ORIGINAL ARTICLE

Reduced posterior cingulate glutamate measured by magnetic resonance spectroscopy in hyperthyroidism

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Abstract

OBJECTIVES: Patients with hyperthyroidism frequently have neuropsychiatric complaints such as lack of concentration, poor performance in memory, depression, anxiety and mania. These symptoms suggest the dysfunction of brain. However, the underlying process of this dysfunction is not well understood. At the same time, glutamatergic system has been considered important in neuropsychiatric process by recent studies. Thus, this study is to investigate the change of glutamate concentration in patients with hyperthyroidism using proton magnetic resonance spectroscopy. **METHODS:** Fifteen untreated patients with hyperthyroidism and fifteen age- and gender- matched controls participated in the study. The region of the posterior cingulate cortex was examined by magnetic resonance spectroscopy with a technique referred as TE-averaged PRESS at 3T field strength. The concentrations of N-Acetylaspartate, creatine, choline and glutamate were assessed using jMRUI v4.0 software. **RESULTS:** Hyperthyroid patients, compared with controls, showed a decrease of glutamate concentration ($P<0.047$) and glutamate/creatine ratios ($P<0.009$) in the posterior cingulate cortex. The decrease of choline concentration ($P<0.004$) and choline/creatine ratios ($P<0.012$) were also discovered. No significant difference was found in the concentrations of N-Acetylaspartate or creatine between patients and controls. **CONCLUSION:** Concentration of glutamate decreased in the region of posterior cingulate cortex in patients with hyperthyroidism. This reduction indicated a possible involvement of glutamate in the brain dysfunction in hyperthyroidism.

Abbreviations:
MRS - magnetic resonance spectroscopy
PET - positron emission tomography
Glu - glutamate
Gln - glutamine
Glx - combination of glutamate and glutamine
NAA - N-acetylaspartate
Cr - creatine
Cho - choline
mI - myo-inositol

INTRODUCTION

Patients with hyperthyroidism may show the neuropsychiatric symptoms, such as lack of concentration, poor performance in memory, depression, anxiety and mania (Maccrimmon *et al.* 1797; Alvarez *et al.* 1983; Trzepacz *et al.* 1988). These symptoms suggest the dysfunction of brain. However, the underlying process of this dysfunction is not well understood.

To explain this process, several studies discovered evidences of metabolic changes in the regional brain of the patients with hyperthyroidism. A previous study, using positron emission tomography (PET), showed the reduction of glucose metabolism in the limbic system. It also discovered a negative correlation between Serum free T3/free T4 levels and regional glucose metabolism in the medial posterior cingulate cortex (Schreckenberger *et al.* 2006). Besides, three magnetic resonance spectroscopy (MRS) studies showed reductions in choline (Cho), myo-inositol (mI) and the mixture (Glx) of glutamate (Glu) and glutamine (Gln) (Bhatara *et al.* 1998; Elberling *et al.* 2003; Danielsen *et al.* 2008) in several regions of brain. Yet, the single level of glutamate, which is considered as a major neurotransmitter in brain, is difficult to analyze in the conventional short echo spectra because of the overlap of Glu, Gln and N-Acetylaspartate (NAA).

Glutamate has been linked to a wide range of brain functions, including learning, memory, development, emotion and so on (Rousseaux 2008). Thus, to evaluate the single level of glutamate without disturbance of other metabolism is important to the understanding of the process of brain dysfunction in hyperthyroidism. Recent studies showed that a new method, referred as TE-averaged PRESS, is a reliable spectroscopic tool of glutamate measurement at 3T field strength (Hurd *et al.* 2004). With this technique, the signal from Glu C4 protons (2.35ppm) is fully resolved from the overlap with Gln and NAA.

Thus, the purpose of this study was to detect glutamate levels in the posterior cingulate cortex, which is the susceptible region indicated by the PET study (Schreckenberger *et al.* 2006), of patients with hyperthyroidism, using the TE-averaged PRESS technique at 3T field strength.

METHODS

Participants

This study included twenty-one consecutively referred, newly diagnosed and untreated patients in the thyrotoxic phase of Graves' disease and Hashimoto's thyroiditis from two endocrinology clinics.

The diagnosis of these diseases was based on the clinical features including hypermetabolism, goiter and/or ophthalmopathy, and examinations including increase of serum thyroid hormones, decrease of thyrotropin (TSH), increase of antibodies and increase of radioiodine uptake. Patients with a previous history of thyroid disease, psychiatric disorders and neurological disorders or with a family history of the diseases above were excluded. Other laboratory examinations including liver function tests were also taken in order to avoid the effect of other etiology on the concentration of the metabolites in brain. Fifteen of the patients (8 female, 7 male; age 31.3±8.0 yr) participated in the study. The reasons of exclusion were neurological disease (n=1) and refusals (n=5) to take the MRI examination.

