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Synaptic mechanisms in the substantia nigra

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INTRODUCTION

(by D.W.S.)

I often wonder whether it was fate (or working in a relatively small subject) that forged my links with the Pharmacology Department at the School of Pharmacy. Thus, my mentor George Brownlee of King's College, London, had been a postgraduate student under Burn from 1937–39; also when I was at the A.R.C. Institute of Animal Physiology at Babraham in the early sixties the Director was J. H. Gaddum who had earlier succeeded Burn at Bloomsbury Square. However, it was Eric Horton's translation from the School of Pharmacy to the Chair of Pharmacology in Edinburgh in 1969 which provided me with the opportunity of moving from Edinburgh to London. Concurrently with these changes in venue I have maintained a continuing interest in the identification of and actions of chemical transmitters. It began with *in vitro* studies in the peripheral nervous system involving measurement of the bulk release of acetylcholine from motor nerve endings in response to electrical stimulation. It has now progressed to studies in the central nervous system *in vivo* and, in particular, the study of synaptic inhibition at the level of the single brain cell and the use of pharmacological antagonists as tools to identify central synaptic transmitters. The pursuit of these interests and the establishment of a group of neuropharmacologists at The Square has been made possible by generous support from the Medical Research Council. From our current research interests I have singled out by way of example the work on the substantia nigra.

The substantia nigra (SN) was first described one hundred and ninety years ago by Vicq d'Azyr (1786). Its name reflects the characteristic pigmentation seen in some species as a result of its high concentration of neuromelanin. The SN is thought to have a key role in the extrapyramidal control of movement. Thus as suggested by Trétiakoff (1919) and Hassler (1938; 1955) the SN shows lesions in Parkinson's Disease—though degeneration of neurons is also seen in the locus coeruleus and globus pallidus, with less marked changes in the striatum, thalamus and subthalamus. Since the SN contains the cell bodies of one of the main dopamine fibre systems, the nigro-striatal pathway (e.g. Fuxe, Hökfelt & Ungerstedt, 1970; Ungerstedt, 1971), this provides an explanation for the depletion of striatal dopamine in Parkinson's Disease (Ehringer & Hornykiewicz, 1960) and for the efficacy of levodopa. Also, it is not surprising that a Parkinson-like state is precipitated by drugs which deplete presynaptic stores of dopamine or block postsynaptic dopamine receptors.

It should also be noted that there is pharmacological and physiological evidence for the involvement of the nigrostriatal dopaminergic system in drug induced locomotor effects and stereotyped behaviour (Arbuthnott, Crow & others, 1970), while extrastriatal dopaminergic mechanisms have been implicated in schizophrenia and its response to neuroleptics and perhaps in the mechanism of action of anorectics and the consolidation of memory.

Because much research interest has been concentrated on the dopaminergic projection from the S N to the striatum, we decided to concentrate on the pharmacology of the S N neurons themselves and on the afferent inputs particularly from the striatum and the raphé to the S N, and the synaptic mechanisms intrinsic to the nigra. Parts of this review describe research done in the Pharmacology Department at The Square by a number of the academic and research staff and Dr Dray and I are grateful to them for their help and collaboration.

Morphology

The S N can be divided into two fairly distinct regions—the zona compacta (Z C) dorsomedially and the zona reticulata (Z R) ventrolaterally. The Z C has mainly dopamine-containing nerve cell bodies some 15–20 μm in diameter which project forwards to the striatum—it seems likely that there are also non-dopaminergic neurons in the Z C projecting to the striatum and these may comprise 20% of the nigrostriatal tract (Ljungdahl, Hökfelt & others, 1975; Fibiger, Pudritz & others, 1972). The Z R has a more extensive neuropil and contains long dendritic projections from the compacta cells

(Björklund & Lindvall, 1975). Most of the afferents to the nigra terminate here, though substantial numbers also end in the Z C. Indeed Fonnum, Grofova & others (1974) calculate 5·9% of the tissue volume of the Z C to be occupied by synaptic boutons c.f. 11·5% in the Z R. In the rat Z R there are larger cells perhaps 25–40 μm diameter (Gulley & Wood, 1971) which may provide the nigro-thalamic projection (Faull & Carman, 1968). This projection goes to the magno-cellular portions of the ventro-medial nucleus of the thalamus in rat and to perhaps analogous structures in higher species, e.g. the ventro-lateral and ventro-anterior nuclei of the thalamus in cat (Rinvik, 1975) and monkey (Carpenter & Peter, 1972). Additionally, dopamine-containing cells are found in the Z R though these are sparse except caudally (Sotelo, 1974). In both the Z C and Z R there also are a number of inter-neurons, but the connections between these, the perikarya and dendrites of the Z C nigrostriatal neurons, the Z R nigrothalamic neurons and the afferents to the S N is not known.

