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Abstract

In the fields of psychiatry and neurology, the dopaminergic system is one of the most important neurotransmitter systems in the brain. Whereas pharmacological and biochemical studies had initially indicated two subclasses of dopamine receptors (DA-R), recent progress in molecular biology techniques has led to the identification of five distinct genes of DA-Rs (D1-R-D5-R) and splice variants. The gene products are classified into the D1-R family (D1-R and D5-R) and D2-R family (D2-R, D3-R and D4-R) based on their structure and pharmacological features. This review summarizes the structure, localization, function and pharmacology of DA-R subtypes on the basis of knowledge obtained during the past few years. The genes encoding the D1-R family have no intron and the D2-R family genes have introns. The distributions of mRNAs encoding these five DA-R subtypes in the brain were different from their respective receptors. The localization of DA-R subtypes to particular brain regions and specific pharmacological profiles of DA-R subtypes allow new insights to be made into the mechanism of action of DA in the control of psychiatric and motor functions. The availability of detailed information about DA-R subtypes will not only clarify their roles in the brain, but will probably also lead to the development of new therapeutic drugs with more specific actions.

KEYWORDS: dopamine receptor subtype, gene, molecular structure, localization, pharmacology

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Review

Molecular and Chemical Neuropharmacology of Dopamine Receptor Subtypes

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In the fields of psychiatry and neurology, the dopaminergic system is one of the most important neurotransmitter systems in the brain. Whereas pharmacological and biochemical studies had initially indicated two subclasses of dopamine receptors (DA-R), recent progress in molecular biology techniques has led to the identification of five distinct genes of DA-Rs (D1-R-D5-R) and splice variants. The gene products are classified into the D1-R family (D1-R and D5-R) and D2-R family (D2-R, D3-R and D4-R) based on their structure and pharmacological features. This review summarizes the structure, localization, function and pharmacology of DA-R subtypes on the basis of knowledge obtained during the past few years. The genes encoding the D1-R family have no intron and the D2-R family genes have introns. The distributions of mRNAs encoding these five DA-R subtypes in the brain were different from their respective receptors. The localization of DA-R subtypes to particular brain regions and specific pharmacological profiles of DA-R subtypes allow new insights to be made into the mechanism of action of DA in the control of psychiatric and motor functions. The availability of detailed information about DA-R subtypes will not only clarify their roles in the brain, but will probably also lead to the development of new therapeutic drugs with more specific actions.

Key words: dopamine receptor subtype, gene, molecular structure, localization, pharmacology

Dopamine (DA), a biologically active monoamine, is present in a relatively limited region of the brain, unlike other monoamines, and plays important roles in psychiatric diseases such as schizophrenia and in extrapyramidal neurodegenerative diseases such as Parkinson's disease. Of the neurotransmitter systems in the brain, the DAergic system has been most extensively studied. Traditionally, studies on the DAergic system have been classified as pertaining to the neurotransmitter phase (1957 to present) or the receptor phase (1972 to present) (1), but we now seem to be in the third phase, the molecular phase. Since abnormal neurotransmission in the brain could result from a defect in the receptor (R) as well as in the neurotransmitter (2), a great deal of effort has gone into the study of DA receptors during past 20 years. Due to the remarkable progress in molecular biology techniques in recent years, the gene encoding D2-R was cloned in 1988 (3). By 1991, the genes for all of the five DA-Rs we know today, D1-R to D5-R, had been successfully cloned (4-9), and the nature of the DA-R molecule, which could not be elucidated in previous pharmacological experiments, has been revealed.

This review summarizes the structure, localization, function and pharmacology of DA-R subtypes, which are classified on the basis of the information obtained during the past several years. The analysis of the structure and function of the DA-R subtypes will not only contribute to the elucidation of their roles in various psychiatric and neurologic diseases, but it will almost certainly lead to the development of drugs which act only on specific receptors subtypes, and thereby reducing the risk of adverse effects.

