FURTHER EVALUATION OF CONTINUOUS-FLOW IONIZATION CHAMBER METHOD FOR DOPA DECARBOXYLASE ASSAY

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A further evaluation of the continuous-flow ionization chamber method with DOPA-carboxyl-^{14}C for DOPA decarboxylase assay in vitro is presented. This method promises to be very useful because the decarboxylation of DOPA and the activity of DOPA decarboxylase seem to be increasing in importance in biochemistry and medicine.

The conversion of 3,4-dihydroxyphenylalanine (DOPA) to dopamine by aromatic L-amino acid decarboxylases (*1,2*) can be determined by various procedures such as manometric and fluorometric (*3,4*) methods as well as radioisotope methods (*5*). Unfortunately, these methods were unable to provide an immediate and continuous measurement of DOPA decarboxylase activity in isolated tissues (*1–5*). In a recent communication (*6*), we have described a reliable method using a modified device containing a vibrating-reed electrometer and an ionization chamber for instantaneous and continuous measurement of DOPA decarboxylation. This paper concerns a further evaluation of this method for DOPA decarboxylase assay in vitro.

METHODS AND MATERIALS

Wistar male rats (Canadian Breeding Laboratories, Quebec) weighing 250–300 gm were killed by decapitation. Individual brains, kidneys, and livers were removed immediately and stored in an ice water bath. The brain, liver, or kidney tissue was homogenized in the cold in 0.1 *M* phosphate buffer, pH 7.0, in a glass homogenizing tube equipped with a Teflon pestle.

Details of the apparatus* devised for an instan-

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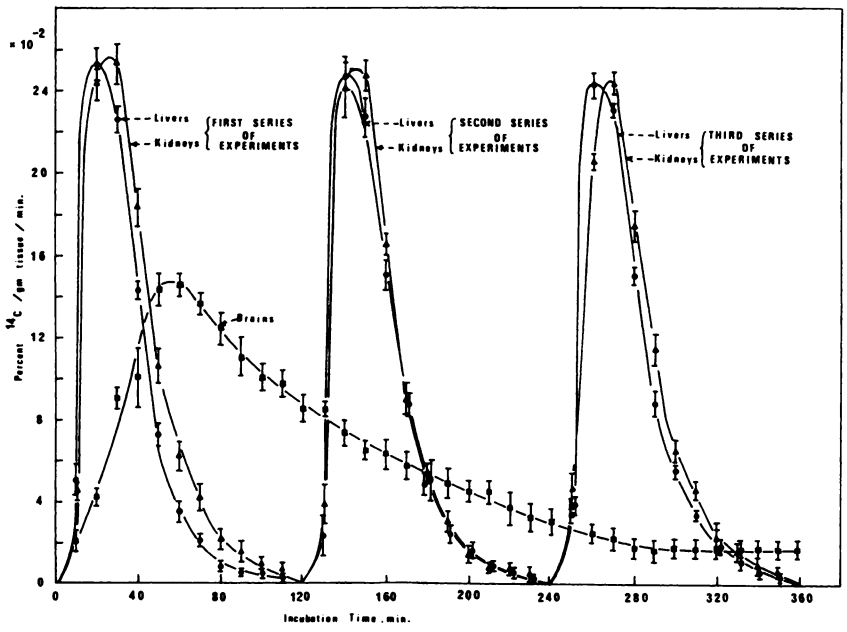

FIG. 1. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with rat liver and kidney homogenates for three periods of 0–120, 120–240, and 240–360 min, respectively, or incubated with brain homogenates for 0–360 min in 0.1 *M* phosphate buffer, pH 7.0, at 37°C, and in N_2 atmosphere. The ordinate is percent of incubated ^{14}C produced as $^{14}CO_2$ per gram tissue per minute, and abscissa is time in minutes after administration of ^{14}C-labeled DOPA. Each point represents mean of $^{14}CO_2$ production of each group of four experiments at given time, and length of vertical bar through each point represents ±1 s.e. of mean.

