METABOLISM OF DOPAMINE AND L-DOPA IN HUMAN BRAIN

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INTRODUCTION

STUDIES concerned directly with the metabolism of dopamine (DA) in the human brain have been limited largely to the use of post mortem material. Despite this limitation, the results leave little doubt that in the human brain the formation and degradation of DA (and other biogenic amines) follows routes well established in laboratory animals. Detailed studies in human brain material obtained at autopsy demonstrated that the distribution pattern of DA and other monoamines, as well as their metabolites, was quite similar to the pattern found in freshly obtained animal brains (cf. HORNYKIEWICZ, 1966, 1972a,b, 1973). Differences between human and animal material in respect to the actual concentrations of the compounds detected are well within the range of interspecies variations observed in fresh animal brain material. An obvious point of caution are certain enzymes whose activity may decline sharply after death. However, animal studies show that the enzymes concerned with the synthesis and catabolism of catecholamines (CA) are comparatively stable in this respect. Therefore, there is at present no reason to doubt that human post mortem brain material represents a valuable and valid source of information on the behaviour and functional role of biogenic amines.

METABOLISM OF DA IN THE NORMAL HUMAN BRAIN

As in all mammalian species studied, in man the nuclei of the basal ganglia complex, notably the caudate nucleus and putamen (these constitute the corpus striatum or striatum), the substantia nigra and the globus pallidus, contain a major portion (80 per cent or more) of the total brain DA (SANO *et al.*, 1959; EHRINGER and HORNYKIEWICZ, 1960; BERTLER, 1961) and its metabolic end product homovanillic acid (HVA) (BERNHEIMER, 1964) (Table 1). The above nuclei are also among those brain regions containing the highest activites of the CA-synthesising enzymes L-tyrosine hydroxylase (TH) (VOGEL *et al.*, 1969; LLOYD, 1972) and aromatic L-amino acid (L-DOPA) decarboxylase (DOPA D) (LLOYD and HORNYKIEWICZ, 1972) (Table 1) as well as the catabolising enzymes monoamine oxidase (MAO) (BIRKHÄUSER, 1940) and catechol-*O*-methyl transferase (COMT) (VOGEL *et al.*, 1969; LLOYD, 1972). In the caudate nucleus and putamen the concentration of HVA is in the same range as that of DA; in contrast, in the substantia nigra and the globus pallidus, the levels of HVA are 5–10 fold higher than those of the parent compound (cf. Table 1). The functional significance of these inter-regional differences in the metabolic rate of DA is not yet established, but it is logical to assume that they reflect the fact that in the striatum DA is contained exclusively in nerve terminals whereas in the substantia nigra this amine is found in neuronal pericarya only. This suggests the possibility

TABLE 1. NEUROCHEMISTRY OF BASAL GANGLIA DISORDERS: PARKINSON'S DISEASE (PD) AND HUNTINGTON'S CHOREA

		Caudate nucleus	Putamen	Substantia nigra	Globus pallidus
DA	Controls	2·64 ± 0·30 (28)	3·44 ± 0·29 (28)	0·49 ± 0·09 (5)	0·42 ± 0·08 (4)
	PD	0·43 ± 0·09 (13)	0·04 ± 0·01 (13)	0·07 ± 0·01 (10)	0·10 (3)
	mild akinesia	0·58 ± 0·12 (13)	0·44 ± 0·21 (13)	—	—
	marked akinesia	0·22 ± 0·08 (9)	0·05 ± 0·02 (9)	—	—
	HemiP right side	1·25	0·93	<0·01	—
	left side	0·59	0·13	<0·01	—
	Huntington's Chorea	1·56 ± 0·20 (10)	2·71 ± 0·35 (10)	0·51 ± 0·15 (5)	—
HVA	Controls	3·23 ± 0·27 (8)	4·29 ± 0·68 (8)	1·79 ± 0·18 (5)	2·12 ± 0·27 (8)
	PD	1·05 ± 0·16 (13)	0·89 ± 0·12 (13)	0·41 ± 0·08 (9)	0·77 ± 0·12 (12)
	mild tremor	1·68 ± 0·35 (7)	2·03 ± 0·55 (7)	—	1·71 ± 0·36 (7)
	marked tremor	1·26 ± 0·20 (11)	1·07 ± 0·22 (11)	—	0·70 ± 0·12 (10)
	Huntington's Chorea	2·02 ± 0·48 (8)	3·88 ± 0·63 (8)	2·09 ± 0·25 (3)	2·02 ± 0·27 (6)
TH	Controls	11·0 ± 0·7 (16)	10·2 ± 0·4 (17)	12·0 ± 2·3 (9)	7·7 ± 1·0 (10)
	PD	7·8 ± 1·1 (11)	6·5 ± 0·8 (12)	7·9 ± 0·5 (3)	4·8 (2)
DOPA D	Controls	364 ± 95 (19)	432 ± 109 (18)	549 ± 294 (15)	22 ± 3 (9)
	PD	54 ± 14 (13)	32 ± 7 (13)	21 ± 6 (10)	18 ± 3 (12)

