

The inhibitory action of L-dihydroxyphenylalanine (L-dopa) in the presence of a monoamine oxidase inhibitor on protein synthesis *in vivo* and the partial amelioration of this by methionine supplementation

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Male Wistar rats of various age groups were injected daily over a period of 3 weeks with iproniazid (10 µg/g body wt.) and L-dihydroxyphenylalanine (L-dopa; 0.1 mg/g body wt.). On the final day 1 h before the termination of the experiment the animals were injected with L-[¹⁴C]valine (0.1 µCi/g body wt.). The specific radioactivity of the valine in the proteins of the subcellular fractions of the tissues examined, relative to the time-integrated mean specific radioactivity of this amino acid in the acid-soluble pools of these tissues, was used to assess protein synthesis. The L-dopa/monoamine oxidase-inhibitor treatment was associated with 30–40% inhibition of protein synthesis. Supplementation of the dietary methionine intake by injection of this amino acid markedly diminished the inhibitory action of the L-dopa/monoamine oxidase-inhibitor treatment on protein synthesis in all fractions examined.

The therapeutic efficacy of L-dihydroxyphenylalanine (L-dopa) in the treatment of Parkinson's disease has been correlated with the efficiency of its uptake by the brain and its physiological role as precursor of the neurotransmitter dopamine. However, a cautionary note regarding its use has been introduced into the literature after the observations of Weiss *et al.* (1972, 1974, 1975) that a marked increase in brain dopamine concentration could lead to brain polyribosome disaggregation with a consequent detrimental effect on brain protein biosynthesis (Taufek, 1977; Bone, 1975). In contrast the electron-microscopic studies by Hartman & Becker (1973) and Taufek (1977) yielded no ultrastructural evidence indicating disaggregation of brain polyribosomes *in vivo* after the administration of L-dopa. Furthermore, King & Beesley (1978), by using procedures that included the gross injection technique of Dunlop *et al.* (1975), concluded that L-dopa causes little or no inhibition of protein synthesis.

In both man and experimental animals given L-dopa much of this amino acid as well as its metabolic products undergo *O*-methylation before excretion (Calne *et al.*, 1969; Wurtman *et al.*, 1970). The therapeutically effective dosage of L-dopa is often quite high and correspondingly large amounts of *S*-adenosylmethionine will be irreversibly utilized for the transmethylation involved. This may cause

the concentration of *S*-adenosylmethionine in the brain and other organs to be substantially decreased. Repetitive doses of L-dopa over a period of several weeks were found to cause a marked depletion of *S*-adenosylmethionine and methionine concentrations in both the brain and other organs, e.g. the liver and kidneys (Taufek & Bone, 1979). The depletion of this amino acid, essential for the initiation of protein biosynthesis and an indispensable component of the majority of the proteins formed, may at least in part be responsible for the impairment of the process of protein synthesis by L-dopa/dopamine.

Materials and methods

The animals used throughout these investigations were male albino rats of the Wistar strain. Observations were made on the following age groups (age given at time of first injection): 20-day-old, 40-day-old and 80–90-day-old. Each group was made up of 20 animals, 10 experimental and 10 controls.

All animals were fed *ad lib.* with the C.R.M. Labsure rat diet with free access to water throughout the investigations.

The experimental animals were injected daily via the caudal vein over a period of 3 weeks with the monoamine oxidase inhibitor iproniazid phosphate

(10 $\mu\text{g/g}$ body wt.) in suspension of concentration 1 mg/ml in physiological saline. This was followed after a period of 5 min by an intraperitoneal injection of L-dopa (0.1 mg/g body wt.) in a warm (37°C) saline suspension containing the non-ionic detergent Tween 80 (0.2%, v/v). This combination of L-dopa and monoamine oxidase inhibitor was sufficient to maintain an increased concentration of L-dopa and dopamine in the tissues over the greater part of the 24 h period after the injection (see Figs. 1 and 2). The controls were treated in the same manner as the experimental animals with the exception that L-dopa was replaced by an equivalent volume of warm physiological saline containing 0.2% (v/v) Tween 80. Experiments were also carried out in which the dietary methionine was supplemented by the daily injection of this amino acid (0.1 mg/day per g body wt.) (dissolved at a concentration of 50 mg/ml) in physiological saline. This was also administered via the intraperitoneal route, contralateral to the site of injection of the L-dopa or placebo.

