Neurotransmitters, Drugs and Brain Function.
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7 Dopamine (DA)

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Dopamine (3:4 dihydroxyphenylethylamine), like noradrenaline and adrenaline, is a catecholamine and in addition to its independent neurotransmitter role in the CNS it is a precursor to noradrenaline (NA) in all central and peripheral noradrenegic neurons.

PATHWAYS

It became possible to visualise neurons which contained catecholamines when it was discovered that these amines reacted with formaldehyde vapour (later replaced by glyoxylic acid) to produce isoquinoline condensation products which emitted a bright-green fluorescence when visualised under ultra-violet light. This was distinguishable from the yellow fluorescence of 5-HT and could be separated from that for NA by appropriate pharmacological manipulations or adjustments to the microscopic techniques. Using this procedure, which is known as the Falk–Hillarp technique, Dahlstrom and Fuxe (1964) located and numbered nuclei in the hindbrain (pons medulla) in which either DA (A8–A12) or NA (1–7) was concentrated. The pathways were then established by axotomy since lesion of the axon is followed by loss of the NT and fluorescence at the neuron's terminals (destination of pathway) but not from its cell bodies (origin).

Most of the DA cell bodies (about 400 000) in the human brain are found in the A9 nucleus which forms the zona compacta (dorsal part) of the substantia nigra (SN), although a few cell bodies are found in the more ventral zona reticulata and in the zona lateralis as well (Fig. 7.1). A8 is lateral, caudal and somewhat dorsal to A9 and A10 whereas A10 is ventral to A9. Axons from A9 form the major contribution, together with some from A8, to the principal DA nigrostriatal pathway running to the striatum (caudate nucleus and putamen) and amygdala. This pathway is lateral to, but runs with, a more medial DA pathway, predominantly from A10, which innervates the nucleus accumbens and olfactory tubercle (mesolimbic pathway) as well as parts of the cortex (mesocortical system) such as the prefrontal and perirhinal cortex. The DA innervation to the anterior cingulate cortex also comes from A10 but with some axons from A9. There is in fact no clear divide between A9 and A10 and some overlap of their pathways. The DA mesolimbic tract and the noradrenergic bundles come together in the medial forebrain bundle before entering the cortex.

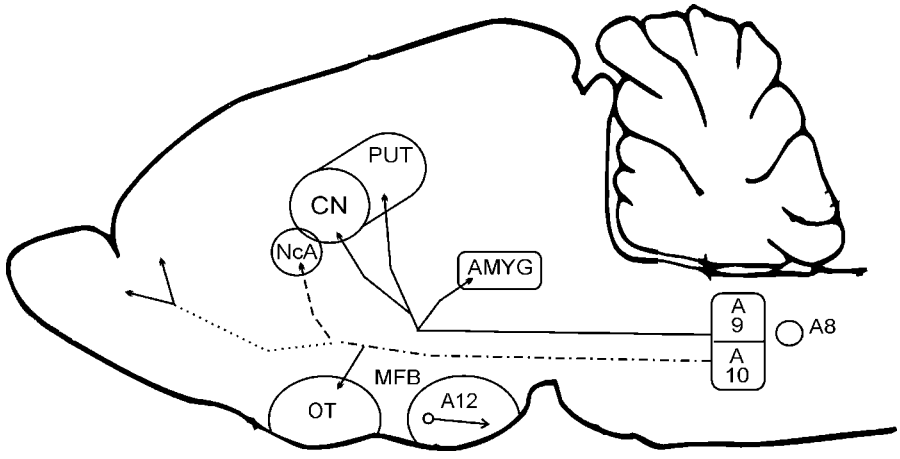

Figure 7.1 Dopamine neuronal pathways. AMYG, amygdala; CN, caudate nucleus; MFB, medial forebrain bundle; NcA, nucleus accumbers; OT, olfactory tubercle; PUT, putamen; SN, substantia nigra. For full details see text and Moore and Bloom (1978) and Lindvall and Bjorkland (1978)

A further totally separate DA pathway arises from A12 in the arcuate nucleus and forms the tuberoinfundibular tract in the median eminence to the pituitary gland for controlling prolactin release. This is partly achieved by DA being released into capillaries of the hypothalamic–hypophyseal portal system and then inhibiting the prolactin releasing cells (lactotrophs) of the anterior pituitary.

While the nigrostriatal pathways are ipsilateral some crossing occurs in fibres from the ventral tegmental A10 nucleus. These pathways are shown diagramatically in Fig. 7.1. Further details can be obtained from Moore and Bloom (1978) and Lindvall and Bjorkland (1978). The nuclei provide distinct loci for activating the dopamine systems for electrophysiological, release and behavioural studies and for their destruction by electrolytic lesion or injection of the toxin 6-hydroxydopamine (6-OHDA).

The concentration of DA in different brain areas of the rat is in keeping with the distribution of its pathways. It is concentrated in the striatum (10 μg/g), nucleus accumbens (5 μg/g) and olfactory tubercle (6 μg/g) but in the cortex there is much less (0.1 μg/g). Cells in the substantia nigra in humans and primates differ from those in other species in containing granules of the lipoprotein pigment called neuromelanin. The melanin granules are free in the cytoplasm and give the SN a distinctive dark colour. Cells in this nucleus can also have hyaline inclusion bodies, the Lewy bodies, which are not common normally but appear to increase dramatically in patients with Parkinsonism. In humans the SN neurons are very closely aligned to blood vessels which could make them readily influenced by blood-borne agents and might explain why they are vulnerable as in Parkinson's disease. Certainly they will require considerable biochemical back-up to maintain function in all their terminals.

NEUROCHEMISTRY

The biochemical pathways in the synthesis and metabolism of dopamine are shown in Fig. 7.2 and their position in the context of the dopamine synapse in Fig. 7.3.

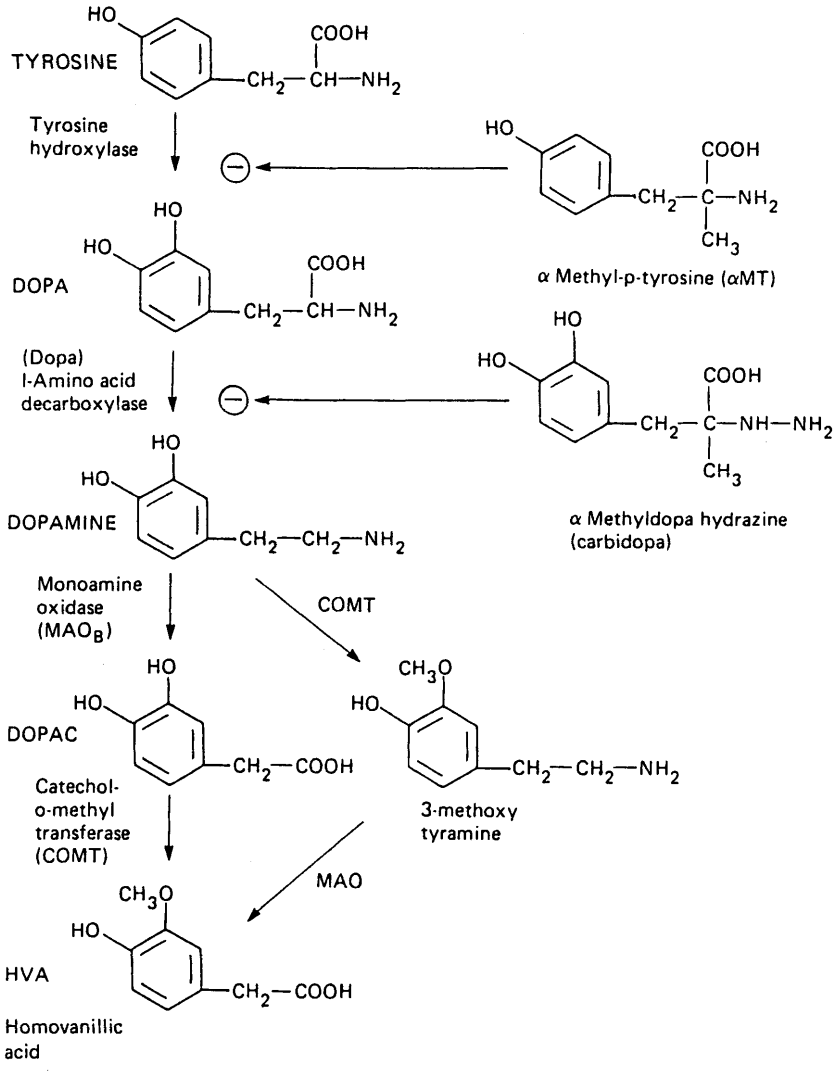

Figure 7.2 Biochemical pathways for the synthesis and metabolism of dopamine. (–) indicates drug inhibition of enzyme activity

SYNTHESIS

The synthesis and metabolism of DA are very similar to that of NA, even when it functions as a NT in its own right. Although both phenylalanine and tyrosine are found in the brain it is tyrosine which is the starting point for NA and DA synthesis. It appears to be transported into the brain after synthesis from phenylalanine (phenylalanine hydroxylase) in the liver rather than from phenylalanine found in the brain. Despite the fact that the concentration of tyrosine in the brain is high (5×10^{-5} M) very little body tyrosine (1%) is used for the synthesis of DA and NA.