The control group contained fifteen healthy volunteers who were age- and gender- matched. Similar standards of exclusion were applied. The study was approved by the local ethics committee. All participants gave their written consent. The participants had standard T1 and T2 images to exclude structural cerebral abnormality.

Magnetic resonance spectroscopy

MRS examinations were performed with the same GE MRI scanner (Signa HDxt) at 3T field strength, using an eight-channel coil for signal reception. T1 and T2 images were acquired as mentioned above. For spectroscopy, one 8 cm^3 volume was carefully placed in the medial posterior cingulate cortex. A single-voxel water-suppressed TE-averaged PRESS technique was chosen to detect the concentrations of glutamate with starting TE of 35 ms and ending at 192.5 ms. TR was set at 2 s, with a spectral width of 5,000 Hz and data points of 2048. The data were collected by 64 steps with an increment of 2.5 ms and NEX of 2. It has been demonstrated that the sequence offers the exquisite accuracy for Glu detection (Hancu *et al.* 2009).

Data analysis

In vivo spectra were processed by jMRUI v. 4.0 software, with the applications of 4-Hz Gaussian apodization, frequency shift and phase correction. The spectra were then fitted using QUEST. Concentrations of NAA, Cr, Cho and Glu (2.35 ppm) were quantified with the basis sets, built by NMR-Scope simulation. The standard GE MRS HD phantom was used as the standard. To correct amplitude of metabolite for relaxation effects, we used the previously published T1 and T2 relaxation times (Mlynárik *et al.* 2001; Sailasuta *et al.* 2009).

Statistical comparisons of the metabolites concentrations and their ratios to creatine between the two groups

were made using Wilcoxon signed rank test with SPSS software. The significance level was set at 0.05.

RESULTS

The characteristics, including age, gender and examinations, of the patients and controls are reported in Table 1.

All of the spectra had good qualities (the full width at half minimum ≤0.07 ppm). The MRS spectrum is shown in the Figure 1. Glutamate was measured as a single peak at 2.35 ppm. Hyperthyroid patients, compared with controls, showed a decrease of glutamate concentration (p<0.047) and glutamate/creatine ratios (p<0.009) in the posterior cingulate cortex. The decrease of choline concentration (p<0.004) and choline/creatine ratios (p<0.012) were also found. However, there was no significant difference in the concentrations of NAA or Creatine between patients and controls. The concentrations of the brain metabolites are shown in Table 2.

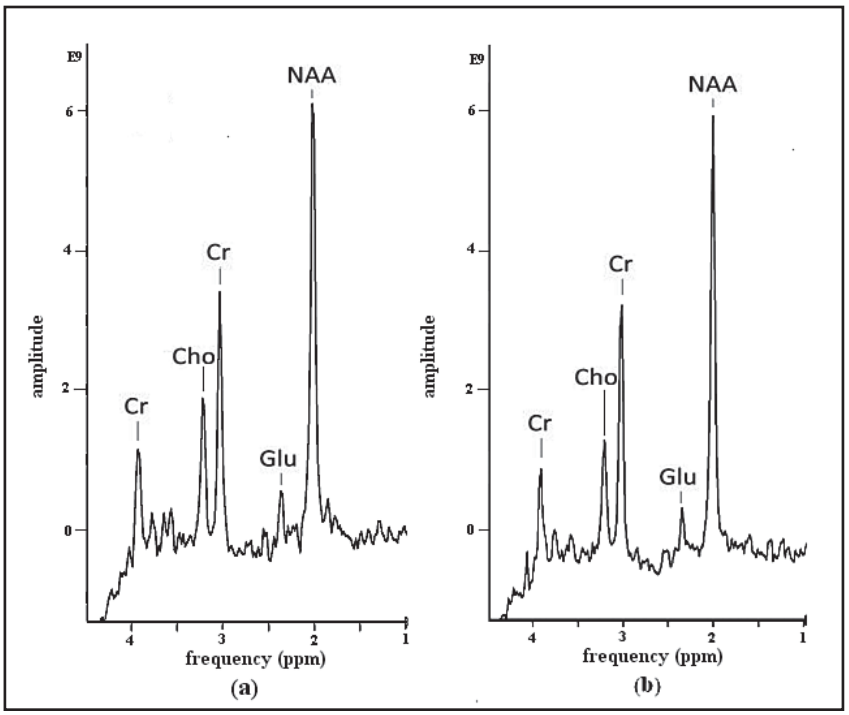

Fig. 1. Representative spectra acquired from the posterior cingulate cortex in a control (a) and a patient with hyperthyroidism (b).

Tab. 1. Characteristics of the patients and controls.