Structure of DA-R Subtypes and Their Genes

DA-R belongs to the G-protein-coupled receptor family which includes many receptors such as adrenergic, serotonin and neuropeptide receptors. The common structural features of the G-protein-coupled receptors (Fig. 1) are: [a] the seven hydrophobic transmembrane domains, [b] the extracellular N-terminus domain with glycosylation sites, [c] the cytoplasmic C-terminus domain and [d] the G-protein-coupling sites in the third cytoplasmic loop. All the known DA-R subtypes consist of a polypeptide chain containing about 400 amino acids (~ 50 kDa) and carbohydrate chains. The size of most receptor molecules detected with anti-D2-R antibodies (anti-D2-R) varies within the range of 90–120 kDa depending on the tissues (10, 11), which is far larger than the molecular weight expected from the amino acid sequence. Therefore, D2-R is likely to contain carbohydrate chains of various sizes. Although these carbohydrate chains are believed to have no effect on ligand affinity, it is important to clarify their roles in the receptor function.

The DA-R was initially classified into various subtypes on the basis of pharmacological properties, but later they have been grouped into two; the D1-R group which activates adenylate cyclase and the D2-R group which inhibits (or has no effect on) the activity (12, 13). Cloning of the receptor genes in recent years led to the identification of new subtypes which had not been identified by the conventional pharmacological and biochemical methods. At present, there are five DA-R subtypes, and they are classified into the D1-R family (D1-R and D5-R) and D2-R family (D2-R, D3-R and D4-R) based on their structures and pharmacological features (Table 1).

The third cytoplasmic loop is short in the D1-R family and long in the D2-R family (Fig. 2). It is generally believed that receptors with a short third cytoplasmic loop couple to stimulatory G-proteis (Gs) and activate adenylate cyclase. On the other hand, receptors with a long third cytoplasmic loop react to Gi and Go, which inhibit adenylate cyclase, and Gq, which couples with phospholipase C (14). D2-R also activates K^+ channels (14).

While the structures of the extra- and intra-cellular loops of the DA-R vary with each receptor, the transmembrane domains are highly homologous among most receptors. The subtypes belonging to the D1-R and D2-R families show overall sequence homology of about 50 % within the families and 30 % between the families (Table 1) (14). It is believed that an aspartate in the third transmembrane domain forms an ion pair with the protonated amine group of DA and that two serines in the fifth transmembrane domain form a hydrogen bonding interaction with two phenol groups of DA (7, 14). The latter interaction is specific for DA and its agonist. On the

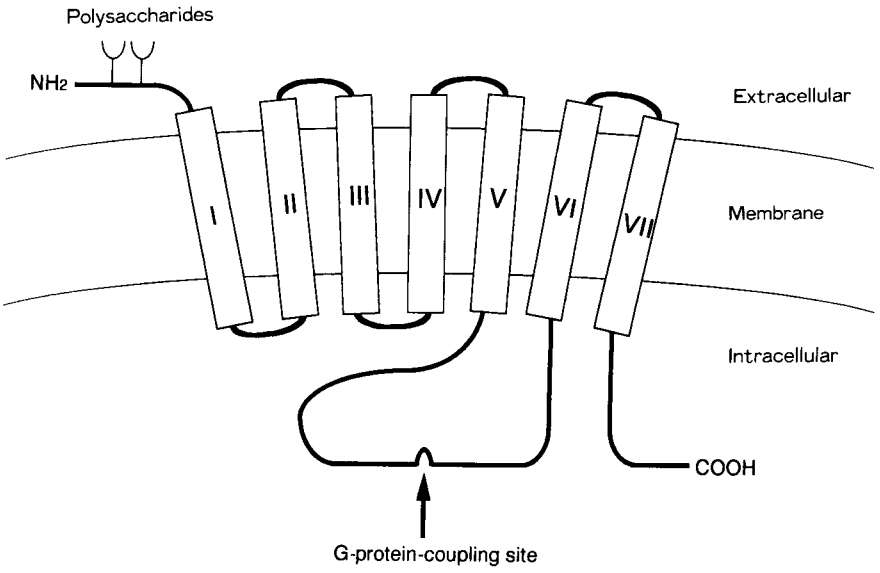

Fig. 1 The common structure of the G-protein-coupled receptors.

