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IN VIVO INHIBITION OF RAT BRAIN PROTEIN SYNTHESIS BY L-DOPA

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Abstract—A study has been made of the effect of a single intraperitoneal dose of L-DOPA on the *in vivo* metabolism of [^{14}C]leucine and [^{14}C]lysine by the brain, and on their uptake into brain protein. Administration of 500 mg DOPA/kg to 40-g rats raised the concentrations of several free amino acids; the only amino acid which underwent a statistically significant increment was alanine. Intracisternally-injected [U-^{14}C]leucine was rapidly metabolized to other labelled compounds; DOPA administration did not influence significantly the rate of its metabolism. No similar metabolic change was observed after administering [U-^{14}C]lysine intracisternally.

Incorporation of [^{14}C]leucine and [^{14}C]lysine into total brain protein was significantly reduced 45 min after DOPA administration. There was also depression of the uptake of labelled amino acid into a supernatant fraction, obtained by high speed centrifugation of the brain homogenate, and into brain microtubular protein (tubulin). Reduced amino-acid incorporation into brain proteins observed 45 min after L-DOPA injection coincided with extensive disaggregation of brain polyribosomes. At 120 min after DOPA treatment, disaggregation was no longer significant and there was a smaller depression in labelled amino aicd incorporation, which disappeared completely 240 min after L-DOPA injection. It is concluded that disaggregation of brain polysomes following DOPA treatment is an accurate reflection of a change in the intensity of brain protein synthesis *in vivo*.

WHEN ribosomes are extracted from rat whole brain after the animal is treated with large doses of various amino acids such as phenylalanine (MCKEAN *et al.*, 1968; AOKI & SIEGEL, 1970; WONG *et al.*, 1972) or L-dihydroxyphenylalanine (L-DOPA; WEISS *et al.*, 1971, 1972, 1973), they are found to be disaggregated from the normal predominantly polyribosome pattern. This has been taken as evidence of an effect of these administered substances on brain protein synthesis.

The mechanisms of action of DOPA and phenylalanine appear to be quite different. The polysome disaggregation produced by injecting 50 mg/kg doses of DOPA into 20-g rats or 500 mg/kg into older rats (WEISS *et al.*, 1971) does not depend on achievement of high levels of DOPA in rat brain, nor on its conversion to 3-*O*-methyldopa (WEISS *et al.*, 1972), nor on depletion of brain *S*-adenosylmethionine by DOPA (WURTMAN, 1970), but is dependent on formation of dopamine and its subsequent action through intact dopamine receptors (WEISS *et al.*, 1973). On the other hand, phenylalanine causes disaggregation of brain polysomes even in rats pretreated with a potent inhibitor of brain aromatic amino acid decarboxylase, so that generation of phenylethylamine is not possible (WEISS *et al.*, 1973). Furthermore, phenylalanine administration causes depression of brain tryptophan levels coincident with polysome disaggregation, whereas brain tryptophan is elevated by DOPA treatment (WEISS *et al.*, 1971).

HARTMANN & BECKER (1973) have recently claimed that disaggregation of brain polyribosomes after treatment with phenylalanine or DOPA is an artifact produced during ribosome isolation because they failed to observe a similar intracellular disaggregation by electron microscopy in the brain cells of rats treated with either of these two amino acids. However, it has been shown that disaggregation of brain polysomes following phenylalanine administration is accompanied by inhibition of amino acid incorporation into brain protein both in the intact rat and *in vitro* (SWAIMAN *et al.*, 1968; AGRAWAL *et al.*, 1969; SIEGEL *et al.*, 1971).

In order to determine whether the polysome disaggregation induced by L-DOPA treatment is also associated with diminished protein synthesis *in vivo*, we have treated rats with DOPA and measured incorporation of [^{14}C]leucine or [^{14}C]lysine into brain protein at times when brain polysomes were known to

be disaggregated. By this criterion, both total brain protein synthesis and synthesis of a particular brain protein (tubulin) were depressed after DOPA administration. Moreover, the time-course of this depression coincided closely with that of polysome disaggregation. A preliminary note of these studies has appeared (MUNRO *et al.*, 1973).