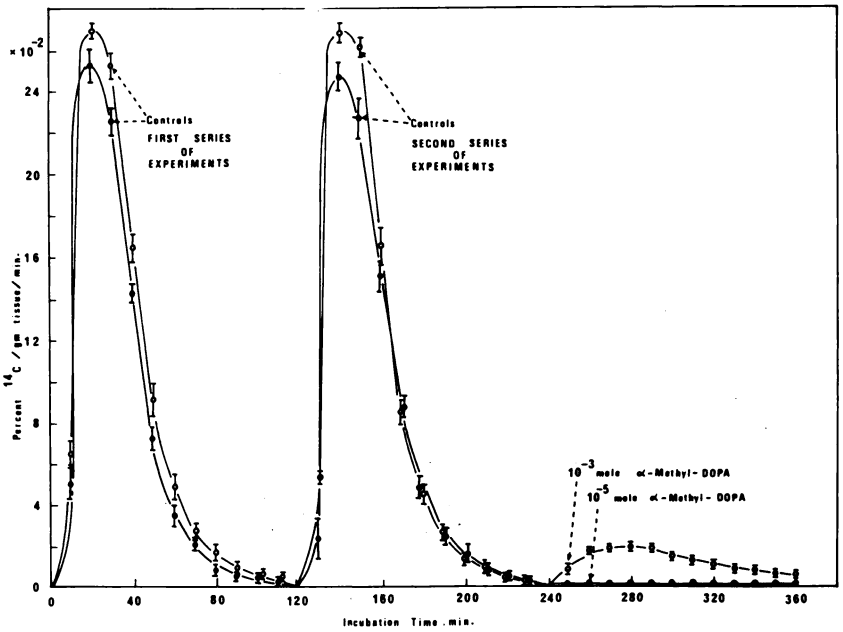

FIG. 2. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with rat liver homogenates for three periods of 0–120, 120–240, and 240–360 min, respectively. Various amounts of DL-α-methyl DOPA were added to enzyme samples at third 240–360-min period of $^{14}CO_2$ studies. See details in Fig. 1.

taneous and continuous measurement of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C (specific activity, 3.4 mCi/m*M*; radiochemical purity >99%; New England Nuclear Co., Boston, Mass.) have been published previously (*6,7*).

For experimental procedure, fresh rat liver or kidney homogenates were incubated with 0.12 μCi DOPA-carboxyl-^{14}C in N_2 at 37°C, and continuous graph plotting of the $^{14}CO_2$ data was achieved by a chart recorder for 120 min. After 120 min of this first $^{14}CO_2$ study, 0.12 μCi DOPA-carboxyl-^{14}C was added to the same enzyme sample, and the experiment was carried out for the next 120 min. Similarly, the last $^{14}CO_2$ study was carried out for another 120-min period. Three experiments were therefore performed in one enzyme sample for 360 min, using a total amount of 0.36 μCi DOPA-carboxyl-^{14}C. Various amounts of DL-α-methyl DOPA (Sigma Chemical Co., St. Louis, Mo.) were added to the incubation chamber of the device at the period of 240–360 min of $^{14}CO_2$ studies.

In the second series of studies, two experiments were carried out in one enzyme sample for 240 min using a total amount of 0.24 μCi DOPA-carboxyl-^{14}C. Various amounts of cupric sulfate ($CuSO_4$) (J.T. Baker Chemical Co., Philipsburg, N.J.) were incubated at the period of 120–240 min of $^{14}CO_2$ studies.

In other experiments, fresh brain homogenates were incubated with 0.12 μCi DOPA-carboxyl-^{14}C and $^{14}CO_2$ production was measured continuously for 360 min.

RESULTS

Figure 1 contains composite data showing the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with fresh brain, liver, and kidney homogenates for 360 min or for the periods of 0–120 min, 120–240 min, and 240–360 min. It is clearly seen that the turnover of ^{14}C-labeled DOPA involved in the decarboxylation in liver or kidney tissues is faster than that obtained in brain tissues.