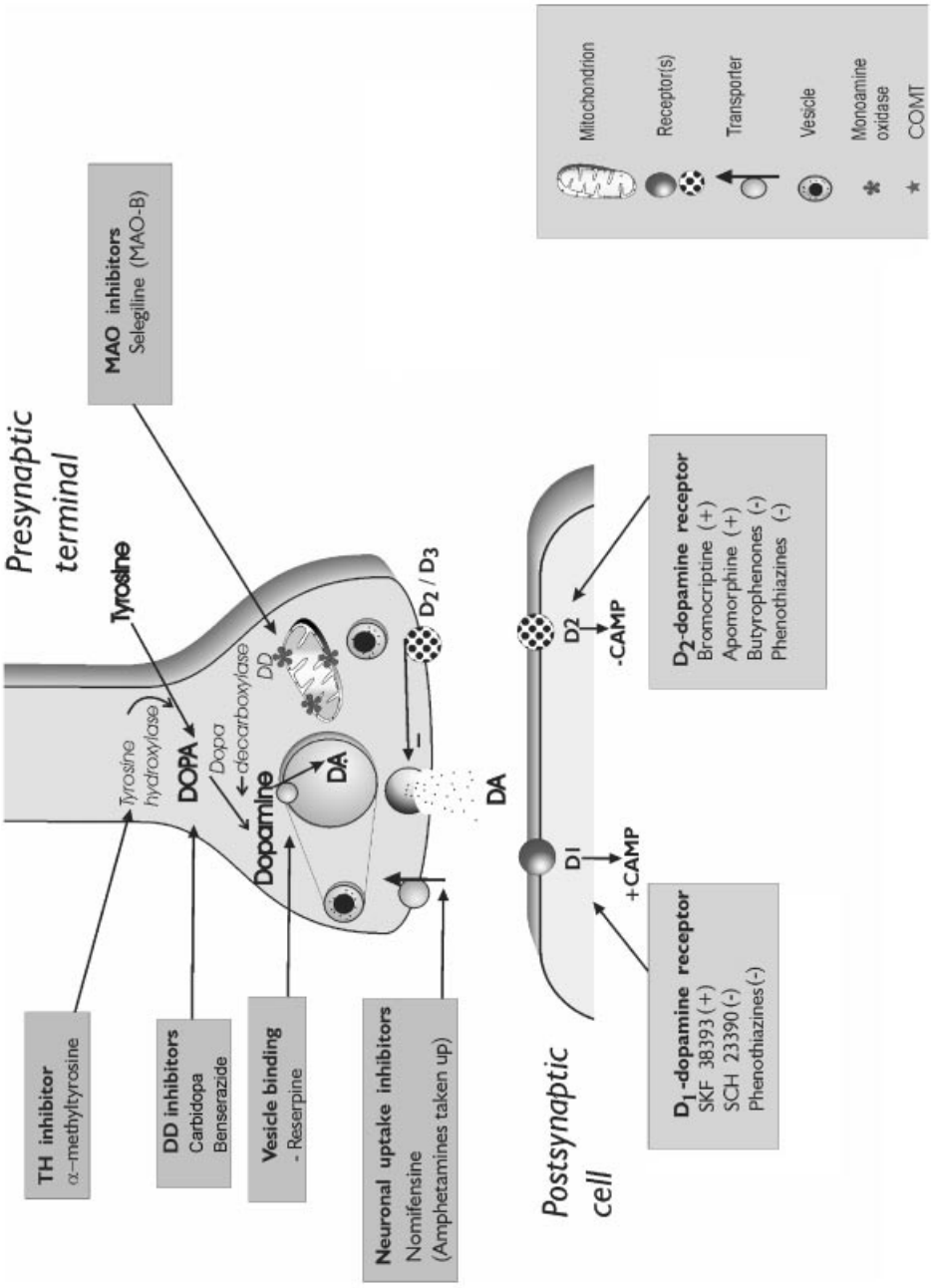

Figure 7.3 Diagrammatic representation of a dompaminergic synapse. (+) = stimulation, agonists; (−) = antagonism

Tyrosine hydroxylase

Tyrosine is converted to dopa by the cytoplasmic enzyme tyrosine hydroxylase. This is the rate-limiting step (K_m 5×10^{-6} M) in DA synthesis, it requires molecular O_2 and Fe^{2+} as well as tetrahydropterine (BH-4) cofactor and is substrate-specific. It can be inhibited by α-methyl-*p*-tyrosine, which depletes the brain of both DA and NA and it is particularly important for the maintenance of DA synthesis. Since the levels of tyrosine are above the K_m for tyrosine hydroxylase the enzyme is normally saturated and so it is not possible to increase DA levels by giving tyrosine.

Dopa decarboxylase

By contrast, the cytoplasmic decarboxylation of dopa to dopamine by the enzyme dopa decarboxylase is about 100 times more rapid (K_m: 4×10^{-4} M) than its synthesis and indeed it is difficult to detect endogenous dopa in the CNS. This enzyme, which requires pyridoxal phosphate (vitamin B6) as co-factor, can decarboxylate other amino acids (e.g. tryptophan and tyrosine) and in view of its low substrate specificity is known as a general L-aromatic amino-acid decarboxylase.

While a number of drugs, e.g. α-methyl dopa, inhibit the enzyme they have little effect on the levels of brain DA and NA, compared with inhibition of tyrosine hydroxylase and they also affect the decarboxylation of other amino acids. Some compounds, e.g. α-methyl dopa hydrazine (carbidopa) and benserazide, which do not easily enter the CNS have a useful role when given in conjunction with levodopa in the treatment of Parkinsonism (see Chapter 15) since the dopa is then preserved peripherally and so more enters the brain.

Controls of synthesis

It is possible to deplete the brain of both DA and NA by inhibiting tyrosine hydroxylase but while NA may be reduced independently by inhibiting dopamine β-hydroxylase, the enzyme that converts DA to NA, there is no way of specifically losing DA other than by destruction of its neurons (see below). In contrast, it is easier to augment DA than NA by giving the precursor dopa because of its rapid conversion to DA and the limit imposed on its further synthesis to NA by the restriction of dopamine β-hydroxylase to the vesicles of NA terminals. The activity of the rate-limiting enzyme tyrosine hydroxylase is controlled by the cytoplasmic concentration of DA (normal end-product inhibition), presynaptic dopamine autoreceptors (in addition to their effect on release) and impulse flow, which appears to increase the affinity of tyrosine hydroxylase for its tetrahydropteridine co-factor (see below).

METABOLISM

Just as the synthesis of DA and NA is similar so is their metabolism. They are both substrates for monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). In the brain MAO is found in, or attached to, the membrane of the intraneuronal mitochondria. Thus it is only able to deaminate DA which has been taken up into nerve endings and blockade of DA uptake leads to a marked reduction in the level of its deaminated metabolites and in particular DOPAC. The final metabolite, homovanillic

acid (HVA), is one that has been both deaminated and O-methylated so it must be assumed that most of any released amine is initially taken back up into the nerve where it is deaminated and then subsequently O-methylated (Fig. 7.2). Certainly the brain contains much more DOPAC (the deaminated metabolite of DA) than the corresponding O-methylated derivative (3-methoxytyramine). It is possible, however, that the high levels of DOPAC, as found particularly in rat brain, partly reflect intraneuronal metabolism of unreleased DA and it is by no means certain that the metabolism of DA to HVA is always initially to DOPAC. Thus released DA that is not taken up into neurons is probably O-methylated initially by COMT.

O-methylation

It is generally accepted that COMT is an extracellular enzyme in the CNS that catalyses the transfer of methyl groups from S-adenylmethionine to the meta-hydroxy group of the catechol nucleus. Until recently the only inhibitors of this enzyme were pyragallol and catechol which were too toxic for clinical use. Now other inhibitors have been developed, e.g. entacapone and tolcapone, but these are used mainly to protect dopa (also a catecholamine) from O-methylation, in the treatment of Parkinson's disease (Chapter 15).

Deamination

Monoamine oxidase exists in two forms, MAO_A and MAO_B. The former is more active against NA and 5-HT than it is against DA, which is a substrate for both, even though, like β-phenylethylamine, it is more affected by MAO_B. It seems likely that MAO_B is the dominant enzyme in human brain and inhibitors of it, such as selegiline, have some value in the treatment of Parkinson's disease by prolonging the action of the remaining endogenous DA as well as that formed from administered levodopa.

Uptake

The removal of released DA from the synaptic extracellular space to facilitate its intraneuronal metabolism is achieved by a membrane transporter that controls the synaptic concentration. This transporter has been shown to be a 619 amino-acid protein with 12 hydrophobic membrane spanning domains (see Giros and Caron 1993). Although it has similar amino-acid sequences to that of the NA (and GABA) transporter, there are sufficient differences for it to show some specificity. Thus DA terminals will not concentrate NA and the DA transporter is blocked by a drug such as nomifensine which has less effect on NA uptake. Despite this selectivity some compounds, e.g. amphetamine and 6-OHDA (but not MPTP), can be taken up by both neurons. The role of blocking DA uptake in the central actions of cocaine and amphetamine is considered later (Chapter 23).