MATERIALS AND METHODS

Animals and treatments. Male Sprague–Dawley rats weighing 40 g (Charles River Breeding Laboratories, Wilmington, Mass.) were injected intraperitoneally with either 500 mg L-DOPA/kg body weight suspended in 1 ml 0·05 N HCl (experimental group), or with the diluent 0·05 N HCl (control group). At various times thereafter, the animals were injected intracisternally with either 1·5 μCi [U-^{14}C]lysine (300 μCi/μmol, New England Nuclear, Boston, Mass.) or with 1·5 μCi [U-^{14}C]leucine (30 μCi/μmol, New England Nuclear) in order to evaluate the effect of DOPA on brain protein synthesis. Three such studies were performed, each being carried out twice. In the first study, the experimental and control groups were injected with [^{14}C]lysine 45 min after being given DOPA or diluent, and rats from each group were killed at 7, 15 or 30 min later (i.e. at 52, 60 or 75 min after DOPA or diluent administration). The second study was similar, but the [U-^{14}C]leucine was the amino acid injected intracisternally. In the third study, the experimental and control groups were injected intracisternally with [^{14}C]lysine at 120 or 240 min after administration of DOPA or the diluent and were killed 30 min later. In each of these studies, six rats from the experimental and the control groups were killed by decapitation at each time-interval. Their brains were rapidly removed, and chilled on dry ice.

Analysis of brain. The brains of six rats from experimental or control groups killed at a given time-interval after isotope injection were homogenized individually in 4 vol. of buffer containing 10 mM $MgCl_2$ and 10 mM sodium phosphate (NaH_2PO_4/Na_2HPO_4, pH 7·0). A portion of this homogenate was centrifuged at 100,000 ***g*** for 1 h at 4°C in a Spinco Model L centrifuge (FEIT *et al.*, 1971), in order to obtain the soluble brain protein fraction.

In order to precipitate the proteins from the whole homogenate and from the soluble supernatant fraction, trichloroacetic acid (TCA) was added to each sample to give a final concentration of 8 per cent; the samples were then heated to 80°C for 20 min to remove nucleic acids, followed by centrifuging for 15 min at 1000 rev./min in an International Centrifuge in order to sediment the precipitated protein. The pellets of precipitated protein were washed several times with water to remove excess TCA and were finally suspended by homogenization in water before counting. The supernatant fluid (acid-soluble fraction) remaining after treatment of the whole homogenate with TCA and harvesting of the protein precipitate was saved for analysis of free amino acid levels in the brain.

In order to estimate the amount of labelled amino acid incorporated into tubulin, the procedures of MARANTZ *et al.* (1969) and of FEIT *et al.* (1971) for precipitating tubulin with vinblastine were applied to the soluble supernatant fraction of the homogenate, omitting the final step of removal of the vinblastine by dialysis. The 100,000 ***g*** supernatant fraction of each homogenate was incubated for 30 min at 37°C with 5×10^{-4} M vinblastine sulphate (a generous gift of The Eli Lilly Co., Indianapolis, Indiana). The resulting precipitate was harvested by centrifuging at 6000 ***g*** for 20 min at room temperature. The pellet of vinblastine and tubulin was washed with the $MgCl_2$-Na_2HPO_4/NaH_2PO_4 buffer used for homogenization of the brain and suspended in 1 ml of this buffer; a Teflon homogenizer or a Biosonik III sonicator (Brownell Scientific, Rochester, N.Y.) was used to disperse the pellet.

Radioactivity was measured in samples of the whole tissue homogenate, the TCA precipitate from the whole homogenate, the TCA precipitate from the soluble supernatant fraction, and the vinblastine precipitate. The samples were solubilized by incubation for 3 h at room temperature in 5 vol. NCS (Nuclear Chicago Corporation, Chicago, Ill.). Duplicate samples were then counted in 10 ml of a toluene phosphor {0·4% PPO [2,5-diphenyloxazolyl], 0·005% POPOP [(1,4-bis[2(5-phenyloxazolyl))] benzene]} after being kept in the darkness for 4 h. Corrections were made for sample quenching by addition of an internal standard. The total radioactivity found in the whole homogenate or soluble fraction was used to correct the incorporation into the protein fractions for differences in labelled amino acid administered intracisternally.

