Effect of hyperphenylalaninemia on polyphosphoinositides content of rat brain[1]

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Summary. Hyperphenylalaninemia (experimental PKU) induced in developing rats by treatment with p-chlorophenylalanine (PCPA) plus phenylalanine (PHE) causes a significant reduction in the triphosphoinositide (TPI) and diphosphoinositide (DPI) content of brain. Since TPI and DPI play an important role in excitable nervous membranes, the functional abnormality in experimental and perhaps in genetic PKU may be related to the reduction in TPI and DPI content.

There is evidence that phosphoinositides play an important functional role in excitable nervous membranes. Firstly, polyphosphoinositides (diphosphoinositide, DPI; and triphosphoinositide, TPI) are localized predominantly in myelin[4]; secondly, of all lipids in myelin only the phosphoinositides have significant and rapid metabolic turnover[5]; and thirdly, polyphosphoinositide metabolism has been linked with the calcium content and permeability of the axonal membrane[6]. In studies from this and other laboratories, it has been shown that hyperphenylalaninemia induced during postnatal development results in deficiency of myelin in the brain[7,8] and the proportion of unsaturated fatty acids in the lipids of brain[9] and myelin membrane is reduced[8,10]. In the present paper we report the effect of hyperphenylalaninemia on the content of inositol phospatides in rat brain.

Method. Hyperphenylalaninemia was induced by treatment with p-chlorophenylalanine (PCPA) + phenylalanine (PHE) as described earlier[11]. Pups of Sprague-Dawley rats maintained in our colony were assigned randomly to control (saline-treated) and experimental (PCPA + PHE-treated) groups. The treatment was begun on the 5th postnatal day and continued for a period of 25 days. At 31 days of age and 24 h after the termination of the treatment, animals were sacrificed by decapitation and the brain removed quickly, weighed and homogenized in 20 vol of ice-cold chloroform methanol (2:1 v/v). The interval between the time of sacrifice and the time at which the brain was homogenized ranged between 2 and 3 min. The homogenate was centrifuged at 800 × g for 10 min and the supernatant lipid extract (neutral solvent) was washed as described by Eichberg and Hauser[12]. Phosphatidyl inositol in lipid extract (neutral solvent) was separated by TLC on a precoated silica gel G plate using methyl acetate : n-propanol : chloroform : methanol : 0.25% KCl (25 : 25 : 25 : 10 : 9 v/v) as developing solvent[13]. Polyphosphionositides (TPI and DPI) were extracted from the damp tissue residue from the neutral solvent extract exactly as described by Hauser et al.[14]. TPI, DPI and PI from lipid extract in acidic solvent were separated by TLC on silica gel H plates impregnated with a 1% solution of potassium oxalate as described by Gonzales-Sastre and Folch-Pi[15]. Phospholipids were visualized by charring with 50% sulphuric acid and were quantified by estimating phosphorous by the method of Bartlett[16].

Results. As observed earlier, the body and brain weights of rats in the experimental (PCPA + PHE-treated) group were found to be significantly lower than those of saline-treated controls. The results in the table show that the amounts of TPI and DPI content in brain from hyperphenylalaninemic rats was significantly lower than the controls. A significant amount of PI was present in the acidic solvent extract, and the amount of PI recovered in the acidic solvent system from brain of hyperphenylalaninemic rats was significantly lower. In lipids extracted by the neutral solvent system, on the other hand, the total phospholipid content of brains from rats in the control and experimental groups did not differ significantly; the amount of PI in the neutral solvent extract from brain of hyperphenylalaninemic rats was, however, significantly higher than that in controls. This indicates that extraction of PI from brain by the neutral solvent system is incomplete, and that hyperphenylalaninemia alters slightly the extractability of PI in a neutral solvent system. The reason for this incomplete extraction of PI by the neutral solvent system, and its increased extractability from the brains of hyperphenylalaninemic rats, is not clear at present. It reflects, however, changes in the solubility of PI. Phosphoinositides are known to complex readily with a variety of proteins, forming, in most cases, water soluble complexes[17]. The data, however, indicate that although the amounts of PI extracted in neutral and acidic solvent systems from the brains of control and experimental rats differed significantly, the total PI content (PI in

Effect of hyperphenylalaninemia on the total phospholipids and polyphosphoinositides of rat brain

Treatment	Saline (A) n = 8	PCPA + PHE (B) n = 8	$\frac{\text{mean B}}{\text{mean A}} \times 100$
Average postmortem period (min)	2.41 ± 0.40	2.65 ± 0.30	
Acidic solvent extract			
Total phospholipids (μmole/g wet wt)	5.58 ± 0.54	5.04 ± 0.20^{+}	90.3
Triphosphoinositides (TPI) (nmole/g wet wt)	443.5 ± 40.6	310.4 ± 18.0^{+++}	70.0
% of acidic extract	7.9 ± 1.5	6.2 ± 1.0	
Diphosphoinositides (DPI) (nmole/g wet wt)	308.5 ± 15.9	219.7 ± 31.0^{+++}	71.2
% of acidic extract	5.5 ± 1.3	4.4 ± 0.5	
Phosphatidyl inositol (PI) (μmoles/g wet wt)	1.33 ± 0.20	0.90 ± 0.07^{+++}	67.7
% of acidic extract	23.8 ± 1.5	17.8 ± 1.0	
Neutral solvent extract			
Total phospholipids (μmoles/g wet wt)	36.0 ± 1.27	35.1 ± 3.0	97.5
Phosphatidyl inositol (PI) (μmoles/g wet wt)	1.84 ± 0.06	2.24 ± 0.18^{++}	122.1
% of neutral extract	5.1 ± 0.8	6.4 ± 1.0	