Figure 2 contains composite data showing changes in the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without DL-α-methyl

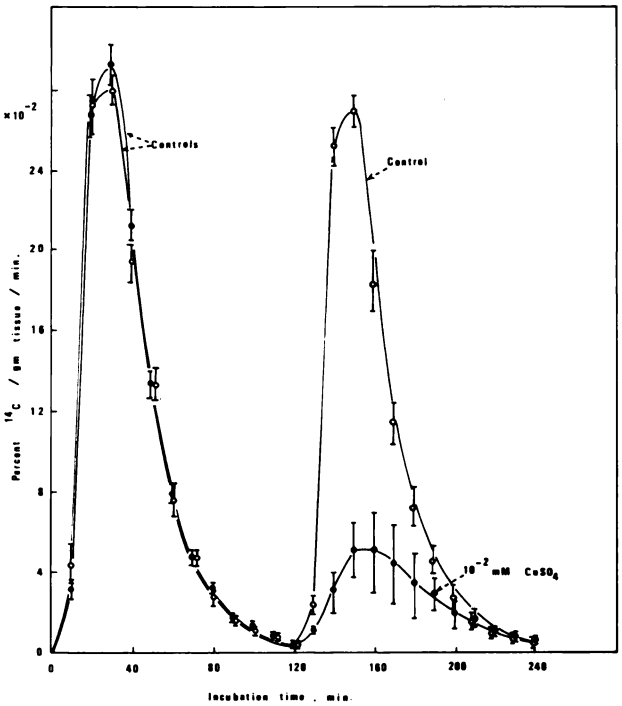

FIG. 3. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with rat liver homogenates for periods of 0–120 and 120–240 min, respectively. SO_4Cu was added to the enzyme samples at second 120–240-min period of $^{14}CO_2$ studies. See details in Fig. 1.

DOPA and liver homogenates. Similarly, Fig. 3 contains composite data showing changes in the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without $CuSO_4$ and liver homogenates.

As shown in Tables 1 and 2, there were no changes in T_{max} ($p > 0.05$) and cumulative percent ^{14}C per gram tissue ($p > 0.05$) among the periods of 0–120, 120–240, and 240–360 min of $^{14}CO_2$ studies, respectively, from DOPA-carboxyl-^{14}C when incubated with the same enzyme sample of rat liver or kidney homogenates. There was a decreased $^{14}CO_2$ production ($p < 0.001$) and a prolonged T_{max} ($p < 0.001$) in the presence of 1×10^{-3} mole DL-α-methyl DOPA. A complete inhibition of DOPA decarboxylation was also seen with 1×10^{-5} mole DL-α-methyl DOPA. In Table 3, a decreased $^{14}CO_2$ production ($p < 0.02$) and a prolonged T_{max} ($p < 0.01$) at the period of 120–240 min of studies were also observed when 1×10^{-2} mM $CuSO_4$ was added to the enzyme sample.

DISCUSSION

A large number of radiometric methods have been used for studies of low activities of DOPA decarboxylation in isolated tissues (*3,8,9*). These methods were based on a modified standard Warburg technique which allowed the collection of $^{14}CO_2$ liberated from DOPA-carboxyl-^{14}C and the determination of the radioactivity of ^{14}C by a scintillation liquid counting (*3,8,9*). They were very time consuming because a very large number of enzyme samples were required for the measurement of $^{14}CO_2$ production curves which were subsequently obtained from the decarboxylation of ^{14}C-labeled DOPA. To overcome such difficulties, we recently used an ionization chamber method for continuous measurement of DOPA decarboxylase activities in vitro (*6*).

TABLE 1. T_{max} OF $^{14}CO_2$ PRODUCTION CURVES FROM DOPA-CARBOXYL-^{14}C INCUBATED WITH KIDNEYS, BRAINS, LIVERS, WITH OR WITHOUT VARIOUS CONCENTRATIONS OF α-METHYL DOPA*