Amino acid analysis. In order to determine whether amino acid pools were affected by DOPA administration, the concentrations of leucine, lysine and seven other amino acids were measured on the supernatant (acid-soluble) fractions of the whole brain homogenate left after precipitation of protein by TCA. Analysis was performed with the Beckman Model 120-C autoanalyser. In order to obtain resolution of the essential amino acid peaks, it was necessary to apply amounts of brain extract to the analyser which were too great to allow the simultaneous resolution and quantification of most of the nonessential amino acids.

Analysis of [^{14}C]amino acid metabolites. To determine whether leucine or lysine undergo metabolic changes in brain following their intracisternal injection, and whether such metabolic changes are affected by L-DOPA pretreatment, any [^{14}C]labelled metabolites present in the supernatant fluid after adding TCA to whole brain homogenate were separated from unchanged amino acids using Dowex 50 × 4 column chromatography. Dowex resin was pretreated by standard procedures, and 4·5 ml columns (1 cm × 4·5 cm) were prepared. For the detection and quantification of leucine metabolites, the columns were equilibrated at pH 6·5 by addition of 10 ml 0·05 M NaH_2PO_4/Na_2HPO_4 (pH 6·5) and 0·1% EDTA followed by a 5-ml water wash. The samples of the TCA supernatant (acid-soluble) fluid were added to the columns after being adjusted to pH 2·0 with 2 N NaOH. Following a wash with 5 ml of water, the columns were eluted with 12 ml 0·05 M $NaPO_4$ (pH 6·5),

and 1 ml fractions of eluate were collected. Samples of each fraction were counted in duplicate in a 1,4-dioxanebase phosphor (0·4% PPO, 0·005% POPOP, 12% naphthalene), and corrections were made for sample quenching by addition of an internal standard.

For the analysis of lysine metabolites, the Dowex columns were equilibrated with 10 ml of a buffer containing 0·027 M citrate, 0·155 M NaH_2PO_4/Na_2HPO_4 (pH 6·6), and 0·1% EDTA, and were washed with 5 ml water. The TCA was removed from the samples by extracting them 6 times with 4 vol. ethyl ether; they were adjusted to pH 2·0 before being added to the columns. The columns were then washed with 5 ml water, and the amino acid eluted with a buffer containing 0·027 M citrate and 0·155 M Na_2HPO_4 (pH 6·6). Twenty one-ml fractions were collected and assayed for radioactivity as described above.

Ribosomal profiles. The procedure of WEISS *et al.* (1971) for resolving brain ribosomes on sucrose gradients was applied to the brains of DOPA-treated and control rats. Rats were injected intraperitoneally with L-DOPA or the diluent HCl and were killed in groups of 6 at specific time-intervals thereafter. The brains were quickly excised and chilled, and the six brains of each group of rats were divided into two pools which were then processed separately for display of their ribosomal profiles on sucrose gradients. Three profiles were run on each of these duplicate samples. The proportion of the profile occupied by polyribosomes was calculated and the average percentage of polyribosomes was obtained from all six profiles of each DOPA-treated or control group of rats.

RESULTS

Effect of DOPA on free amino acid concentrations in brain. WEISS *et al.* (1971) measured the free amino acid concentrations in the brains of rats treated with DOPA and observed an increase only in tryptophan level. However, these animals were larger and thus less sensitive to DOPA than the rats in our present study, and we have accordingly examined free amino acid levels in our 40-g rats receiving the large dose of DOPA. Nine free amino acids were assayed in the brains of DOPA-treated and control animals 75 min after injection (Table 1). Five amino acids showed a substantial increase in concentration, but only in the case of alanine did this attain statistical significance. However, it should be noted that the average level of lysine was 34 per cent higher in the DOPA-treated animals, whereas the average concentration of leucine changed only 7 per cent. This implies that treatment with DOPA may tend to cause a greater dilution of free [^{14}C]lysine given intracisternally compared to administration to the control group, whereas injected [^{14}C]leucine will not undergo this differential effect on its specific activity. As noted below, this phenomenon is related to the greater depression in labelled amino acid incorporation into brain protein observed after DOPA administration when lysine is the labelled precursor than when leucine is used.

