Lower Plasma Neuropeptide Y Level in Patients With Atypical Febrile Convulsions

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Febrile convulsion (FC) is the most common neurological disease in children. Cases with seizures that persist for more than 15 minutes or recurrent seizures within the same febrile illness are considered to be atypical and may have a different prognosis. Neuropeptide Y (NPY), an endogenous anticonvulsant that is widely distributed throughout the central nervous system, including the hippocampus, is known to prevent seizures by increasing the seizure threshold. Based on our previously finding that patients with atypical FC have lower concentrations of NPY, we hypothesized that the concentration of NPY may play a role in the development of atypical FC. To investigate this hypothesis, we used a radioimmunoassay to measure the plasma NPY concentration of 60 children with FC (typical FC, $n=46$; atypical FC, $n=14$) and 56 age-matched controls. The atypical FC group had significantly lower concentrations of NPY than children with typical FC and controls (66.47 ± 19.11 pmol/L *vs*. 88.68 ± 28.50 pmol/L and 86.82 ± 22.66 pmol/L, respectively). Very low NPY levels were found in two patients; one patient (NPY level: 44.75 pmol/L) experienced prolonged seizures lasting for up to 1 hour and the other had recurrent seizures (three seizures) during the same febrile illness (NPY level: 33.53 pmol/L). These results suggest that patients with inadequate NPY inhibitory activity are more susceptible to atypical FC.

Key Words: febrile convulsion, neuropeptide Y
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The most common seizure disorder in children is febrile convulsion (FC), which occurs in 1–10% of children [1]. Neuropeptide Y (NPY), which is widely distributed throughout the central nervous system, including the hippocampus, is known to prevent seizures in rats [2]. Woldbye et al observed that NPY administered through the lateral ventricle of rats inhibited kainic acid-induced motor and electroencephalographic seizures [3]. Dubé et al also found that endogenous NPY prevented experimentally induced seizures in rats by increasing the seizure threshold [4]. NPY receptor knockout mice have also been shown to be sensitive to kainic acid-induced seizures [5]. Taken together, these animal studies suggest that NPY is critical in modulating the excessive synaptic excitation associated with an epileptic seizure. However, the relationship between NPY and FC has not been established in humans. In a previous study, we found that plasma concentrations of NPY may be lower in patients with atypical FC than those with typical FC [6]. To test our hypothesis, we performed this observational study to further investigate the role of plasma NPY concentrations in children with typical FC, atypical FC and age-matched controls.

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Materials and Methods

For this study, we enrolled 60 children who were clinically diagnosed with FC and 56 age-matched controls. All FC patients met stringent FC criteria based on those used by Freeman [7]. The children were further classified into those with either typical FC or atypical FC, the latter defined as those patients having seizures persisting for more than 15 minutes, repeated seizures during the same febrile illness or a focal seizure. Our controls were recruited from patients who were admitted to our department for non-FC diseases such as pneumonia or acute gastroenteritis. A responsible adult provided written informed consent for each child. Blood samples were taken from the participants within 3 days after a seizure and from our controls within 3 days after admission. The protocol for this study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital. Blood samples underwent electrolyte, blood sugar and gas analysis in the hospital's clinical laboratory. Plasma was obtained by centrifugation for 5 minutes at 3,000 rpm at 4°C and stored at –70°C until required for the NPY assay.

The plasma concentration of NPY was measured by radioimmunoassay, as previously described [8]. Briefly, 50 μL of plasma was added to disposable reverse-phase mini columns (Sep Column; Peninsula Laboratories Inc., Belmont, CA) and eluted. The eluted fractions were lyophilized and reconstituted in radioimmunoassay buffer. The NPY concentration in each sample was measured using a commercially available radioimmunoassay (Peninsula Laboratories).

Statistical analysis

Data are shown as mean ± standard error. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Two-sample *t* tests and χ^2 tests were used to compare the means and the proportions between the two groups. A value of $p < 0.05$ was considered significant.

Results

Forty-six of the 60 patients with FC (76.7 %) had typical FC and 14 (23.3%) had atypical FC. The mean ages of the typical and atypical FC patients, and controls, were 25.98 ± 15.57 months, 31.14 ± 18.48 months and 30.0 ± 14.91 months, respectively. There was no significant difference in age between the three groups. Nineteen FC patients (31.67%) had a family history of FC. The female-to-male ratio was 1:2.29 for the control group, 1:1.42 for the typical FC group, and 1:3.66 for the atypical group (Table). Of the 60 patients with FC, 11 (18.33%) had experienced two or more FC seizures by the time of our study.

The results of the blood sample analysis showed no significant differences in the levels of sodium, potassium or sugar between patients with typical and atypical FC, although patients with atypical FC had significantly lower levels of ionized calcium (4.54 ± 0.39 mg/dL *vs*. 5.00 ± 0.33 mg/dL, $p = 0.011$) and acidosis (7.289 ± 0.096 *vs*. 7.357 ± 0.051, $p = 0.041$) than the typical group (Table).

