DOPAMINE RECEPTORS

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Professor Philip Strange has worked on the structure and function of G protein coupled receptors for a number of years. His lab is currently examining dopamine, serotonin and chemokine receptors with a particular emphasis on the mechanisms of agonism and inverse agonism.

History

It was not until the late 1950's that dopamine was recognised as a neurotransmitter in its own right when the demonstration of its non-uniform distribution in the brain suggested a specific functional role for dopamine. Interest in dopamine was intensified by the realisation that dopamine had an important role in the pathogenesis or drug treatment of certain brain diseases e.g. Parkinson's disease, schizophrenia.[1] This lead to much research on the sites of action of dopamine, the dopamine receptors. A milestone in this was the suggestion, based on anatomical, electrophysiological and pharmacological studies by Cools and Van Rossum, that there might be more than one kind of receptor for dopamine in the brain.[2] In the 1970's, biochemical studies on dopamine receptors based on second messenger assays, e.g. stimulation of cAMP production, and based on ligand binding assays supported the idea of more than one kind of dopamine receptor. This idea was given a firm foundation by Kebabian and Calne in their 1979 review,[3] in which they extended an earlier suggestion by Spano,[4] and proposed that there were two classes of dopamine receptor, D_1 and D_2, with different biochemical and pharmacological properties and mediating different physiological functions. Some of the properties of these two subtypes are summarised in Table 1. Selective agonists and antagonists exist to define the two subtypes in functional assays and some of these are shown in Table 1. Both the D_1 and D_2 subtypes are G-protein coupled receptors but

Table 1. Dopamine receptor subtypes defined from physiological, pharmacological, and biochemical studies

	D_1	D_2
Pharmacological characteristics		
Selective antagonists	**SCH 23390** SKF 83566	**(-)-sulpiride** nemonapride
Selective agonists	**SKF 38393** **dihydrexidine**	**quinpirole** N-0437
Specific radioligands	[^{3}H]SCH 23390* [^{125}I]SCH 23982	[^{3}H]nemonapride [^{3}H]raclopride [^{3}H]spiperone**
Physiological functions	aspects of motor function (brain), cardiovascular function	aspects of motor function and behaviour (brain), control of prolactin and γ MSH secretion from pituitary, cardiovascular function
Biochemical responses	adenylyl cyclase↑ phospholipase C↑	adenylyl cyclase↓ K^+ channel↑ Ca^{2+} channel↓
Localisation	caudate nucleus, putamen, nucleus accumbens, olfactory tubercle, cerebral cortex (brain), cardiovascular system	caudate nucleus, putamen, nucleus accumbens, olfactory tubercle, cerebral cortex (brain), anterior and neurointermediate lobes of pituitary gland, cardiovascular system

(Bold text denotes compounds available from Tocris)

With the advent of molecular biological studies (Table 2), these subtypes should be termed D_1-like and D_2-like receptors. The localisation data are from functional and ligand-binding studies on dispersed tissues and tissue slices. *[^{3}H]SCH 23390 can also bind to 5-HT_2 receptors if present; **[^{3}H]spiperone can also bind to 5-HT_{1A}, 5-HT_2 receptors, and α_1-adrenoceptors if present. For more details see reference 52.

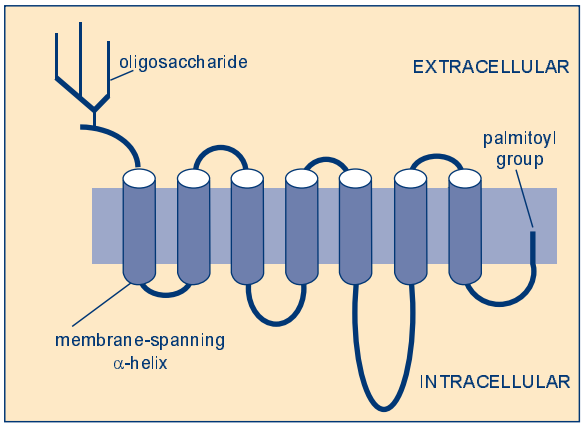
different G-proteins and effectors are involved in their signalling pathways (Table 1).

Although biochemical studies gave some indications of further heterogeneity of these dopamine receptor subtypes, it was not until the late 1980's that the true extent of this was revealed by the application of gene cloning techniques to the dopamine receptors. This showed that there were at least five dopamine receptors (D_1-D_5) and they may be divided into two subfamilies whose properties resemble the original D_1 and D_2 receptors defined pharmacologically and biochemically.[5-8] The two subfamilies are often termed D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) and some of their key properties are summarised in Table 2. There may be other subtypes yet to be discovered, for example additional D_1-like receptors have been cloned from *Xenopus*, chicken and drosophila.[9-11] In subsequent discussion, receptor subtypes defined from cloned genes will be referred to as D_1, D_2, D_3, D_4, D_5 and where only the subfamily of receptor has been defined pharmacologically, the D_1-like and D_2-like nomenclature will be used.