Metabolism of [^{14}C]leucine and [^{14}C]lysine by rat brain. The total amount of radioactivity recovered from the brains of DOPA-treated and control rats was estimated at 7, 15, and 30 min after intracisternal injection of either [^{14}C]leucine or [^{14}C]lysine. Table 2 shows that there was an apprciable reduction in recovered activity in the soluble fraction by 30 min after injection. This indicates that DOPA treatment did not accelerate the rate of disappearance of radioactivity from the acid-soluble fraction after either [^{14}C]leucine or [^{14}C]lysine injection, and thus cannot account for the reduced labelling of protein in the brains of DOPA-treated animals reported below.

TABLE 1. BRAIN FREE AMINO ACID CONCENTRATIONS IN DOPA-TREATED AND CONTROL RATS

Amino acid	Control group (μg/g wet wt.)	Dopa group (μg/g wet wt.)	Difference (%)
Alanine	0·727 ± 0·059	1·010 ± 0·043	+39
Arginine	0·330 ± 0·079	0·392 ± 0·072	+17
Glycine	1·093 ± 0·132	1·097 ± 0·060	+1
Histidine	0·130 ± 0·011	0·130 ± 0·017	±0
Isoleucine	0·076 ± 0·018	0·077 ± 0·011	+1
Leucine	0·149 ± 0·044	0·158 ± 0·033	+7
Lysine	0·346 ± 0·046	0·467 ± 0·063	+34
Phenylalanine	0·072 ± 0·021	0·094 ± 0·019	+30
Tyrosine	0·074 ± 0·013	0·114 ± 0·015	+54

The concentrations of the amino acids were measured in the acid-soluble fraction remaining after precipitation of brain protein from brain homogenate with TCA. The animals were killed 75 min after administration of DOPA or diluent, and the brains of six rats were analysed per treatment. The values are reported ± S.E. Only alanine is significantly increased by DOPA ($P < 0·02$).

TABLE 2. TOTAL AND ACID-SOLUBLE [^{14}C]RADIOACTIVITY IN THE BRAINS OF DOPA-TREATED AND CONTROL RATS: RECOVERIES PER BRAIN AND AFTER PASSAGE THROUGH DOWEX-50 RESIN

[^{14}C]labelled amino acid	Time after injection (min)	Total [^{14}C] radioactivity per brain (c.p.m. × 10^{-3})		Soluble [^{14}C] radioactivity per brain (c.p.m. × 10^{-3})		Dowex-50 recoveries from soluble fraction: Applied activity recovered in eluate (%)		Dowex-50 recoveries from soluble fraction: Eluted activity in form of injected amino acid (%)	
		Control	DOPA	Control	DOPA	Control	DOPA	Control	DOPA
Leucine	7	140	111	62	60	81 ± 23	77 ± 36	61 ± 3	53 ± 3
	15	243	155	79	80	97 ± 27	86 ± 3	43 ± 7	38 ± 7
	30	152	79	39	36	62 ± 13	82 ± 4	33 ± 2	24 ± 1
Leucine standard						93		99	
Lysine	7	430	463	391	453	32 ± 3	38 ± 2	92 ± 0	90 ± 3
	15	429	415	375	395	39 ± 2	40 ± 3	90 ± 1	89 ± 1
	30	314	274	119	247	38 ± 0·1	40 ± 4	86 ± 1	79 ± 3
Lysine standard						36		94	

Rats were injected intraperitoneally with L-DOPA (500 mg/kg in 0·05 N HCl) or with 0·05 N HCl (control) and 45 min later were injected intracisternally with 1·5 μCi [^{14}C]leucine or 1·5 μCi [^{14}C]lysine. Groups of four rats were killed at the times indicated after intracisternal injection and the brains were removed and homogenized. Aliquots of the homogenates were solubilized in NCS, and counted as described in Materials and Methods. TCA was added to the homogenates, which were heated at 80°C and then centrifuged to remove the protein precipitates. Acid-soluble counts were determined as the difference between total homogenate counts and total TCA-precipitable counts. The remaining supernatant (acid-soluble) fractions were applied to Dowex-50 columns, as described in Materials and Methods. The table shows the total amount of the applied radioactivity that was recovered in the combined eluate fractions, and the percentage of the eluted radioactivity that appeared in the fraction known to contain the injected amino acid. Recoveries of radioactivity are also shown for authentic [^{14}C]leucine and [^{14}C]lysine treated with TCA and then passed through the column of resin.

