The Effect of Ketogenic Diet on Phosphorylated CREB/CREB in the Hippocampus after Prolonged Seizure in Immature Rat Brain

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This certifies that the Doctorial Dissertation
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Abbreviations

ß-OHB	β-OH butyrate
CREB	Cyclic adenosine 3',5'-monophosphate (cAMP) response element (CRE) binding protein
g	gram
GABA	gamma-aminobutyric acid
hr	hour
IP	intraperitoneum
KA	kainic acid
KD	Ketogenic diet
Li-PC	lithium-pilocarpine
min	minute
ND	Normal diet
P	Postnatal day
pCREB	phosphorylated CREB
SQ	subcutaneous
SE	Status epilepticus
sec	second

Abstract

The Effect of Ketogenic Diet on Phosphorylated CREB/CREB in the Hippocampus after Prolonged Seizure in Immature Rat Brain

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Purpose: The aim of this study was to investigate the relationship between cognitive effects of ketogenic diet (KD) and phosphorylated CREB/CREB in the hippocampus after lithium-pilocarpine (Li-PC) induced prolonged seizure in immature rat brain.

Materials and Methods: Spraque-Dawley rats were subjected to Li-PC induced seizure or normal saline injection. Pilocarpine injected rats (n=24) progressed to prolonged seizure and seven rats (29%) died. On P21, the rats were assigned to four groups: prolonged seizure with normal diet (SE-ND, n=8) or KD (SE-KD, n=9), no seizure with normal diet (Cont-ND, n=8) or KD (Cont-KD, n=8). Spatial learning and memory test using the Morris water maze was performed from P51 to P55. Ketosis was assayed by spectrophotometrically measuring the serum β-OH butyrate (ß-OHB) levels. The hippocampi of sacrificed rats were treated to detect the phosphorylated

$CREB^{Ser-133}$.

Results: In the water maze test, **e**ach group significantly had reduced escape latency during four days. At the testing day 1, Cont-ND group showed shorter latency than the other three groups, but there was no statistical differences among the others. At the day 4, however, Cont-ND group performed with shorter latency in only comparison to SE-ND group. The probe test showed that the Cont-ND, Cont-KD, and SE-KD groups spent more time in the target quadrant in comparison to SE-ND group. Serum β-OHB levels were significantly greater in KD group than in normal diet. The total CREB in hippocampus was increased by prolonged seizure (SE-ND, SE-KD) and ketogenic diet (Cont-KD), but phosphorylation of CREB was decreased in the Cont-KD, SE-ND, and SE-KD groups.

Conclusion: The Li-PC induced prolonged seizure to immature rat deteriorated the spatial learning and memory. Ketogenic diet on prolonged seizure in immature brain improved the spatial learning and memory, even though decreased the phosphorylated CREB/CREB.

Keywords: Ketogenic diet; pilocarpine; prolonged seizure; learning and memory; CREB

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I. Introduction

The ketogenic diet (KD) - high fat, low protein and low carbohydrate diet - has been used clinically for several decades to control refractory epilepsy in children [1-7]. Recently, the expectations of improvement in the cognition, learning, mood, and behavior are important as well as seizure reduction by

KD treatment [8]. Nevertheless, the long-term effects of KD on cognition are uncertain.

The seizure-induced effects on the brain can be regarded as a three-stage process, in which a rapid neuronal death induced by an excitotoxic effect of glutamate is the initial phase. The second phase is the neuroprotective responses mediated by activation of apoptosis and cytokine-activated inflammatory processes. The third, long-lasting stage is epileptogenesis characterized by changes in cellular connectivity, synaptic reorganization, and functions of the hippocampal circuitry. The gradual progression of cellular and molecular changes finally leads to other long-term deleterious consequences, such as learning and memory impairment [9]. On the other hand, KD was found to affect several hippocampal processes associated with diminished neural excitability and to alter epileptogenesis [10]. However, The cellular and molecular mechanisms underlying KD effects on the cognitive function remain unknown.