STORAGE

Most DA (up to 75%) is stored in vesicles like NA. This can be disrupted by the rauwolfia alkaloid, reserpine and by drugs like tetrabenazine. It should be emphasised that these drugs deplete the neurons of amines by stopping their incorporation into

vesicles so that it leaks out and is deaminated. They do not cause an active release of amine.

RELEASE AND TURNOVER

Short-term control (autoreceptors)

As with many neurons (e.g. NA) there are presynaptic autoreceptors on the terminals of dopamine neurons whose activation attenuate DA release. Although most of these receptors appear to be of the D_2 type, as found postsynaptically, D_3 receptors are also found. It is possible that in addition to the short-term control of transmitter release they may also be linked directly to the control of the synthesising enzyme tyrosine hydroxylase. It seems that autoreceptors are more common on the terminals of nerves in the nigrostriatal (and possibly mesolimbic) than mesocortical pathway.

Autoreceptors are also found on the cell bodies of DA neurons, in the substantia nigra (A9) and ventral tegmentum (A10) where their activation leads to a reduction in cell firing. To what extent they are stimulated by endogenous DA is uncertain but systemic DA agonists certainly activate them to inhibit the neuron, and since DA antagonists alone can increase the firing of DA neurons that implies that the autoreceptors could be tonically active. This can have important implications, as we shall see later when considering the mode of action of DA antagonists in the treatment of schizophrenia (Chapter 17).

There is much evidence (e.g. Cheramy, Leviel and Glowinski 1981) from both *in vitro* and *in vivo* perfusion studies that DA is released from the dendrites of DA neurons in both A9 and A10 even though those dendrites do not contain many vesicles compared with axon terminals. The release and changes in it may also be slower and longer than that at axon terminals and the synaptic arrangement between the releasing dendrites and postsynaptic target is not clear. DA receptors also appear to be on neurons other than dopamine ones and on the terminals of afferent inputs to A9 (and A10). It seems that the activation of the DA neurons may partly be controlled by the effects of the dendritically released DA on such inputs.

Long-term control

Generally the concentration of DA remains remarkably constant irrespective of the level of neuronal activity. One reason for this is that nerve stimulation increases tyrosine hydroxylase activity and DA synthesis. It is thought that tyrosine hydroxylase can exist in two forms with low and high affinities for its tetrahydropteredine co-factor (BH-4) and that nerve traffic increases the high-affinity fraction.

Certainly the activity of tyrosine hydroxylase is greater in the DA neurons of the substantia nigra (17.5 nmol dopa synthesised/mg protein/h) than in the NA neurons of the locus coeruleus (4–5), as is the turnover of the amine itself (1.7 μg/h) compared with that of NA (1.0) (see Bacopoulus and Bhatnager 1977). In the caudate nucleus and nucleus accumbens the turnover of DA is even higher at 7.4 and 2–6 μg/g/h respectively.

NEUROTOXINS

The 6-hydroxylated form of DA, 6-hydroxydopamine (6-OHDA) is taken up into both DA and NA nerve terminals where it is readily oxidised to compounds that cause

degeneration of the terminals over a period of days. To produce a central effect it must be administered directly into the brain by intracerebroventricular (icv) injection. NA terminals can be protected by prior injection of the NA uptake inhibitor desmethylimipramine. Alternatively, small amounts may be injected directly by stereotaxic techniques into particular DA nuclei where uptake takes place even if terminals are not present.

Recently much interest has centred on a very specific toxin for DA neurons. This is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). It was discovered when a student, who was addicted to pethidine, tried to manufacture 1-methyl-4-phenyl-4-propionoxy-piperidine (MPPP) but took a short-cut in synthesis and produced MPTP. When he administered this to himself he developed Parkinsonism. MPTP destroys DA neurons. Again this process depends on the neuronal uptake mechanism, since MPTP itself is not the active material. It needs to be deaminated to MPP^+ which is then taken up by DA nerve terminals.

DOPAMINE RECEPTORS

CLASSIFICATION

The original discovery and classification of DA receptors was based on the results of three distinct studies:

(1) Stimulation of adenylate cyclase
(2) Ligand binding
(3) Inhibition of prolactin release

The adenylate cyclase discovered originally in bovine superior cervical ganglia, and then found in homogenates of rat striatum, was specific to DA, in that it was activated by other DA agonists like ADTN, but not greatly by NA or 5-HT. Some other drugs with established DA-like effects proved, however, to be either partial agonists (apomorphine) or ineffective (bromocriptine). Also while some neuroleptic (anti-psychotic) drugs that are DA antagonists in behavioural studies, such as the thioxanthenes and phenothiazines, antagonised this effect with a relative potency that compared with their antipsychotic activity, other potent neuroleptics like the butyrophenones were relatively ineffective. Overall there was a poor correlation between antipsychotic activity and DA antagonism as measured by blockade of DA-induced cAMP production.

Ligand-binding studies, originally with [^{3}H] dopamine and [^{3}H] haloperidol but subsequently using [^{3}H] spiperone, demonstrated the existence of a specific binding site for them in membrane preparations from mammalian striatum. Displacement studies with a whole range of neuroleptic drugs also showed that not only was the rank order different from that for blocking the adenylate cyclase but also correlated much better with antipsychotic activity. Additionally DA agonists like bromocriptine, which were ineffective in increasing cAMP production, showed appropriate binding.

When tested on prolactin release in isolated mammatrophs of bovine anterior pituitary, apomorphine appeared a full agonist (inhibiting release) while antagonism of the inhibition of prolactin release by the neuroleptics showed a potency more similar to that for binding than for blocking cAMP production. Also the inhibition of prolactin

release by DA was not accompanied by any change in intracellular cAMP and therefore was not linked to it.

Thus the establishment of two clear dopamine effects, one directly linked to stimulation of adenylate cyclase and the other inhibition of prolactin release, which was independent of adenylate cyclase stimulation but associated with distinct binding sites led to the concept, formulated by Kebabian and Calne (1979), that DA effects were mediated through two distinct receptors. One was linked to stimulation of adenylate cyclase (D_1) while the other (D_2) did not appear to be associated with the enzyme but had distinct binding sites. The justification for this classification was subsequently enhanced by the synthesis of two compounds, SKF 38393 and SCH 23390. The former activated the DA adenylate cyclase without affecting prolactin release or spiperone binding, i.e. it was a D_1 agonist, while the latter blocked the stimulation of adenylate cyclase, again without affecting prolactin release or binding. It was a D_1 antagonist. The basis for this early classification is shown in Table 7.1.

Although some subsequent pharmacological studies suggested that perhaps there could be a subdivision of both the D_1 and D_2 receptors, the paucity of appropriate agonists and antagonists (and indeed of test responses) precluded its justification until molecular biology took over. Cloning studies show that structurally there are two distinct groups of DA receptors, D_1 and D_2. There is a D_5 variant of D_1 as well as D_3 and D_4 forms of D_2. The D_1 and D_5 receptors are linked to activation of adenylate cyclase and the D_2 group to its inhibition, although this is not its main effect on neurons (see later).

Despite this profusion of receptors the D_1 and D_2 predominate (over 90% of total) and most known effects of DA, its agonists and antagonists are mediated through the D_2 receptor. Although the above nomenclature is now accepted it might have been better, as suggested by Sibley and Monsma (1992), to retain D_1 and D_2 to represent the two families and then subdivide them as D_{1A} for (D_1), D_{1B} for (D_5), then D_{2A} for (D_2), D_{2B} for (D_3) and D_{2C} for (D_4), even though variants of all five have been found. Their

Table 7.1 Evidence for the initial basic classification of D_1 and D_2 dopamine receptors

(a) **Discovery of specific DA stimulated striatal adenylate cyclase**
  But (i) Effect only antagonised by some neuroleptics,
    phenothiazines–YES
    thioxanthenes–YES
    butyrophenones–NO
    (metoclopromide inactive)
  (ii) Effect not reproduced by DA agonists like bromocriptine.

(b) **Binding studies: ^{3}H dopamine ^{3}H haloperidol**
  Displacement studies with a wide range of neuroleptics showed good correlation with their clinical potency in schizophrenia.
  DA agonists such as bromocriptine show binding.

(c) **Prolactin release from isolated mammatrophs from anterior pituitary**
  DA agonists — bromocriptine, apomorphine and ADTN decrease release.
  Blocked by neuroleptics — similar in effectiveness to their binding affinities (b).
  Not linked to stimulation of adenyl cyclase.