Table 1 Synopsis dopamine receptor subtypes

	D1-R family		D2-R family		
	D1	D5	D2	D3	D4
Amino acids (human)	446	477	443/414	400	387
(rat)	446	475	444/415	446	368
Homology					
D1-R	100%	50%	30%		30%
D2-R	30%	30%	100%	50%	
Locus (human)	5q34-35	4p16	11q22-23	3q13.3	4p16
Intron	−	−	+ (6)	+ (5)	+ (4)
Polymorphism		T→C(Pro^{326})*	*Asu* I C→G(Ser^{311}→Cys)	*Bal* I A→G(Ser→Gly)	Repeated sequences of 48 bases (third cytoplasmic loop)
mRNA					
Size (kb)	3.8	3	2.5	8.3	5.3
Localization	Striatum Nucleus accumbens Olfactory tubercle	Hippocampus Thalamus	Striatum Nucleus accumbens Olfactory tubercle	Islands of Calleja Nucleus accumbens Olfactory tubercle	Frontal cortex Amygdala Hippocampus Hypothalamus Medulla oblongata
Substantia nigra	−	−	+	+	+
G-protein coupling	Gs	Gs	Gi	?	?
Adenylate cyclase	↑	↑	↓	−	−
K^+ channel			↑		
Pharmacological profile					
Affinity for DA	μM	$<\mu$M	μM	**nM**	$<\mu$M
Agonist	SKF-38393	SKF-38393	**Bromocriptine**	**7-OH-DPAT** **Quinpirole** (Pergolide)	(Quinpirole)
Antagonist	SCH-23390	SCH-23390	Haloperidol	**UH-232**	**Clozapine**

* Silent mutation (ref. 79)

other hand, an aspartate in the second transmembrane domain of D2-R has been shown to interact with antagonists (15).

In humans, the genes of the five DA-R subtypes are located on different chromosomes (Table 1). In general, the genes for G-protein-coupled receptors have no introns. The genes of the D1-R family (D1-R and D5-R) also lack introns. On the other hand, a specific feature of the genes of the D2-R family is the presence of introns in their coding regions; the D2-R, D3-R and D4-R genes have 6, 5 and 4 introns, respectively (Table 1) (16, 17). The presence of these introns strongly suggests that the gene products of each subtype of the D2-R family undergo post-translational splicing, resulting in a greater number of receptor isoforms.

D1-R Family

D1 receptor

Using the homology to the D2-R gene which had already been cloned, the D1-R gene was cloned, and reported concurrently by three different groups in 1990 (6–8). The human and the rat D1-Rs consist of 446 amino acids with 91% overall homology and 96% homology in the transmembrane domains. The D1-R activates adenylate cyclase and shows high affinity for

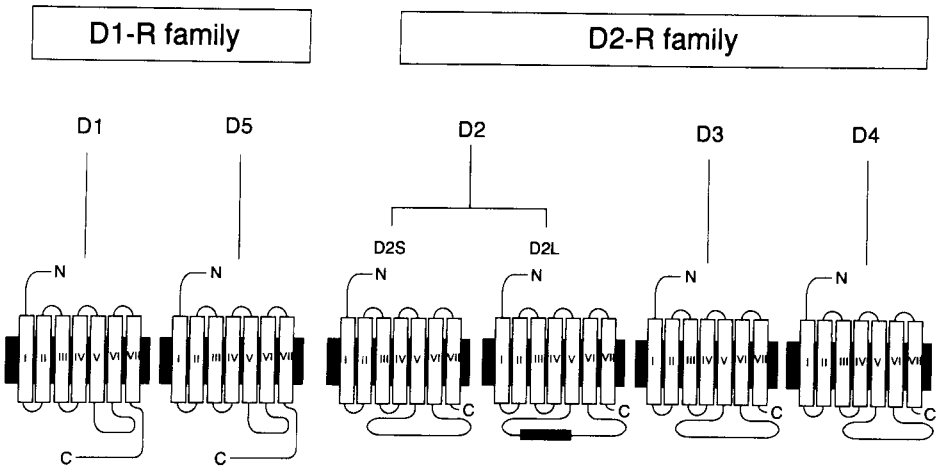

Fig. 2 Schematic diagrams depicting dopamine receptor subtypes.