All values are means ± S.E.M. (number of cases in brackets); dopamine (DA) and homovanillic acid (HVA) in μg/g, L-tyrosine hydroxylase (TH) and L-DOPA decarboxylase (DOPA D) in nmol/100 mg protein/2 hr. HemiP = Hemiparkinsonism (symptoms on right side of the body). The data are taken from: EHRINGER and HORNYKIEWICZ (1960); BERNHEIMER and HORNKYIEWICZ (1965); BAROLIN *et al.* (1964); BERNHEIMER *et al.* (1965; 1973), LLOYD (1972); LLOYD and HORNYKIEWICZ (1972); LLOYD *et al.* (1973).

that cell bodies are less effecient than the nerve endings in storing the newly synthesised, and recapturing the released, amine.

METABOLISM OF BRAIN DA IN BASAL GANGLIA DISORDERS

Parkinson's disease

Parkinson's disease is a disorder of the basal ganglia, characterised morphologically by degeneration of the melanin-containing nerve cells in the substantia nigra: the same nerve cells that give rise to the nigrostriatal DA pathway. The main extrapyramidal symptoms of Parkinson's disease are: akinesia, rigidity and tremor.

Neurochemically, Parkinson's disease is characterised by a marked decrease in the concentrations of DA (EHRINGER and HORNYKIEWICZ, 1960) and HVA (BERNHEIMER and HORNYKIEWICZ, 1965) as well as TH (LLOYD, 1972) and DOPA D (LLOYD and HORNYKIEWICZ, 1970) in the nigro-striato-pallidal system (Table 1). These neurochemical changes are characteristic of all Parkinsonian syndromes (EHRINGER and HORNYKIEWICZ, 1960; BERNHEIMER *et al.*, 1965; 1973) regardless of etiological factors involved (including the reversible condition produced by neuroleptic drugs such as reserpine, phenothiazines and butyrophenones). Therefore, Parkinsonism can be regarded, from a neurochemical point of view, as a "Striatal Dopamine Deficiency Syndrome" (HORNYKIEWICZ, 1972c). In Parkinson's disease proper there exists a significant correlation between the degree of cell loss in the substantia nigra and the degree of DA and HVA deficiency in the striatum (BERNHEIMER *et al.*, (1965; 1973).

The severity of the main symptoms also correlates significantly with the degree of striatal DA deficiency. This is demonstrated by the observations (Table 1) that (1) in a case with Hemiparkinsonism, the DA deficiency was markedly more severe in the striatum contralateral to the side of the symptoms (BAROLIN *et al.*, 1964); (2) the degree of akinesia was significantly correlated ($P < 0{\cdot}05$) with the degree of DA deficiency in the caudate nucleus (with a similar trend in putamen and globus pallidus) (BERNHEIMER *et al.*, 1973); and (3) the degree of tremor was significantly correlated ($P < 0{\cdot}01$) with the degree of HVA decrease in the globus pallidus (and possibly putamen, but not caudate nucleus) (BERNHEIMER *et al.*, 1973).

