Multiple dopamine receptors: relevance for neurodegenerative disorders

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In several neurodegenerative disorders such as Parkinson’s disease and Huntington’s disease, neuronal systems employing the neurotransmitter dopamine have been implicated in either the pathology or the drug treatments of the disease (for review see [1]). It is therefore of some importance to understand the mechanism of action of dopamine in controlling brain function, both in the normal state and in disease states.

**Multiple dopamine receptors**

Dopamine mediates its actions via binding to receptors. Dopamine receptors have been studied intensively over the last 30 or more years. From biochemical and pharmacological studies, two distinct dopamine receptor subtypes were identified (D₁ and D₂) [2]. The application of molecular biological techniques showed, however, that there were at least five dopamine receptor subtypes (D₁–D₅) [3, 4]. These may be divided into ‘D₁-like’ (D₁ and D₅) and ‘D₂-like’ (D₂, D₃ and D₄) subgroups on the basis of structural and functional properties reflecting the earlier classification.

When the amino acid sequences of the receptor subtypes, derived from the DNA sequence, were analysed, each conformed to the now classical model, typical of a G-protein-linked receptor: seven putative transmembrane spanning (α-helical) regions, linked by loops of protein outside the membrane. The seven α-helices are thought to cluster within the membrane to form the ligand-binding site and models for this have been proposed (for example, see [5]). There is significant amino acid homology between the dopamine receptor subtypes; this is greater between members of one subgroup (e.g. D₂/D₃) than between members of different subgroups (e.g. D₁/D₂).

| Abbreviations used: GABA, γ-aminobutyrate; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; L-DOPA, 3,4-dihydroxyphenylalanine; SCH 23390, 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine. |

The pharmacological and biochemical properties of the subtypes defined by gene cloning have been established by expressing the cloned gene in a suitable host system, such as Chinese hamster ovary (CHO) cells (see for example [6, 7]). The D₁-like receptors (D₁ and D₅) have similar pharmacological properties (high affinity for SCH 23390, low affinity for sulpiride) but dopamine shows a ten-fold higher affinity for D₁. The D₂-like receptors (D₂, D₃ and D₄) all have high affinities for drugs such as haloperidol and sulpiride, and a low affinity for SCH 23390, but each subtype has a unique pharmacological profile. In particular, D₁ has a higher affinity than D₂ or D₃ for certain agonists, for example, dopamine, pergolide and quinpirole.

The distribution of the dopamine receptor subtypes has been defined in different brain regions from the mRNA distribution. This showed that D₁ and D₂ receptors were the principal dopamine receptor subtypes in brain, being present at high levels in regions such as the neostriatum (caudate nucleus/putamen), nucleus accumbens and olfactory tubercle. D₃, D₄ and D₅ are present at lower levels in brain, each with different distributions. The distribution of D₁ is of some interest as it is localized largely in limbic regions of brain.

The D₂-like receptors exist in variant forms based on alternative splicing of the gene to produce variants with third intracellular loops of different lengths: D₂short/D₂long [8]; D₃short/D₃long [9]; and polymorphic variants of D₄ [10]. Although these variants do exhibit slightly different pharmacological properties [7, 10], and have different distributions in brain, their functional properties are not sufficiently different or defined to warrant consideration as separate subtypes and I shall consider them below as single subtypes.

**Neuronal localization of dopamine receptors in the neostriatum**

In order to understand the role of dopamine and dopamine receptors in normal brain function and in
neurodegenerative disorders, it is important to define the neuronal localization of the subtypes. Here I shall confine the discussion to the basal ganglia, and in particular the neostriatum (caudate nucleus/putamen), since this part of the brain is important for understanding Parkinson's and Huntington's diseases. This discussion will also largely concern D₁ and D₂ dopamine receptors as these are the principal dopamine receptor subtypes in the neostriatum.