Content and distribution of putative transmitters

There are significant levels of a number of putative transmitter substances in the S N: these are outlined in Table 1. As would be expected the bulk of the dopamine is contained in the Z C. However, in man one third of the total dopamine is in the Z R and this can be attributed to dopamine cell bodies, compacta cell dendrites and perhaps to axon collaterals from the nigrostriatal output. Histochemical studies cited by Gulley & Smithberg (1971) also suggest a small number of noradrenaline-containing nerve endings in the neuropil of the Z R.

In contrast to the low noradrenaline levels, the levels of 5-HT in the S N are amongst the highest in the brain (Palkovits, Brownstein & Saavedra, 1974). Histochemical studies show 5-HT to be located predominantly in nerve terminals in the Z R (Fuxe, 1965) and it was suggested that the 5-HT input to the S N was from the raphé nuclei by way of axon collaterals from a main projection to the striatum (Poirier, Bédard & others, 1969). However, in the rat it seems likely that there is a discrete projection from the nucleus raphé medianus to the S N not involving the striatum. Thus seven days after making a small electrolytic lesion in the nucleus raphé medianus there was a more than 50% reduction in the 5-HT concentration in the S N, but no change in GABA concentration. In the same animals there was an increase in striatal dopamine levels, but significant changes in the concentrations of 5-HT, noradrenaline and GABA in the striatum were not seen (Dray, Gonye & others, 1976).

The concentration of GABA in the S N is the highest of any region in the nervous system. Fonnum & others (1974) calculate the GABA concentrations in the Z R to be 9 mM and the intraterminal concentration to be at least 60 mM. There are also high

Table 1. *Putative neurotransmitters in the substantia nigra, their concentration; effects on single substantia nigra neurons, antagonists and changes in disease.*

Putative neurotransmitters	Concentration in substantia nigra per g tissue		Effects on single neurons			Antagonists administered electrophoretically or systemically	Changes in diseased states
	Human	Rat	Z C	Z R	Un-defined		
Dopamine	3·2 nmol[9]	9·1 nmol[1]	−[2,7]	±[7]	−[8]	i.v. Haloperidol[3,7]* i.v. Chlorpromazine[3] α-Flupenthixol[7]* Methiothepin[7]*	− Parkinsonism[9]
5-Hydroxytryptamine	5·8 nmol[9]	10·9 nmol[7]	−[7,4] +[7]	±[7,4]	±[6]	α-Flupenthixol[7]* Methiothepin[7]*	− Parkinsonism[9] + Huntingtons Chorea[9]
Noradrenaline	0·24 nmol[9]		−[7]	±[7]	±[6]		
Acetylcholine		8·8 nmol[5]	+[7]	+[7,2]	−[8] +[8]	Atropine[7]* *Scopolamine*[3]*	− (ChAc,) Parkinson[13]
GABA	5.3 μmol[10]	11·0 μmol	−[7]	−[7]	−[6,8]	i.v. Picrotoxin[13] Picrotoxin[6]* Bicuculline[7]*	− (GAD) Parkinsonism − Huntingtons Chorea[10]
Glycine	2·3 μmol[10]	1·2 μmol[7]	−[7]	−[7]	−[6]	Strychnine[7]*	
Taurine	1·2 μmol[10]	3·5 μmol[7]	−[7]	−[7]			
Substance P	0·37 μmol[11]		+[7]	+[7]		β-pCPG[7]*	

− = depression. + = excitation. * Studies where another agonist was used.