Category	T_{max} (min ± s.e.) A	B	C	D
Kidney homogenates (4)	24.625 ± 0.554	27.250 ± 0.478 ($p > 0.05$)	26.875 ± 0.426 ($p > 0.05$)	—
Liver homogenates (4)	24.250 ± 0.478	24.000 ± 0.070 ($p > 0.05$)	24.500 ± 0.644 ($p > 0.05$)	—
Liver homogenates + 10^{-5} mole α-methyl DOPA (4) (at 240–360 min)	23.875 ± 0.426	24.250 ± 0.248 ($p > 0.05$)	43.750 ± 1.600 ($p < 0.001$)	—
Liver homogenates + 10^{-3} mole α-methyl DOPA (4) (at 240–360 min)	24.425 ± 0.544	27.175 ± 0.445 ($p > 0.05$)	—	60.750 ± 2,954
Brain homogenates (4)	—	—	—	—

* A, B, C, and D represent experimental periods of 0–120 min, 120–240 min, 240–360 min, and 0–360 min respectively. Number of enzyme samples for each series of studies was noted in parentheses.

TABLE 2. CUMULATIVE PERCENTAGE OF $^{14}CO_2$ PRODUCTION FROM DOPA-CARBOXYL-^{14}C INCUBATED WITH OR WITHOUT VARIOUS CONCENTRATIONS OF α-METHYL DOPA*

Category	^{14}C production within 0–120 min or 0–360 min (% ^{14}C ± s.e./gm tissue) A	B	C	D
Kidney homogenates (4)	9.257 ± 0.698	9.428 ± 0.261 ($p > 0.05$)	9.584 ± 0.183 ($p > 0.05$)	—
Liver homogenates (4)	8.394 ± 0.338	8.780 ± 0.342 ($p > 0.05$)	8.935 ± 0.107 ($p > 0.05$)	—
Liver homogenates + 10^{-5} mole α-methyl DOPA (4) (at 240–360 min)	9.479 ± 0.287	9.304 ± 0.344 ($p > 0.05$)	1.482 ± 0.064 ($p < 0.001$)	—
Liver homogenates + 10^{-3} mole α-methyl DOPA (4) (at 240–360 min)	9.260 ± 0.677	9.321 ± 0.255 ($p > 0.05$)	0	—
Brain homogenates (4)	—	—	—	20.604 ± 1.784

* See Table 1 for details.

TABLE 3. T_{max} AND CUMULATIVE PERCENTAGE OF $^{14}CO_2$ PRODUCTION FROM DOPA-CARBOXYL-^{14}C INCUBATED WITH OR WITHOUT $CuSO_4$

Category	T_{max} (min ± s.e.)		^{14}C production within 0–120 min (% ^{14}C ± s.e./gm tissue)	
	A	B	A	B
Liver homogenates (4)	25.125 ± 0.509	25.000 ± 0.812	11.050 ± 0.176	10.463 ± 0.507
Liver homogenates + 10^{-2} mM $CuSO_4$ (4) (at 120–240 min)	24.375 ± 1.024 ($p > 0.05$)	36.750 ± 2.461 ($p < 0.01$)	11.482 ± 0.407 ($p > 0.05$)	3.290 ± 1.294 ($p < 0.02$)

* A and B represent experimental periods of 0–120 min and 120–240 min respectively. Number of enzyme samples for each series of studies was noted in parentheses.

The present study is a further evaluation of this method. We found that there were no changes in T_{max} and cumulative $^{14}CO_2$ production from DOPA-carboxyl-^{14}C for the periods of 0–120, 120–240, and 240–360 min of three series of experiments performed at the same enzyme sample. This result indicates the stability of DOPA decarboxylase in these rat tissues and also improves the reliability of our technique. As shown in Fig. 1, the turnover of ^{14}C-labeled DOPA involved in the decarboxylation for the production of dopamine in liver or kidney homogenates is faster than that observed in brain homogenates. An inhibition of DOPA decarboxylation by DL-α-methyl DOPA (*10*) for a 240–360-min period or by $CuSO_4$ (*11*) for a 120–240-min period of $^{14}CO_2$ experiments shows furthermore that the ionization chamber is an outstanding method for in vitro studies of the continuous effect of inhibitors on DOPA decarboxylase in the unique tissue sample. In fact, for the first time, one enzyme sample can be used for both the control and the experiment.

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