Notes:
Studies with various agonists and antagonists showed that the effects on (a) differed in potency from both (b) and (c) and were thus associated with a receptor (D_1) different from that (D_2) linked to (b) and (c). See text for detail.

structure has been established in both rat and human brain and they are generally similar in the two. The human D_2 receptor shows a protein sequence which is 96% identical to that of the rat D_2 and although the similarity is only 91% between the human and rat D_1 receptor, it is 96% in the transmembrane region. It is differences in the amino-acid sequences in this region that primarily justify the classification into two groups (D_1 and D_2) rather than their total amino-acid number. Basically the D_1 (and D_5) receptors differ from the D_2 (D_3, D_4) in having a much shorter third cytoplasmic loop and a much longer intracellular C-terminus (Fig. 7.4), which appears to be a feature of receptors linked to G_s and the stimulation of adenylate cyclase. Based on amino-acid sequencing the D_3 receptor is only 53% homologous with the D_2 (but 75% in the transmembrane region) while with D_4 it is only 41% (56%). The D_5 receptor shows 50% homology with the D_1 rising to 80% in the transmembrane region. So-called short and long variants of the D_2 receptor (D_{2S} and D_{2L}) have also been discovered, differing by the presence or absence of a run of 29 amino acids in the third intracellular loop. For more detail see Sibley and Monsma (1992).

DISTRIBUTION AND MECHANISMS

The potential value of the discovery, classification and subdivisions of any NT receptors rests on the knowledge that

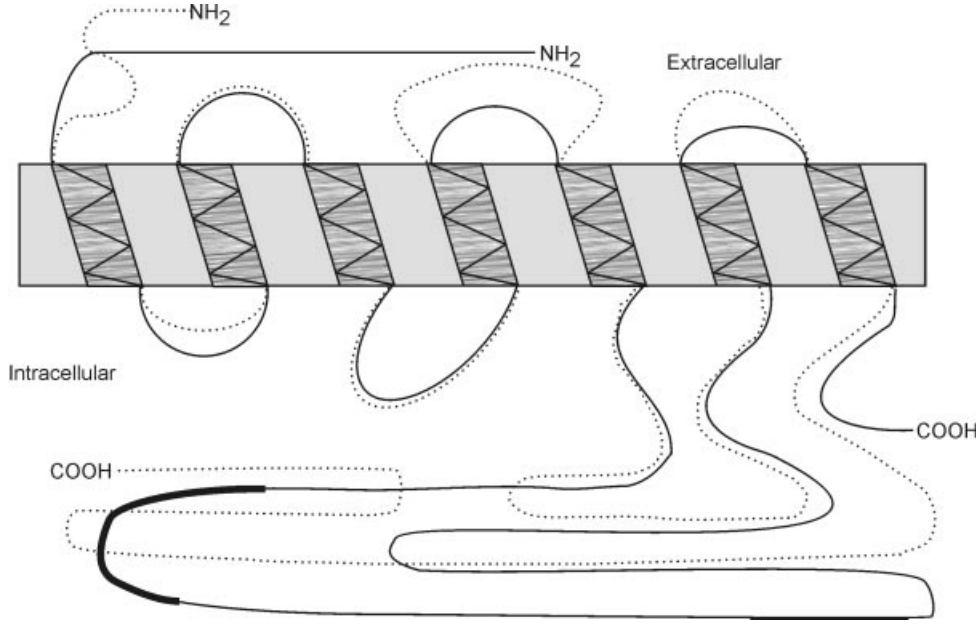

Figure 7.4 Comparative schematic representation of the D_1 (· · ·) and D (—) dopamine receptor. The figure attempts to highlight the major differences between extra- and intracellular loops, especially the intracellular loops between transmembrane sections 5 and 6 and the much longer C terminal of the D_1 compared with the D_2 receptor. It is based on the proposed topography of Sibley and Monsma (1992). The thickened length of the D_2 receptor represents the amino-acid sequence missing in the short form of the receptor. No attempt has been made to show differences in amino-acid sequencing or transmembrane topography

(1) Those receptors are linked to different cellular actions and/or are located in different brain regions or parts of the neuron so as to produce different functional effects.
(2) There are appropriate specific agonists or antagonists to establish and exploit those differences.

To some extent these requirements are cyclic since the establishment of different functions (1) depends on the availability of appropriate drugs (2). There is no shortage of drugs, especially antagonists, but since the main difference in structure between DA receptors is intracellular, rather than at the binding or recognition site, very specific drugs may be hard to produce. Since receptors can be expressed in cell lines the affinity of drugs for the different receptors can, however, be established, as can their cellular actions. Detection of appropriate mRNA also makes it possible to map the distribution of the receptors. The main characteristics of the DA receptors are summarised below and in Table 7.2.

D_1 receptor family

D_1 Highest expression in human striatum and nucleus accumbens and olfactory tubercle but also some in cortex and hypothalamus. In the striatum 50% of medium sized striato-nigral neurons, which also express substance P, express them. They are

Table 7.2 Dopamine receptor characteristics

Main class	D_1		D_2		
Subclass	D_{1A}	D_{1B}	D_{2A}	D_{2B}	D_{2C}
Named	D_1	D_5	D_2	D_3	D_4
No. amino acid (human)	446	477	414 (s) 443 (l)	440	387
DA affinity	Low	Moderate	Moderate	High	High
K_1 (nM) approx.	2000	200	599	20	20
Effector					
Activation of adenylate cyclase	↑	↑	↓	(↓)	(↓)
IP_3 turnover	(↑)	–	↑	–	–
Ca^{2+} influx	(↑)	–	↓	↓	↓
K^+ efflux	–	–	↑	–	–
Agonists	✓		✓	7-OHDPAT?	
Antagonists	✓		✓		Clozapine
Number	High	Low	High	Low	Low
Distribution					
Striatum	✓		✓		
Nuc. accumbens	✓		✓	✓ (Mainly)	
Frontal cortex					
Hippocampus		✓		✓	✓
Hypothalamus		✓		✓	(Midbrain)
Substantia nigra			✓	✓	
VTA (auto-receptors)			(✓)	(✓)	

Note:
S = short, L = long versions of D_2 receptor, ↑ = main effect observed, (↓) = some evidence of an effect. See Sibley and Monsma (1997), Sokoloff and Schwartz (1995) and Strange (1996).

linked primarily to stimulation of adenylate cyclase but also increase IP_3 turnover. They have a low micromolar affinity for DA ($K_1 \sim 2\,\mu M$).

D_5 Highest concentration in hippocampus and hypothalamus but much lower expression overall. Also linked to stimulation of adenylate cyclase but higher submicromolar affinity for DA ($K_1 \sim 200\,nM$). Also found in rat striatum and nucleus accumbens.

D_2 receptor family

D_2 Mostly in striatum, nucleus accumbens and olfactory tubercle but also on neuron cell bodies in substantia nigra and ventral tegmentum where they are the autoreceptors for locally (dendritic) released DA. The loss of specific D_2 antagonist binding in the striatum after lesions of the afferent nigro-striatal tract indicates their presynaptic autoreceptor role on terminals there. Other lesion studies have also established D_2 receptors on other inputs such as the cortico striatal tract.

As with D_1 receptors some 50% of striatal medium-sized cells contain them but they are different neurons as they co-express enkephalin rather than substance P. The importance of this difference in the therapy of Parkinsonism is taken up later (Chapter 15). D_2 receptors are also expressed on larger cells — probably cholinegic. Although linked to inhibition of adenylate cyclase (and IP_3 turnover) this is not their primary action. They increase K^+ conductance (hyperpolarise neurons) but also inhibit Ca^{2+} entry through voltage-sensitive channels, probably directly. When functioning as autoreceptors, these effects would also reduce DA release. The affinity for DA is slightly higher for the D_2 ($K_1 \sim 400\,nM$) than for D_1 receptors. No pharmacological differences have been established between the long or short forms of the D_2 receptor.

D_3 Much less abundant than D_2. Mainly in limbic regions (nucleus accumbens and olfactory tubercle) but also in hypothalamus. Some in caudate and cortex and also expressed on DA neurons in substantia nigra, presumably as autoreceptors. No effect on adenylate cyclase but inhibits Ca^{2+} entry (autoreceptor role). High affinity for DA ($K_1 \sim 25\,nM$).

D_4 Again very few in number compared with D_2 but located in frontal cortex, midbrain and amygdala. High affinity for DA ($K_1 \sim 20\,nM$) and a number of variants in humans.

Comparison of the K_1 values of various agonists and antagonists for the different receptors (Table 7.3) shows that whereas there are a number of drugs that readily distinguish between the D_1 and D_2 families and can be used to study their function, none distinguish between D_1 and D_5 and there is little to choose between D_2, D_3 and D_4 activities. Some differences that have been exploited are the low affinity of raclopride for D_4 receptors (compared with D_2 and D_3), the high affinity of clozapine and the benzamide derivative YM 43611 for the D_4 (cf. D_2, D_3) and that of 7-OH-DPAT for D_3. Since only the latter is an agonist, however, their value in establishing the roles of the D_3 and D_4 receptors is limited, although the high affinity of clozapine for D_4 receptors and their location in the frontal cortex has been considered, somewhat controversially, to be of significance in the aetiology and therapy of schizophrenia (see Chapter 17).

