Effects of Pyridoxal Phosphate and L-Dopapyridoxal Phosphate on DOPA Decarboxylase Activity

The conversion of 3, 4-dihydroxyphenylalanine (DOPA) to dopamine by DOPA decarboxylase is known in animal[1] and human[2] tissues. The maximum DOPA decarboxylase activity is obtained by the addition of pyridoxal phosphate (PLP) to the reaction mixture, but the degree to which the enzyme is activited depends on PH, substrate, source of enzyme, and other factors[3]. There have been many reports concerning compounds which inhibit the enzymatic decarboxylation, particularly of DOPA[4]. In the present study, we wish to report our observations on the continuous effect of PLP and L-DOPA-PLP solution on the decarboxylation of L-DOPA in the rat liver, respectively.

Methods and materials. Details of the apparatus devised for an instantaneous and continuous measurement of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C have been published previously[5,6]. For experimental procedure, rat liver homogenates were incubated with or without PLP, PLP plus L-DOPA, and L-DOPA in N_2, at 37 °C, for 10 min. After 10 min of incubation, 0.12 μCi DOPA-carboxyl^{14}C (specific activity: 3.4 μCi/mmole) was added to the incubation chamber with an enzyme concentration of 2.5 mg tissue/ml. The total volume of incubation mixture was 40 ml. Continuous plotting of the $^{14}CO_2$ data was achieved by a chart recorder.

Results. The Figure represents data showing changes in rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C when this labeled substrate was incubated with rat liver homogenates with or without PLP, L-DOPA, and L-DOPA plus PLP.

As shown in the Table, there was no change in $^{14}CO_2$ production ($p > 0.05$) when 0.6×10^{-3} *M* PLP was incubated with liver homogenates as compared to values obtained without PLP.

Similarly, an unchanged $^{14}CO_2$ production ($p > 0.05$) was obtained in the presence of 15×10^{-6} mole L-DOPA. A decreased $^{14}CO_2$ production was obtained in the presence of either 2.5 m*M* PLP ($p < 0.001$) or 0.6 m*M* PLP plus 15×10^{-6} mole L-DOPA ($p < 0.001$), respectively.

Discussion. It is known that L-DOPA was generally added to the enzyme sample incubated with ^{14}C-labeled DOPA for the measurement of DOPA decarboxylase activity[2]. L-DOPA is also known to inhibit the decarboxylation of DOPA both in vivo and in vitro studies[7]. We have observed, however, that small doses of L-DOPA did not influence DOPA decarboxylase activity of rat liver homogenates in 0.1 *M* phosphate buffer at PH 7.0. But a

Cumulative $^{14}CO_2$ production during initial 120 min from DOPA-carboxyl-^{14}C incubated with rat liver homogenates, with or without L-DOPA, pyridoxal phosphate, and L-DOPA plus pyridoxal phosphate, respectively. Each series of studies consist of 4 experiments. ^{14}C production in 120 min is expressed as (percentage ± S.E.)/g tissue. Results of *t*-test for probability of significance are made between means of each experimental group compared to the control group.

Category	^{14}C production in 120 min
Control	9.160 ± 0.398
0.6 m*M* PLP	7.895 ± 0.426 ($p > 0.05$)
0.6 m*M* PLP + 15 μmoles L-DOPA	4.375 ± 0.187 ($p < 0.001$)
15 μmoles L-DOPA	8.863 ± 0.932 ($p > 0.05$)
2.5 m*M* PLP	3.443 ± 0.142 ($p < 0.001$)

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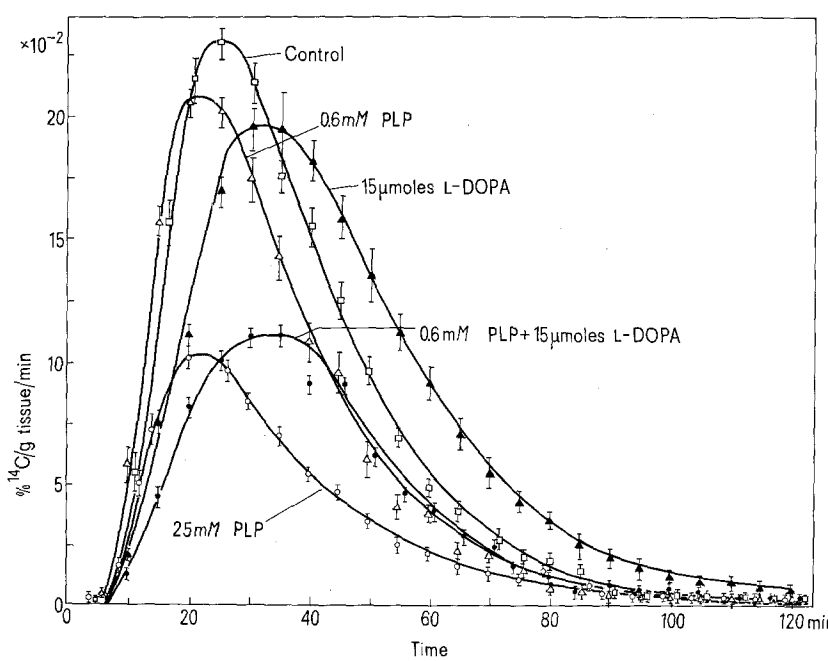