	Patients	Controls	*p*-value
Gender (M/F)	7/8	7/8	
Age (y)	31.3±8.0	30.5±8.0	0.27
TSH (0.27-4.2 μIU/ml)	0.015±0.037	2.9±1.5	<0.001
Total T4 (66-181 nmol/L)	296.5±24.3	102.2±14.4	<0.001
Free T4 (12-22 pmol/L)	88.5±12.5	17.2±2.0	<0.001
Total T3 (1.15-3.1 nmol/L)	7.6±1.6	1.9±0.3	<0.001
Free T3 (3.1-6.8 pmol/L)	31.1±9.2	4.4±0.5	<0.001
Anti-TPO (0-34 IU/ml)	288.0±242.1	17.9±4.3	<0.001
ALT (8-40 IU/L)	36.9±18.1	21.7±12.8	0.131
AST (5-40 IU/L)	30.6±7.6	22.0±3.4	0.035
ALP (40-140 IU/L)	181.6±57.1	59.8±12.8	<0.001

All values are presented as mean ± SD.
Abbreviations: TSH, thyrotropin; T4, thyroxin; T3, tri-iodothyronine; Anti-TPO, anti-thyroid peroxidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

Tab. 2. Concentrations and ratios of metabolites in posterior cingulate cortex of patients and controls.

Data	Patients	Controls	*p*-value
Metabolite concentrations (mmol/L)			
N-Acetylaspartate (NAA)	11.98±1.39	11.51±0.97	0.23
Creatine (Cr)	9.29±1.37	9.36±1.19	0.50
Choline (Cho)	1.32±0.29	1.59±0.36	0.004
Glutamate (Glu)	7.80±1.38	8.86±1.32	0.047
Ratios to creatine			
N-Acetylaspartate (NAA)	1.31±0.21	1.24±0.16	0.28
Choline (Cho)	0.15±0.04	0.17±0.04	0.012
Glutamate (Glu)	0.84±0.12	0.95±0.10	0.009

Values are mean±SD. Reductions of concentrations of glutamate and glutamate/creatine ratios were shown. Also, reductions of concentrations of choline and choline/creatine ratios were shown in the posterior cingulate cortex in patients.

DISCUSSION

Our study showed that glutamate decreased in the posterior cingulate cortex in patients' brain. One of the previous studies using short echo time MRS in 1.5T reported the reduction in the combination (Glx) of Glu and Gln in the parieto-occipital white matter (Danielsen *et al.* 2008). This result indicated the disturbance of the glutamatergic system in brain. In addition, our study provided a direct evidence of regional reduced glutamate when we chose the posterior cingulate cortex as the region of interest and used the technique of TE-averaged PRESS.

We also analyzed the ratios of Glu/Cr, because the ratios to Cr could diminish the deviance, which was inevitable, caused by the phase correlation, a step in the spectra processing. The radios of Glu/Cr also showed a significant reduction in posterior cingulate cortex in hyperthyroidism.

We compared two groups in pair in order to avoid the effect of the age-dependent decreases of the glutamate levels on the comparisons (Linda *et al.* 2009).

The reason why glutamate reduced in hyperthyroidism is unclear, but there is a possible explanation based on the glutamate-glutamine cycle. Glutamate is released by neuron, cleared from the extracellular space, transported into the astrocytes and converted to glutamine. Glutamine is then retaken by neuron and converted back into glutamate (Sibson *et al.* 1997; Erecinska *et al.* 1990). It's possible that tri-iodothyronine (T3) influenced the glutamate level by its effect on glutamate transporters which transported glutamate into astrocytes. The evidence from a study of rats showed that astrocytes treated by T3 increased mRNA levels and protein expression of the glutamate transporters (GLAST and GLT1), and regulated the extracellular glutamate content (Mendes-de-Aguiar *et al.* 2008). However, the alterations of the intracellular glutamate content remain unclear.

Glutamate is the most abundant excitatory neurotransmitter in brain. Evidences from the studies of glutamate receptors suggest that glutamatergic system is associated with synaptic plasticity (Rousseaux *et al.* 2008), which plays an important role in cognition and emotion (Manji *et al.* 2000; Sanacora *et al.* 2008). Thus, the reduction of glutamate that we found in this study may indicate the possibilities of the involvement of glutamate in brain dysfunctions of hyperthyroidism.

The reduction of choline was also found in this study, which confirmed the previous studies. The reason for its reduction is still unclear. Though the increase of choline mainly indicates the membrane turnover, it's not likely that decrease of membrane turnover is the reason for the decrease of choline, because the choline-containing phospholipids, which are involved in membrane turnover, are not the main part of the total choline in normal brain which can be detected by MRS (Bhatara *et al.* 1998). One possible reason, mentioned by previous study, is that the pool of choline-containing compounds bound to the membrane is increased. Thus less choline-containing compounds, which can be detected by MRS, are left (Danielsen *et al.* 2008).

The extent of brain dysfunctions and impairments in hyperthyroidism is still not clear. There is potential requirements for the diagnosis of the exact extent of the brain dysfunction and treatment for it. After all, the neuropsychiatric symptoms persist in some patients even after more than 12 months of euthyroidism (Fahrenfort 2000). Thus, it has not escaped our attention that the involvement of glutamate in the brain dysfunction suggests the potential means of diagnosis and treatment of such dysfunction in hyperthyroidism.

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