The behaviour of [^{14}C]leucine and [^{14}C]lysine following intracisternal injection was explored by analysing the distribution of acid-soluble radioactivity eluted from Dowex 50 (Table 2). In the case of leucine, approximately 80 per cent of the radioactivity applied to Dowex 50 was recovered in the total eluate irrespective of the time after leucine injection or of treatment of the rats with DOPA, and was similar to recovery of authentic leucine. However, the proportion of this radioactivity eluting as unchanged leucine diminished rapidly from approximately 60 per cent at 7 min after injection, to about 20 per cent at 30 min after injection. The rate at which brain [^{14}C]leucine was thus being metabolized was not significantly greater in the DOPA-treated animals.

In the case of lysine, the recovery of added radioactivity from the Dowex columns was low in the presence of TCA, even when an authentic standard of pure lysine treated with TCA was used or when the TCA extracts of brain homogenates were repeatedly extracted with ether to remove the TCA. Nevertheless, essentially all of the recovered radioactivity was eluted from the Dowex as authentic lysine at all times after intracisternal injection, irrespective of DOPA pretreatment.

Ribosome profiles and protein synthesis following L-*DOPA administration.* In confirmation of previous findings (WEISS *et al.*, 1971), brains of rats killed 45–75 min after L-DOPA treatment exhibited gross polyribosomal disaggregation on sucrose gradients (Fig. 1 and Table 3). This effect was temporary and disappeared after 120 min (Table 3) when brain DOPA and dopamine concentrations fell to normal levels (WEISS *et al.*, 1972).

Incorporation of [^{14}C]lysine into total brain protein and some of its fractions was studied both at the time of maximal polyribosome disaggregation by L-DOPA and after recovery had set in. First, the time-course of [^{14}C]amino acid uptake into total brain protein was examined after giving pulse doses of [^{14}C]lysine (Fig. 2a) or [^{14}C]leucine (Fig. 2b) intracisternally to rats 45 min after an intraperitoneal dose of L-DOPA; that is, at the time of maximal polyribosomal disaggregation. At 7, 15 and 30 min after injection of [^{14}C]lysine, uptake into the protein of the DOPA-treated group was depressed by 67, 64 and 67 per cent respectively below the control values (Fig. 2a). Since DOPA administration causes a 34 per cent expansion in the pool of free lysine in brain (Table 1), it seems likely that about half of the reduction in radioactivity after DOPA is due to a reduction in incorporation, the other half being the consequence of dilution of the injected [^{14}C]lysine. At all these time intervals, there was extensive disaggregation of the

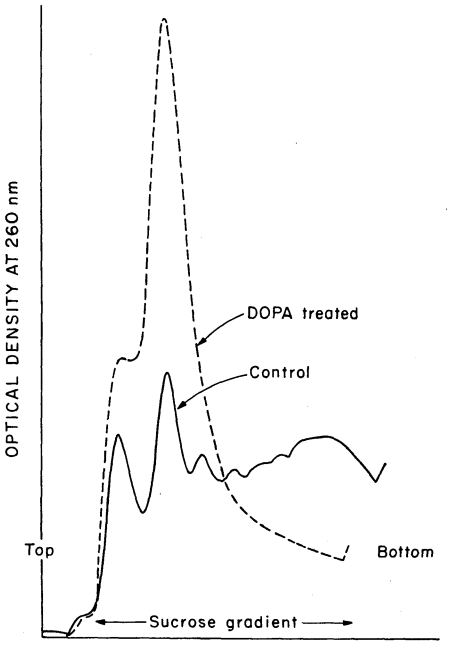

FIG 1. Profiles of rat brain ribosomes on continuous sucrose density gradients (10–40% sucrose) 60 min after treatment of rats with 500 mg DOPA/kg body weight. Control animals received the diluent. The ribosomes were prepared from the pooled brains of three rats in each group by the procedure of WEISS *et al.* (1971). (----) DOPA-treated rats; (——) control rats.

polysomes in the DOPA-treated group. When [^{14}C]leucine was injected intracisternally (Fig. 2b), the DOPA-treated animals showed 34, 32 and 36 per cent less uptake of radioactivity into whole brain protein. Since the concentration of free leucine in brain is unchanged by DOPA administration (Table 1), this estimate of reduced brain protein synthesis by DOPA required no correction for dilution. It is notable, however, that uptake into protein from [^{14}C]lysine is linear over the 15-min time intervals (Fig. 2a), whereas that for [^{14}C]leucine is curvilinear (Fig. 2b). This latter effect can be attributed to rapid metabolism of [^{14}C]leucine to other acid-soluble products (Table 2), whereas [^{14}C]lysine is not subject to catabolism in the brain and remains a [^{14}C] donor (Table 2).