Control experiments for these analyses were of two types: (a) animals that received supplementary methionine and monoamine oxidase inhibitor in saline; (b) animals that received monoamine oxidase inhibitor in saline only.

On day 21 of treatment animals to be used for protein-synthesis studies were injected immediately after and contralateral to the L-dopa or placebo injection with L-[^{14}C]valine of specific radioactivity 10 mCi/mol (0.1 $\mu\text{Ci/g}$ body wt.). The utilization of valine of this low specific radioactivity and high

dosage was based on the work of Taufek (1977) and Dunlop *et al.* (1975). The method overcomes the problem of the rapid catabolism of radiolabelled amino acids of high specific radioactivity at low dosage. The animals were then maintained in a warm environment ($25 \pm 1^\circ\text{C}$) for exactly 1 h. They were then lightly anaesthetized with $\text{N}_2\text{O}/\text{O}_2$ (1:1) mixed with fluothane. The chest was opened and the liver was perfused briefly with physiological saline through the portal vein. The liver, kidneys and brain were then rapidly excised, rinsed with cold (5°C) saline and frozen in liquid N_2 . All tissues were weighed in the frozen state, taking appropriate precautions to prevent moisture condensation. The specimens were maintained at -20°C until analysed. The extraction and fluorimetric assay of the L-dopa and dopamine content of the tissues was carried out by the method of Anton & Sayre (1962).

The extraction, partial purification and fluorimetric assay of monoamine oxidase activity in the tissues was carried out by the method of Krajl (1965). This assay is based on the formation of a hydroxyquinone from kynuramine under the action of the enzyme. The degree of inhibition of monoamine oxidase activity by iproniazid under the conditions used in the protein-synthesis studies was thereby found to be $\geq 98\%$ in all tissues examined.

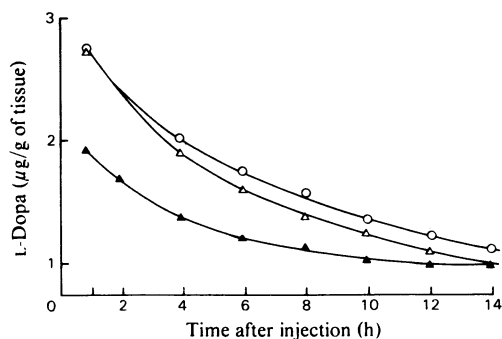


Fig. 1. Changes in tissue concentrations of L-dopa after its intraperitoneal injection (0.1 mg/g body wt.) in conjunction with the monoamine oxidase inhibitor iproniazid (10 $\mu\text{g/g}$ body wt.)

Each graphical point is based on duplicate assays on each of two animals. The standard deviation of the means was in each case less than 11% of the mean value. The concentrations of L-dopa in the tissues of control animals given iproniazid (10 $\mu\text{g/g}$ body wt.) alone were: whole brain (▲), $0.06 \pm 0.004 \mu\text{g/g}$; liver (○), $0.04 \pm 0.006 \mu\text{g/g}$; kidney (△), $0.03 \pm 0.003 \mu\text{g/g}$.

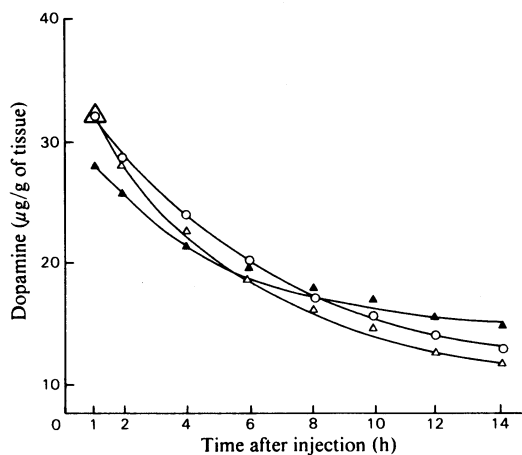


Fig. 2. Changes in tissue concentrations of dopamine after the intraperitoneal injection of L-dopa (0.1 mg/g body wt.) in conjunction with the monoamine oxidase inhibitor iproniazid (10 $\mu\text{g/g}$ body wt.)