SKF-38393 and SCH-23390, features common to the conventional D1-R as defined pharmacologically (18).

D1-R mRNAs are distributed abundantly in the striatum, nucleus accumbens and olfactory tubercle (Table 1), where D1-R itself is also abundant (6, 7, 18-23). In the substantia nigra, however, D1-R mRNAs have not been detected even though the D1-R itself is present which suggests that the D1-Rs in the substantia nigra are located at the terminal regions of neurons as inputs (19, 23-25).

D5 receptor

The D5-R gene was cloned from a human genomic library using homology to the D1-R gene (9, 26, 27). The sequence homology between the D5-R and D1-R is 50 % throughout the protein and 80 % in the transmembrane domains. The D5-R has properties common to the D1-R, *i.e.*, it activates adenylate cyclase and shows high affinity for the DA agonist SKF-38393 and antagonist SCH-23390. However, the affinity of D5-R for DA is over tenfold higher than that of D1-R, which is the greatest difference between the D1-R and D5-R.

The distribution of D5-R mRNAs in the brain was initially reported to be similar to that of D1-R mRNAs, but later D5-R mRNAs were shown to be present mainly in the hippocampus and thalamus uning a highly specific probe (28). D5-R mRNAs are sparsely present in the striatum, nucleus accumbens and olfactory tubercle regions (Table 1) where D1-R mRNAs are highly expressed.

D1B-R, which Tiberi *et al.* (29) found in rats, is considered to be the equivalent of D5-R in humans. It is proposed that D1-R and D5-R should be called D1A-R and D1B-R, respectively, and D2-R, D3-R and D4-R should be called D2A-R, D2B-R and D2C-R, respectively (30), although this nomenclature has not been generally used yet.

D2-R Family

D2 receptor

D2-R was the first subtype of the DA-R to be cloned. In 1988, taking advantage of the high homology among the G-protein-coupled receptors, Bunzow *et al.* cloned the D2-R gene from a rat genomic library using a part of the base sequence of the gene encoding the β_2 adrenergic receptor as a probe (3). The D2-R thus cloned showed the pharmacological characteristics of D2-R, *i.e.*, it had a high affinity for haloperidol, spiperone and bromocriptine.

In the brain, the D2-R mRNA was abundant in the striatum, nucleus accumbens, olfactory tubercle and substantia nigra (Table 1) (31-35). In the striatum, the D2-R mRNA is reportedly expressed in most of the GABA/enkephalin neurons and acetylcholine neurons (20, 36-38), and also in DA nerve cell bodies in the substantia nigra. The D2-R mRNA is decreased significantly in the substantia nigra and on the ventral side of the tegmental field by the destruction of DA nerves by treatment with 6-hydroxydopamine (6-OHDA), which suggests that D2-R in these domains is an autoreceptor.

A new species of D2-R (D2-long: D2L; 443 amino acids) has been discovered, which has an insert of 29 amino acids in the third cytoplasmic loop (39); thus, there are now two kinds of D2-Rs, the old one (D2-short: D2S; 414 amino acids) and the new one. Although the ratio of these two kinds of D2-R varies with tissues, they coexist in all the tissues in which D2-Rs are present (40). Usually, there are more D2L-Rs than D2S-Rs, and in the striatum, D2L-Rs are particularly abundant. There are no differences between the D2L-R and D2S-R in terms of their effects on the second messenger systems such as inhibition of the adenylate cyclase (41), activation of K^+ channels, enhancement of phosphatidyl inositol metabolism, or induction of arachidonic acid release (Table 1) (42, 43). No difference has been noted in the affinity for ligands between the D2L-R and D2S-R, until the recent report showing a higher affinity of D2S-R for the atypical anti-psychotic, clozapine (44). It has also been shown that the D2S-R mRNA level markedly changed after prolonged administration of neuroleptic drugs such as haloperidol (45, 46).