These experiments were extended to determine whether incorporation of [^{14}C]amino acids into the proteins of the soluble supernatant fraction of the brain homogenate and into tubulin are similarly affected by L-DOPA administration, and whether reaggregation of polyribosomes at 120 min or longer after DOPA administration is accompanied by recovery or amino acid incorporation. For these studies, [^{14}C]lysine was given intracisternally at 45, 120, and 240 min after L-DOPA administration and the animals were killed 30 min later. Table 3 shows that the reduction in uptake of [^{14}C]lysine into homogenate protein 45 min after DOPA administration is shared by

TABLE 3. INCORPORATION OF [^{14}C]LYSINE INTO BRAIN PROTEIN AND POLYRIBOSOME ABUNDANCE AT VARIOUS TIMES AFTER L-DOPA INJECTION

Time after L-DOPA (min)	Incorporation of [^{14}C] into brain protein (%) Total brain protein	Supernatant protein	Tubulin	Polyribosomes (% of profile)
45 Min				
Control[1]	22·1 ± 2·9	10·2 ± 2·0	—	69
DOPA	9·5 ± 1·1 (−57%*)	3·2 ± 0·5 (−69%*)	—	22 (−47%)*
Control[2]	—	3·3 ± 0·4	2·5 ± 0·4	—
DOPA	—	1·7 ± 0·3 (−48%*)	1·3 ± 0·3 (48%*)	—
120 Min				
Control[1]	21·1 ± 1·6	8·5 ± 0·6	—	69
DOPA	16·9 ± 1·5 (−20%)	4·9 ± 0·6 (−42%*)	—	54 (−15%)
240 Min				
Control[1]	16·6 ± 1·1	6·2 ± 0·6	—	—
DOPA	20·7 ± 1·9 (+24%)	6·1 ± 0·4 (−1%)	—	—

The table compares incorporation of [^{14}C]lysine into brain protein with the proportion of polyribosomes in rat brain at various times after L-DOPA administration. To measure the effect of [^{14}C]lysine incorporation, two studies, designated [1] and [2] in the table, were performed. Groups of six rats received 500 mg L-DOPA/kg body weight or the diluent intraperitoneally and then 1·5 μCi [^{14}C]lysine intracisternally at the times indicated in the table. They were killed 30 min later and uptake of radioactivity was measured into total homogenate protein, into soluble supernatant protein and into tubulin harvested from the supernatant fraction. Uptake into whole homogenate protein is expressed as radioactivity recovered in protein as a percentage of total [^{14}C]radioactivity in the same volume of brain homogenate. The amount of radioactivity recovered in the protein of the supernatant fraction or in tubulin is expressed as a percentage of the total radioactivity in the supernatant fraction. The data are given as mean values ± S.E., and significant differences between DOPA-injected groups and controls are indicated by an asterisk (*). The table also shows the percentage of polyribosomes in the ribosomal profile at different times after DOPA injection. The profiles were run on samples of three pooled brains; the data are the average of three determinations on each pooled sample.