Properties common to the different dopamine receptor subtypes

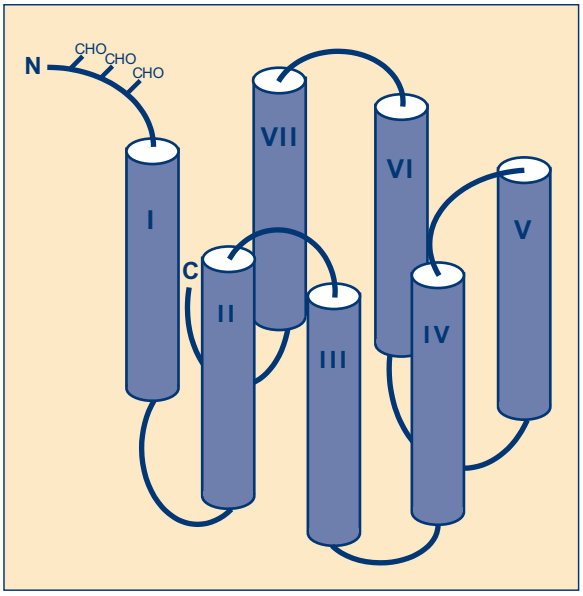
Analysis of the amino acid sequences of the dopamine receptor subtypes has shown that significant homologies exist among the subtypes with the greatest homologies being found between members of either subfamily.[7] Each receptor has been shown to contain seven stretches of amino acids that are hydrophobic and long enough to span the membrane. It seems therefore that each of the dopamine receptors conforms to the general structural model for a G-protein coupled receptor,[12] with an extracellular amino terminus and seven putative membrane spanning-helices linked by intracellular and extracellular protein loops (Figure 1). One or more potential sites for glycosylation are found on the amino terminus and second extracellular

Figure 1. Schematic representation of a G-protein coupled dopamine receptor



loop. The helices are bundled together in the membrane to form the ligand binding site (Figure 2) and some information is available on the residues that make contacts with ligands.[13,14] There is an intracellular carboxyl terminus probably bearing a palmitoyl residue which may form a further link to the membrane. The D_1-like receptors have short third intracellular loops and long carboxyl terminal tails whereas the D_2-like receptors have long third intracellular loops and short carboxyl terminal tails. This provides a structural basis for the division of the receptors into two subfamilies but is also likely to have a functional significance possibly related to the specificity of receptor/G-protein interaction.

Figure 2. Bundling of the helices in a G-protein coupled dopamine receptor to form the ligand binding site



Indeed the third intracellular loop of these receptors is thought to be important for the interaction of receptor and G-protein and for the D_2-like receptors, variants of these subtypes exist based on this loop. For example there are short and long variants of the D_2 and D_3 receptors with the long forms having an insertion (29 amino acids for D_2long) in this loop.[15,16] Polymorphic variants of the D_2 receptor have been described with single amino acid changes in this loop.[17] For the D_4 receptor there are polymorphic variants in the human population with different length insertions in this loop[18]. In some cases these D_2-like receptor variants may have differential abilities to couple to or activate G-proteins[17,19,20] and may also exhibit slightly different pharmacological properties.[21,22] The variants of the D_4 receptor have not been found to exhibit any differences in the binding of ligands or in coupling to G proteins.[23]

The individual properties of the different subtypes have been probed by expressing the receptors in recombinant cells and by examining the localisation of the subtypes at the mRNA and protein level.

Individual properties of the different dopamine receptor subtypes

The dopamine receptor subtypes exhibit different properties in terms of their pharmacological profiles, localisation and mechanisms of action and the following sections will summarise these for the two subfamilies.

D_1-like receptors

Both the D_1 and D_5 receptors show pharmacological properties similar to those of the original pharmacologically defined D_1 receptor, i.e. a high affinity for the benzazepine ligands SCH 23390 and SKF 83566 which are selective antagonists for these subtypes. Thioxanthines, e.g. flupenthixol, and phenothiazines, e.g. fluphenazine, also show high affinity but are not selective for D_1-like over D_2-like receptors. The D_1-like receptors also show moderate affinities for typical dopamine agonists such as apomorphine, and selective agonists such as SKF 38393, SKF 82526 and dihydrexidine are now available. There are differences in the affinities of some compounds for the D_1 and D_5 receptors (higher agonist and lower antagonist affinities[24,25]) but no truly selective compounds are as yet available.

