Metabolic fingerprints of altered brain growth in a Rett syndrome mouse model : a ^{31}P and ^{1}H MRS study of tissue extracts

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Introduction

Rett syndrome (RS) is the leading cause of profound mental retardation of genetic origin in girls [1]. In most cases, RS is caused by mutations or deletions in the *MECP2* gene [2]. Several behavioral, neuroanatomical and neurochemical features are consistently associated with RS. Among these are decelerating head growth in childhood, resulting in microcephaly, and cerebellar atrophy in adult RS patients [3]. Phospholipid (PL) metabolism plays an essential role in cell growth since PL form the matrix of cell membranes. We therefore performed a comprehensive phospholipidomic study in an RS mouse model with *Mecp2* deletion to analyze metabolic processes underlying reduced brain size.

Methods

Experiments were performed using the mouse model strain B6.129P2(C)-*Mecp2*$^{tm1\text{-}1Bird}$ as described previously [4]. Freeze-clamped brains were extracted with methanol/chloroform/water. The resulting aqueous and organic phases underwent ^{1}H and ^{31}P MRS, respectively. Spectra were acquired on an AVANCE 400 spectrometer (Bruker, Wissembourg), using acquisition parameters described previously [5]. Spectra were referenced using trimethylsilyl tetradeuteropropionate and methylene diphosphonate as external standards, respectively, and evaluated with Bruker's deconvolution software (Topspin 1.3). The metabolite concentrations obtained, as well as selected metabolite ratios, were statistically analyzed employing the Mann-Whitney *U* test (n=4 for each mutants and controls), using Statview 5.0.1 (SAS, Cary, NC, USA).

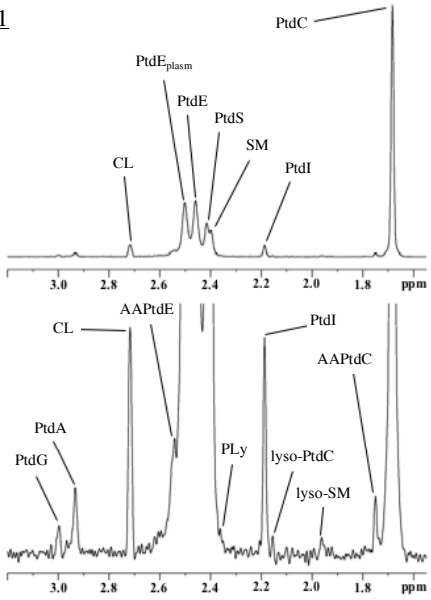
Results

Figure 1 shows a typical ^{31}P MR spectrum representing PL from the *Mecp2*-null brain (lower spectrum magnified). PtdC, phosphatidylcholine; PtdI, phosphatidylinositol; SM, sphingomyelin; PtdS, phosphatidylserine; PtdE, phosphatidylethanolamine; $PtdE_{plasm}$, ethanolamine plasmalogen; CL, cardiolipin; AAPtdC, alkyl-acyl phosphatidylcholine; AAPtdE, alkyl-acyl phosphatidylethanolamine; PtdA, phosphatidic acid; PtdG, phosphatidylglycerol; PLy, unassigned PL. Concentrations of glycerophosphocholine (GPC) lipids and their metabolites (µmol/g tissue wet weight, means ± s.d.) are listed in Table 1 with significance levels (p values). The most prominent PL, PtdC, is enhanced in *Mecp2*-null brain vs. controls, while the PtdC degradation product, lyso-PtdC, is decreased. Phosphocholine (PC) remains constant, while the PC/GPC ratio is increased owing to GPC reduction. The choline (Cho) decrease is of borderline significance, and none of the other PL or PL metabolites are significantly changed.

Table 1

^{31}P MRS :	PtdC	AAPtdC	$PtdC_{plasm}$	lyso-PtdC
controls	17.82 ± 1.28	0.21 ± 0.01	0.50 ± 0.13	0.13 ± 0.03
Mecp2-/y	21.99 ± 1.82	0.24 ± 0.02	0.35 ± 0.03	0.06 ± 0.04
p	0.034*	0.043*	0.021*	0.043*
^{1}H MRS :	Cho	PC	GPC	PC/GPC
controls	0.13 ± 0.03	0.39 ± 0.06	0.53 ± 0.07	0.74 ± 0.03
Mecp2-/y	0.08 ± 0.02	0.38 ± 0.11	0.41 ± 0.15	0.97 ± 0.15
p	0.077(*)	1.000	0.289	0.034*

Fig. 1



Discussion

The PL metabolite pattern observed for *Mecp2*-null mouse brain vs. controls is consistent with a well-established model suggesting a reduced PtdC turnover rate, limiting the ability of cells to grow [6,7], according to the following scheme :

PtdC↑, lyso-PtdC↓ PC/GPC↑ → reduced PtdC turnover → reduced PtdC production → reduced cell growth

Further experiments will reveal to what extent neurons, astrocytes and/or other neural cells are affected by growth reduction due to *Mecp2* mutation. These results may open new avenues for the identification of molecular targets for early and efficient pharmacological RS treatment.

References

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