Values derived from:

[1] Andén & others (1966)
[2] Aghajanian & Bunney (1974a)
[3] Aghajanian & Bunney (1974b)
[4] Aghajanian & Bunney (1975)
[5] Cheney & others (1975)
[6] Crossman & others (1974)
[7] From the authors' laboratory
[8] Feltz (1971)
[9] Lloyd & Hornykiewicz (1974)
[10] Perry & others (1973)
[11] Powell & others (1973)
[13] Precht & Yoshida (1971)
[13] Lloyd & others (1975)

concentrations of glutamic acid decarboxylase (GAD) which are maximal in the region of the Z C–Z R junction and fall off in the medial part of the Z C and lateral part of the Z R. Lesions in the caudate nucleus, putamen and globus pallidus cause a marked decrease in GABA in the rat S N, and GAD in the rat and cat S N (Kim, Bak & others, 1971; McGeer, McGeer & others, 1971; Hattori, McGeer & others, 1973; Fonnum & others, 1974), but the exact origin of these striato-nigral fibres, particularly an origin in the globus pallidus, is not resolved. This evidence is thought to be compatible with a descending monosynaptic inhibitory pathway as the changes after lesioning are perhaps too gross to be due to loss of transynaptic influences on inhibitory interneurones within the S N. It is difficult to exclude the possibility that some or all the S N GABA is contained in intrinsic inhibitory interneurons (Wolman, 1971) which are excited principally by striato-nigral impulses—that is, that the descending inhibitory pathway is polysynaptic.

Acetylcholine and its synthetic enzyme choline-acetylase (ChAc) occur in the S N. On the basis of histochemical studies on acetylcholinesterase (AChE), Olivier, Parent & others (1970), suggested that there was a descending cholinergic pathway from the caudate nucleus to the S N. However, both Fonnum & others, and McGeer & others, have been unable to show significant depletion of ChAc in the S N following a variety of striatal lesions which caused a marked depletion of GAD in the S N. Indeed, the ratio between AChE and ChAc at about 1000 is sufficiently large to suggest that the AChE is not a reliable marker for cholinergic neurons in the S N (Fonnum & others, 1974). Thus, until an afferent cholinergic pathway can be confirmed, it must be assumed that the cholinergic system is intrinsic to the S N.

The levels of substance P in the S N are higher than in any other part of the brain (Powell, Leeman & others, 1973). Immunohistochemical studies suggest that substance P in brain is predominantly associated with nerve fibres, and with synaptosomal fractions. In the rat, substance P appears to be mainly in the Z C of the S N and parallels the immunohistochemical distribution of tyrosine hydroxylase (Hökfelt, Kellerth & others, 1975).

Pharmacology

There have been relatively few pharmacological studies of S N neurons; some have utilized the technique of iontophoresis where drug ions are expelled by the passage of appropriate current from the tips of multibarrelled glass micropipettes into the environment of single neurons whilst simultaneously recording changes in cell activity (usually extracellularly) from another barrel of the micropipette. The advantages of this technique in avoiding the hazards of metabolism and of the blood brain barrier and of action at sites very distant from the neuron under study are clear. Some of the disadvantages should also be stressed, particularly (i) that it is possible to achieve high and possibly unphysiological concentrations of drugs in the neuronal environment and (ii) that a demonstration of responses to and receptors for putative transmitters is not by itself proof that such substances are released as transmitters (see also Kelly, Simmonds & Straughan, 1975).

The earliest pharmacological studies were made in the S N of Dial-anaesthetized cats by Feltz (1971) who showed that glutamate strongly excited and GABA strongly depressed all the cells to which they were applied. Similar results have been obtained for the rat S N by Crossman, Walker & Woodruff (1974) and Dray & Gonye (1975). Feltz also reported glycine to be totally ineffective as a depressant and this contrasts with the results obtained in rat by Crossman & others, and by Dray & Gonye, showing glycine to be a potent depressant on most neurons tested. The GABA receptor in rat S N appears to be similar to that encountered in most other areas of the nervous system in that it is readily blocked by iontophoretic picrotoxin or bicuculline methochloride (Crossman & others, 1974; Dray & Gonye, 1975). Also, small iontophoretic ejections of strychnine can selectively block the glycine, but not the GABA-induced depression of S N neurons (Dray & Gonye, 1975). The lack of selectivity of strychnine reported by Crossman & others (1974) is perhaps attributable to non-specific effects consequent upon the high ejecting currents they used for strychnine.