Composite data of the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without PLP, L-DOPA, and PLP plus L-DOPA in a 0.1 *M* phosphate buffer, pH 7.0. The ordinate represents the percent of incubated ^{14}C produced as $^{14}CO_2$/min/g tissue, and the abscissa represents time in minutes after the administration of DOPA-carboxyl-^{14}C. Each point represents the mean of the $^{14}CO_2$ production of 4 experiments at the given time and the length of the vertical bar through each point represents ± 1 standard error of the mean.

marked inhibition of the enzyme was found after the administration of 0.6 m*M* PLP plus the same amount of L-DOPA. Such an enzyme inhibition was possibly caused by condensation products formed from L-DOPA and PLP in the incubation chamber, such as L-DOPA-PLP complex[7]. It is well established that L-DOPA and m-hydroxyphenylethylamines react nonenzymatically with aldehydes including PLP to corresponding tetrahydroisoquinoline derivatives[8]. These products are stable[7,8] and can account for the inhibition of enzymic decarboxylation activity obtained in our in vitro studies. Such an inhibition of DOPA decarboxylase by L-DOPA plus PLP may not be caused by an enlarged pool of L-DOPA subsequent to the addition of carrier L-DOPA to the enzyme mixture, since no change in the decarboxylation of DOPA was found with such a very small amount of L-DOPA (Figure).

The fact that DOPA decarboxylase activity is inhibited by L-DOPA plus PLP, possibly by condensation products formed from these substrates[7,8] may support previous clinical[9] and experimental[10] studies showing that pyridoxine antagonized the activity of L-DOPA in the treatment of parkinsonism. Interestingly, the salutary effects as well as the serious side effects observed in patients treated with L-DOPA were also reversed by pyridoxine[10,11].

Sommaire. Les résultats obtenus démontrent une inhibition de l'activité de la DOPA decarboxylase par de haute concentration de PLP ou de faible concentration de L-DOPA plus PLP. Ceci pourrait expliquer les observations cliniques et expérimentales précédentes démontrant que la pyridoxine antagonise l'effet de L-DOPA utilisé dans le traitement de la maladie de Parkinson.

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Sterols of the Brine Shrimp, *Artemia salina*, from Mono Lake, California

Mono Lake, California, has no outlet for its waters, consequently its salinity is almost twice that of the sea. In addition to their high salt content (ca. 2.23 *M*) and high content of dissolved organic matter (ca. 62 mg/l), the waters of Mono Lake have a fairly basic pH (ca. pH 9.6) with 95% of the buffer capacity contributed by the carbonate-bicarbonate system and the remaining 5% contributed mainly by borate. As a result of this extreme environment, the biological composition of the lake is relatively simple and the highest organism found in the lake waters is the brine shrimp, *Artemia salina* (L.). MASON[1] who obtained the data given above, has surveyed the limnology of Mono Lake showing the extreme environmental conditions in the habitat of these shrimp. Others[1,2] have shown that the brine shrimp in Mono Lake are physiologically distinct from the populations found in San Francisco Bay, California, and the Great Salt Lake of Utah.

Sterols have been implicated as having major roles in maintaining the stability of biological membranes[3–5] and variations in the permeability of erythrocyte membranes have been observed as a result of removing part of their sterol compliment or replacement of their cholesterol content with other sterols[6]. Since cellular membranes serve as an interface between an organism and its environment and, since sterols are an important component of these membranes, it was possible that the sterol composition of *A. salina* from Mono Lake would be altered as a result of its adaptation to this unusual environment.

Fresh-frozen brine shrimp, harvested from Mono Lake, were obtained from the California Koi Company (Glendora, California), thawed in tap water, and washed several times with cold 0.1 *M* KCl to remove debris and adhering material. The shrimp were initially extracted with diethyl ether-ethanol (2:1, v/v), and the residue was re-extracted 3 times with diethyl ether, after which the combined extracts were concentrated on a rotary evaporator and taken up in petroleum ether (B.P. 30–60 °C).

The concentrate was chromatographed on silicic acid[7], and the sterol containing fractions were collected. The sterol ester fraction was saponified and the sterols were combined with the free sterol fraction from the silicic acid column. The combined sterol fraction was further purified on Florisil[8]. A yellowish contaminant was eluted with the sterol fraction from Florisil.

Preparative thin-layer chromatography on Silica Gel G was used for the final isolation of brine shrimp sterols. The developing solvent contained: petroleum ether, diethyl ether, and acetic acid (50:50:0.25; v/v/v). This sterol fraction was completely precipitated by digitonin[9] and gave a positive Lieberman-Burchard reaction[10]. There was no evidence for 'fast-acting' sterols.

Sterol acetates were prepared by reacting the sterols with acetic anhydride in pyridine, and stored in petroleum ether. Cholesterol acetate prepared from commercial cholesterol gave m.p. 114°–115°. The brine shrimp sterol acetate gave m.p. 111°–112° and a mixture of the shrimp sterol acetate and cholesterol acetate gave m.p. 113°–114°. The brine shrimp sterol acetate had an IR-spectrum virtually identical with that of cholesterol acetate.

Gas chromatography of the brine shrimp sterol acetate was performed on a glass column containing 1% XE-60

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