_3 receptor possess certain limitations, several themes are gradually emerging from the body of data collected thus far.

Locomotor activity

Although the D_3 receptor has been implicated in numerous behaviors, the receptor is most widely cited in the modulation of locomotor activity. In contrast to the D_2 receptor, where stimulation is believed to increase locomotion, stimulation of the D_3 site appears to inhibit locomotor activity. This effect was initially reported in several studies using the D_3-preferring drug 7-OH-DPAT. This drug produced a biphasic effect on locomotor activity in rats in which locomotion was inhibited at lower doses and stimulated at higher doses (Daly and Waddington 1993; McElroy et al. 1993; Ahlenius and Salmi 1994; Svensson et al. 1994a,b; Khroyan et al. 1995; Depoortere et al. 1996; Kagaya et al. 1996). The inhibitory effects of the drug were attributed to activity of the drug at the D_3 receptor; the stimulatory effects to the actions of higher doses of the drug at the D_2 receptor (Daly and Waddington 1993; Ahlenius and Salmi 1994; Svensson et al. 1994a). This interpretation was supported by the demonstration that the inhibitory effects of 7-OH-DPAT were produced by doses of the drug which do not produce significant occupancy of D_2 receptors in vivo (Levant et al. 1996). Several studies observed inhibition of locomotor activity after microinjection of 7-OH-DPAT into the nucleus accumbens (Gilbert and Cooper 1995; Kling-Petersen et al. 1995). Inhibition of locomotor activity by 7-OH-DPAT has also been reported in mice (Starr and Starr 1995). The D_3-preferring agonist PD 128907 produced similar biphasic effects on locomotor activity in rats (Pugsley et al. 1995).

Consistent with the effects of D_3 agonists on locomotor activity, the D_3-preferring antagonist nafadotride produced biphasic effects on locomotor activity in rats, stimulating locomotion at lower doses, inhibiting at higher doses (Sautel et al. 1995b). As with 7-OH-DPAT, doses of nafadotride which increased locomotor activity were shown to produce negligible occupancy of D_2 receptors, while those which inhibited locomotion produced significant D_2 occupancy (Levant and Vansell 1997). Another D_3-preferring antagonist, U-99194A, has also been reported to increase locomotor activity (Waters et al. 1993, 1994). Finally, increased locomotor activity, rearing behavior, and hyperactivity in an exploratory test were observed in one strain of D_3 "knock-out" mice (Accili et al.

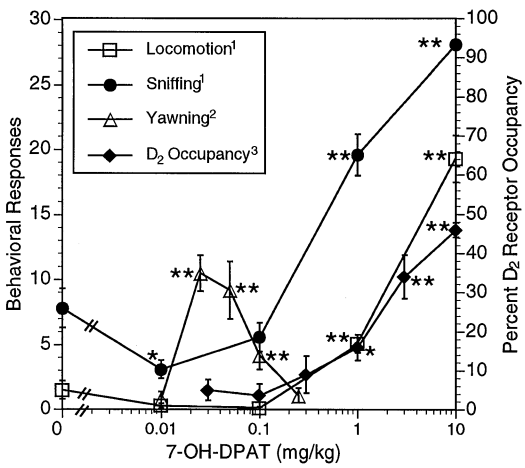

Fig. 1 Behavioral effects of putative D_3-preferring agonist 7-OH-DPAT in rat. Comparison with in vivo occupancy of D_2 dopamine receptors. Data are reported as number of exhibitions of a behavior during observation periods. In vivo occupancy of D_2 dopamine receptor by systemically administered (SC) 7-OH-DPAT was defined as percent protection of receptors from inactivation by EEDQ as determined by ex vivo [^{3}H] spiperone binding in striatal membranes. $^{**}P < 0.01$, $^{*}P < 0.05$, [1]Daly and Waddington (1993). [2]Kurashima et al. (1995). [3]Levant et al. (1995)

1996). Likewise, a preliminary report on another strain of D_3- deficient mice indicated a transient increase in activity in a novel environment compared to wild-type mice although no alterations in agonist stimulated locomotor behavior were observed (Koeltzow et al. 1995; Xu et al. 1995). Taken together, these findings indicate the probable involvement of the D_3 receptor in the modulation of locomotor activity in a manner opposite of that of the D_2 receptor.

Reinforcement and reward

Stimulation of the D_3 receptor has also been implicated in intriguing behavioral effects involving reinforcement and reward. Of note, 7-OH-DPAT has been reported to decrease self-administration of cocaine (Caine and Koob 1993; Parsons et al. 1996; Caine et al. 1997) and self-stimulation of the ventral tegmental area (Depoortere et al. 1996). Likewise, stimulation of D_3 sites is implicated in blocking the reinforcing effects of cocaine and *d*-amphetamine (Kling-Petersen et al. 1994), decreasing the rate of food-reinforced responding in a fixed-ratio operant paradigm (Sanger et al. 1996), and producing an aversive effect in a conditioned place preference paradigm (Chaperon and Thiebot 1996). In addition, the subjective effects of 7-OH-DPAT and other D_3-preferring agonists generalize to cocaine in drug-discrimination paradigms (Lamas et al. 1996). These observations have important implications for the understanding and treatment of drug addiction. However, as discussed above, further study must determine the role of specific dopamine receptor subtypes in these observations.