D3 receptor

Sokoloff *et al.* cloned D3-R, a new type of DA-R similar to the D2-R, from a rat brain library using part of the base sequence of the D2-R gene as a probe (47). Of all the DA-R subtypes, D3-R shows the greatest difference in the number of amino acids between humans and rats (400 and 446, respectively). Unlike D2-R, D3-R is not coupled to Gi and does not inhibit cAMP synthesis induced by forskolin (48). It is not known to which second messenger systems D3-R is linked.

Pharmacologically, the affinity of apomorphine and bromocriptine for D3-R is similar to their affinity for D2-R whereas both of these drugs had previously been thought to be specific for D2-R. *R*-(+)-7-Hydroxy-2-(*N*, *N*-di-*n*-propylamino)tetralin (7-OH-DPAT) (49), quinpirole and pergolide bind to D3-R with high affinity (Table 1). The affinity of D3-R for UH-232, which is regarded as a selective ligand of autoreceptors, is higher than that of D2-R (14, 47). The afinity of the typical anti-psychotic, haloperidol, for the D2-R is higher than that for the D3-R, and the affinity of sulpiride for the D3-R is similar to that for the D2-R (14). The most outstanding pharmacological feature of the D3-R is that the affinity for DA is at a nM level, which is far higher than the affinity at a μM level of the other DA-R subtypes to DA (50).

Although the amount of D3-R mRNA is much smaller than that of D2-R mRNA, it is typically concentrated in the limbic system (28), *i.e.*, in the islands of Calleja, nucleus accumbens and olfactory tubercle. This suggests that the D3-R may be involved in the cognitional and emotional functions (28).

D4 receptor

The D4-R was cloned using the rat D2-R cDNA as a probe (5). Although the structure of the D4-R is similar to that of the D2-R, it has no effect on adenylate cyclase, and the second messenger system coupled to the D4-R has not yet been found. Pharmacologically, D4-R resembles D2-R and D3-R, and its affinity for the atypical anti-psychotic, clozapine, which scarcely causes side effects in the extrapyramidal system, is about tenfold higher than that of D2-R and D3-R (Table 1) (5, 14).

The D4-R mRNA level as well as the D3-R mRNA level is significantly lower than the D2-R mRNA level. D4-R mRNAs are distributed in the frontal cortex, amygdala, hippocampus, hypothalamus and medulla oblongata, but are weakly expressed in the basal ganglia (28, 51). Since the D4-R is distributed in more limbic than motor structures, it is suggested that the D4-R may be important in schizophrenia in adition to D2-R. They are present in the substantia nigra, which is a common feature of the D2-R family.

The D4-R has repeated sequences of 48 bases in the third cytoplasmic loop, and the number of repeats varies among individuals from 2 to 8 comprising 7 kinds in all (Table 1) (52). This is unique in that it is caused not by the differential splicing but by polymorphism of the genome itself. The pharmacological profie of the D4-R is reported to vary depending on the number of the 48 base-repeats in the third loop, which suggests that genetic polymorphism causes differences among individuals in the onset of neuropsychiatric diseases and in the sensitivity to anti-psychotics.

Pharmacology of DA-R Subtypes and Development of New Therapies

The pharmocology of each DA-R subtype has been described in the previous chapters. The DA-R subtypes will now be examined with respect to ligands (Fig. 3). Considering the extremely high affinity of the endogenous ligand DA for the D3-R (50) and the possibility that the D3-R is the main factor of the manifestation of psychic