As could be expected, the degeneration of the nigrostriatal DA pathway produces a denervation supersensitivity of the striatum to DA. This is shown by the observation that in Parkinsonian patients the sensitivity of the akinesia to L-DOPA, DA's immediate precursor substance, was inversely related to the DA content of the striatum: cases with mild akinesia (i.e. significantly milder degree of striatal DA deficiency) responded less promptly to an i.v. test dose of L-DOPA than more severe cases (BERNHEIMER *et al.*, 1973). The presence of a (denervation) supersensitivity of the Parkinsonian striatum to DA explains the high susceptibility of the extrapyramidal symptoms to doses of L-DOPA that have hardly any significant effect on the basal ganglia in normal subjects.

Huntington's chorea

The extrapyramidal symptomatology of Huntington's chorea is dominated by the presence of a hyperkinetic-hypotonic state, that is abnormal involuntary movements associated with decreased muscle tone. This disturbance of the basal ganglia function can be related, on the morphological level, to the severe neuronal degeneration (especially loss of the small neurons) regularly observed in the caudate nucleus and putamen.

In Huntington's chorea the only abnormality of DA metabolism within the basal ganglia so far detected (Table 1) was a mild, but statisically significant ($P < 0{\cdot}05$), decrease (by approximately 40%) of DA and HVA in the caudate nucleus (BERNHEIMER and HORNYKIEWICZ, 1973; BERNHEIMER *et al.*, 1973). The levels of DA and HVA in the putamen and the other DA-containing nuclei of the basal ganglia remained essentially unchanged. Thus, it can be postulated that in Huntington's chorea there is a significant shifting of the dopaminergic balance "caudate-putamen" in favour of the putamen. In view of the clinical observations showing that antidopaminergic drugs (reserpine, phenothiazines, butyrophenones) depress the abnormal involuntary movements in the patients with Huntington's chorea and L-DOPA exacerbates them, it is tempting to speculate that the dopaminergic predominance of the putamen over the caudate nucleus may be responsible for the choreatic hyperkinesias.

Dopaminergic balance "caudate–putamen"

The possibility of an unbalanced dopaminergic relationship between the caudate nucleus and putamen as the cause of choreatic hyperkinesias, although speculative at the present time, is not lacking in indirect experimental support. Neurophysiological evidence shows that DA may have opposite actions in the caudate nucleus and putamen with the activity of caudate units inhibited (BLOOM *et al.*, 1965; CONNOR, 1970), and

those in the putamen facilitated (YORK, 1970) by this amine. Thus from the point of view of striatal DA, there might well exist a functional differentiation between the caudate nucleus and putamen, with caudate nucleus subserving mainly inhibitory, and putamen facilitatory functions for the dopaminergic control of motor functions.

METABOLISM OF L-DOPA IN THE BRAINS OF PATIENTS WITH PARKINSON'S DISEASE

Major metabolites of L-DOPA—DA *replacement*

Chemical analyses in brains of Parkinsonian patients treated chronically with high oral doses (2–6g daily) of L-DOPA until death disclosed (DAVIDSON *et al.*, 1971) that, in principle, the Parkinsonian brain metabolises L-DOPA along the same chemical pathways as the brain of untreated, normal laboratory animals. Thus, small amounts of DOPA and 5–10 fold higher levels of 3-*O*-methyl-DOPA were found throughout the brain (Table 2). The quantitative differences between the concentrations of DOPA and 3-*O*-methyl-DOPA reflect the fact that 3-*O*-methyl-DOPA has a much longer half-life (12–14 hr) as compared to the short half-life of L-DOPA (30 min) (BARTHOLINI and PLETSCHER, 1968). In contrast to the diffuse occurrence of these compounds in L-DOPA treated patients, DA accumulated exclusively, and HVA predominantly, in the striatal areas (Table 2). The magnitude of DA's increase in the caudate nucleus and putamen was strictly dependent on the size of the last dose of L-DOPA, and even more so on the time this dose was given before death; the latter fact is in agreement with the comparatively short half-life (2 hr) of the striatal DA (COSTA and NEFF, 1966.)

