Free fatty acid inhibition of α-glycerophosphate dehydrogenase activity in rat brain

α-Glycerophosphate dehydrogenase is known to play an important rate-limiting role in phospholipid synthesis and intracellular hydrogen ion transport (Kornberg & Pricer, 1953; Kennedy, 1953). A recent report from this laboratory demonstrated that the free fatty acid, octanoate, inhibited competitively the activity of α-glycerophosphate dehydrogenase in both adipose tissue and heart (Vijayvargiya & Singhal, 1970). We now report that octanoate produces competitive inhibition of α-glycerophosphate dehydrogenase in rat cerebral cortex and cerebellum.

Female Sprague-Dawley rats, approximately 250 g, were killed by decapitation and bled. The cerebral cortex and cerebellum were rapidly excised and supernatant fluids were obtained as described previously (Singhal, Valadares & Ling, 1967; Singhal, Valadares & Schwark, 1969). The activity of α-glycerophosphate dehydrogenase was determined in the supernatant fluid under strictly linear kinetic conditions by measuring the formation of NAD+ from NADH in an assay system coupled with aldolase (Vijayvargiya & Singhal, 1970). Changes in extinction were recorded for a period of 5 min and enzyme activity was calculated as μmol of substrate metabolized per g of tissue per h at 37°.

The effects of various concentrations of sodium octanoate on the activity of α-glycerophosphate dehydrogenase in the cerebral cortex and cerebellum are shown in Table 1. A definite inhibition of the enzyme activity was observed with 10 mM concentration of the free fatty acid in both regions of the brain. α-Glycerophosphate dehydrogenase in cerebellum and cerebral cortex declined further when the concentration of sodium octanoate was increased and was almost completely inhibited in the presence of 80·0 mM octanoate.

Table 1. *Effect of sodium octanoate on α-glycerophosphate dehydrogenase activity in rat cerebral cortex and cerebellum.* Various concentrations of sodium octanoate (pH 7·4) were added directly to the reaction mixture just before the addition of the substrate, fructose 1,6-diphosphate. Each value is the mean ± s.e. of three determinations of enzyme activity. Values in parentheses indicate the percentages of control values which are taken as 100%.

Sodium octanoate (mM)	α-Glycerophosphate dehydrogenase activity (μmol/g h^{-1})	
	Cerebral cortex	Cerebellum
None (control)	284 ± 20 (100)	365 ± 2 (100)
2·5	245 ± 22 (86)	340 ± 3 (93)
5·0	207 ± 26 (73)	301 ± 3 (82)*
10·0	178 ± 12 (63)*	255 ± 8 (70)*
20·0	133 ± 11 (47)*	196 ± 9 (54)*
40·0	84 ± 14 (30)*	108 ± 4 (30)*
80·0	12 ± 0 (4)*	11 ± 2 (3)*

* Statistically significant difference compared with the control values ($P = <0·05$).

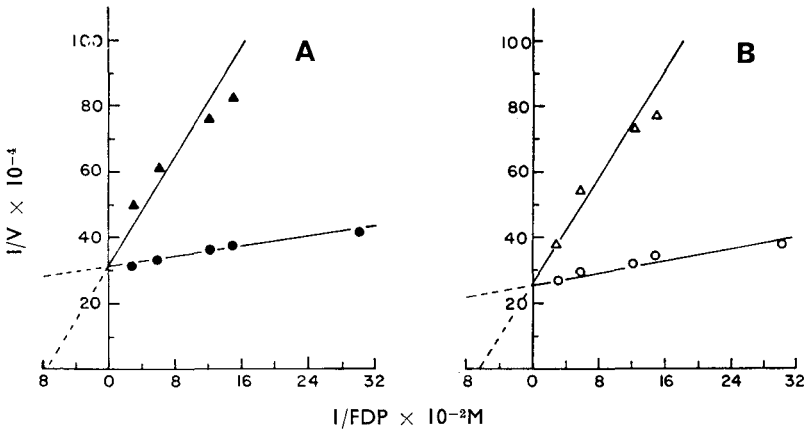

FIG. 1. Lineweaver-Burk plots showing competitive inhibition of α-glycerophosphate dehydrogenase by sodium octanoate in rat cerebral cortex (A) and cerebellum (B). The final concentration of sodium octanoate in the reaction mixture was 10·0 mM. ▲—▲, $K_m = 1{\cdot}35 \times 10^{-2}$M; ●—●, $K_m = 1{\cdot}19 \times 10^{-3}$M; △—△, $K_m = 1{\cdot}56 \times 10^{-2}$M; ○—○, $K_m = 1{\cdot}67 \times 10^{-3}$M.

