sense that they intervene at the level of the formation of cross-linkages between the fibrils. They inhibit the lysine-oxydase which, oxidizing the amine group in some of the lysine residues, creates the prior condition for the formation of the cross-linkages. A reduced formation of these linkages leads to an increase in the fragility of the connective tissue and can explain the various manifestations of lathyrism due to an abnormal structure of the connective tissue itself, including the vascular changes.

In the light of these facts, the action of the two flavonoids in question, tending to maintain the level of the soluble collagen at values approximating to normality, appears to signify a protection of the lysine-oxydase against the effect of the lathyrogens. It can be suggested as a hypothesis that IDPN and AAN may block the enzyme, acting directly on the active areas or complexing the Cu^{++}ion, which is indispensable for the oxidation of the amino group of the lysine. If, in fact, either the flavonoids or the aminonitriles may complex the Cu^{++} ion[12], the positive action could be due to the fact that they interfere in some way in the distribution of the Cu^{++} ion between the aminonitriles and the lysine-oxydase.

If, on the other hand, the lathyrogens inhibit the enzyme, acting at the level of the active areas, one might consider as a second hypothesis the removal, through the agency of the flavonoids, of the lathryrogens, which, once blocked, would leave the lysine-oxydase free to pursue its normal function.

Riassunto. È stato determinato il collagene neutro solubile dell'aorta in ratti trattati con latirogeni (*β*, *β*-iminodiproprionitrile, aminoacetonitrile) e flavonoidi [*O*-(*β*-idrossietil)rutosidi o (+)-catechina]. Il contenuto in collagene solubile è aumentato negli animali trattati con i latirogeni, mentre non risulta modificato qualora i latirogeni vengano somministrati ad animali trattati con flavonoidi.

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L-DOPA Treatment in Parkinson's Disease: Effect on Dopamine and Related Substances in Discrete Brain Regions

It is well established that in Parkinson's disease the concentrations of dopamine (DA) and its metabolite homovanillic acid (HVA) are greatly decreased in the striatum (= caudate nucleus and putamen), substantia nigra and globus pallidus[1]. Based on these findings L-3,4-dihydroxyphenylalanine (L-DOPA), DA's immediate precursor, was successfully introduced in the treatment of Parkinson's disease[2–4]. The present study reports on the metabolic fate of L-DOPA in the brains of Parkinsonian patients who chronically received large daily doses (2–6 g) of this drug until death. It was thought that such an investigation would contribute to our understanding of the mechanisms through which L-DOPA exerts its effects in patients with Parkinson's disease.

Results and discussion. The brains of control patients (9 cases) and patients with Parkinson's disease (3 non-DOPA treated cases, 4 cases with L-DOPA treatment) were obtained and processed as previously described[5]. The following substances were estimated[6] in several discrete brain regions: DOPA, 3-*O*-methyl-DOPA, DA and HVA. The results of the study are summarized in the Table.

In control and non-DOPA treated patients neither DOPA nor 3-*O*-methyl-DOPA could be detected in any examined brain area. In these groups of patients the values for DA and HVA in the caudate nucleus and putamen agree well with earlier reports[7–10] illustrating the striatal DA deficiency as characteristic of Parkinson's disease. In contrast, in DOPA treated patients the DA concentrations in the caudate nucleus and putamen were 4–8 times higher than in the non-DOPA treated group of the present and previously reported studies. In the caudate nucleus of the DOPA-treated groups the mean DA concentration (2.2 μg/g) approached control values. The higher levels of striatal DA in the DOPA-treated group are most probably related to L-DOPA's transfromation to DA; they cannot be explained by assuming that this group of patients had a milder degree of degeneration of the nigro-striatal DA neurons than the non-DOPA-treated group. This follows from our observations[11] that a) in both groups of patients the depigmentation of the substantia nigra and the decrease in striatal L-DOPA decarboxylase activity were of the same magnitude, and b) the levels of DA in the striatum of the DOPA-treated patients were directly related to the administered dose of L-DOPA (which varied from 2–6 g daily) and the time interval between the last dose of the drug and death of the patient (4–24 h). This latter observations also help to explain the large scatter of the values for DA (and the other substances estimated; see Table) in this group of patients, which in most instances precluded a statistical evaluation of the results obtained; from the point of view of statistical analysis, the patient material that constituted the DOPA-treated group was by necessity non-homogeneous.

In analogy to DA, the mean concentrations of HVA in the caudate and putamen in the DOPA-treated group were 8–20 times higher (8–11 μg/g) than in the non-DOPA treated Parkinsonian patients, being about twice as high as the control values. In addition, substantial though lower amounts of HVA (3–5 μg/g) were also detected in many extra-striatal brain regions; in these regions no DA was detected as result of treatment with L-DOPA. This finding shows that in the extra-striatal regions there is formation of, but no significant storage capacity for, DA. The preferential accumulation of DA and HVA in the Parkinsonian striatum indicates that a certain degree of regional selectivity and specificity of L-DOPA's metabolic transformation is still preserved in the brain of the diseased patients. However, nothing certain can at present be said about the cellular structures, in the striatum or other regions of the Parkinsonian brain, in which the therapeutically administered L-DOPA is metabolized. It is possible that, besides the still preserved DA neurons, the serotonin and noradrenaline neurons are involved[12].