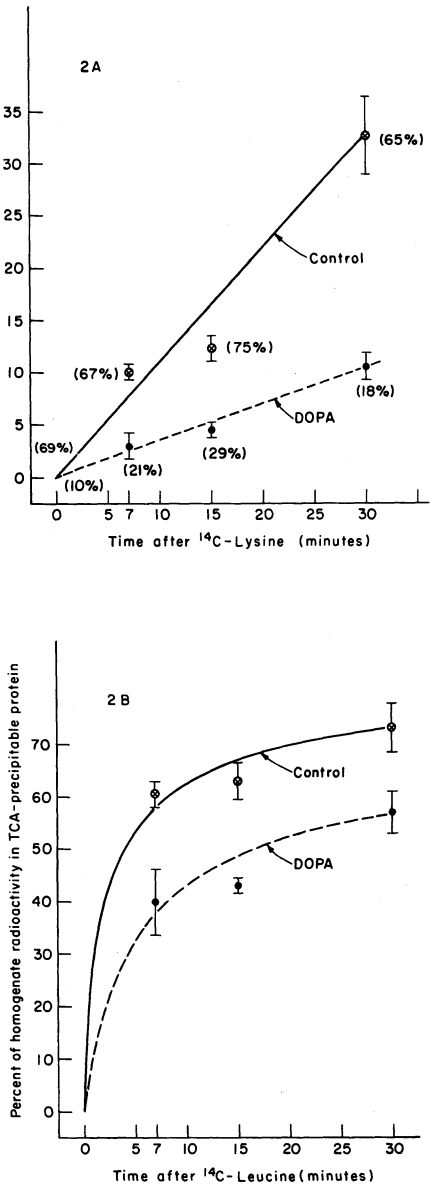

FIG. 2. Effect of L-DOPA administration on incorporation of [^{14}C]lysine (2a) and [^{14}C]leucine (2b) into brain protein. Groups of six rats received 500 mg L-DOPA/kg body weight or the 0·05 N HCl diluent intraperitoneally and 45 min later were given 1·5 μCi of either [^{14}C]lysine or [^{14}C]leucine intracisternally. They were killed at the times indicated after [^{14}C]amino acid injection. Radioactivity recovered in brain protein is expressed as a percentage of total radioactivity in the homogenate. The standard errors are shown as bars above and below each point. Ribosomes were also prepared at these times and the percentage of polyribosomes in the profiles is shown in parentheses beside each point in 2a.

the supernatant protein and the tubulin, the depression being the same extent in all three proteins and coinciding with extensive polysome disaggregation. At 120 min after DOPA administration, the reduction in [^{14}C]lysine uptake is less and is significant only in the case of the soluble protein fraction; polysome aggregation is almost complete at this time. Finally, 240 min after DOPA administration, [^{14}C]lysine incorporation into total brain protein and supernatant protein is no longer depressed below the control levels. Thus recovery of amino acid incorporation into brain protein coincides with reaggregation of brain polyribosomes.

DISCUSSION

Recently HARTMANN & BECKER (1973) have reported, on the basis of electron micrographs, that phenylalanine-induced and L-DOPA-induced brain polysome disaggregation is an artifact of preparation, and does not reflect the state of polysome disaggregation *in vivo*. Our earlier studies (WEISS *et al.*, 1971) indicate that the dose of L-DOPA used by Hartmann and Becker may have been inadequate to disaggregate brain polyribosomes in 40-g rats. Their finding is also in conflict with our present observations on [^{14}C]amino acid incorporation *in vivo*, which demonstrate a close parallel between polysomal aggregation states and uptake of labelled amino acids into the whole brain protein *in vivo*. At a time after L-DOPA administration when brain polysomes are known to be disaggregated, the *in vivo* uptake of amino acids into brain protein is markedly depressed, whether all TCA-precipitable proteins, the soluble proteins of the supernatant fraction, or tubulin are examined (Table 3). At 120 min after DOPA administration, polysomes are reaggregated and protein synthesis has returned to control values (Table 3). A significant inhibition of the synthesis of soluble brain proteins persists somewhat longer than the inhibition of total homogenate proteins, possibly reflecting a difference in the population of proteins synthesized (Table 3). It has been reported that free polysomes are more sensitive to protein synthesis inhibitors and disaggregation agents than are bound polysomes (SARMA *et al.*, 1969; HEMMINKI, 1972).

Our observation of rapid metabolism of L-leucine in the brain is in agreement with the findings of OGAWA *et al.* (1970), who have observed a very active branched-chain amino acid transaminase in rat brain. However, lysine does not appear to be attacked, in agreement with the general concept that its site of metabolic degradation is limited to the liver (ELWYN,

1970). ROBERTS (1974) has also observed that leucine but not lysine are metabolized following intracranial administration.

It is concluded that L-DOPA disaggregation of brain polysomes is a physiologically significant phenomenon, reflecting altered rates of *in vivo* protein synthesis. L-DOPA therefore provides the neurochemist with a potentially useful experimental tool for transient suppression of brain protein synthesis. The repeated disaggregation of brain polysomes and suppression of brain protein synthesis that might be caused by repeated doses of L-DOPA could affect the growth and development of the brain. This possibility should be considered in the treatment of pediatric populations with this drug.

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