The nature of octanoate-induced inhibition of α-glycerophosphate dehydrogenase was examined by determining the enzyme activity in presence of varying amounts of the substrate, fructose 1,6-diphosphate, with or without the addition of octanoate (10·0 mM). Lineweaver-Burk plots of the results obtained are shown in Fig. 1. These plots, which extrapolate to the same point on the ordinate, indicate that octanoate produces a competitive inhibition of α-glycerophosphate dehydrogenase in cerebral cortex and cerebellum. The Ki values calculated from these data were found to be $9{\cdot}7 \times 10^{-4}$M for the cerebro-cortical and $1{\cdot}2 \times 10^{-3}$M for the cerebellar enzyme.

It has been demonstrated that preincubation of supernatant fluids with free fatty acids produces marked inhibition of hepatic and cardiac pyruvate kinase as well as that of α-glycerophosphate dehydrogenase in heart and adipose tissue (Weber, Convery & others, 1966; Tsutsumi & Takenaka, 1969; Vijayvargiya & Singhal, 1970). Fig. 2 shows the effects of preincubation with octanoate on the activity of

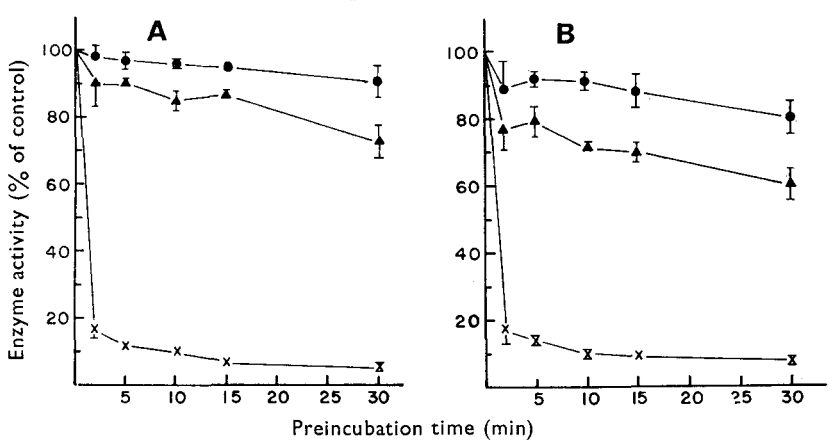

FIG. 2. Effect of preincubation of supernatant fluids with octanoate on α-glycerophosphate dehydrogenase activity in cerebral cortex (A) and cerebellum (B). 0·9 ml of the supernatant fluid was incubated at 37° for various periods of time with either 0·3 ml distilled water or sodium octanoate (1·0 or 5·0 mM). At the end of incubation, a 0·4 ml aliquot from this mixture was added to the assay system for determining enzyme activity. Each point represents the mean ± s.e. of 2 or 3 enzyme determination. ●—●, control; ▲—▲, 1 mM octanoa; ×—×, 5 mM octonoate.

α-glycerophosphate dehydrogenase in the rat cerebral cortex and cerebellum. Incubation at 37° without octanoate for 30 min decreased enzyme activity to 80% of the control values in cerebral cortex and to 91% in the cerebellum. When 1·0 mM sodium octanoate was included in the incubation mixture, the enzyme activity was inhibited to 60% in the cerebral cortex and to 73% of the control values in the cerebellum following 30 min incubation. However, in the presence of 5·0 mM octanoate, a more rapid and pronounced inhibition of the enzyme activity was observed. In this case, α-glycerophosphate dehydrogenase was inhibited by 80% in both cerebral cortex and cerebellum with 2 min preincubation and by 90% when the supernatant fluids were incubated with octanoate for a period of 5 min.

α-Glycerophosphate dehydrogenase plays an important role in nervous tissue since it provides glycerol phosphate for the synthesis of myelin lipids (DeVellis, Schjeide & Clemente, 1967). Laatsch (1962) has shown that the period of most active myelination in the brain is accompanied by a marked rise in the activity of α-glycerophosphate dehydrogenase. DeVellis & others (1967) reported that myelin deficiency observed in the brains of neonatal rats subjected to head X-irradiation was accompanied by a marked decrease in the activity of α-glycerophosphate dehydrogenase. It is generally believed that a reciprocal relation exists between the consumption of carbohydrates and free fatty acids since glycolysis is inhibited while free fatty acids are being utilized (Shipp, Opie & Challoner, 1961; Opie, Evans & Shipp, 1963). Demonstration of the inhibitory effects of free fatty acids on hepatic and cardiac glycolytic enzymes (Weber & others, 1966; Tsutsumi & Takenaka, 1969) led to the suggestion that the free fatty acids may function physiologically in acute adaptation as a "metabolic directional switch" to restrict the flow of glycolysis. The present study demonstrates that octanoate inhibits the activity of α-glycerophosphate dehydrogenase in cerebral cortex and cerebellum of the rat. While the precise significance of these findings must await the demonstration that physiological concentrations of other free fatty acids can produce inhibition of this brain enzyme, it is conceivable that an increase in the intracellular level of free fatty acids may affect the process of myelin formation in nervous tissue.

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