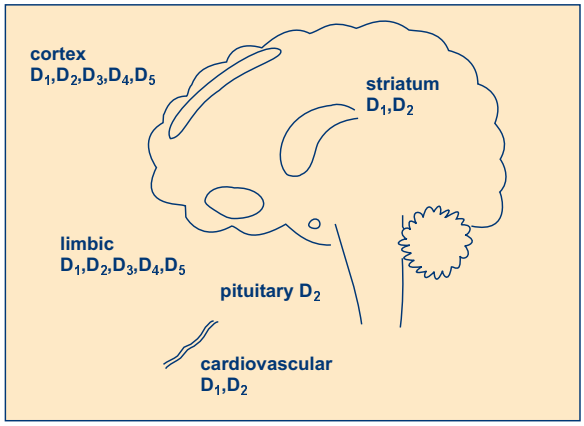
D_1 receptors are found at high levels in the typical dopamine rich regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercle, whereas the distribution of the D_5 receptors is much more restricted (Figure 3, Table 2); this subtype is found generally at much lower levels. Both receptors are able to stimulate adenylyl cyclase (Figure 4) and the D_5 receptor shows some constitutive activity for this response.[25] Inverse agonist activity at the D_1 and D_5 receptors is seen in recombinant systems with some compounds such as butaclamol;[25] compounds which were previously considered to be antagonists.

The function of the D_5 receptor is not understood but the D_1 receptor seems to mediate important actions of dopamine to control movement, cognitive function and cardiovascular function. Interaction between D_5 receptors (G protein coupled) and $GABA_A$ receptors (ion channel linked) has been described[26] which may point towards a functional role for the D_5 receptor.

D_2-like receptors

Overall the D_2, D_3 and D_4 receptors exhibit pharmacological properties similar to those of the original pharmacologically defined D_2 receptor, i.e. they all show high affinities for drugs such as the butyrophenones, e.g. haloperidol, and the substituted benzamides, e.g. sulpiride, and these classes of drug provide selective antagonists for the D_2-like receptors. As indicated above, the D_2-like receptors also show high affinities for phenothiazines and thioxanthines.

Figure 3. Dopamine receptor distribution in the brain and periphery



Each D_2-like receptor does have its own pharmacological signature so that there are some differences in affinities of drugs for the individual D_2-like receptors (Table 2). For example raclopride shows a high affinity for the D_2 and D_3 receptors but a lower affinity for the D_4 receptor. Clozapine shows a slight selectivity for the D_4 receptor. More selective antagonists have been synthesised and these will be invaluable in determining the functions of these subtypes. For example L-741,626, PD 58491 and L-745,870 are D_2 selective (~40 fold), D_3 selective (~100 fold) and D_4 selective (~2000 fold) antagonists respectively.[27-29] The aminotetralins UH 232 and AJ 76 have been reported to be selective D_2-like

Table 2. Dopamine receptor subtypes from molecular biological studies

	'D1-like'		'D2-like'		
	D_1	D_5	$D_{2(short)/(long)}$	D_3	D_4
Amino acids	446(h,r)	477(h) 475(r)	414/443(h) 415/444(r)	400(h) 446(r)	387(h,r)
Pharmacological characteristics (K_d, nM)	**SCH 23390** (0.35) dopamine (2340)	**SCH 23390** (0.30) dopamine (228)	**spiperone** (0.05) raclopride (1.8) **clozapine** (56) dopamine (1705)	**spiperone** (0.61) raclopride (3.5) **clozapine** (180) dopamine (27)	**spiperone** (0.05) raclopride (237) **clozapine** (9) dopamine (450)
Homology with D_1 receptor with $D_{2(short)}$	 100 44	 82 49	 44 100	 44 76	 42 54
Receptor localisation	caudate/putamen, nucleus accumbens, olfactory tubercle, hypothalamus, thalamus, frontal cortex	hippocampus, thalamus, lateral mamillary nucleus, striatum, cerebral cortex (all low)	caudate/putamen, nucleus accumbens, olfactory tubercle, cerebral cortex (low)	nucleus accumbens, olfactory tubercle, islands of Calleja, cerebral cortex (low)	frontal cortex, midbrain, amygdala, hippocampus, hypothalamus, medulla (all low), retina
Response	adenylyl cyclase↑	adenylyl cyclase↑	adenylyl cyclase↓	adenylyl cyclase↓	adenylyl cyclase↓
Introns in gene	none	none	yes	yes	yes
Organisation of amino acid sequence putative third intracellular loop carboxyl terminal tail	 short long	 short long	 long short	 long short	 long short
Reference (examples)	45	24, 46	47	31	48