Dopamine (DA), homovanillic acid (HVA), DOPA and 3-*O*-methyl-DOPA (*O*-Me-DOPA) in control brains and non-DOPA treated and DOPA-treated Parkinson's disease brains

Region	Controls				Parkinson's disease							
					Non-DOPA treated patients				DOPA-treated patients			
	DOPA	*O*-Me-DOPA	DA	HVA	DOPA	*O*-Me-DOPA	DA	HVA	DOPA	*O*-Me-DOPA	DA	HVA
Caudate nucleus	nd (4)	nd (5)	3.50 ± 0.55 (9)	2.94 ± 0.65 (9)	nd (3)	nd (3)	0.47[a] ± 0.33 (3)	1.18[a] ± 0.10 (3)	0.24 ± 0.14 (4)	6.65[b] nd (3)	2.22 ± 0.93 (4)	8.14[a] ± 3.10 (4)
Putamen	nd (3)	nd (3)	4.76 ± 0.86 (7)	4.24 ± 0.63 (7)	nd (3)	nd (3)	0.26[a] ± 0.09 (3)	0.67[a] ± 0.27 (3)	0.25 ± 0.18 (4)	3.00 ± 2.43 (4)	2.06 ± 0.71 (4)	11.46[a] ± 3.28 (4)
Thalamus, ventral	nd (3)	nd (3)	nd (3)	0.40 ± 0.08 (8)	nd (2)	nd (2)	nd (3)	0.43 ± 0.16 (3)	1.06[b] nd (2)	6.34[b] nd (2)	nd (3)	4.79[a] ± 1.51 (4)
Medial	nd (2)	nd (2)	nd (2)	nd (9)	nd (3)	nd (3)	nd (3)	nd (3)	0.70 ± 0.47 (3)	nd (3)	nd (3)	5.02 ± 2.17 (3)
Dorsal	nd (2)	nd (3)	nd (3)	nd (9)	nd (2)	nd (3)	nd (2)	nd (2)	5.77[b] nd (3)	1.42 ± 1.28 (4)	nd (4)	3.77 ± 1.06 (4)
Temporal cortex	nd (3)	nd (3)	nd (4)	nd (9)	nd (2)	nd (2)	nd (2)	nd (2)	1.01 ± 0.54 (3)	3.28 ± 2.56 (3)	nd (3)	3.01 ± 0.92 (4)
White matter[c]	nd (1)	nd (1)	nd (1)	nd (7)	nd (2)	nd (2)	nd (2)	nd (2)	0.29 ± 0.19 (4)	2.51 ± 2.24 (4)	nd (4)	3.32 ± 1.07 (4)
Cerebellar cortex	nd (1)	nd (1)	nd (1)	nd (4)	nd (3)	nd (2)	nd (2)	nd (2)	0.57 ± 0.35 (3)	3.46 ± 2.47 (3)	nd (3)	2.96 (2)

All values, except those marked[b], are expressed as mean (μg/g) ± S.E.M. The number of cases analyzed is indicated in parentheses. nd, not detectable (< 0.2 μg/g). [a] Significantly different from control ($p < 0.01$). [b] Single value. [c] Album centrale.

Although DOPA and 3-*O*-methyl-DOPA were absent in the brains of control patients and non-DOPA-treated Parkinsonian patients (see above), in the DOPA-treated patients these compounds were found to occur in detectable amounts, the concentrations of 3-*O*-methyl-DOPA being, as a rule, distinctly higher than those of DOPA (see Table). However, in contrast to DA and HVA, the distribution pattern of DOPA and 3-*O*-methyl-DOPA throughout the brain was fairly uniform. The fact that the concentrations of DOPA, although detectable, were low as compared to those of its metabolites, shows that, as in the normal brain, DOPA in the Parkinsonian brain is mainly a metabolic intermediate in the formation of other compounds. The observation that comparatively large amounts of 3-*O*-methyl-DOPA accumulated during L-DOPA therapy throughout the brain is interesting in view of the recent report[13] that 3-*O*-methyl-DOPA may be re-converted (by demethylation) to DOPA thus serving as a renewed source for DA.

In conclusion, it is shown that in the limited number of cases studied, L-DOPA treatment preferentially increased the DA and HVA concentrations in the caudate nucleus and putamen in patients suffering from Parkinson's disease. From this, it can be concluded that increased formation of DA in the striatum represents one of the important mechanisms through which L-DOPA exerts its therapeutic effects in Parkinsonism. Since, however, small amounts of DOPA and larger amounts of 3-*O*-methyl-DOPA and HVA were spread diffusely throughout the brain of these patients, an involvement of these substances (especially 3-*O*-methyl-DOPA) as well as brain areas other than the striatum in L-DOPA's various pharmacological effects or side effects, or both, can not at present be excluded[14]

Zusammenfassung. Bei L-DOPA behandelten Parkinsonpatienten wird gefunden, dass Dopamin und Homovanillinsäure besonders im Striatum akkumuliert wurden. Damit wird die Möglichkeit, dass Dopamin an der Antiparkinson-Wirkung des L-DOPA entscheidend beteiligt ist, in direkter Weise gestützt.

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