Each graphical point is based on duplicate assays on each of two animals. The standard deviations of the means were in each case less than 5% of the mean values. The concentrations of dopamine in the tissues of control animals given iproniazid alone (10 $\mu\text{g/g}$ body wt.) were: whole brain (▲), $0.11 \pm 0.03 \mu\text{g/g}$; liver (○), $0.62 \pm 0.13 \mu\text{g/g}$; kidney (△), $0.75 \pm 0.07 \mu\text{g/g}$.

The specific radioactivity of the L-[^{14}C]valine in, and the amino acid content of, the acid-soluble pools of cerebral, hepatic and renal tissues were determined as previously described for brain tissue by Sabri *et al.* (1974).

Homogenates of whole-brain tissue were separated by discontinuous gradient centrifugation into mitochondrial, synaptosomal and microsomal fractions. The hepatic and renal tissue was separated into mitochondrial and microsomal fractions. The procedures used in these subcellular fractionations were those of Tamir *et al.* (1974) and Gurd *et al.* (1974). The protein content and the specific radioactivity of these fractions were assayed, after dissolving in N.C.S. solution, by the method of Schmukler & Yiengst (1968).

The time-integrated mean specific radioactivity of the amino-acid pools of the tissues examined over the period of the experiments was evaluated graphically from the plot of specific radioactivity (d.p.m./nmol of valine) in the HClO_4 extract of tissues of animals maintained for periods of 15, 30 and 60 min after L-[^{14}C]valine injection under the conditions already described. An example of such a plot is given in Fig. 3.

If α is the rate of protein synthesis, S the specific radioactivity of the total protein of the tissue after a fixed time t , T the time-integrated mean specific radioactivity of the amino-acid pool of the tissue over the period t , then α is proportional to $S/(Tt)$. By using the suffixes c and e to specify these factors for the control and the experimental (L-dopa-treated)

animals the percentage inhibition of protein synthesis is:

$$\frac{\alpha_c - \alpha_e}{\alpha_c} \times 100 = \left(1 - \frac{S_e \times T_c}{S_c \times T_e}\right) \times 100$$

Results and discussion

In the present series of experiments further evidence has been obtained of the inhibitory effect of prolonged increased concentrations of L-dopa + dopamine on the synthesis of the proteins of a variety of animal tissues *in vivo* (Table 1). The experiments of Weiss *et al.* (1972) indicate that it is the elevated concentrations of dopamine rather than those of L-dopa that are responsible for this inhibitory action. The enzymes, necessary for the resynthesis of methionine from homocysteine utilizing serine as the source of the methyl groups, are functional within the rat brain (Ordonez & Wurtman, 1974). These workers found that L-dopa treatment depleted brain methionine only when there was a concomitant folate deficiency to decrease this resynthesis. In the present experiments the maintenance of increased concentrations of both L-dopa and dopamine over prolonged periods led to depletion of the brain and other tissue methionine pools even in the rats that were given a normal folate intake (Taufek & Bone, 1979).

The percentage inhibition of protein biosynthesis (as defined above) in the various subcellular fractions of the organs of the animals given L-dopa + iproniazid ranged from 27 to 36%. The degree of inhibition was markedly diminished by supplementation of the methionine intake of the treated animals. The improvement was somewhat greater in the more adult animals (Tables 1–3). Although not strictly comparable, owing to differences in experimental procedure, these results contrast markedly with those of King & Beesley (1978). These workers concluded from a rather limited number of experiments that L-dopa treatment has little effect on brain protein synthesis. The discrepancy may result from the far longer period of maintenance of the increased L-dopa/dopamine tissue concentrations in our own experiments. However, in most of their experiments the animals were decapitated and brain protein synthesis was examined within 15 min of the labelled amino-acid injection. The specific radioactivity of the brain amino-acid pool is undergoing rapid change over such a short experimental period, which is dependent on the transport of the labelled amino acid to the brain and its transfer across the blood/brain barrier. To define the specific radioactivity of the precursor amino-acid pool as that found at the time of