As shown in Figure 1, the NPY concentration was significantly lower in patients with atypical FC than in those with typical FC (66.47 ± 19.11 pmol/L *vs*. 88.68 ± 28.50 pmol/L, $p = 0.01$) or the control group (86.82 ± 22.66 pmol/L, $p = 0.019$). Two of the patients with atypical FC had particularly severe FC episodes, with one having experienced a seizure that persisted for 1 hour and the other having three seizures during the same febrile illness. The average NPY concentration

Table. Demographic characteristics and clinical laboratory investigations of patients with febrile convulsion*

	Typical FC ($n = 46$)	Atypical FC ($n = 14$)	*p*
Age (mo)	25.98 ± 15.57	31.14 ± 18.48	0.303
Sex (male *vs*. female)	27:19	11:3	0.175
Family history of FC	13 (28.3)	6 (42.9)	0.306
Na (mmol/L)	135.5 ± 3.0 ($n = 40$)	135.5 ± 2.8 ($n = 10$)	1.000
K (mmol/L)	4.26 ± 0.35 ($n = 40$)	4.40 ± 0.41 ($n = 10$)	0.292
Ca (mg/dL)	5.00 ± 0.33 ($n = 17$)	4.54 ± 0.39 ($n = 6$)	0.011†
Sugar (mg/dL)	113 ± 34 ($n = 38$)	120 ± 51 ($n = 12$)	0.582
pH	7.357 ± 0.051 ($n = 15$)	7.289 ± 0.096 ($n = 7$)	0.041†

*Data presented as mean ± standard error or *n* (%); †$p < 0.05$; FC = febrile convulsion; Na = Sodium; K = Potassium; Ca = Calcium.

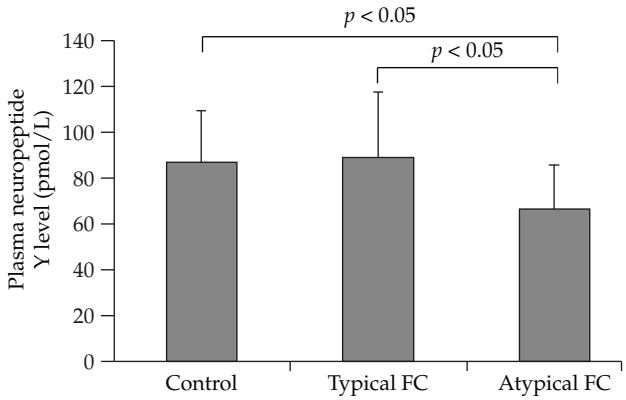

Figure 1. *Plasma neuropeptide Y level of patients with atypical febrile convulsions (n = 14) was significantly lower than in those with typical febrile convulsion (n = 46) and control group (n = 56). FC = febrile convulsions.*

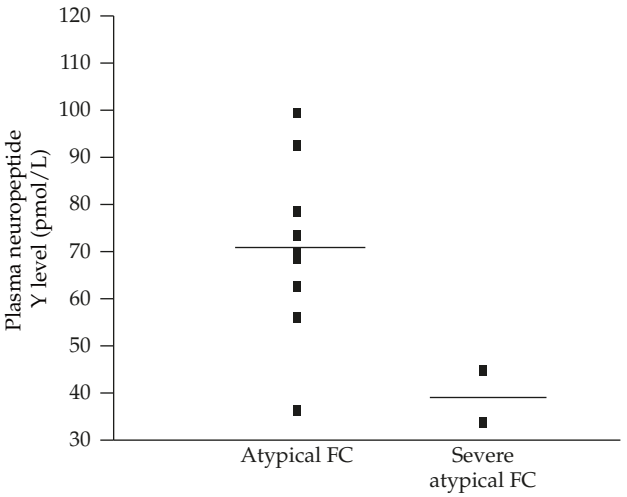

Figure 2. *Plasma neuropeptide Y levels in two patients with severe atypical febrile convulsion compared with other patients with atypical febrile convulsion. FC = febrile convulsions.*

in the two patients with severe atypical FC was 39.14 ± 7.93 pmol/L (Figure 2), which was lower, although of borderline significance, than the levels in other patients in the atypical FC group ($n = 12$, $p = 0.055$). We compared the differences in NPY levels of the boys and girls in our FC group (typical and atypical), and found no significant difference between sexes (males *vs*. females, 83.56 ± 26.80 pmol/L *vs*. 84.73 ± 29.69 pmol/L).

Discussion

In this study, we found a significantly lower plasma concentration of NPY in patients with atypical FC than in those with typical FC. The two patients with prolonged seizures (> 30 minutes) or with more than two seizures during the same febrile illness had even lower plasma NPY concentrations.

