^{31}P-NMR Study of Brain Metabolism in the Rat Model of the Ketogenic Diet

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INTRODUCTION

The ketogenic diet is a non-drug therapy, which is effective in many forms of intractable epilepsy. Its anticonvulsant actions are dramatic in some cases when all other treatments have failed (1). The exact mechanism by which the ketogenic diet works to ameliorate or eliminate seizures is unknown (2). Possibilities include adaptational changes in brain metabolism, changes in electrolytes and water balance. Most researchers favour ketosis as the casual factor. Several authors reported changes in cerebral metabolites extracted from ketotic animals (for review see 3) or detected in human patients using ^{31}P-NMR technique (4). This study reports ^{31}P-NMR in vivo analysis of brain in the rats with various levels of ketosis induced using different versions of the ketogenic diet.

METHODS

Male, Long-Evans rats (Charles River Canada, La Prairie, Quebec) weighing about 400g were used in this study. The rats were divided into 4 groups. The first group (n=4) was assigned to the control diet which had 1:12 ratio by weight of fat:(protein+carbohydrate). Groups 2 to 4 were assigned to 3.6:1 ratio ketogenic diets formulated with butter (n=4), flax (n=9) and medium-chain triglycerides (MCT, n=4) oils as the source of fat. Rats remained on the diet for 90 days before NMR experiment. Samples of tail vein venous blood for measurement of plasma β-hydroxybutyrate (β-HBA) were obtained right before NMR analysis. In vivo ^{31}P-NMR spectroscopy was performed using a Bruker AM-300 spectrometer and a probehead with the surface coil operating at 121.5 MHz for phosphorous. Data were processed using 25 Hz/400 Hz exponential broadening/convolution difference. Brain intracellular pH and free [Mg^{2+}] were calculated from spectra according to (5). For NMR experiment rats were anesthetized with ketamine.

RESULTS and DISCUSSION

Rats consuming the ketogenic diets had significantly higher blood plasma levels of β-HBA than rats on the control diet. MCT-fed rats were most ketotic followed by the rats from flax and butter groups (Table 1). Table 1 also shows some of the parameters measured in ^{31}P spectra of the rat brain. Significant changes compared to control ($p<0.05$) were found in PCr/Pi ratio for all ketogenic groups. However, while PCr/Pi ratio increased in the butter and flax groups, this ratio in MCT rats was significantly lower than in any other group. Free [Mg^{2+}] in the brains of MCT rats was also remarkably low. Brain intracellular pH in the ketogenic rats was within the normal range.

Table 1.

	Control	Butter	Flax	MCT
PCr/Pi	5.4±1.5	6.8±1.1	6.8±1.2	2.7±0.6
PCr/γATP	2.3±0.7	2.3±0.3	2.2±0.3	1.7±0.3
[Mg^{2+}]μM	385±55	336±54	341±54	239±31
β-HBA mmol/L	0.1to 0.3 (range)	0.5 to 1.2 (range)	0.5 to 2.3 (range)	5.6 to 7.8 (range)

Mean±SD

Our spectroscopic data on changes of PCr/Pi ratio obtained for butter and flax groups of the ketogenic rats agree with analogous observation (4) in human patients on the ketogenic diet. Previous reports have shown that brain concentrations of ATP, glucose-6-phosphate, and others were higher and concentrations of such metabolites as ADP, creatine, cyclic nucleotides, were lower in ketogenic diet-fed animals (3). This suggests that some of the observed differences could be explained by the higher energy state of the ketotic brain. However, spectral changes in PCr/Pi ratio for MCT rats were opposite to that in the butter and flax groups. Free [Mg^{2+}] was also low and these could be related to extremely high level of ketosis observed in MCT rats. Although some studies have indicated that plasma levels of ketones correlate with anticonvulsant effect of the diet (6), the optimal level / range of ketosis and the precise way in which the observed metabolic changes may rise the seizure threshold remain unclear.

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