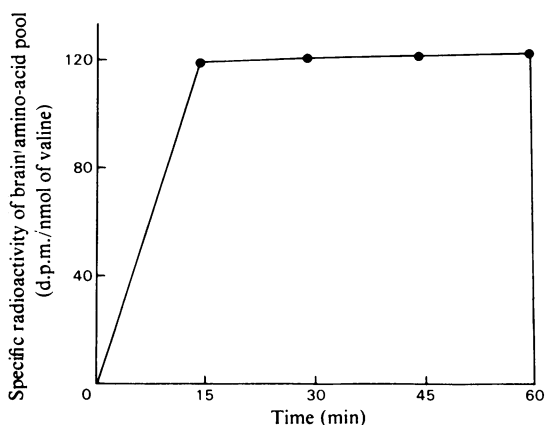


Fig. 3. Typical curve for assay of time-integrated mean specific radioactivity of the brain amino acid pool. Rats (20-days old) were injected with $10\mu\text{g}$ of iproniazid/g body wt. 5 min before injection of $0.1\mu\text{Ci}$ of [^{14}C]valine/g body wt. Animals were kept at an environmental temperature of 30°C during the period of incorporation.

Table 1. *Effect of L-dopa in the presence of a monoamine oxidase inhibitor, with and without methionine supplementation, on protein synthesis in the tissues of 20-day-old rats*

The protein specific radioactivities are given as means \pm s.e.m. (10 animals per group). The time-integrated mean specific radioactivities of the L-[14 C]valine in the brain, liver and kidney amino-acid pools were assessed graphically by the method of Sabri *et al.* (1974). An example of this assay is given in Fig. 3. The control refers to animals given iproniazid alone. 'Experimental' refers to animals given iproniazid + L-dopa. 'Experimental with methionine' refers to animals given iproniazid + L-dopa + methionine. In each example of the protein-synthesis study there is a significant decrease (two-tail *t* test $P < 0.002$) in the incorporation of the labelled amino acid into the proteins of each of the subcellular fractions of the experimental (iproniazid + L-dopa-treated) animals compared with the controls. This effect is partially alleviated by methionine supplementation.

Tissue	Subcellular fraction	Specific radioactivity of protein (d.p.m./mg of protein)			Specific radioactivity of amino-acid pool (d.p.m./nmol of valine)			Inhibition of protein synthesis (%)					
		Control	Experimental	Experimental with methionine	Control	Experimental	Experimental with methionine	Experimental	Experimental with methionine				
Brain	Myelin	90 \pm 2.0	70 \pm 1.7	85 \pm 1.5	106	121	118	31.7 \pm 1.5	15.0 \pm 1.5				
	Mitochondria	120 \pm 2.2	98 \pm 2.0	114 \pm 1.6						28.3 \pm 1.0	14.5 \pm 0.9		
	Synaptosomal	118 \pm 2.0	92 \pm 1.8	110 \pm 1.8								31.5 \pm 1.0	16.0 \pm 1.2
	Microsomal	114 \pm 1.8	84 \pm 1.8	106 \pm 1.6									
Liver	Myelin	120 \pm 1.8	99 \pm 1.5	113 \pm 1.4	108	123	119	27.4 \pm 2.0	15.3 \pm 1.0				
	Microsomal	117 \pm 3.0	92 \pm 2.7	112 \pm 2.2						31.0 \pm 1.6	14.0 \pm 1.2		
Kidney	Mitochondrial	114 \pm 1.4	94 \pm 1.2	108 \pm 1.0	106	121	118	28.9 \pm 1.4	14.0 \pm 0.8				
	Microsomal	120 \pm 2.4	87 \pm 2.0	114 \pm 2.0						29.2 \pm 2.0	14.0 \pm 0.8		

Table 2. *Effect of L-dopa in the presence of a monoamine oxidase inhibitor, with and without methionine supplementation, on protein synthesis in the tissues of 40-day-old rats*
Experimental details are as given in the legend to Table 1.