Table 7.3 (a) Dissociation constants (K_i) for various agonists and antagonists at the different dopamine receptors. (b) Indication of specificity for D_2 compared with D_1 receptors and between D_2 (i.e. D_2, D_3 and D_4) receptors

(a)

	D_1	D_5	D_2	D_3	D_4
Agonist					
Bromocriptine	440*	440*	8*	5*	290*
Quinpirole	1900	–	5	24*	30*
7-OH-DPAT	5000*	–	10	1*	650
SKF 38393	1	0.5*	150*	5000*	1000*
Apomorphine	0.7	–	0.7*	32*	4*
Antagonist					
Chloropromazine	90*	130*	3	4	35
Haloperidol	80*	100*	1	7*	2
Clozapine	170*	330*	230*	170*	21
Raclopride	18 000		1.8	3.5	2400
Spiperone	350*	3500*	0.06	0.6	0.08
S-Sulpiride	45 000	77 000	15*	13*	1000
YM 43611	10 000+	10 000+	43	11	2
SCH 23390	0.2*	0.3	1100*	800*	3000*

(b)

	Specific for D_2 family		Ratio to D_1 activity
Agonist	$D_2 = D_3 > D_4$	Bromocriptine	50
	$D_2 > D_3 = D_4$	Quinpirol	< 400
	$D_3 > D_2 >> D_4$	7-OH-DPAT	< 5000
Antagonist	$D_2 = D_3 >> D_4$	Raclopride	10 000
	$D_2 = D_4 > D_3$	Spiperone	6000
	$D_2 = D_3 > D_4$	S-Sulpiride	3000
	$D_4 > D_3 = D_7$	YM 43611	250
	Specific for D_1 family		Ratio to D_2 activity
Agonist	$D_1 = D_5$	SKF 38393	150+
Antagonist	$D_1 = D_5$	SCH 23390	1000

Note:
All values shown are taken from Seeman and Van Tol (1994) except for those for YM 43611 (Hidaka *et al*. 1996). Asterisked values are considered approximate.

SYNAPTIC EFFECTS

Because DA is very much localised to one brain area (striatum) and as there is such a pronounced DA pathway from the substantia nigra to the striatum it would be reasonable to assume that the effect of this pathway on striatal neuron activity is well established. Unfortunately this is not the case.

Over the years a large number of studies using extracellular recording in the striatum have shown that iontophoretic DA depresses 75–100% of all neurons responding to it, irrespective of whether spontaneous, excitatory amino acid-induced, or synaptic-evoked activity was being monitored. This inhibitory response is slow in onset (up to 15 s) and long in duration (possibly minutes). Stimulation of the substantia nigra can produce inhibition, excitation or mixed effects but it is possible, despite the high proportion of

DA neurons in this nucleus, that not all the effects are elicited by the release of DA. Most neuroleptics block the inhibitory effects of applied DA but some, e.g. haloperidol, are less active against SN-evoked inhibition. Generally these studies lacked specific agonists and antagonists used microintophoresis which is not really quantitative and with extracellular recording gave little information on the state of polarisation of the neuron.

Unfortunately the picture was not clarified by intracellular recordings from striatal neurons which, as these need to be large to take an electrode, are not necessarily typical (only 10%) of most striatal neurons innervated by DA afferents. Stimulation of the substantia nigra invariably produces a monosynaptic depolarisation in them that is blocked by haloperidol, but which may proceed to a hyperpolarisation, if the stimulus is strong enough. DA iontophoresced onto the same neuron may also cause depolarisation (Kitai, Sugimori and Kocsis 1976) but can still reduce its discharge. Mixed effects are often seen with DA and when it is infused in increasing concentrations into the striatum through a push–pull cannula it generally depresses extracellularly recorded cell firing but low concentrations can produce excitation or bimodal excitation–inhibition (Schoener and Elkins 1984). Voltammetry studies with an electrode that can also be used for recording neuronal firing have shown that increasing nigrostriatal stimulation induces not only an increase in DA release but also an inhibition of neurons (after some initial but variable excitation of large neurons), which outlasts the rise in extracellular DA. Thus the effects of endogenous DA appear to be critically dependent on its extracellular concentration and it may be that while synaptic effects can be excitatory, extrasynaptic ones are inhibitory. Some of this effect may also be indirect through reducing the release of excitatory NTs such as glutamate from cortico-striatal fibres or ACh from intrinsic neurons.

In view of the known cellular actions of DA, such as increased K^+ efflux and reduced Ca^{2+} currents associated with D_2 receptor activation in cell lines, inhibition would be the expected response to DA, especially as cyclic AMP, which is increased by D_1 receptor activation also inhibits striatal neurons. In fact although many DA synaptic effects are blocked by D_2 antagonists like haloperidol, the role of D_1 receptors should not be overlooked.

Iontophoretic studies on rat striatal neurons (Hu and Wang 1988) showed that while the release of DA by low currents facilitated glutamate-induced activation, high current efflux inhibited it. Although these effects were reduced by the D_2 antagonist haloperidol it was the D_1 agonist SKF 38393 which mimicked them, causing activation when released by low currents but inhibition at higher ones. Both effects were abolished by the D_1 antagonist SCH 23390. By contrast, the D_2 agonist quinpirol produced a less marked biphasic effect in which inhibition dominated.

A number of studies in fact show clear D_1 effects. Intracellular recording from striatal neurons in rat brain slices show a cAMP-mediated D_1-dependent (blocked by SCH 23390) suppression of a voltage-dependent sodium current which make the cell less responsive.

Repetition of some of these approaches using more modern techniques, e.g. whole cell and patch-clamp recording from dissociated striatal neurons, shows a similar mixed picture. An observed D_1-sensitive suppression of the sodium current and a shift of the inactivating voltage in a hyperpolarising direction, together with a depression of certain Ca^{2+} currents, would make the neuron less excitable. The D_2 effect in these measures is less clear with reports of both a depolarising and hyperpolarising shift of the

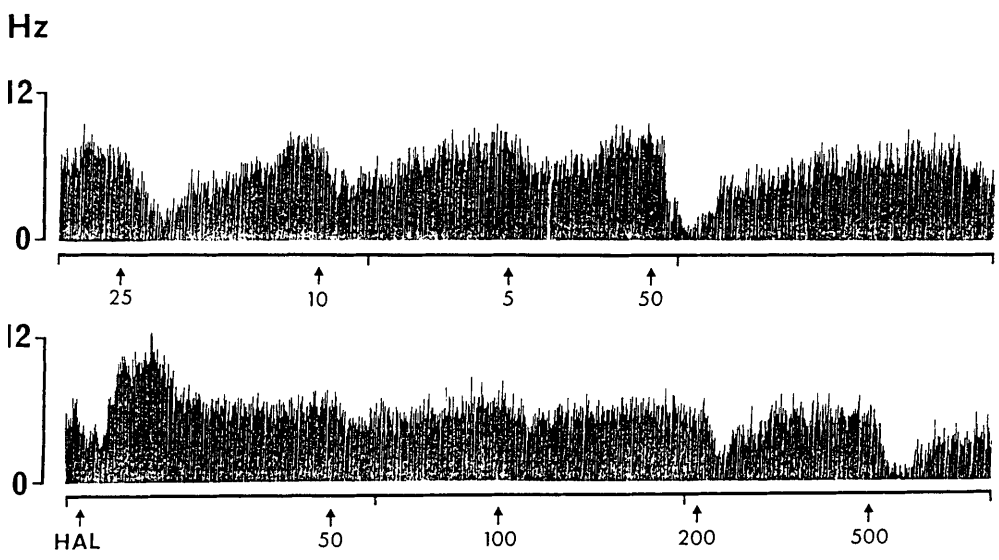

Figure 7.5 Rate recording of the dose-dependent inhibitory effects of apomorphine (μg/kg) on the spontaneous activity of a neuron in the medial prefrontal cortex of the halothane anaesthetised rat and its antagonism by haloperidol (HAL, 0.5 mg/kg). Time scale is 50 min intervals. Reproduced by permission from Dalley (1992)

inactivation curves and an increased opening of a potassium conductance (see Calabresi *et al.* 1987).

What is clear from all these experiments is that DA can have a bimodal effect depending on how much is applied or released, and which receptors are involved. Excitation is more common at low concentrations and inhibition at higher ones. What happens *in vivo* is not clear but *in vivo* voltammetry certainly suggests that the extracellular concentration of DA can be very high and this would favour the more commonly observed inhibition.

In other brain areas which receive a DA input, such as the nucleus accumbens and prefrontal cortex, it appears to be inhibitory and predominently D_2-mediated. This is clear from Fig. 7.5 which shows inhibition by apomorphine (mixed D_2, D_1 agonists) of the firing of neurons in the medial prefrontal cortex of the anaesthetised rat and its antagonism by the D_2 antagonist haloperidol.

These are, of course, extracellular recordings but more recent intracellular studies in both rat and guinea pig accumbens slices show that DA produces a D_2-mediated depolarisation and a D_1 hyperpolarisation which appear to be dependent on decreased and increased K^+ conductances respectively. This would certainly fit in with the belief that DA mediates the positive effects of schizophrenia by a D_2-mediated stimulation of the nucleus accumbens (see Chapter 17).

It is perhaps not surprising that DA produces such mixed effects. The D_1 receptor is primarily linked to the activation of adenylate cyclase and then protein kinase A. The response to its activation will therefore depend on the ion channels and other proteins modulated by the kinase which can vary from one neuron to another. Since the D_2 receptor is not so closely associated with just one G-protein, this gives it the potential for even more effects (see Greenhoff and Johnson 1997).