The neostriatum contains several neuronal cell types. The predominant neuronal cell type is the medium spiny cell, which forms the principal outflow pathway of the neostriatum and constitutes about 95% of the neuronal cells in the neostriatum [11]. Medium spiny cells contain the neurotransmitter γ-aminobutyrate (GABA). About half the cells also contain the co-existing neuropeptide substance P, the other half additionally contain the neuropeptide enkephalin. This chemical differentiation of medium spiny cells seems to parallel an anatomical differentiation. GABA/substance P-containing medium spiny neurones form the so-called 'direct' pathway, linking the neostriatum to the two principal outflow nuclei of the basal ganglia, the substantia nigra pars reticulata and the globus pallidus internal segment (SN/GP_i). GABA/enkephalin-containing medium spiny cells form the first part of the 'indirect' pathway, linking the neostriatum to SN/GP_e and subthalamic nucleus. In addition to the medium spiny cells, there are a number of minor neuronal populations including a population of aspiny cholinergic cells. For more detailed reviews of this see [12–14].

The distribution of dopamine receptor subtypes within the neostriatum has been defined in several ways. Studies at the mRNA level using in situ hybridization have shown that the D₁ and D₂ receptors are located preferentially on the GABA/substance P- and GABA/enkephalin-containing medium spiny neurones [15, 16], although there may be some medium spiny neurones containing both receptor subtypes respectively [15, 17, 18]. Evidence for D₂ receptors on the aspiny cholinergic cells has also been obtained [19].

Functional studies have also provided evidence for the segregation of D₁ and D₂ receptors on different populations of medium spiny neurones. The regulation of expression of c-fos in the direct and indirect neostriatal outflow pathways corresponds to the effects of dopamine on neostriatal D₁ (stimulatory) and D₂ (inhibitory) receptors respectively [20]. Studies of the effects of selective D₁ and D₂ receptor agents on the expression of neuropeptide genes or glucose utilization by neostriatal neurones are also consistent with this segregation (reviewed in [12, 14]).

These studies, therefore, suggest a differential expression of dopamine receptor subtypes on neuronal populations in the neostriatum. In contrast, recent work has been reported that suggests extensive colocalization of dopamine receptor subtypes on single neurones in the neostriatum. Amplification of mRNA, aspirated from single striatongiral neurones, showed the presence of D₁, D₂ and D₃ receptor mRNA, although the levels of D₁ receptor mRNA were the highest [21]. Since mRNA for a species may be present but not expressed as active receptor protein, several groups have also been working to obtain antibodies specific for the different dopamine receptor subtypes (see for example [22, 23]). Immunofluorescent analysis of the distribution of D₁ and D₂ receptor showed that medium spiny neurones expressed both D₁ and D₂ receptor protein whether the chemical subtype was GABA/substance P or GABA/enkephalin [24]. These suggestions of colocalization of several dopamine receptor subtypes on single neurones, are consistent with some biochemical and functional studies which show that dopamine acting on D₁ and D₂ receptors can influence the activity of the same neurone (reviewed in [25]).

It is difficult at present to reconcile these two conflicting views on receptor localization in the neostriatum. Surmeier et al. [25] have proposed that, whereas dopamine receptor subtypes are colocalized in a large proportion of medium spiny neurones, this colocalization is only expressed at the neuronal cell body and dendrites. At the nerve terminals there is segregation of receptor subtypes so that the terminals of GABA/substance P-containing cells contain D₁ receptors and the terminals of GABA/enkephalin cells contain D₂ receptors. This then provides a mechanism for selective effects of D₁ and D₂ agonists on neurotransmitter release from the two neuronal populations. It is not clear, however, how this relates to normal effects of dopamine released from mesostriatal dopamine neurones. Nevertheless, the studies of Robertson et al. [20] on the expression of c-fos did show clearly segregated effects of D₁ and D₂ dopamine receptors within the neostriatum, rather than at nerve terminals. At present it seems prudent to reserve judgment on this issue, but both sets of studies do provide mechanisms for selective effects of D₁ and D₂ receptor agonists on the activities of the different neostriatal neuronal efferent populations.
Role of the neostriatum in control of movement

The studies outlined above are important for understanding movement disorders because of the critical role of the neostriatum in controlling voluntary movement. The direct and indirect neostriatal efferent pathways play opposing roles; activity in the direct pathway ultimately facilitates movement, activity in the indirect pathway inhibits movement. Therefore, some kind of balance between the two neostriatal efferent pathways needs to be maintained if movement is to be facilitated overall, in favour of the direct pathway. Dopamine (released from mesostriatal neurones projecting to the neostriatum from the substantia nigra pars compacta) may achieve this balance. Dopamine has been shown to facilitate neuronal activity in the direct pathway and inhibit that in the indirect pathway. Evidence for this comes from studies on the electrophysiological activity of neostriatal neurones in animals with dopamine lesions (reviewed in [26]), glucose utilization by neostriatal neurones [27], peptide gene expression in neostriatal neurones [15] and c-fos expression in neostriatal [20] neurones. Therefore, dopamine may be important in maintaining the balance between the direct and indirect pathways, in favour of the former.