Long-term potentiation (LTP) in the hippocampus is considered as a cellular mechanism underlying learning and memory and several studies have implicated the hippocampus in learning and memory consolidation [11-13]. In adult animals, status epilepticus (SE) is known to cause neuronal loss in the hippocampus that results in long-term deficits in learning, memory, and behavior [14]. Prolonged seizure induced by lithium-pilocarpine (Li/PC) in immature rats can cause long-term cognitive deficits and histological damages in hippocampus [15]. The cyclic adenosine 3',5'-monophosphate (cAMP)

response element (CRE) binding protein (CREB) is a transcription factor, which localized in the nucleus in all brain cells. CREB in the hippocampus is largely regulated by phosphorylation at Ser-133, which results in activation of gene transcription associated with learning and memory [12, 16]. The CREB in hippocampus could be a potential therapeutic target to improve cognitive deficits that accompany with certain disease states such as prolonged seizure.

Thus, this study aimed to address (1) whether KD improves learning and memory after Li/PC induced prolonged seizure in immature rat brain and (2) whether effects of KD on learning and memory arise from increased the ratio of phosphorylated CREB/CREB.

II. Materials and Methods

2.1. Animals and induction of seizure

Sprague-Dawley rats (Orient Bio, Korea) were used to make the lithium-pilocarpine (Li-PC) induced status epilepticus model, which mimics features of human temporal lobe epilepsy [17, 18]. All of the animal studies were in compliance with the National Institutes of Health's Guidelines for care and experimental procedures.

On postnatal day(P) 19, Sprague-Dawley male rats (n=40) received lithium chloride (3mEq/kg, IP). Pretreatment with lithium potentiates the epileptogenic action of pilocarpine and reduces mortality [17]. Twenty hours later, 24 rats among them were injected with pilocarpine hydrochloride (60mg/kg, SQ) and the rest with the same amount of normal saline [18, 19]. The rats were monitored in observation cages for at least 6 hours after the last injection. All pilocarpine injected rats (n=24) progressed to prolonged seizure. Seven of prolonged seizure-induced rats (29%) and none of the control died. On P21, the rats which experienced prolonged seizure were assigned to either normal (SE-ND, n=8) or ketogenic (SE-KD, n=9) diet group. The saline-injected control rats were also divided into two groups: normal (Cont-ND, n=8) and ketogenic (Cont-KD, n=8) diet.

2.2. Diets

For the normal and ketogenic diets, rodent chow (Purina #5001; Purina Korea, Inc.) and DYET #180853 (Dyets Inc., Bethlehem, PA, USA) were used

respectively (Table 1). The estimated composition (g/100g) of the KD was approximately 67.4 fat, 15.3 protein, and 0.6 carbohydrate ([fat] : [protein + carbohydrate] = 4.3:1). Body weight was checked three days per a week.

2.3. Water maze test

Between P51 and P55, the Morris water maze test was taken [19]. A circular steel tank (117cm diameter × 50cm height) was filled with water (26±1℃) to a depth of 25cm. The water was made opaque by addition of 100ml evaporated milk to prevent visualization of the platform. An 8×8cm plexiglass platform, onto which the rat could escape, was in constant location in the center of one of quadrants, 1cm below the water surface. For four days each rat was trained for 24 trials (six trials per day) to search the submerged platform in the water maze. The rat was immersed points in which varied in each trial, so was not able to predict the platform location. On mounting the platform, the rats were given a 30 sec rest period. If the rat failed to find the platform within 120 sec, then it was manually placed on the platform for a 30 sec rest. The time from immersion to escaping on the platform was recorded for each trial. After four days of training, rats underwent the probe test. The platform was removed and the rats were immersed in the water maze of the opposite site to where the platform had been placed. The path and time spent in the quadrant of platform were recorded.

2.4. Ketosis assessment

Blood samples (0.2-0.3ml) from the tail vein of each rat at P56~P57 were collected in EDTA tubes containing heparin. The serum was isolated by

centrifugation at 2,000 rpm for 5 min and kept at 4℃. Ketosis was spectrophotometrically measured serum β-OH butyrate (β-OHB) by using the Stanbio Laboratory kit (ß-Hydroxybutyrate LiquiColor® Stanbio laboratory, Boerne, TX, USA) in according to manufacture instructions.

