EFFECT OF DEAMINATED METABOLITES OF PHENYLALANINE ON DESATURATION OF STEARYL CoA BY CELL FREE PREPARATIONS FROM DEVELOPING RAT BRAIN

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1. Introduction

In hyperphenylalaninemic rats the ratio of mono-unsaturated to saturated fatty acids in lipids of whole brain and myelin is lower as compared with saline treated controls [1,2]. In phenylalanine-treated rats the rise in cerebral phenylalanine levels is accompanied by a rise in the levels of deaminated metabolites of phenylalanine, namely phenylpyruvate, phenyllactate and phenylacetate [3,4]. Cook and Spence [5] have shown that in brain, the supply of mono-unsaturated fatty acids is regulated by a desaturation system. Thus, the reduction in unsaturated fatty acids in the brains of phenylalanine-treated rats could be due to an interference with the desaturation process by either phenylalanine or by one of its metabolite(s). We have therefore investigated the in vitro effects of phenylalanine and its metabolites on the desaturation of stearyl CoA by preparations from brain tissue. The results indicate that the desaturation process is inhibited by phenyllactate and phenylacetate but not by phenylpyruvate and phenylalanine, and thereby suggest that the reduction in the proportion of unsaturated fatty acids in the brains of phenylalanine-treated rats is due to increases in cerebral levels of phenyllactate and phenylacetate.

2. Materials and methods

1-[^{14}C]stearyl CoA (sp. act. 57 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.). Its radiochemical purity was checked by thin-layer chromatography of the methyl ester. Nonradioactive stearyl CoA, stearic acid, oleic acid and NADH were purchased from Sigma Chemical Company (St. Louis, Mo.). Bovine serum albumin (BSA) was purchased from Pentex Corporation (Kankakee, Ill.).

Sprague-Dawley rats (13–20 day-old) were sacrificed, and the brains were removed, weighed and homogenized in 9 vol of 0.32 M sucrose containing 1.0 mM EDTA. The homogenate was spun at 800 *g* for 10 min, and the resulting supernatant was employed as the source of enzyme. Protein content was determined by the biuret method [7]. Incubation mixtures for the assay of stearyl CoA desaturase contained 8 mg of enzyme protein, 0.7 mM NADH, 10 mM citrate–phosphate buffer (ph 6.4), 0.3 ml of 1% BSA, 1-[^{14}C] stearyl CoA (100 000 cpm) and 70 μM unlabeled stearyl CoA in a final vol of 2.0 ml. Incubations were carried out aerobically with gentle shaking for 15 min at 37°C. Reactions were terminated by the addition of 3.0 ml of 15% KOH in 50% ethanol, and the mixtures saponified for 20 min at 80°C. Nonsaponifiable lipids were extracted with petroleum ether and discarded. The aqueous phase was then acidified with 6 N HCl and fatty acids extracted twice with petroleum ether. The extract containing fatty acids was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated under nitrogen. The fatty acids were methylated with 10% BF_3 in methanol and the resulting methyl esters were separated by thin-layer chromatography on silver nitrate impregnated silica gel H as described by Morrison and Smith [8]. The spots were visualized under ultraviolet light after spraying with a 0.5% solution of Rhodamine 6G in ethanol. R_f values for methyl stearate and methyl oleate in this system were 0.90 and 0.50, respectively. The areas correspon-

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ding to the respective methyl esters were scraped and the esters were eluted twice with ethyl ether. The [^{14}C] content in the ester fractions was assayed by using a liquid scintillation spectrometer. The amount of stearyl CoA desaturated was calculated from the radioactivity recovered from the region of the chromatogram containing the methyl oleate. Gas liquid chromatography of the material from this region gave the major peak corresponding to $C_{18:1}$ and a small peak corresponding to $C_{20:1}$.