TABLE 2. NEUROCHEMISTRY OF L-DOPA IN PARKINSON'S DISEASE (PD)

		Caudate nucleus	Putamen	Substantia nigra	Globus pallidus
DA	PD untreated	0·43 ± 0·09 (13)	0·04 ± 0·01 (13)	0·07 ± 0·01 (10)	0·10 (3)
	PD + L-DOPA	1·76 ± 1·10 (4)	2·06 ± 0·72 (4)	—	—
	poor responders	0·22 (3)	0·05 (3)	—	—
	good responders	1·94 (3)	1·04 (3)	—	—
HVA	PD untreated	1·05 ± 0·16 (3)	0·89 ± 0·12 (13)	0·41 ± 0·08 (9)	0·77 ± 0·12 (12)
	PD + L-DOPA	9·14 ± 2·59 (4)	2·69 ± 3·84 (4)	7·98 (1)	10·93 (1)
	poor responde	2·69 (3)	4·18 (3)	—	—
	good responders	6·61 (3)	7·22 (3)	—	—
DOPA	PD + L-DOPA	0·53 + 0·09 (5)	0·58 ± 0·14 (5)	—	—
Me-DOPA	PD + L-DOPA	2·76 ± 1·14 (5)	3·58 ± 1·78 (5)	—	—
GAD	PD untreated	641 (2)	583 (2)	526 (2)	776 (2)
	PD + L-DOPA <8 months	339 ± 53 (4)	249 ± 24 (4)	300 ± 102 (4)	504 ± 50 (4)
	PD + L-DOPA >12 months	1172 ± 173 (5)	887 ± 95 (5)	1210 ± 110 (4)	1210 ± 109 (5)

All values are means ± S.E.M. (number of cases in brackets); dopamine (DA) and homovanillic acid (HVA), DOPA and 3-*O*-methyl-DOPA (Me-DOPA) in μg/g, L-glutamic acid decarboxylase (GAD) in nmol/100 mg protein/2 hr. Except for GAD, the data for the L-DOPA treated patients with Parkinson's disease (PD + L-DOPA) are from a group of patients who received the last dose of L-DOPA (0·5–1·5 g orally) no more than 2½ to 9 hr before death. Compiled from: LLOYD (1972); LLOYD and HORNYKIEWICZ (1973); LLOYD *et al.* (1973).

Although no DA could be detected, in the L-DOPA treated cases, in any of the other brain regions analysed, substantial concentrations of HVA accumulated also in extrastriatal brain areas. This shows that (1) in analogy to normal laboratory animals the Parkinsonian patient metabolises L-DOPA to DA essentially in all brain regions containing significant levels of DOPA D activity, and (2) only the basal ganglia appear to possess any significant storing capacity for DA, and, although heavily damaged, this DA-storing capicity is not completely lost in Parkinson's disease.

In conclusion, from the point of view of its main therapeutic activity, L-DOPA treatment in Parkinson's disease can be regarded as a DA replacement therapy. This conclusion is strongly supported by the observation that good response to L-DOPA could be related to the accumulation of markedly higher amounts of DA in the striatum than poor response to the drug (cf. Table 2, "good responders" and "poor responders") (LLOYD *et al.*, 1973).

L-DOPA *and the dopaminergic balance "caudate-putamen"*

There is evidence to show that the DA formed from L-DOPA is used up in the putamen of the treated cases significantly faster than in the caudate nucleus. This is probably due to the more severe damage of the DA storage and inactivation (re-uptake) mechanisms in the former region. Thus, in contrast to what is seen shortly after the administration of the drug, (cf. Table 2) in cases receiving the last dose of L-DOPA 10–24 hr before death, the concentration of DA in the putamen declined to very low (pre-DOPA) values whereas in the caudate nucleus the amine level was still elevated (approximately 1 μg/g); this was accompanied by an opposite behaviour of HVA (LLOYD and HORNYKIEWICZ, in preparation). This suggests the possibility that in Parkinsonian patients chronic L-DOPA treatment may result in a dopaminergic predominance of the putamen (cf. faster "utilisation" of DA) over the caudate nucleus. Thus, it is possible that L-DOPA's major side effect of abnormal involuntary (choreiform) movements may be due to such a putamenal predominance. It will be remembered that this factor has been considered as a possible cause of the hyperkinetic behaviour in patients with Huntington's chorea (see above).