Other differences between the cat and the rat S N are evident with respect to ACh and dopamine. Thus, Feltz reported a weak slow depression from these putative neurotransmitters which was difficult to distinguish from the effect of the iontophoretic current itself (whether the neurons were Z C or Z R was not indicated). However, in rat S N ACh readily excites Z R neurons, but there is disagreement as to whether Z C cells are also excited by ACh (Dray, Gonye & Oakley, unpublished results) or relatively unaffected (Aghajanian & Bunney, 1974a). The reported ACh excitations are readily and selectively blocked by iontophoretic atropine (Dray, Gonye

& Oakley, unpublished observations) and scopolamine (Aghajanian & Bunney, 1974a). Dopamine readily depresses Z C cells in the rat but again there is disagreement as to dopamine actions on Z R cells, Aghajanian & Bunney (1974a) reporting that they are relatively unaffected while Dray & others (1976) find Z R cells are usually depressed, but sometimes also excited by dopamine. The reasons for these differences between laboratories is not clear. Studies using monoamine antagonists have not been extensive; systemically administered haloperidol (a butyrophenone neuroleptic) blocks dopamine- and apomorphine-induced depression of S N cells (Aghajanian & Bunney, 1974b), but unfortunately, no control agonists were used to test the specificity of this effect. This is important as we find iontophoretic applications of α-flupenthixol (a thioxanthene neuroleptic) to be only marginally more selective for dopamine-induced depression of S N neurons than for 5-HT induced depression. Where dopamine excitation occurs, it appears to be more readily blocked than dopamine depression.

In the rat S N the predominant effect of 5-HT is one of depression in the Z C, but in the Z R both depression and excitation by 5-HT are seen (Aghajanian & Bunney, 1975; Dray, Oakley & Straughan, 1975).

Iontophoretic applications of methiothepin, which has been reported to be an effective antagonist of 5-HT-induced depression in other areas of brain (Tebecis, 1972), blocked 5-HT induced depression in the S N and was marginally less effective against dopamine-induced depression. Selectivity between monoamine and amino acid effects is much better; iontophoretic applications of methiothepin and α-flupenthixol which block monoamine depression are without effect on GABA depression.

Recent studies by Davies & Dray (1976) show that substance P produces a slow and small excitation of S N neurons as does the analogous polypeptide eledoisin. These polypeptides did not have the great excitant potency expected from studies in other areas, e.g. the spinal cord. The centrally acting muscle relaxant β-chlorophenyl-GABA (Baclofen) has been reported by Saito, Konishi & Otsuka (1975) to antagonize selectively the excitant effects of substance P in rat spinal cord. Iontophoretic applications of this agent had intrinsically depressant effects on all S N neurons tested and while a selective effect against sP could sometimes be seen this was not consistent and on other occasions the excitatory effects of ACh and glutamate were also reduced.

Perhaps the clearest pharmacological differences between the cells in the Z C and those in the Z R occurs in response to parenterally administered (+)-amphetamine. This causes a prolonged depression of Z C cell firing (Aghajanian & Bunney, 1974b), sometimes after an initial excitation (Rebec & Groves, 1975) but an increased firing in the Z R as well as in the mid-brain reticular formation. These effects appear to be exerted in the striatum since lesions interrupting the nigrostriatal pathway block the amphetamine but not apomorphine depression of S N cell firing. Also, iontophoretic (+)-amphetamine has weak effects in contrast to the powerful inhibitory effects of iontophoretic apomorphine on Z C cells. However in other experiments (Groves, Wilson & others, 1975) intranigral infusions of amphetamine are effective and thus amphetamine may after all act within this area.

Physiological and pharmacological analysis of synaptic inputs into the substantia nigra

We have already seen from the deafferentation-neurochemical experiments that there seems to be a direct descending GABA releasing pathway from the striatum to the S N which would be inhibitory on the target cells in the S N. However, in cat and rat, though stimulation of the caudate nucleus elicits a constant latency inhibition of S N neurons which is believed to be monosynaptic, on many occasions inhibition is preceded by excitation (Frigyesi & Purpura, 1967; Feltz, 1971; Feger & Ohye, 1975; Dray & Gonye, 1975). The mechanism of this excitation and the identity of the transmitter involved has not been investigated and cannot thus be discussed here. However, the inhibition of nigral cells produced by caudate stimulation has been subjected to pharmacological investigation; in the rat, synaptic inhibition and the effect of iontophoretic GABA are blocked by iontophoretic picrotoxin (Crossman, Walker & Woodruff, 1973) or by bicuculline methochloride at a time when the responses to glycine are unaffected (Dray & Gonye, 1975). Characteristically, the applications of bicuculline methochloride required to block synaptic inhibition in the S N were about twice as large as those needed to block the effects of iontophoretic GABA, whilst the evoked inhibition was unaffected by iontophoretic applications of strychnine twice those needed to block the effects of iontophoretic glycine (Dray & others, 1976). These results thus confirm and extend those in the cat described by Precht & Yoshida (1971) where intravenous injections of picrotoxin blocked the caudate evoked inhibition of S N neurons (which were resistant to intravenous