3. Results and discussion

Initial studies indicated that desaturation of stearyl CoA was linear up to 20 min of incubation, and was optimal at 100 μM stearyl CoA. The amount of stearyl CoA desaturated increased in proportion to an increase in supernatant protein concentration of up to 10 mg. The results presented in table 1 show that phenyllactate and phenylacetate inhibited the desaturation of stearyl CoA by 50% at concentrations of 3 mM. On the other hand, phenylalanine and phenylpyruvate even at concentrations as high as 5 mM had little or no effect on the reaction. We next examined the effect of phenyllactate and phenylacetate at varying concentrations on the desaturation of stearyl CoA. The inhibition was proportional to the inhibitor concentrations

Table 1
Effects of phenylalanine and its metabolites on desaturation of stearyl CoA by 800 *g* (10 min) supernatant from rat brain

Additions	Stearyl CoA desaturated	
	p mol/mg protein/min	% of Control
None	40.2	100
Phenylalanine (3 mM)	36.0	89.4
" (5 mM)	35.4	88.1
Phenylpyruvate (3 mM)	36.4	90.5
" (5 mM)	35.6	88.6
Phenyllactate (3 mM)	17.0	42.2
Phenylacetate (3 mM)	13.8	34.4

The details of the assay conditions are given in text. The solutions of inhibitors were adjusted to pH 6.4 with 1 M NaOH prior to their additions to the incubation mixtures. The results of a typical experiment are presented in the table. Each value is an average of closely agreeing duplicate determinations.

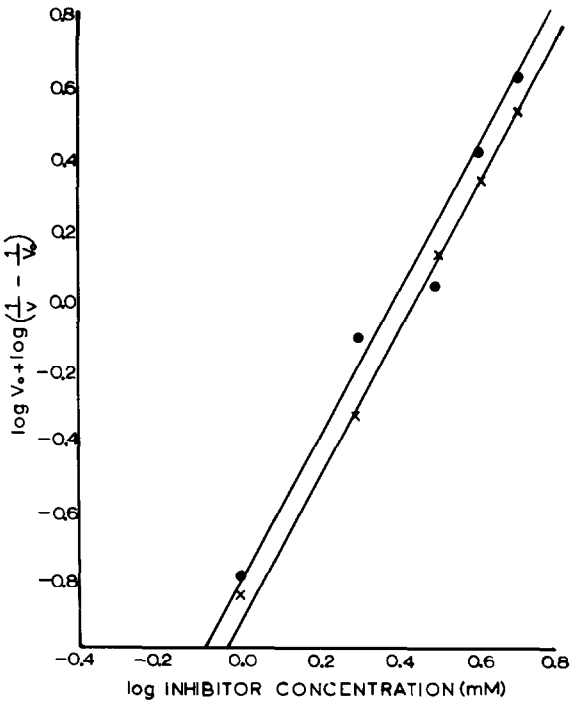

Fig.1. Inhibition of stearyl CoA desaturation as a function of inhibitor concentration. The details of assay conditions are described in text. The solutions of phenylacetate and phenyllactate were adjusted to pH 6.4 with 1 M NaOH prior to their addition to the incubation mixture. V_0 and v are the uninhibited and inhibited rates of reaction respectively. The calculated value for the slope as a function of phenyllactate is 1.91, and that of phenylacetate is 2.0. Each plotted point is an average of closely agreeing duplicate determinations. (●—●—●) phenyllactate, (X—X—X) phenylacetate.

over the range of 1 to 5 mM. Hill type plots of the inhibition (fig.1) by phenyllactate and phenylacetate were linear with slope values of 1.91 and 2.00, respectively, suggesting a second order interaction between the inhibitor molecules and the desaturase system at a single rate limiting step [9].

In brain tissue, as in other animal tissues, desaturation of stearyl CoA requires molecular oxygen and reduced pyridine nucleotide. Phenylacetate does not inhibit oxygen utilization by brain mitochondria [10], thus the site of inhibition is not associated with the reaction(s) utilizing molecular oxygen. In view of the similarities in the kinetics of phenylacetate and phenyllactate inhibition we would presume their mechanism of inhibition to be similar.

The results of the present study show that phenyllactate and phenylacetate added in vitro inhibit the desaturation of stearyl CoA but that phenylalanine added in vitro has no effect in the reaction. The cerebral levels of these metabolites are elevated, in phenylalanine-treated rats, and in genetic phenylketonuria. Although the concentrations of phenyllactate and phenylacetate required for in vitro inhibition of the desaturase are considerably higher than the concentrations likely to be encountered in in vivo situations, prolonged exposure of the brain tissue to these metabolites may result in in vivo inhibition of stearyl CoA desaturase. This in turn would cause a reduction in monounsaturated fatty acids. Such a reduction has indeed been observed in brain tissue of rats subjected to chronic hyperphenylalaninemia [1,2] and in brains from phenylketonuric individuals [11–13].

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