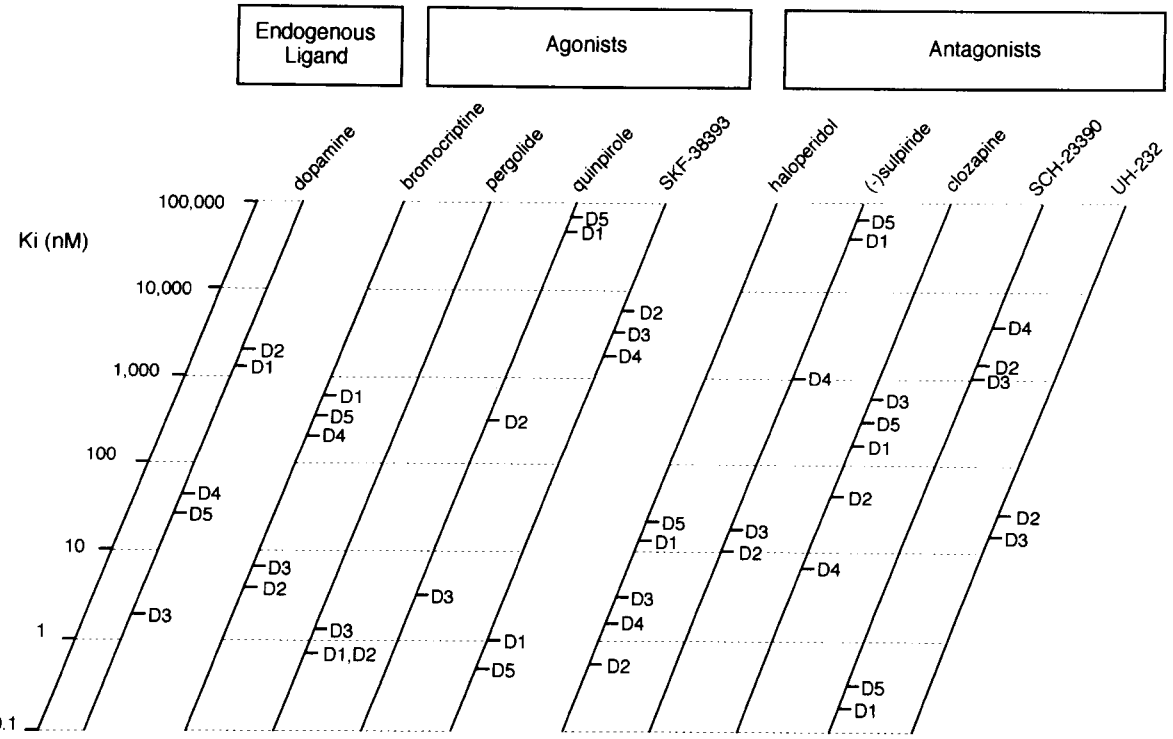

Fig. 3 Dissociation constants (Ki) for endogenous ligand, agonists and antagonists at dopamine receptor subtypes. This figure was depicted based on data from Ref. 14 and 52, and our original data.

symptoms, excessive DA is likely to cause psychic symptoms.

Among the agonists of the DA-R, quinpirole which has been thought to be specific for D2-R, was found to have high affinity for D3-R (14, 47). Among the DA agonists belonging to the ergot alkaloid family, bromocriptine shows a high affinity for D2-R, but its affinity for D3-R is sligthly low, and it negligibly reacts with D1-R (14). In contrast, pergolide shows very high affinity for D3-R and its affinities for D2-R and D1-R are also high (53). Among the antagonists of DA-R, haloperidol shows a high affinity for the D2-R family and a rather high affinity for the D1-R family as well (14). Since the atypical anti-psychotic, clozapine, specifically reacts with D4-R, drugs such as clozapine that would antagonize DA on D4-R are being developed, and are expected to have less side effects (5, 14). A drug that selectively blocks the D3-R would also be a novel anti-psychotic.

As we have seen, detailed studies on the DA-R subtypes lead to the development of completely new therapies highly specific for each subtype. They are also expected to facilitate the development of new treatments of disease by controlling receptor expression and by specific inhibition with antisense mRNAs.

Localization of D1-R and D2-R and Its Significance

In the brain, D1-R and D2-R mRNAs are much more abundant than mRNAs of the other three subtypes. To clarify the pathology and drug therapy of various neuropsychiatric diseases, particularly diseases in the kinetic systems such as Parkinson's disease, studies on D1-R and D2-R are important.

D2-R agonists induce hyperactivity and stereotyped movement in test animals, whereas D2-R antagonists induce catalepsy. D1-R antagonists also induce catalepsy and antagonize the hyperactivity and stereotyped movements associated with D2-R agonists. There are a number of reports on the pharmacological interference between D1-R and D2-R (54), and it is well-known that the stimulative effect on D2-R by an agonist is significantly

enhanced if D1-R has been previously stimulated (55). In such a case, excessive stimulation of D1-R may cause side effects because the stimulatory effect on D2-R also becomes too large. A significant additive effect is observed on the release of arachidonic acid when D1-R and D2-R are expressed in a single cell (43). Thus, the close interaction between D1-R and D2-R is well-known.

