Decarboxylation of [1-^{14}C] L-dopa after intravenous and intraventricular administration to rats as measured by expired $^{14}CO_2$

L-Dopa administered orally or intravenously is metabolized largely in peripheral tissues to form dopamine and noradrenaline. In treating parkinsonism with L-dopa large amounts must be given to enable sufficient quantities to reach the brain. Dopaminergic and noradrenergic pathways have also been postulated to be involved in the aetiology of schizophrenia. Experiments with rabbits by Pletscher & Gey (1962) have shown that DL-dopa is metabolized within brain, preferentially in areas known to be rich in noradrenergic and dopaminergic structures. The [2-^{14}C] DL-dopa reached a peak in the brain within 5 min after intravenous injection, but it is not clear whether this early peak was due to rapid passage through the blood-brain barrier of both isomers or of only the D-isomer. Bartholini, Pletscher & Tissot (1966) found that when [2-^{14}C]L-dopa was administered to cats intravenously, [^{14}C]labelled homovanillic acid (HVA), a dopamine metabolite, appeared in the cerebrospinal fluid (csf). The labelled HVA did not reach a maximum in the csf until about 60 min after injection, although it was at a maximum in 10 min in the plasma. The authors concluded that at least part of the ^{14}C-HVA in the csf resulted from metabolic transformation of [2-^{14}C]L-dopa within the brain. In a study of [^{14}C]L-dopa injected peripherally into patients, [^{14}C]metabolities were found to reach a peak in the plasma at 10 min and then declined, but in the occipital csf the peak of the labelled metabolites was not reached until 2 to 4 h after injection (Pletscher, Bartholini & Tissot, 1967). It remains unclear from the two latter experiments whether the relatively slow appearance of L-dopa metabolites in the csf was due to slow diffusion of L-dopa into, or to a slower rate of metabolism after, entry into the brain.

With the aim of determining whether L-dopa is more slowly metabolized in the brain than peripherally, we have measured the decarboxylation rate of [1-^{14}C]L-dopa by measuring the expired $^{14}CO_2$ after administering the labelled compound intravenously and intraventricularly to rats. Carboxyl-labelled [1-^{14}C]L-dopa was obtained from Amersham-Searle at a specific activity of 9·1 mCi mM^{-1}; 1·4 μCi (30 μg) in 4 μl of isotonic solution was injected into the tail vein or lateral ventricle of the brain of female Buffalo rats weighing approximately 200 g each. The lateral ventricle was located and the injection performed stereotactically, using a Hamilton microliter syringe. Injections were done under light ether anaesthesia and the animal was placed immediately in a 2 litre metabolism cage. Air from the cage passed through a 400 cc ionization chamber with vibrating reed electrometer and through an infrared detector for measurement of CO_2. ^{14}C and CO_2 were recorded continuously on a chart recorder, and 1 min integral values were read and punched on IBM cards for computer analysis. To correct for variations in expiration rate of CO_2, the $^{14}CO_2/CO_2$ specific activity of each point was multiplied by the mean CO_2. The final curve is expressed as fraction of injected dose expired as $^{14}CO_2$ as a function of time. These curves are shown in Fig. 1.

If we assume that the injected ^{14}C moves through various physiological and physical compartments in a fashion described by standard compartment theory, we may, without specifying details of the compartments or their connections, write an equation which describes the concentration of ^{14}C which leaves the system as a function of time. Such an equation is a sum of exponentials:

$$F(t) = Ae^{-r_1 t} - B(e^{-r_2 t} + e^{-r_3 t})$$

Table 1. *Parameters of fitting functions for $^{14}CO_2$ respiration curves after i.v. and intraventricular injection of [1-^{14}C]L-dopa in rats*

	Rat Number	r_1 (min^{-1})	r_2 (min^{-1})	r_3 (min^{-1})	A	B	120 min integral	T_{max} (min)
	1	·0219	·153	·153	00682	·690	·341	18
	2	·0203	·281	·282	·00811	4·68	·403	14
Intravenous	5	·0222	·182	·183	·00864	2·43	·395	18
	mean	·0215	·205	·207	·00786	2·600	·380	16·7
	mean $T_{\frac{1}{2}}$	32 min	3·4 min	3·3 min				
	3	·00537	·0571	·0587	·00234	·664	·498	30
Intra-	7	·0231	·139	·141	·00739	1·15	·333	23
ventricular	8	·0290	·135	·142	·0123	·189	·397	24
	mean	·0192	·110	·114	·00734	·668	·409	25·3
	mean $T_{\frac{1}{2}}$	36 min	6·3 min	6·1 min				
	t value	·265	1·65	1·62	·143	1·33	·572	3·10
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	·05>*P*>
		n.s. = *P*>·05						·025

The terms r_1, r_2 and r_3 are the slopes of the components of the multi-exponential expiration curve, and A and B represent the intercepts of these components at zero time.