Other behaviors

Based on the somewhat limited in vivo and in vitro pharmacological data currently available, it is possible that the D_3 site may play a role in several additional behaviors. These include agonist-induced yawning and hypothermia (Damsma et al. 1993; Ahlenius and Salmi 1994; Millan et al. 1994, 1995a,b; Ferrari and Guiliani 1995; Khroyan et al. 1995; Kurashima et al. 1995), decreased sniffing (Daly and Waddington 1993), decreased alcohol consumption (Meert and Clincke 1994), and increased penile erection and ejaculatory behavior (Ahlenius and Larsson 1995; Ferrari and Guiliani 1995). Clearly, further study must confirm the role of the D_3 receptor in these behaviors, particularly in light of the significant variability in the in vitro pharmacological profile of the D_2 and D_3 sites on which much of the interpretation of these data is based.

In addition to the pharmacological effects discussed above, a variety of other behavioral and physiological effects of putatively D_3-preferring compounds have been reported. These effects include conditioned taste aversion (Bevins et al. 1996), disruption of huddling behavior in rats (Kagaya et al. 1996), decreased grooming (Khroyan et al. 1995), alterations in performance in an elevated maze test (Rodgers et al. 1996), decreased prepulse inhibition (Caine et al. 1995), catalepsy (Millan et al. 1995b; Sautel et al. 1995b), enhancement of morphine-induced conditioned place preference (Rodriguez De Fonseca et al. 1995), inhibition of pilocarpine-induced limbic seizures (Alam and Starr 1994), induction of depressant EEG patterns (Popoli et al. 1996), increased oxytocin secretion (Uvnas Moberg et al. 1995), and decreased gastric acid secretion (Glavin 1994). The D_3 receptor has also been suggested to play a role in emesis in the dog (Yoshida et al. 1995) and decreased climbing in mice (Sautel et al. 1995b). The involvement of dopamine receptors in these effects is likely; however, evidence for the selective involvement of the D_3 site is currently lacking.

Role in development

Expression of the D_3 receptor in the brain occurs quite early in development. In rat, D_3 receptor mRNA is detectable by polymerase chain reaction as early as embryonic day 11 and is clearly detectable by embryonic day 14 (Cadoret et al. 1993). Similarly, in the mouse, D_3 receptor mRNA is detectable on embryonic day 9.5, 4 days before the detection of D_2 receptor mRNA (Fishburn et al. 1996). D_3 receptor binding, as assessed with [^{3}H]7-OH-DPAT, is detectable in the islands of Calleja and olfactory tubercle at birth in the mouse brain. D_3 binding in the nucleus accumbens is detectable on postnatal day 4, substantia nigra on postnatal day 8, and in the vestibulocerebellum on postnatal day 11. Binding in these brain areas was observed to increase in density through development until adult levels were reached. In addition, transient expression of D_3 binding was observed in the dorsolateral parietal cortex between postnatal days 6 and 15 (Demotes-Mainard et al. 1996). In contrast to the mouse, D_3 receptors in the developing rat appear to be expressed later in development than D_2 receptors in the forebrain, islands of Calleja, and nucleus accumbens. D_2 receptor binding occurred as early as postnatal day 3, whereas, significant levels of [^{125}I]*trans*-7-OH-PIPAT-labeled D_3 sites were not detected until postnatal days 14 –21 (Stanwood et al. 1997).

Interestingly, during the second trimester of gestation, D_2-like receptors are transiently expressed in the cortical plate of the developing human (Todd 1992; Unis 1993). Preliminary reports also indicate transient, dense expression of D_3 receptor mRNA in the cortical plate of human brain at midgestation (Unis and Dorsa 1993; Unis et al. 1995) suggesting that these receptors are of the D_3 subtype. This transient expression of D_3 receptors suggests a role for dopamine in orchestrating

neuronal migration and differentiation during this period of accelerated cortical development that is mediated by the D_3 receptor. This hypothesis is supported by the observation that stimulation of D_3 sites induces increased branching and extension of neurites in both mesencephalic MN9D cells and primary mesencephalic neuronal cultures (Swarzenski et al. 1994).

Role in neuropsychiatric disorders

Schizophrenia

Investigation into alterations in the expression of the D_3 receptor in disease is still in the early stages. Accordingly, data available at present is relatively limited. Initial studies examining the densities of the D_2-like dopamine receptors in post-mortem putamen tissues from schizophrenic patients using relatively non-selective radioligands suggested an elevation in D_4 sites but not D_2 or D_3 (Seeman et al. 1993). Another post-mortem study of schizophrenic brain reported that D_3 mRNA was lost in certain cortical regions of their brains, whereas a mRNA encoding a splice variant of the D_3 receptor protein (D_{3nf}) could readily be detected in the same anatomic regions (Schmauss 1996; Schmauss et al. 1993). D_{3nf}, a D_3-like protein with a different C terminus, results from a deletion of 98 nucleotides that constitute the C-terminal region of the putative third cytoplasmic domain of the D_3 receptor (Schmauss et al. 1993; Liu et al. 1994b). The predominance of D_{3nf} protein in schizophrenic brain appears to result from increased D_{3nf}-specific splicing activity which could potentially contribute to the pathogenesis of the disease.