Around 50–60 % of the middle-sized cells in the striatum are considered to express D1-R and D2-R mRNAs (25, 56, 57). Although it is interesting that D1-R and D2-R, which affect adenylate cyclase in opposite ways, coexist in a single neurocyte, it has been proposed that one or the other of the two subtypes plays the principal role at the nerve ending in each cell (57). The coexistence of D1-R and D2-R in the striatum and substantia nigra neurons agree with the results of electrophysiological studies (58–61).

With regards to the functions of the basal ganglia, Albin *et al.* have proposed the following hypothesis (62). Namely, there is a direct pathway consisting of GABA and substance P (SP) containing neurons as outputs from the striatum and directly projecting onto the inner pallidus and reticular formation of the substantia nigra, and an indirect pathway which links the GABA/enkephalin (ENK)/dynorphin (Dyn) neurons and the reticular formation of substantia nigra via the outer pallidus and subthalamic nucleus. D1-R is present on the SP neuron and D2-R on the ENK neuron (23, 63). At present, it is generally thought that different DA-R subtypes mediate the direct and indirect pathways. Most acetylcholine neurons in the striatum express D2 mRNAs (20, 36–38). Although, direct synapse formation between the DA neurons and the acetylcholine neurons in the striatum has not been confirmed, interaction without synapse between the nerve ending of the DA neuron and acetylcholine neuron in the vicinity has been suggested (64).

DA-R and Psychiatric Diseases

An increase of D2-Rs has been observed at autopsy even in untreated patients with schizophrenia (65), and there are reports on the families with frequent occurrence of schizophrenia (66), which suggest that the D2-R gene is an etiological factor in the disease. Recently, it has been reported that the variation frequency of the D2-R gene at codon 311 ($Ser^{311} \rightarrow Cys$) was significantly higher in the schizophrenic patients than in the controls (Table 1) (67). Furthermore, some reports demonstrated a relationship between shizophrenia and polymorphism of the D3-R which is regarded as the action point of antipsychotics (4, 47). In this case, there is a point mutation from A to G in the 5' terminal region of the D3-R gene causing an amino acid substitution from Ser to Gly (48). Since polymorphism has been shown to occur in this region using the restriction enzyme *Bal* I (Table 1), changes in the properties of the D3-R caused by mutation in this region are being investigated (68). The distribution of D4-R suggests that it may be more closely associated with limbic rather than motor systems. Quite recently, Seeman *et al.* reported that receptor bindings for the D4-R subtype were especially high in the postmortem brain of patients with schizophrenia (69).

DA-R and Dependence

It is reported that there is a significantly high correlation between the restriction fragment length polymorphism (RFLP) in the D2-R gene induced by the restriction enzyme *Taq* I and alcoholism (70), and that this correlation is higher in more severe cases (71). However, there are also many reports which failed to confirm this correlation (72, 73).

Since substances causing addictions are related to the DA system, the relationship with DA-R has long been implicated. Although there are a few reports suggesting a correlation between drug addictions and the polymorphism in the D2-R gene, others failed to confirmed such a correlation.

DA-R and Neural Diseases

Parkinson's disease is mainly caused by a lesion in the presynaptic DA neurons, and therefore is unlikely to be related to the DA-R subtypes, however its relationship with the receptors is very important in connection with drug therapy and its side effects.

Among the DA-R subtypes, the D1-R and D2-R are much more abundant than the others, in terms of mRNA as well as protein (28), and clinical and experimental studies have shown that they play a central role in producing the defects in the motor systems and the effects of drug therapy. As the D2-R is the principal transmitter in the motor neuron systems, and as there is interaction between D1-R and D2-R as described above, the effects of D1-R should be considered secondary.