Originally, it was proposed that the dual effects of dopamine could be via differential subcellular distributions of dopamine receptors on neostriatal neurones, or segregated D₁ and D₂ dopamine receptors on the cell bodies of different populations of medium spiny neostriatal neurones, influencing the direct and indirect pathways respectively [14, 28, 29]. Because the idea of segregation of D₁ and D₂ receptors on cell bodies has been questioned (see above), it is not clear how secure this argument is.

Nevertheless, there is evidence that dopamine does facilitate activity in the direct pathway, and inhibit activity in the indirect pathway. There is also evidence that occupancy of D₁ and D₂ receptors is required for full expression of movement [30]. As outlined earlier, whether D₁ and D₂ dopamine receptors are segregated or not, mechanisms can be proposed for selective effects of D₁ and D₂ agonists on different neostriatal neuronal populations and hence the direct and indirect pathways respectively. It seems that for adequate control of movement, an appropriate balance must be set between the activities of the direct and indirect neostriatal efferent pathways and both D₁ and D₂ dopamine receptors must be occupied. These two requirements may be linked as outlined above.

Relevance to neurodegenerative disorders

Parkinson's disease

In Parkinson's disease the principal neurodegenerative change is the loss of the mesostriatal dopamine innervation from the substantia nigra pars compacta to the neostriatum. If dopamine is important in maintaining a balance between the direct and indirect neostriatal output pathways, as indicated above, then loss of dopamine will shift the system towards a state where the indirect pathway predominates. Since this is inhibitory for movement, the relative paucity of movement in Parkinson's disease can be rationalized (see for example [3, 29, 31]).

Given that the control of normal movement by dopamine requires occupancy of D₁ and D₂ receptors on neostriatal neurones, treatment of Parkinson's disease will require that this occupancy be re-established. l-DOPA (converted to dopamine) presumably achieves this, as do certain mixed D₁/D₂ agonists such as apomorphine and pergolide. The efficacy of bromocriptine, a selective D₂ agonist, in treating parkinsonism may require the release of endogenous dopamine from surviving mesostriatal terminals to achieve D₁ receptor occupancy. Trials with selective D₁ and D₂ receptor agonists (reviewed in [29]) have provided some support for the idea that occupancy of both D₁ and D₂ dopamine receptors is critical for restoring movement control in humans or monkeys with a dopamine-denervated neostriatum.

The effects of D₁ and D₂ receptor occupancy on the treatment of movement defects in Parkinson's disease can be considered in the light of the selective localization of these receptor subtypes on the direct and indirect neostriatal output pathways [29], or selective effects of D₁ and D₂ receptor activation on the terminals of these pathways [25].