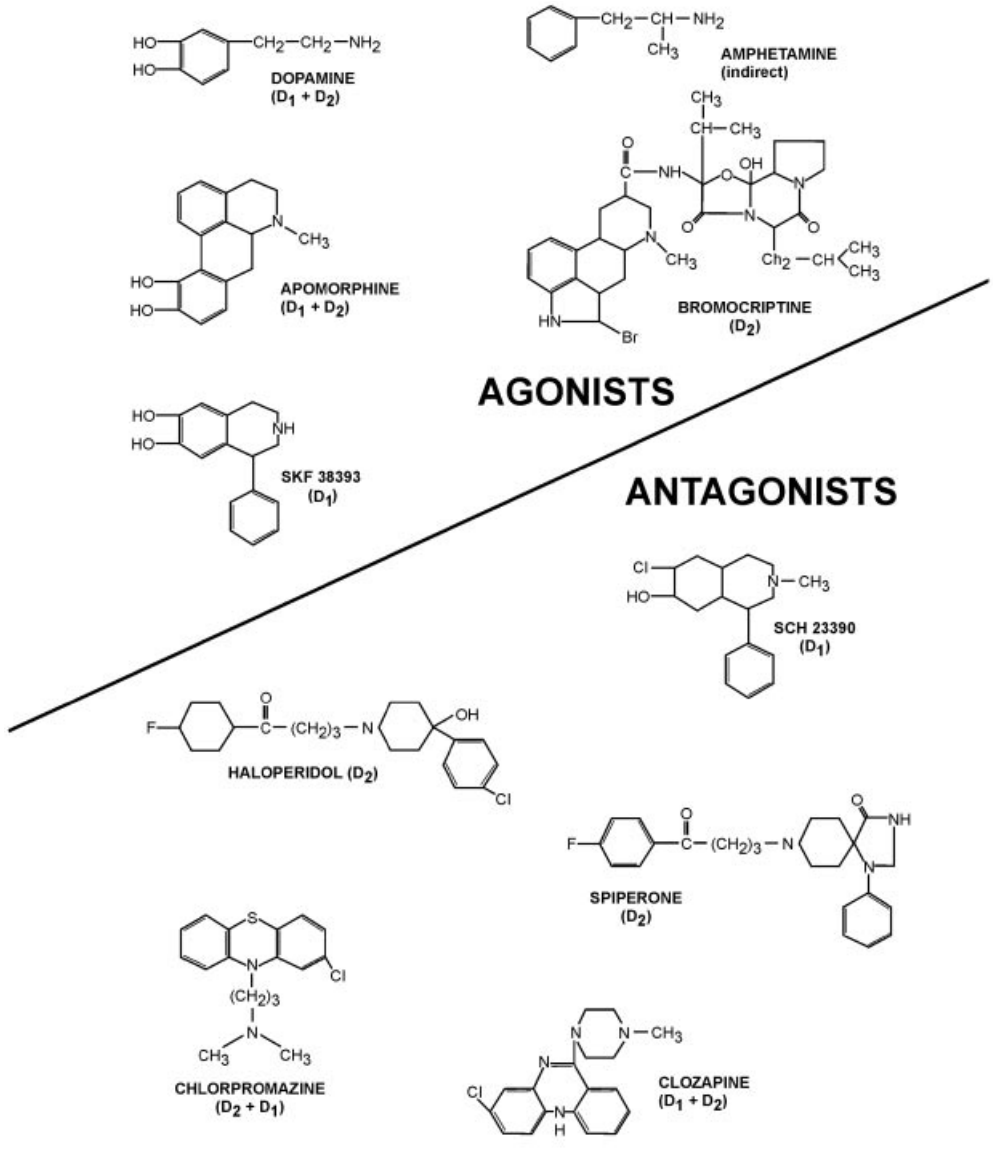

Figure 7.6 Chemical structures of some dopamine agonists and antagonists

PHARMACOLOGY OF THE DOPAMINE SYNAPSE

The sites of action of drugs affecting the dopamine synapse are indicated in Fig. 7.3. Those modifying the synthesis, storage, release, uptake and metabolism of DA have been covered above in the appropriate sections on neurochemistry. The actions and uses of agonists and antagonists are outlined in Table 7.4 and covered in detail in appropriate chapters. Their structures are given in Fig. 7.6.

AGONISTS

Those for the D_2 receptor (e.g. bromocriptine) have a particular value in the treatment of Parkinson's disease by reproducing the effects of the dopamine lost through degeneration of the nigrostriatal tract (Chapter 15). They are also used to reduce the undesirable effects of prolactinaemia (high plasma prolactin), such as amenorrhoea and galactorrhoea.

ANTAGONISTS

Again it is the D_2 compounds (e.g. chlorpromazine haloperidol) that generally have some benefit in and are the mainstay of therapy for schizophrenia although D_2 antagonism alone appears inadequate (Chapter 17). They also reduce dyskinesias such as those seen in Huntington's Chorea (Chapter 15). Some are used to control drug- and fever-induced vomiting and although any D_2 antagonist is effective, proclorperazine, metoclopramide and domperidone are more generally used. The latter two have fewer central effects since domperidone does not cross the blood–brain barrier while metaclopromide has the additional peripheral effect of increasing gastric emptying possibly by stimulating 5-HT receptors.

AMPHETAMINE

This is generally known as an indirectly acting sympathomimetic amine because peripherally it mimics activation of the sympathetic system although centrally it primarily reproduces the effect of DA. Chemically (Fig. 7.6) it is a phenylamine not a catecholamine and so it has no direct effect on any DA or NA receptor. It is, however, a substrate for the high-affinity DA (and NA) neural membrane transporter and is taken up into nerve terminals by that process. When released from the transporter into the cytoplasm its place is taken by DA which is then transported out (exchange diffusion). It is said to have released DA. In addition, amphetamine increases cytoplasmic DA by weakly reducing its uptake into vesicles. As a reasonably lipophilic compound amphetamine can enter the vesicles where being a weak base it takes up H^+ ions. This makes the vesicle interior less acidic and the reduction in pH gradient across the vesicle membrane appears to inhibit DA uptake into the vesicle. In addition, amphetamine is an inhibitor of MAO but it preferentially attacks the A form so its effect is greater on the breakdown of NA than of DA.

Most of the motor effects of amphetamine, especially stereotypy, are due to the release of DA as are its psychotic effects such as hallucinations. Its ability to mimic the action of DA in reward and reinforcement behaviour may contribute to its abuse potential (see Chapter 22) but its arousal (stimulant) properties also involve NA release.

CENTRAL FUNCTIONS

It is perhaps easier to identify some of the central functions of DA than that of the other monoamines because not only does it have distinctive central pathways associated with particular brain areas, but it has few peripheral actions. Also the actions of its antagonists reveal its central effects. These are summarised in Table 7.4.

Table 7.4 Summary of dopamine function

Function	Pathways	Effect of DA agonist	Effect of DA antagonist	Receptor
Control of motor function	Nigrostriatal tract from substantia nigra (A9)	Animals: Stereotypy. Rotation if one tract is lesioned Humans: Induces dyskinesias Effective in Parkinsonism	Animals: Catalepsy Humans: Reduces dyskinesias Induces Parkinsonism	Mainly D_2 some D_1
Initiation of behaviour	Mesolimbic pathway to nucleus accumbens from VTA (AIO)	Animals: Increases locomotor activity and intracranial self-stimulation	Animals: Decreases activity and self-stimulation	D_2
	Mesocortical pathways to prefrontal cortex from VTA (AIO)	Humans: Hallucinations, psychoses (reward, reinforcement)	Humans: Reduces positive symptoms of schizophrenia	
Control (inhibition) of prolactin release	Tuberoinfundibular tract from A12 in the arcuate nucleus of the median eminence to pituitary	Humans: Hypoprolactaemia	Humans: Hyperprolactaemia Galactorrhoea Amenorrhoea	D_2
Emesis	No distinct pathway DA receptors in chemoreceptor pathway zone	Vomiting	Anti-emetic (not motion sickness)	D_2

DA antagonists are anti-emetic, elevate plasma prolactin and have major motor and behavioural effects. Thus DA must be involved in the initiation of vomiting, the secretion of prolactin and control of motor and behavioural activity. Its role in emesis and as the prolactin release inhibitory factor have been adequately covered above. Its motor and behavioural function will now be considered.