It has been reported that the D2-R level is increased

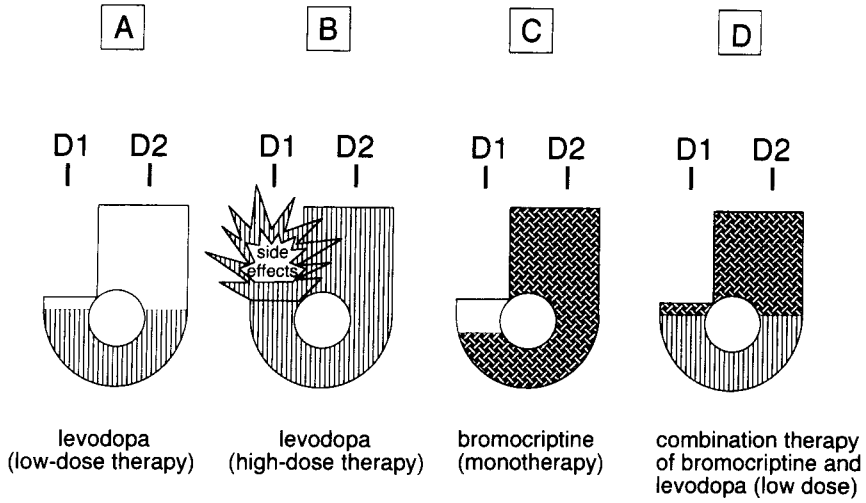

Fig. 4 Theoretical combination therapy of levodopa and dopamine agonist bromocriptine. A: low-dose therapy of levodopa. B: high-dose therapy of levodopa, C: monotherapy of bromocriptine, D: combination therapy of bromocriptine and low-dose levodopa. Most desirable therapeutic efficacy will be achieved by D (see text). ▥: levodopa effects; ▩: bromocriptine effects.

in untreated patients with Parkinson's disease whereas levodopa therapy normalizes the level (74). However, examination by positron emission tomography (PET) of patients with Parkinson's disease has shown that the D2-R level is increased only in the early phase of the disease but is otherwise normal or slightly decreased (75). It therefore seems that the D2-R level in Parkinson's disease varies with the phase of the disease. On the other hand, the D1-R level is known to remain unchanged (74, 75). The D2-R level in the patients showing fluctuations or a wearing-off is generally low (76). Murata *et al.* found that the transient increase in the D1-R and D1-R mRNA levels seen in the early phase of levodopa therapy diminishes after the prolonged administration of levodopa (77), and that this may be the cause of the wearing-off phenomenon, one of the problems with the long-term levodopa therapy. It has also been reported that, in a dyskinesia model, D1-R and D2-R mRNA levels are both decreased in the striatum but can be up-regulated by lowering the secretion of DA (78). As these reports indicate, the D1-R and D2-R mRNA levels are readily affected by the condition of the disease and by drug therapy, and therefore care must be taken when drug therapy is performed.

Considering the properties of the new DA-R subtypes hitherto described, one can envisage desirable drug therapies which elevate the DA system in patients with Parkinson's disease, as discussed in the following. Although D2-R and D1-R are the predominant DA-Rs, DA reacts with other DA-Rs as well under physiological conditions. Therefore levodopa, which reacts with all the DA-R subtypes, is indispensable as a basic drug. However, if administered at a low dose in order to prevent side effects, it will not be sufficiently effective for symptoms in Parkinson's disease (Fig. 4A). If the dosage is too high, levodopa is likely to cause side effects because of excessive stimulation of the D1-R, which acts additively with the D2-R (Fig. 4B). In addition, excessive DA reacts with D3-R, which is likely to cause psychic symptoms. On the other hand, the DA agonist bromocriptine, which is selective to the D2-R, does not stimulate the D1-R, and therefore is less effective (Fig. 4C). The best method at the moment will therefore be to use a low dosis of levodopa to stimulate the D1-R and D2-R to appropriate levels and bromocriptine to supplement the insufficient D2-R stimulation (Fig. 4D). Pergolide may be used instead of bromocriptine, but pergolide has far stronger stimulative effects on D1-R and D3-R (53), which may cause psychic symptoms such as hallucination. An antagonist highly selective for D3-R, if developed, would suppress the drug-induced psychic symptoms which often cause problems in the treatment of Parkinson's disease.

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