Several biochemical and electrophysiological findings in animal models and in tissues from humans with epilepsy suggest that NPY plays an important role in suppressing seizures. One study found that epileptic rats treated with a recombinant adeno-associated viral vector expressing the human *NPY* gene had much fewer seizures than the control group, an effect that was correlated with overexpression of NPY in the hippocampus [9]. Two other studies have suggested that the absence of inhibitory control by NPY may contribute to epileptogenesis [10,11]. One animal study of rat models of seizures revealed that adding agonists of the Y_1, Y_2, and Y_5 NPY receptors to hippocampal cultures reduced seizure-like activity [12]. In a study of patients with mesial temporal lobe epilepsy, NPY was found to possibly mediate the inhibition of glutamate release through up-regulation of NPY Y_2 receptors [13]. Based on this evidence, it is clear that NPY plays an important role in suppressing seizures in epilepsy. Our study found significantly lower plasma NPY concentrations in patients with atypical FC. However, there was no significant difference in the plasma NPY concentration between patients with typical FC and the control group. It was reported that patients with atypical FC show increased risk for epilepsy [14]. Therefore, our results suggest that patients with typical FC and normal NPY concentrations are not at increased risk of epilepsy. Besides, patients with low NPY concentrations may be more susceptible to long-lasting or recurrent seizures and are at higher risk of developing epilepsy. Accordingly, the concentration of NPY could be used as a marker to differentiate between typical and atypical FC.

There is a low possibility that our results could be confounded by sex because one of our previous studies and a study by another group have revealed that the majority of people with FC in Taiwan are male [6,15], raising the possibility that gender may play a role in the pathogenesis of FC. However, our current study and a study performed by Doniec et al in asthmatic patients found no differences in plasma concentrations of NPY between male and female [16].

Our results might also be confounded by the imbalance in electrolyte levels. We found that patients with atypical FC had a lower average ionized calcium level than those with typical FC but, because the average was within the normal range (reference value, 4.0–5.5 mg/dL), it is unlikely that the persistent or recurrent seizures were induced by lower levels of ionized calcium. We did find a significant difference in acidosis, a finding similar to another study, which reported acidosis to be common in patients with status epilepticus [17]. These findings suggest that there might be an association between acidosis in patients with atypical FC and their prolonged seizures.

One limitation of our study is that we did not analyze NPY concentrations in cerebrospinal fluid (CSF). One study of patients under anesthesia reported that the NPY concentration was much higher in CSF than in peripheral blood [18]. However, another study found no association between plasma and CSF concentrations of NPY in obese women [19]. Therefore, the relationship between central and peripheral NPY remains poorly understood. Because there was no clinical indication for lumbar tapping in our patients, we did not analyze the NPY concentration in CSF, representing a possible limitation or our study. The association between the central and peripheral NPY levels will need further investigation. Our finding that patients with atypical FC have lower of plasma NPY concentrations than those with typical FC suggests that low NPY levels renders FC patients more susceptible to long-lasting seizures or recurrent FC, and increased the risk for epilepsy, independent of sex.

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非典型熱性痙攣病患具有較低的血漿神經胜肽 Y

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熱性痙攣是小兒科最常見的神經疾患，病患若抽痙超過 15 分鐘或是在同一次發燒疾患中發生兩次以上的抽痙都被稱為非典型的熱性痙攣，這種非典型的熱性痙攣跟典型的熱性痙攣通常有不一樣的預後。神經胜肽 Y 是一種內生性的抗痙攣物質，它廣泛的分布於中樞神經系統包含海馬回中，被認為有增加痙攣的閾值而達到減少痙攣發生的功用，我們過去的研究發現非典型熱性痙攣病患有較低的血漿神經胜肽 Y 濃度，因此假設神經胜肽 Y 濃度在非典型熱性痙攣扮演一定的角色，為了證明此一假說，我們用放射免疫分析來測量 60 個熱性痙攣兒童的血漿神經胜肽 Y 濃度，其中包含 14 個非典型熱性痙攣及 46 個典型熱性痙攣，並與 56 個年齡相近的對照組做比較。結果顯示非典型熱性痙攣兒童的血漿神經胜肽 Y 濃度顯著的比典型熱性痙攣兒童及對照組低 (66.47 ± 19.11 pmol/L *vs.* 88.68 ± 28.50 pmol/L 及 86.82 ± 22.66 pmol/L)，尤其有兩個非典型熱性痙攣病患血漿神經胜肽 Y 濃度更低 (44.75 pmol/L 及 33.53 pmol/L)，臨床上，一個痙攣持續一個小時，另一個在同一次的發燒疾患發生三次痙攣，這些結果表示病患若缺乏足夠的神經胜肽 Y 抑制作用比較容易發生非典型熱性痙攣。

關鍵詞：熱性痙攣，神經胜肽 Y

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