MOTOR ACTIVITY

People with Parkinson's disease show a specific degeneration of the nigrostriatal tract so DA must be linked in some way to the control of motor function. It is also known that an imbalance of DA function on the two sides of the rat brain, either by stimulation or lesion of one SN, causes off-line or rotational movement (Ungerstadt and Arbuthnott 1970). This is best shown some days after 6-OHDA lesion of one substantia nigra and its nigrostriatal pathway when systemic apomorphine (DA agonist) causes animals to turn away from the lesioned side (contraversive), presumably

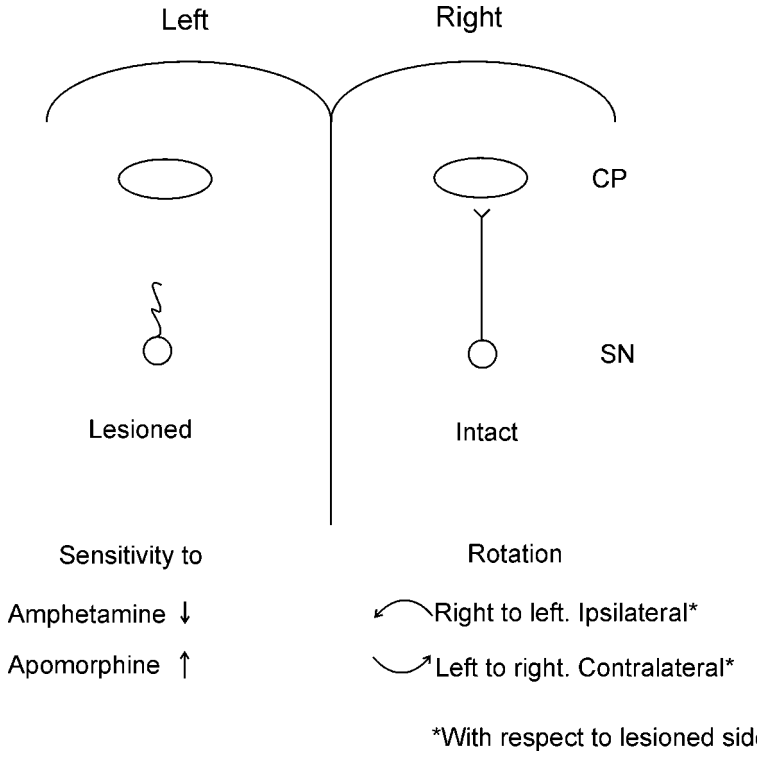

Figure 7.7 Dopamine-induced rotation in the rat in which one (left) nigrostriatal dopamine pathway from the substantia nigra (SN) to the caudate putamen (CP) has been lesioned by a prior injection (14 days) of 6-hydroxydopamine. Amphetamine, an indirectly acting amine, releases DA and so can only act on the right side. Since the animal moves away from the dominating active side it induces ipsilateral rotation (i.e. towards the lesioned side). By contrast, the development of postsynaptic supersensitivity to DA on the lesioned side ensures that apomorphine, a directly acting agonist, is actually more active on that side and so the animal turns away from it (contralateral rotation)

because the denervated striatum has become supersensitive and therefore more responsive than the control side to the DA agonist. Conversely, the indirectly acting amine amphetamine promotes movement towards the lesioned side (ipsiversive) because it can only release DA in the intact striatum (Fig. 7.7). Thus animals move away from the side with the most responsive and active striatum. These drugs also produce other motor activity including increased locomotion and a so-called 'stereotype' behaviour in which rats sniff avidly around the cage and spend much time licking and rearing. It appears that stereotypy is due to activation of the nigrostriatal pathway as it is absent after lesion of the substantia nigra and follows apomorphine and amphetamine injection into the striatum, whereas locomotor responses to amphetamine are reduced by lesions to A10 and can be induced by its injection into the nucleus accumbens.

Another indication of the importance of DA in motor control is the observation that in humans its precursor levodopa, and DA agonists like bromocriptine, not only overcome the akinesia of Parkinsonism but in excess will actually cause involuntary movements, or dyskinesia (Chapter 14). Also it is well known that DA antagonists like chlorpromazine and haloperidol produce Parkinsonian-like symptoms in humans (and catalepsy in animals) and, as indicated above, reduce the dyskinesia of Huntington's Chorea. Thus DA seems to sit on a knife edge in the control of motor function (Fig. 7.8).

PSYCHOSES

The main use clinically of DA antagonists is in the treatment of schizophrenia (Chapter 17) and the control of mania. Since psychotic symptoms are also a side-effect of levodopa therapy in Parkinsonism and as amphetamine causes hallucinations and schizophrenic-like symptoms in humans, presumably by releasing DA, it appears that DA also has an important part to play in the control and induction of psychotic symptoms. It is possible that the role of DA in psychosis is mediated primarily through the mesolimbic and mesocortical pathways and its control of motor function through the striatum, and there is evidence that the neurons from which these pathways arise have different characteristics. Although there is some overlap between the various DA nuclei in respect of the location of the cell bodies of the neurons that give rise to the different DA pathways, neurons can be identified by antidromic activation of their terminal axons in the appropriate projection areas. Recordings from neurons so identified show that they have differing firing patterns. Those cells innervating the prefrontal cortex fire at a much higher rate (9.7 Hz) than those to the cingulate and piriform cortex (5.9 or 4.3) and the striatum (3.1). Unlike the NA neurons they are also remarkably little affected by the state of the animal, i.e. its wake–sleep cycle. The cells in A10 which form the mesocortical pathway are also less easily inhibited by DA agonists suggesting that they probably have fewer autoreceptors. Unfortunately it seems that the DA postsynaptic receptor is the same at both sites so it has been difficult to divorce the antischizophrenic from the extrapyramidal-inducing activity of DA antagonists (see Chapter 16).

REWARD AND REINFORCEMENT

We expect reward to be pleasurable and it is assessed in animals by their willingness to seek and approach something, such as a lever linked to either food dispensing or brain

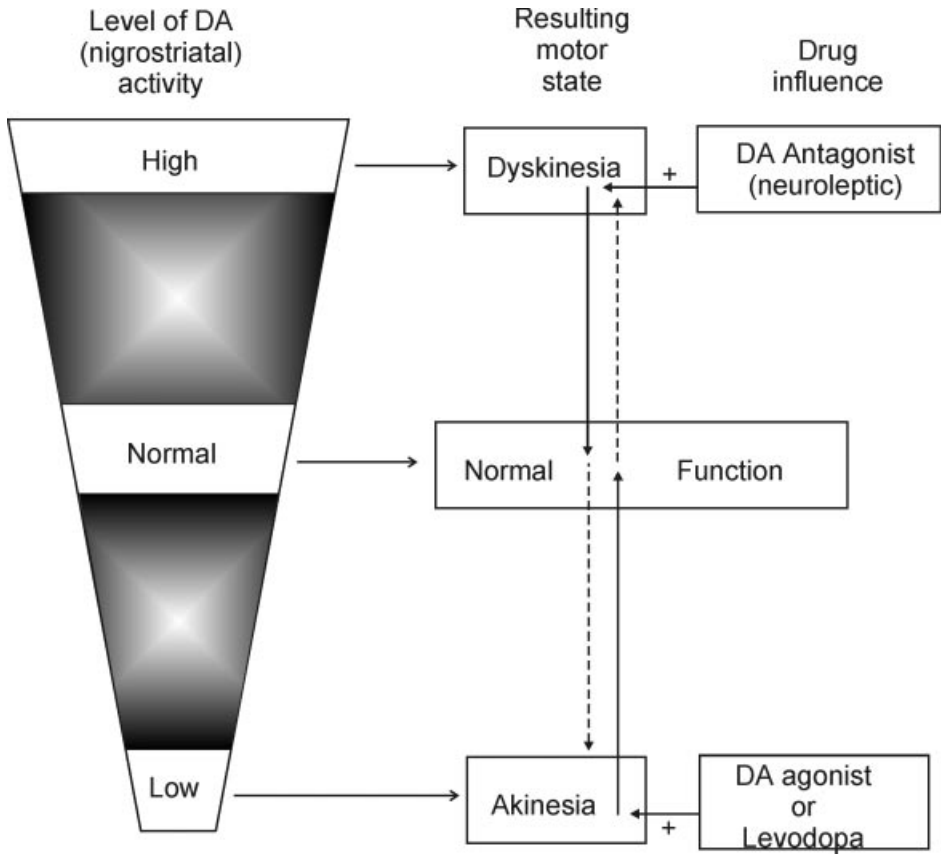

Figure 7.8 Dopamine and motor function. When nigrostriatal dopamine activity is normal so is motor function. Any reduction in this DA activity, as in Parkinson's disease, results in reduced motor activity, i.e. akinesia. By contrast, too much DA activity, as in Huntington's Chorea, produces abnormal motor function, i.e. dyskinesia. The latter may be controlled by neuroleptic drugs (DA antagonists) but they can swing the balance in DA activity sufficiently to produce akinesia (Parkinsonism). DA agonists (and levodopa) may overcome akinesia but can induce DA overactivity and dyskinesia (peak dose effect) (see Chapter 15)

stimulation. Reinforcement is the manner in which one event (stimulus) strengthens the likelihood of its repetition, i.e. repeated lever pressing for a pleasurable reward. In this instance, of course, reinforcement is rewarding but it need not be. Reward and reinforcement are considered by some to be the basis of motivation.

In 1954, Olds and Milner first described the effects of intracranial self-stimulation (ICSS). Rats with electrodes implanted in certain brain regions appeared to find the stimulation mediated through them to be rewarding (pleasurable) and so would seek out whatever part of their surroundings they associated with it. In addition, such self-stimulation reinforced the animal's inclination to indulge in other activity such as pressing a lever for a food reward. Since it was thought that the brain pathways and NTs mediating ICSS could also be responsible for more natural pleasurable rewards such as food, drink and sex their identification generated much interest.