Although at present there is no evidence of genetic linkage between the D_3 gene and schizophrenia (Coon et al. 1993; Wiese et al. 1993; Nanko et al. 1994a; Sabate et al. 1994), several studies suggest that a distinct polymorphism in the first coding exon of the D_3 gene, the *Bal*I or *Msc*I restriction fragment length polymorphism, may increase the suspectibility to disease (Crocq et al. 1992; Mant et al. 1994; Kennedy et al. 1995; Griffon et al. 1996; Shaikh et al. 1996). This polymorphism corresponds to a point mutation that results in a serine-to-glycine substitution in the receptor's extracellular N-terminal domain. This substitution is likely to affect the way in which the protein is incorporated into the membrane and appears to increase the affinity for dopamine (Lundstrom and Turpin 1996). Although other studies have failed to support an association between the *Bal*I polymorphism and schizophrenia (Jonsson et al. 1993; Nanko et al. 1993; Nothen et al. 1993; Yang et al. 1993; Bella et al. 1994; Macciardi et al. 1994; Saha et al. 1994; Higuchi et al. 1995; Gaitonde et al. 1996; Nimgaonkar et al. 1996; Rietschel et al. 1996; Tanaka et al. 1996; Chen et al. 1997), this genotype may be associated with certain subsets of schizophrenics. For example, the association between the *Bal*I polymorphism and schizophrenia was found to be particularly strong in patients with a good response to antipsychotic treatment (Asherson et al. 1996). Other studies found a positive association only in male subjects (Asherson et al. 1996) or in patients with a familial history of schizophrenia (Nimgaonkar et al. 1993). Likewise, the *Bal*I polymorphism has been associated with the onset of the disease in certain age groups (Durany et al. 1996; Maziade et al. 1997).

Parkinson's disease

Investigation into alterations in the D_3 receptor in Parkinson's disease suggest that while decreases in expression of D_3 receptor mRNA are observed in certain brain regions, such as the olfactory tubercle, during aging (Valerio et al. 1994), densities of both D_3 receptor mRNA and D_3 binding are not altered in post-mortem brain from patients afflicted with Parkinson's disease (Hurley et al. 1996a). Although no significant alterations in D_3 receptor expression in the brains of Parkinson's patients have yet been reported, a decrease in D_3 receptor mRNA expression in lymphocytes of Parkinson's patients has been observed. In fact, the magnitude of the decrease in D_3 receptor mRNA expression correlated with the severity of the disease suggesting that this might serve as a marker for monitoring disease progression (Nagai et al. 1996). The detection of D_3 receptor mRNA in human lymphocytes, however, has not been observed in some studies (Vile and Strange 1996). No association has been made between the D_3 receptor gene and polymorphisms in its alleles in the incidence of Parkinson's disease (Nanko et al. 1994b; Higuchi et al. 1995).

Cocaine use

Finally, alterations in the density of the D_3 receptor have been reported with cocaine use. No changes in D_3 receptor mRNA expression were observed in the dorsal or ventral striata of post-mortem brains of chronic cocaine abusers (Meador-Woodruff et al. 1995). However, the density of D_3 sites was observed to be increased from 2- to 3-fold in the caudate, nucleus accumbens, and substantia nigra of persons who died from a cocaine over-dose (Staley and Mash 1996). In addition, a recent study of gene polymorphisms associated with reward dependence suggests that the *Bal*I D_3 receptor polymorphism, as well as D_4 receptor polymorphisms, appears to interact with certain serotonin receptor polymorphisms to produce this personality trait which may contribute to the susceptibility to drug addiction (Ebstein et al. 1997).

Clearly, investigation of alterations in the D_3 receptor in disease is in the early stages. Further study must verify whether alterations in the density or the regulation of this receptor exist in various pathological conditions.

Conclusion

In the short time since the identification of the dopamine D_3 receptor, considerable progress has been made towards understanding the function of this novel site. Although some avenues of investigation have yielded more definitive results than others, studies to date indicate the D_3 receptor is localized preferentially in limbic brain areas and affects locomotion. The receptor may also play a role in reinforcement and reward. A subpopulation of the receptors appear to be autoreceptors which modulate dopamine synthesis, release, and neuronal activity. These observations have led to the hypothesis that the D_3 receptor may be an appropriate target in the treatment of neuropsychiatric disorders, such as schizophrenia and drug addiction. The role of D_3 sites in disease, however, remains to be established. Genetic association of D_3 receptor polymorphisms with neuropsychiatric disorders have been proposed but remain controversial. Alterations in expression of D_3 sites may occur in some diseases. Although some study of this receptor is clearly in the early stages, these findings lay the foundation for future investigation. Further study may ultimately aid in the elucidation of the role of the D_3 receptor in health and disease and their potential utility in the treatment of neuropsychiatric disorders.

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