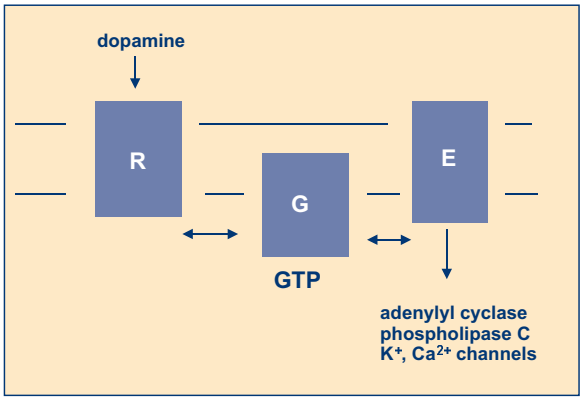
(Bold text denotes compounds available from Tocris)

The properties of the principal dopamine receptor subtypes identified by gene cloning are shown. They are divided into 'D_1-like' and 'D_2-like' groups to reflect amino acid homology, functional similarity, structural similarity, and pharmacological properties. This grouping conforms with a previous classification based on pharmacological and biochemical properties (Table 1). h and r refer to human and rat sequences, respectively. $D_{2(short)}$ and $D_{2(long)}$ refer to different alternatively spliced forms of the D_2 receptor gene as outlined in the text. The K_d values represent the dissociation constants (nM) of selected ligands for rat or human receptors as quoted in the literature. The figures for dopamine are in the presence of Gpp(NH)p. $D_{2(short)}$ and $D_{2(long)}$ do not differ greatly in their pharmacology regarding antagonist affinities, although small differences have been reported for the substituted benzamide drugs.[21,22] The homology values are for the transmembrane-spanning regions and are taken from reference 7. The localisations shown are the principal ones known at present from *in-situ* hybridisation and use of the polymerase chain reaction. Recent reviews of the field are references 52-54..

autoreceptor antagonists[30] but they also possess some selectivity for the D_3 receptor[31] where UH 232 is a partial agonist.[32] Most antagonists show a higher affinity for the D_2 receptor compared with the D_3 and D_4 receptors. The D_2-like subtypes show moderate affinities for typical dopamine agonists with the D_3 receptor generally showing higher affinities for agonists than the other subtypes. There are compounds available that are selective agonists for the D_2-like receptors, e.g. NO 437 and quinpirole. There are no highly selective agonists for the individual subtypes as yet.

The D_2 receptor is the predominant D_2-like subtype in the brain and is found at high levels in typical dopamine rich brain areas. D_3 and D_4 receptors are found at much lower levels and in a more restricted distribution pattern and they are found predominantly in limbic areas of the brain (Figure 3, Table 2). The D_2-like receptor subtypes have each been shown to inhibit adenylyl cyclase (Figure 4) when expressed in recombinant cells[33-36] although the signal via the D_3 receptor has proven more difficult to demonstrate and is generally lower than for the other two subtypes. The D_2-like receptors will also stimulate mitogenesis[33,37] and extracellular acidification[33] in recombinant systems. Effects have been shown on arachidonic acid release and MAP kinase for the D_2 receptor[38,39] and on Ca^{2+} channels for the D_2 and D_3 receptors.[40] The relationship of these effects to the *in vivo* responses is entirely unclear. Many compounds that were thought to be antagonists at D_2-like receptors have been shown to possess inverse agonist activity at D_2 and D_3 receptors.[41,42]

Figure 4. Signal transduction mechanism of dopamine receptors – showing the binding of dopamine to the receptor (R) and the interaction with the G protein (G) and effector (E).