The brain area most commonly, but not uniquely, associated with ICSS is the medial forebrain bundle (MFB). This includes the axons of noradrenergic as well as dopaminergic neurons but it appears to be the ventral tegmentum area and the A10 DA neurons innervating the nucleus accumbens (and prefrontal cortex) which is most active, as evidenced by 2-deoxyglucose autoradiography during self-stimulation. In fact effective, i.e. rewarding, self-stimulation through electrodes in the VTA is accompanied by DA release in the nucleus accumbens (Fiorino *et al.* 1993). The threshold current (or frequency) of stimulation for the initiation of ICSS is lowered by amphetamine and raised by DA antagonists while the rate of lever pressing in response to a particular level of ICSS is potentiated or reduced by the same respective procedures. Animals also learn to press a lever to initiate the administration (injection) of certain drugs in preference to obtaining food or water and will continue this to a point of intoxication. Dopamine uptake blockers and D_2 (or mixed) agonists are all strongly sought in self-administration trials and it became generally accepted that DA was paramount in mediating the reinforcing effects not only of ICSS but also of drug abuse and sex.

As a result of these observations it has been suggested that DA released in the nucleus accumbens is important in motivation by linking reward (especially when it is food) with the motor activity required to achieve it (Mogenson, Jones and Yim 1980). It is difficult, however, to distinguish a pure behavioural role for DA in actually initiating the sense of reward and motivation from its undisputed part in facilitating the motor response necessary to obtain the reward, e.g. a lever press in rats.

Salamone, Cousins and Snyder (1997) in fact suggest that the function of DA in the nucleus accumbens should not be described by terms such as motivation, reinforcement and reward. Rather it should be considered to mediate the higher-order motor and sensory processes that are important for the activation of aspects of motivation and responsiveness to conditioned stimuli.

DOPAMINE RECEPTORS, FUNCTION AND SYNERGISM

With the discovery of five distinct DA receptors within two major families, one might hope that the effects of the different DA pathways would be mediated through different receptors. Unfortunately this is not the case. As indicated above, it is generally the D_2 receptor that is important. Thus only D_2 antagonists have anti-emetic activity and only D_2 agonists, like bromocriptine, reduce plasma prolactin levels. In schizophrenia it is again the D_2 antagonists that are effective, although D_1 and D_4 receptors have been implicated (see Chapter 16) while in Parkinson's disease the symptoms can be alleviated by D_2 but not D_1 agonists if they are given alone. In this condition, however, some D_1 stimulation may augment the effect of the D_2 agonists (Chapter 14), suggesting a synergism between the two receptors. This synergism has been observed in both electophysiological studies on striatal neuron activity and some animal behaviours.

The electrophysiological studies of DA function in the striatum, reported above, suggest some similarities between D_1- and D_2-mediated effects. A clear synergism has been seen in fact, on the so-called type I DA neurons in the ventral tegmentum *in vitro* (Momiyama, Naoyuki and Saso 1993). The D_2 agonist quinpirole produced hyperpolarisation ($\uparrow K^+$ conductance) and reduced-action potential numbers but the D_1 agonist SKF 38393 had no effect alone. When given with quinpirole, however, it increased the ability of the D_2 agonist to raise the threshold for action potential

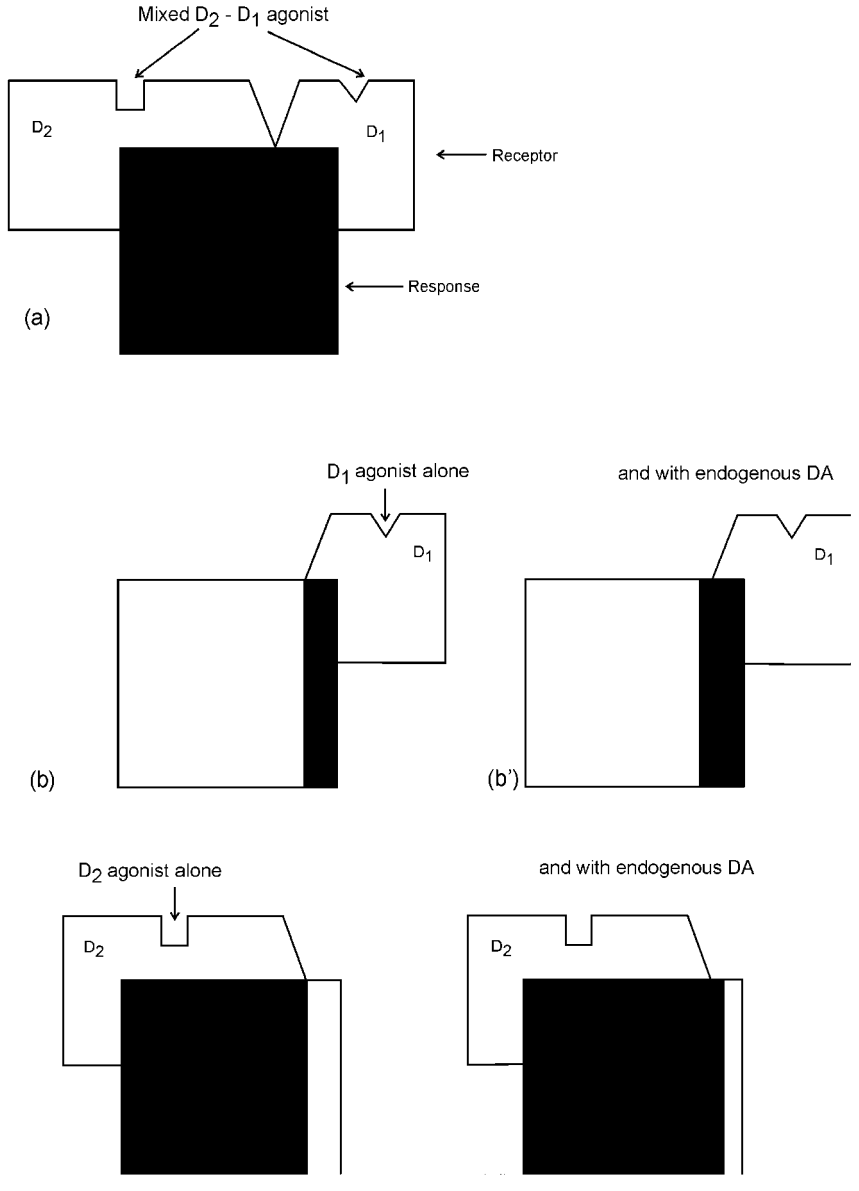

Figure 7.9 Synergism between dopamine D_1 and D_2 receptor activation. The level of behavioural and motor response to dopamine agonists is shown by the extent of shading in the response box. A mixed agonist, including DA itself, can produce a full response (a) by activating both D_1 and D_2 receptors. A D_1 agonist alone (b) has little effect compared with a D_2 agonist alone (c). The effect of the D_1 agonist is greater in the presence of endogenous DA acting synergistically on D_2 receptors (b′) and so can be partly reduced by a D_2 antagonist that has no D_1 receptor activity. While the effect of the D_2 agonist can also be augmented by DA acting on the D_1 receptor (c′) this increase is less marked and a D_1 antagonist has little effect on D_2 activity. The need to activate, or block, both the D_1 and D_2 family of DA receptors in order to reproduce or elimate full endogenous DA activity has implications in the treatment of Parkinson's disease and schizophrenia

generation and reduced firing and this potentiation was abolished by the D_1 antagonist SCH 23390.

Behaviourally, a D_1 agonist like SKF 38593 has few effects alone in rats apart from inducing grooming and some sniffing. By contrast, the D_2 agonist bromocriptine produces a marked behavioural stereotypy during which animals move avidly around the cage sniffing, gnawing, digging and then rearing. This is even more pronounced with a mixed D_2 and D_1 agonist like apomorphine. Surprisingly the activity of the D_1 agonist was severely attenuated by a D_2 antagonist (haloperidol) while the D_1 antagonist SCH 23390 slightly reduced the stereotypy of the D_2 agonist bromocriptine (Waddington 1989). Also, the effect of bromocriptine was increased by addition of the D_1 agnonist.

On this evidence it appears that a D_1 agonist is only fully effective if endogenous DA is present to act on D_2 receptors while a D_2 agonist also requires, although not to the same extent, some DA to act on D_1 receptors. Of perhaps more importance is the fact that a full DA effect depends on the activation of both D_1 and D_2 receptors even though the latter is dominant (Fig. 7.9).

At a time when pharmacological endeavour is aimed at producing drugs with limited and specific receptor profiles, the possibility that more than one receptor needs to be activated in order to replicate an action of an endogenous NT like DA is disturbing. Its significance in the therapy of Parkinson's disease is considered in Chapter 15. It must be remembered, however, that despite the links between D_1- and D_2-receptor mediated effects and the equality in their number, no D_1 agonist or antagonist produces or blocks any of the main known effects of DA either in humans or animals, whereas their D_2 counterparts are active.

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