The data were submitted to a CDC 7600 computer with a least-squares minimization program which uses a series of iterations to find the values of the parameters of this function for which it most closely fits each data curve. These parameters, and the total ^{14}C expired in 120 min, are shown in Table 1. Also shown is the time at which maximum excretion of radioactivity occurred, obtained by inspection of the curves. For a more complete description and further references with regard to the instrumentation, theoretical considerations of compartment analysis and the computer program the reader is referred to Pierson, Kusubov & others (1974).

Analysis of the significance of difference between the means for each of the parameters, in comparing intravenous *vs* intraventricular injection, is shown in Table 1. The values of r_1, A and the 120 min integral are clearly not different. The values of r_2, r_3 and B are lower after intraventricular than after intravenous administration, but in no case is the difference significant at the 95% confidence level. The T_{max}, however, occurs significantly later after intraventricular injection.

Winchell, Stahelin & others (1970) have studied the metabolism of [^{14}C]labelled compounds and bicarbonate in man, using essentially the same method employed here. On the basis of their concepts of CO_2-HCO_3^- compartment models, we can form tentative ideas about the decarboxylation of L-dopa measured here. The 32–36 min half time component (r = ·0215 and ·0192 intravenous and intraventricular, respectively) undoubtedly represent the turnover time of CO_2-HCO_3^- compartment in muscle and other relatively poorly vascularized tissues. The two shorter half-times then must relate to exchange between the above compartment and the CO_2-HCO_3^- in the rapidly turning over blood pool, and to the rate of decarboxylation of L-dopa. A complete solution of the compartment model is not possible with the limited data presented here. Comparison of the fitting parameters of the $^{14}CO_2$ respiration curves (Table 1) shows that they are not significantly different for the two routes of administration. We can deduce from this that the rate of decarboxylation of L-dopa in the brain is not very different than that which occurs peripherally. The total fraction of the injected dose which is decarboxylated within 2 h is also the same.

There are several factors which might be responsible for the delayed T_{max} which occurred after intraventricular injection. In the brain, unlike most other areas of the body, the enzyme that decarboxylates L-dopa is either membrane bound or sequestered (Udenfriend, 1965). It is reasonable to expect that the transport of L-dopa to this enzyme via the csf, where circulation occurs by ciliary action of the ependymal cells, would be slower than via blood in peripheral circulation. Transport of $^{14}CO_2$ or $H^{14}CO_3^-$ across the ependyma might also be slower.

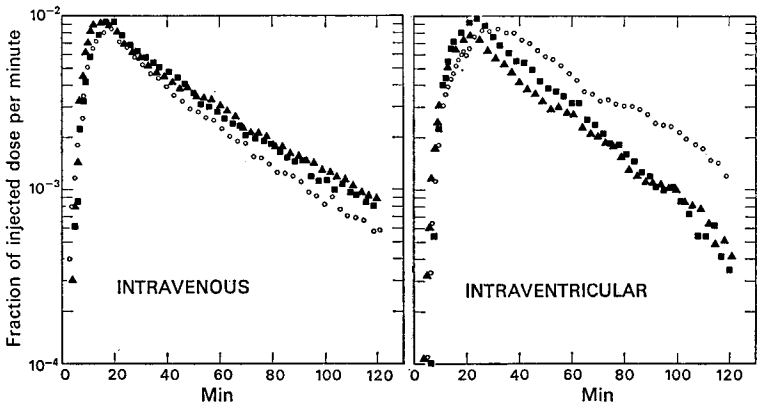

FIG. 1. $^{14}CO_2$ respiration curves after intravenous and intraventricular injection of [1-^{14}C]-L-dopa into rats at zero min. After the first 10 min only every third data point is shown.

It is possible that the intraventricularly injected [^{14}C]L-dopa in our experiments diffused out of the csf into the peripheral circulation and that the decarboxylation measured was peripheral rather than intracerebral. The rate of removal of L-dopa from the csf of monkeys measured by ventriculocisternal perfusion by Shaywitz, Gormley & others (1972) was slower than the slowest rate found here for decarboxylation. We cannot say from the results of Shaywitz & others, who used a different species and a different method, that there would be no appreciable efflux of the labelled [^{14}C]L-dopa from brain back into peripheral circulation. We feel it suggests, however, that it would not occur fast enough to affect significantly the rates measured in our intraventricular experiment.

We conclude from this study that the rate of decarboxylation of L-dopa within the cns of rats is not significantly different from that which occurs when the L-dopa is given peripherally. If these results are referrable to cats and humans, the apparently slow appearance of dopa metabolites (Bartholini & others, 1966; Pletscher & others, 1967) in csf after parenteral administration must then reflect either a relatively slow rate of passage of dopa across the blood brain barrier or a hold-up of dopamine in storage sites which delays appearance of the labelled HVA in the csf.

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