"Minor" metabolites of L-DOPA*—possible "false" transmitters or* DA *antagonists*

In the animal organism, L-DOPA can give rise, via DA, to small quantities of complex condensation products (with aldehydes) such as tetra-hydroisoquinoline and tetrahydropapaveroline derivatives (SANDERS *et al.*, 1973). In the light of this possibility it is important to note that such compounds, if accumulated in high enough amounts might interfere with DA's effectiveness at the level of the striatal receptors, either by virtue of their being "false transmitters" within the CA terminals (cf. COHEN *et al.*, 1972) or partial agonists of DA at the receptors. The latter possibility is clearly borne out by observations that apomorphine, which may serve as the prototype of some of these complex L-DOPA metabolites and which has direct DA receptor simulating properties, is a potential DA antagonist (i.e. partial agonist), both in the periphery (GOLDBERG and MUSGRAVE, 1971; SIMON and VAN MAANEN, 1971; FERRINI and MIRAGOLI, 1972) and the CNS (BIEGER *et al.*, 1972). Thus, the possibility should be kept in mind that accumulation of pharmacologically effective concentrations of these complex L-DOPA metabolites might be involved in some puzzling side effects of

L-DOPA therapy, for example the "on-off" phenomenon; this side-effect is characterised by a sudden and transient loss of L-DOPA's therapeutic activity during the course of an otherwise successful chronic treatment with the drug. At present there is no pharmacological evidence that any of these complex L-DOPA condensation products possess significant direct antiakinesia and antirigidity activity of their own.

L-GLUTAMIC ACID DECARBOXYLASE (GAD) IN PARKINSON'S DISEASE—A POSSIBLE DA-GABA LINK IN THE BASAL GANGLIA

Subnormal GAD *levels in Parkinson's disease—effect of* L-DOPA

In parkinson's disease, the activity of GAD is significantly decreased in the nuclei of the nigro-striato-pallidal complex (BERNHEIMER and HORNYKIEWICZ, 1962; LLOYD and HORNYKIEWICZ, 1973) (Table 2). Recent observations disclosed that prolonged treatment of the patients with high doses of L-DOPA had a significant influence on the levels of GAD in the basal ganglia (LLOYD and HORNYKIEWICZ, 1973). Thus, in patients receiving L-DOPA for 12 months or longer the GAD levels in the caudate nucleus, putamen, globus pallidus and substantia nigra (but not in other brain areas) were well within the range of control values, being significantly higher than in patients who received L-DOPA for shorter periods of time (8 months or less) (Table 2). An attractive possibility to explain these findings is that the decrease in GAD activity in the basal ganglia in Parkinson's disease may in fact be secondary to the severe degeneration of the dopaminergic neurons in this brain region. One may speculate that the striatal and pallidal GAD (GABA)-containing neurons might be under a continuous "trophic" influence of the dopaminergic system. Degeneration of the latter system might then result in a biochemical atrophy of these GAD-containing neurons. This possibility, though hypothetical at present, would help to explain the decrease of GAD in the basal ganglia in Parkinson's disease and the positive effect of prolonged L-DOPA administration on the activity of this enzyme.

GABA *neurons in the basal ganglia—possible relation to tremor in Parkinson's disease*

From a clinical point of view, the possibility of a DA-GABA link in the basal ganglia may have a significant bearing on the etiology and neurochemistry of the Parkinsonian tremor. It is known that tremor responds rather late in the course of chronic L-DOPA treatment, thus paralleling to some extent the course of L-DOPA's effect on GAD; in addition, the antitremor effect of L-DOPA requires particularly high doses of the drug. Thus, it is possible that a deficiency of a GABA-containing neuronal system in the basal ganglia, via changes originating in the dopaminergic system, may be directly involved in the etiology of Parkinsonian tremor; this possibility would furnish, for the first time, a neurochemical basis for this symptom. It may be relevant in this context that recently a GABA-like compound (3,4,5-trimethoxybenzoyl-1,4-aminobutyrate) has been reported to exert a prompt effect on tremor in patients with Parkinson's disease (CURCI and PRANDI, 1972).

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