injections of strychnine); however, it must be noted that in Precht & Yoshida's experiments no check was made on the sensitivity of the S N neurons to GABA or glycine, thus non-specific block was not excluded. The pharmacological evidence therefore supports the view that descending inhibitory impulses from the caudate release GABA as an inhibitory transmitter in the S N. The functional connection of GABA releasing nerves in the S N is unknown. It is assumed that they directly inhibit the dopaminergic Z C cells and reduce the nigrostriatal output. However, recent experiments in our laboratory are in conflict with this view. Thus experiments have been made (Dray, Oakley & Simmonds, 1975) in which the concentration of GABA was elevated unilaterally in the Z R of the rat S N by focal injection of ethanolamine-*O*-sulphate, an irreversible active site directed inhibitor of GABA transaminase, into the Z R. This caused a significant decrease in ipsilateral striatal dopamine content (Dray, Fowler & others, 1975) and an increase in homovanillic acid. This, coupled with ipsilateral turning in response to apomorphine and amphetamine injection was suggestive of an ipsilateral increase in impulse traffic in ascending nigrostriatal dopaminergic neurons. The implication is that GABA inhibits some other inhibitory influence on the dopaminergic neurons.

From the neurochemical and iontophoretic experiments made in our laboratory, it appears that 5-HT might be an inhibitory (or an excitatory) transmitter in the S N. To evaluate this, several hundred spontaneously active S N cells have been studied during focal stimulation in the median raphé nucleus. About 68% showed some response and of these responsive cells 52% showed a simple inhibition; 31% an inhibition preceded by excitation and only 17% of the S N cells responded to raphé stimulation with simple excitation. There was a highly significant correlation between the response of S N cells to raphé stimulation and their response to 5-HT. This correlation was not seen when raphé stimulation was compared with the response to dopamine. This then provides additional supporting evidence for the belief that 5-HT is a transmitter in the S N. However, pharmacological confirmation of this, at the single neuronal level is not available (cf. the studies with GABA antagonists). Thus, though we have shown that methiothepin blocks the effects of raphé stimulation and those of 5-HT on many S N neurons, the selectivity of methiothepin is poor since it also reduces dopamine responses and indeed similar effects can be achieved with α-flupenthixol. Additionally, methiothepin affects synaptic responses in the S N at the same time as responses to iontophoretically applied 5-HT, in contrast to the relative resistance of striatonigral inhibition to GABA antagonists. Clearly there is a need for more extensive pharmacological studies on S N cells with more selective 5-HT antagonists.

A number of neuropharmacological and neurochemical problems remain with regard to identification of the chemical transmitters in the S N particularly those mediating excitation from the striatum and the need to establish more firmly the suggested intrinsic cholinergic system. Also the complex synaptic organisation of the S N needs elucidating, and this will require afferent stimulation combined with simultaneous recording from identified neuron types, particularly nigrostriatal and nigrothalamic 'output' cells. The nigrothalamic pathway provides attractive possibilities for additional links between the striatum and cerebellum and the significance of this pathway needs to be determined.

Perhaps the basic research studies will provide a basis for understanding the role of nigral and striatal GABA in the extrapyramidal control of movement. The depletion of GABA and GAD in Parkinson's Disease (? disuse atrophy) and their recovery with prolonged dopa therapy should be noted. The marked depletion of nigrostriatal GABA in Huntington's Chorea should further increase interest in the role of GABA. More speculatively the work we have described is not incompatible with a role for a raphé-nigral system in extrapyramidal control. It is of interest that Parkinson's Disease and other dyskinesias (Barbeau, 1962; Chadwick, Reynolds & Marsden, 1974) in man respond to 5-hydroxytryptophan treatment and also that substantial increases in 5-HT concentration in the S N and striatum have been reported in Huntington's Chorea (Lloyd & Hornykiewicz, 1974).

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