The D_2 receptor is important for mediating the effects of dopamine to control movement, certain aspects of behaviour in the brain and prolactin secretion from the anterior pituitary gland. The functions of the D_3 and D_4 receptors are currently unknown although their localisations in limbic areas of brain suggest roles in cognitive, emotional and behavioural function. The D_2-like receptors show high affinities for most of the drugs used to treat schizophrenia (antipsychotics) and Parkinson's disease (e.g. bromocriptine). The distribution of the D_3 and D_4 receptors in limbic brain regions has made them particularly attractive targets for the design of potential selective antipsychotic drugs. L-745,870 was the first highly selective D_4 antagonist synthesised and it has proven to be inactive against the psychosis of schizophrenia.[29]

Future

In order to understand the functions of the individual dopamine receptor subtypes and their roles in behaviour it will be essential to have selective compounds for each subtype. Although some compounds are now available[27-29] we are a long way away from achieving this aim. Transgenic "receptor knock out" animals may help to further elucidate the roles of the subtypes.[43] It is also important to try to understand the mechanism of binding of ligands to these receptors and this is currently being actively researched using modelling and mutagenesis techniques.[49] The recent description[50] of the structure of the prototypical G protein coupled receptor rhodopsin, should act as a major spur to work in the area.

Finally, it is also important to understand the mechanism of action of the receptors and how the binding of an agonist generates the signal leading to the efficacy of agonist action.[44] The recently described phenomenon of G protein coupled receptor dimerisation[51] may apply to the dopamine receptors and the relevance of this to receptor function needs to be determined.

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DOPAMINERGICS AVAILABLE FROM TOCRIS

D_1-like receptor selective compounds

1249	CY 208-243	Selective D_1-like agonist
0884	Dihydrexidine	Selective D_1-like agonist
0925	SCH 23390	Standard selective D_1-like antagonist
0922	SKF 38393	Selective D_1-like agonist

D_2-like receptor selective compounds

Agonists

0427	Bromocriptine	D_2-like agonist
0474	Dihydroergocristine	Partial dopamine receptor agonist. Also partial adrenergic agonist and 5-HT antagonist
0475	Dihydroergotamine	Partial D_2-like agonist. Also partial α agonist and 5-HT antagonist
0706	7-HydroxyDPAT	Dopamine agonist ($D_3 \geq D_2 > D_4$)
1031	Piribedil	Dopamine agonist
1061	(-)-Quinpirole	D_2-like agonist

Antagonists

0678	(+)-AJ 76	Antagonist; preferential action at D_2-like autoreceptors
0524	AMI-193	D_2-like receptor ligand
0782	2-Chloro-11-(4-methylpiperazino)dibenz[b,f]oxepin	Ligand with high affinity for D_4
0444	Clozapine	Dopamine antagonist. Some D_4 selectivity. Also muscarinic antagonist and 5-HT ligand
0701	3´-Fluorobenzylspiperone	Potent D_2-like receptor ligand
0931	Haloperidol	Antagonist, partly D_2 selective
0679	(1S,3R)-*cis*-5-Methoxy-1-methyl-2-(dimethylamino)tetralin	Antagonist at pre- and postsynaptic sites
0937	Pimozide	D_2-like antagonist
0916	Remoxipride	Selective D_2-like antagonist
0894	(RS)-(±)-Sulpiride	Standard selective D_2-like antagonist
0895	(S)-(-)-Sulpiride	Standard selective D_2-like antagonist
0775	(+)-UH 232	D_2-like autoreceptor antagonist. D_3 partial agonist

D_2 selective compounds

1003	L-741,626	High affinity D_2 antagonist

D_3 selective compounds

1109	GR 103691	D_3 antagonist (> 100-fold selective)
0719	7-HydroxyPIPAT	D_3 agonist ($D_3 > D_2$)
1243	(+)-PD 128907	D_3 agonist ($D_3 \geq D_2 > D_4$)
1357	U 99194	Potent D_3 antagonist

D_4 selective compounds

1005	L 741,741	Potent, selective D_4 antagonist
1004	L 741,742	Highly selective D_4 antagonist
1002	L 745,870	Highly selective D_4 antagonist
1065	PD 168077	High affinity, selective D_4 agonist

Dopamine uptake / release inhibitors

0717	1-(2-Benzo[b]thienyl)-N-butylcyclo hexanamine	Uptake inhibitor
0720	1-[1-(2-Benzo[b]thienyl)cyclohexyl]pyrrolidine	Uptake inhibitor
0718	1-Benzo[b]thien-2-yl-N-cyclopropyl methylcyclo-hexanamine	Uptake inhibitor
0918	3α-Bis-(4-fluorophenyl)methoxytropane	Uptake inhibitor
0702	BTCP	Uptake inhibitor
0917	3α-[(4-Chlorophenyl)phenylmethoxy]-tropane	Uptake inhibitor
0513	GBR 12783	Uptake inhibitor
0421	GBR 12909	Selective DA uptake inhibitor
0514	GBR 12935	Uptake inhibitor
0420	GBR 13069	Uptake inhibitor
0730	4-Phenyl-1,2,3,4-tetrahydroisoquinoline	DA release inhibitor

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