A notable feature of certain drugs used to treat Parkinson's disease is their high affinity for D₁ receptors, for example, dopamine, pergolide. D₁ dopamine receptors are localized predominantly in the limbic regions of brain, e.g. nucleus accumbens, olfactory tubercle, which themselves receive a dopamine innervation from the mesostriatal or mesocortical dopamine pathways. The activity of dopamine in the nucleus accumbens is thought to be important in providing motivation for actions. It may be, therefore, that drugs used to treat parkinsonism occupy D₁ and D₂ receptors in the neostriatum, providing basic control of movement, and occupy D₁ receptors in the nucleus accumbens (in addition to D₁ and D₂ receptors) to provide motivation for movement.
In experimental animals, dopamine denervation of the neostriatum leads to changes in the number of dopamine receptors. These changes may be important in humans suffering from Parkinson's disease. Unfortunately, data on the numbers of \( D_1 \) and \( D_2 \) receptors in the neostriatum of patients with Parkinson's disease are variable and this may reflect the variable state of the patients and the post mortem material. In untreated parkinsonian patients, the number of \( D_1 \) and \( D_2 \) receptors measured post mortem tends to be raised in some, but not all, studies [32–34] and treatment with \( L-DOPA \) tends to return the receptor numbers to normal levels. In MPTP-lesioned monkeys, clear increases of neostriatal \( D_2 \) dopamine receptors have been reported whereas \( D_1 \) receptors are either elevated or unchanged [35, 36]. Using positron emission tomography in living patients, variable results were again seen [33]. Untreated patients with Parkinson's disease showed normal, or mildly elevated, levels of \( D_2 \) receptors, whereas in treated patients levels were normal or reduced. For \( D_1 \) receptors, no changes were seen in either treated or untreated parkinsonian patients [37, 38]. There may therefore be a trend towards greater changes in \( D_2 \) receptors in the neostriatum in Parkinson's disease. This may be relevant to the effects of drugs although the changes in receptor number are not great. It is unclear to what extent this will alter the sensitivity of certain neurones to the effects of \( D_2 \) receptor stimulation.

**Huntington's disease**

Although in the late stages of Huntington's disease there are extensive neuronal losses and brain shrinkage (especially in the neostriatum), in the early stages of the disease quite selective neuronal changes have been described [12]. Early neuronal losses seem to be in the medium spiny neostriatal neurones forming the indirect pathway. This will shift the balance of neostriatal output in favour of the direct pathway so that there may be excessive facilitation of movement. This may account for the abnormal involuntary movements (chorea) these patients exhibit.

Although there is no effective treatment for Huntington's disease, some relief of choreic symptoms may be achieved by treatment with dopamine antagonists. Since dopamine tends to poise neostriatal output pathways in favour of the direct pathway, dopamine antagonism will reduce this effect and may partially reduce the predominance of the direct pathway.

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Scrapie, Creutzfeldt-Jakob disease and bovine spongiform encephalopathy: the key role of a nerve membrane protein (PrP)

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Introduction

Astrocytosis, vacuolation of the neuropil and a progressive loss of neurones are the most common histological signs of scrapie, a transmissible (infectious) disease of sheep and goats [1]. This type of neurodegenerative disorder is found in other mammalian species: Creutzfeldt-Jakob disease (CJD, in man); Gerstmann-Straussler syndrome (GSS, man); bovine spongiform encephalopathy (BSE, cattle), transmissible mink encephalopathy (mink); chronic wasting disease (mule, deer and elk); and feline spongiform encephalopathy (cats). The incubation period of the disease, following transmission by inoculation or feeding, varies with dose of exposure, route of infection, species (and genotype) of host or recipient and strain isolate of pathogen. These factors may also influence the average age of onset of natural disease (given in parentheses) and this varies with species: for example, sheep (3.5 years) [2], cattle (4–5 years) [3] and man (kuru, 5–40 years; CJD, sporadic and familial, 50–75 years; GSS, 35–55 years) [4]. Clinical and biochemical indices of disease can also differ widely and for many years this biological variation confounded attempts to provide a molecular description of the neurodegenerative process.

In the 1960s, transmission of scrapie to inbred strains of mice simplified experimental approaches to these diseases and revealed a genetic locus (Sinc, with two alleles, s7 and p7) that determined the overall rate of pathogen replication and incubation period [5, 6]. Concurrently, over 20 different strains of scrapie were characterized by ranking the incubation periods, and the severity and location of vacuolar pathology which they produced in mice, of the three Sinc genotypes [1]. In some of these models, notably the 87V strain of scrapie in MB/DK (Sincp7) mice, large (40–100 μm) plaque-like deposits of congophilic amyloid were observed, similar (in morphology) to the amyloid plaques of Alzheimer’s disease [7]. In retrospect, these congregations of sparsely soluble protein fibrils held the key to a molecular understanding of this type of neurodegenerative disease.

The PrP protein

The plaque fibrils are composed of PrPSc, an abnormal form of a phosphoinositol-glycolipid-anchored membrane glycoprotein (PrPζ) found in the brain and, to a lesser extent, in other tissues [8]. In contrast to PrPζ, PrPSc is relatively resistant to proteolysis by proteinase K in low, non-denaturing,