Phospholipids were extracted and separated by TLC as described in the text. Results are expressed as mean ± SD. The number of animals in each group whose brain tissue were analyzed. $^{+}$ Significantly different from control (A): $p < 0.025$; $^{++}$ significantly different from control (A): $p < 0.010$; $^{+++}$ significantly different from control (A): $p < 0.005$.

acidic plus neutral solvent extract) in brains from control and experimental rats was not significantly different.

Discussion. The data presented here indicate that experimental hyperphenylalaninemia induced in rats by treatment with PCPA + PHE caused a significant reduction in the DPI and TPI content of brain but no change in the PI content. This reduction in polyphosphoinositide content could occur as a result either of an inhibition of its synthesis or of increased degradation. Since the PI content in brains from experimental rats did not increase it is unlikely that the reduction in TPI and DPI observed in hyperphenylalaninemic rat brain is related to increased degradation of polyphosphoinositides. Since the enzymes which phosphorylate PI are present in myelin[18], and a reduction in brain myelin content occurs in experimental hyperphenylalaninemia[7,8], the reduced levels of DPI and TPI may not only reflect reduction in brain myelin levels but could also be the result of their reduced synthesis due to the reduction of PI phosphorylating enzymes. It has been suggested that interconversion of TPI and DPI is responsible for the permeability changes in the membrane during excitation[17,19]. Another possibility is that changes in the permeability properties of myelin and other membranes in brain, which are caused by a reduction in the portion of the unsaturated fatty acids in brain lipids of animals with experimental hyperphenylalaninemia[8-10] are responsible for the reduction in polyphosphoinositide in the brains of hyperphenylalaninemic rats. Since the myelin deficit and the reduction of unsaturated fatty acids in brain also occurs in genetic PKU[20], our present finding raises the possibility that in genetic PKU the brain polyphosphoinositide levels are altered and that the functional abnormalities in the PKU brain could in part be related to the reduction in brain polyphosphoinositides.

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Effect of 5-thio-D-glucose on testicular lipids of mice

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Summary. Spermatogenesis is reported to be completely inhibited by 5-thio-D-glucose in mice. In an investigation of this inhibition, testicular lipid constituents, namely, total lipids, phospholipids, triacylglycerol, free and total cholesterol, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and NADPH generators like glucose-6-phosphate dehydrogenase, isocitrate dehyrogenase and malic enzyme were estimated in mice fed with 5-thio-D-glucose (33 mg/kg) by gastric intubation for 21 days. Significant increase in cholesteryl ester, glucose-6-phosphate dehydrogenase activity and malic enzyme and a decrease in free cholesterol and phospholipids were observed.

A structural analogue of D-glucose, 5-thio-D-glucose[2,3] (5-TG) inhibits glucose uptake and glycolysis not only in liver, kidneys and diaphragm[4,5] but also in the testes[6]. The feeding of 5-TG at a daily dose of 33 mg/kg for 3 weeks inhibited spermatogenesis completely[7]. Our earlier work showed that oral feeding of 5-TG inhibited spermatogenesis, but not i.p. administration[6]. The widespread distribution and the high levels of the lipids are indicative of their importance in the function of testes. The concentration of lipids is very important for spermatogenesis and steroidogenesis. When there is active spermatogenesis, little lipid is evident in the testis but during periods when spermatogenesis is impaired, lipids in various forms accumulate in the interstitial cells[8,9]. As spermatogenesis is inhibited in the testis of mice treated with 5-TG, it was of interest to investigate the lipid constituents and some enzymes which offer reducing equivalents of $NADP^+$ for lipogenesis.

Materials and methods. Male albino mice weighing 25-30 g were used. The animals were maintained on the stock laboratory diet (Hindustan Lever, India) and water ad libitum. 5-TG and glucose-6-phosphate (di-sodium salt) were obtained from Sigma Chemical Co. (St. Louis, USA); DL-isocitrate (barium salt) from Nutritional Biochemical Corporation, digitonin from BDH Laboratory, England; malic acid from Seelse-Hannover, Germany. All others were A.R. grade chemicals. Saline-treated control and experimental animals treated with 5-TG (33 mg/kg) for 21 days were used. The 21-day feeding by gastric intubation was the minimum period for arresting the spermatogenesis completely[6]. All animals were fasted for 24 h and sacrificed by decapitation. The testes were taken for various estimation. The total lipid was estimated by the method of Folch et al.[10]. The free and total cholesterol were estimated by the method of Schoenheimer and Sperry modified by Venugo-