Tissue	Subcellular fraction	Specific radioactivity of protein (d.p.m./mg of protein)			Specific radioactivity of amino-acid pool (d.p.m./nmol of valine)			Inhibition of protein synthesis (%)					
		Control	Experimental	Experimental with methionine	Control	Experimental	Experimental with methionine	Experimental	Experimental with methionine				
Brain	Myelin	91 \pm 2.0	73 \pm 1.8	86 \pm 1.6	108	125	115	30.5 \pm 1.0	11.3 \pm 2.0				
	Mitochondrial	115 \pm 1.0	95 \pm 0.7	109 \pm 0.8						28.6 \pm 1.5	11.0 \pm 2.0		
	Synaptosomal	116 \pm 2.0	90 \pm 1.6	108 \pm 1.6								32.9 \pm 2.0	12.5 \pm 2.0
	Microsomal	114 \pm 2.5	84 \pm 2.2	104 \pm 2.0									
Liver	Mitochondrial	120 \pm 2.0	100 \pm 1.8	114 \pm 1.6	106	123	114	27.9 \pm 1.0	10.8 \pm 2.0				
	Microsomal	116 \pm 2.0	92 \pm 1.8	110 \pm 1.8						31.5 \pm 1.0	11.0 \pm 1.0		
Kidney	Mitochondrial	114 \pm 2.0	95 \pm 1.7	107 \pm 1.5	109	126	115	37.9 \pm 1.0	11.8 \pm 2.5				
	Microsomal	118 \pm 3.0	93 \pm 2.6	112 \pm 2.7						31.7 \pm 2.0	10.8 \pm 1.0		

decapitation of the animal is, under these circumstances, misleading.

Methionine plays an indispensable role in the biogenesis of proteins because of its function in the initiation of the process and as an essential constituent of the majority of tissue proteins. The role of *S*-adenosylmethionine is fundamental to the process of methylation in the animal body, e.g. in the formation of the 7-methylguanosine triphosphate 'caps' at the 5'-termini of most mRNA molecules

and necessary for their binding to the smaller ribosomal subunit. The process of methylation is also essential for the formation of mature ribosomes. If the methylation process is deficient normal derivation of the 32S rRNA and 18S rRNA units from the 45S ribosome subunit occurs. However, maturation of the 18S rRNA to form the smaller ribosomal subunit is defective and it undergoes degradation (Starr & Sells, 1969).

There is also an apparent conflict regarding our

Table 3. *Effect of L-dopa in the presence of a monoamine oxidase inhibitor, with and without methionine supplementation, on protein synthesis in the tissues of 80–90-day-old rats*
Experimental details are as given in the legend to Table 1.

Tissue	Subcellular fraction	Specific radioactivity of protein (d.p.m./mg of protein)			Specific radioactivity of amino-acid pool (d.p.m./nmol of valine)			Inhibition of protein synthesis (%)	
		Control	Experimental	Experimental with methionine	Control	Experimental	Experimental with methionine	Experimental	Experimental with methionine
Brain	Myelin	90 ± 1.6	77 ± 1.4	85 ± 1.2	104	130	112	31.6 ± 1.2	12.1 ± 2.0
	Mitochondrial	120 ± 2.0	106 ± 1.8	114 ± 1.6				29.4 ± 1.0	11.8 ± 2.0
	Synaptosomal	116 ± 1.6	101 ± 1.3	108 ± 1.2				30.3 ± 1.8	13.0 ± 2.0
	Microsomal	114 ± 1.7	100 ± 1.5	106 ± 1.4				30.0 ± 1.7	13.3 ± 2.0
Liver	Mitochondrial	115 ± 2.0	103 ± 1.8	104 ± 1.7	103	129	110	28.4 ± 1.0	15.9 ± 1.5
	Microsomal	118 ± 3.0	103 ± 2.6	112 ± 2.6				30.2 ± 1.3	11.5 ± 1.6
Kidney	Mitochondrial	112 ± 2.0	100 ± 1.7	106 ± 1.6	104	130	112	28.5 ± 1.5	12.0 ± 2.0
	Microsomal	115 ± 2.0	100 ± 1.7	110 ± 1.8				30.4 ± 1.5	11.4 ± 1.0

own conclusion that methionine + S-adenosylmethionine depletion is in part responsible for the inhibition of protein synthesis and the observations by Weiss *et al.* (1972) that depletion of S-adenosylmethionine by use of D-dopa does not lead to polyribosomal disaggregation. The conflict, however, is more "apparent" than real, owing to the differences in experimental conditions used. Whereas in our own experiments the concentrations of both S-adenosylmethionine and of methionine remained decreased over a period of 3 weeks in those of Weiss *et al.* (1972) no depletion of methionine occurred in brain tissue and depletion of S-adenosylmethionine was maintained only for a 1 h period.

These processes may be responsible for the methionine-reversible component of the inhibitory effect of dopamine on protein biosynthesis. They may also explain the apparently different effects observed on polyribosome profiles when examined by different techniques, because of their influence on the stability of the polyribosome structure.

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