2.5. Western blotting of pCREB/CREB

The whole hippocampus from each sacrificed rat was lysed at 4℃ with a lysis buffer (pH 7.4) composed of 50mM Tris-HCl, 150mM NaCl, 10mM NaF, 10mM EDTA, 10% NP-40, 1mM sodium orthovanadate, 10mM sodium diphosphate decahydrate, 0.5mM DTT, 0.2mM PMSF, 4mg/ml pepstatin, 4mg/ml aprotinin and 4mg/ml leupeptin. Then it was centrifuged at 8000×g for 10 min. For preparation of nuclear extracts including CREB, it was homogenized in five volumes of a buffer (pH 7.2) containing 15mM HEPES, 0.25M sucrose, 60mM KCl, 10mM NaCl, 1mM phenylmethylsulfonyl fluoride (PMSF) and 2mM NaF. Cell debris were seperated by centrifugation at 14,000×g for 30 min and the supernatant was used as nuclear extracts [12].

For analysis of phosphorylated CREB, 50mg of protein was boiled in a sample buffer composed of 0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 20% 2×TBE (90mM Tris, 64.6mM boric acid, 2.5mM EDTA, pH 8.4) and applied onto a 7.5% polyacrlyamide gel and transferred to PVDF (Millipore, Bedford, MA, USA), and blocked with Detector Block Kit (KPL, Gaithersburg, MD, USA). Membranes were incubated with anti-phospho-CREB (1:1000 dilution; Upstate Biotechnology, Lake Placid, NY, USA), and washed with TBST (10mM Tris-HCl, pH 7.4, and 150mM NaCl, 0.1% Tween20) three times for 10 min. The immune complex was detected by ECL (Amersham

Pharmacia Biotech, Piscataway, NJ, USA) and exposed to X-ray film. For quantifying non-phosphorylated form of CREB, the same membranes were stripped with stripping buffer (100mM 2-mercaptoethanol, 2% sodium dodecyl sulphate (SDS), 62.5mM Tris-HCl, pH 6.7) at 50℃ for 10 min, and incubated with anti-CREB (1:2000 dilution; Upstate Biotechnology, Lake Placid, NY, USA).

Data obtained by CREB experiments were analyzed semiquantitatively, using a computer-assisted image analysis by UniPACS viewer (version March 2007, UniPACS Inc., http://www.unipacs.com) as DICOM (Digital Imaging and Communications in Medicine) viewer software. The band intensities on the film were scanned digitally, and converted to DICOM files. One observer reviewed all the band intensities in the DICOM files. The densities of bands of the phosphorylated and non-phosphorylated form of CREB were measured on circular operator-defined ROI (regions of interest) with an electronic cursor on each DICOM file. And also the reference densities of bands were measured by the same way. Corrected density values of bands could be obtained from the densities of bands and the reference densities. For comparison, the phosphorylated CREB densities were normalized on respective total CREB densities in order to assess the actual fraction of phosphorylated protein

2.6. Statistical analysis

Results were expressed as means±SD. Body weights were evaluated using *t*-test. During four days water maze test, the improvement in escape latencies in each individual was analyzed with two-way repeated measure ANOVA. For comparison among four groups, escape latencies to the platform for each day

of testing and times to spend in the target quadrant during the probe test were assessed by *t*-test. All statistical analysis were performed with SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). The *P* value less than 0.05 was regarded as statistically significant.

III. Results

3.1. Characteristics of the seizures

Approximately 10-15 min after pilocarpine injection, rats exhibited head bobbing, scratching, chewing, and exploratory behavior. Recurrent seizures started about 30 min after pilocarpine administration with episodes of head and bilateral forelimb myoclonus, rearing and falling, with progression to prolonged seizure at around 50 min after pilocarpine injection. Prolonged seizure was characterized by repetitive clonic seizures involving the whole body and limbs, leading to repeated rearing and falling. Such status episodes occurred intermittently up to 5 hours, after which rats gradually recovered to baseline behavior.

3.2. Body weight

In the normal diet group (Fig. 1A), the mean weight of SE-ND group was significantly lower than that of Cont-ND at the P23 (SE-ND 43.9±4.7g, Cont-ND 50.4±4.7g, $p<0.05$) and P26 (SE-ND 61.1±6.9g, Cont-ND 68.1±4.8g, $p<0.05$), but after then there was no difference between two groups (weights at P42 ; SE-ND 173.2±7.4g, Cont-ND 182.6±14.8g, $p>0.05$). In the ketogenic diet group (Fig. 1B), SE-KD group was significantly lighter than Cont-KD. At the P23 (SE-KD 44.8±5.1g, Cont-KD 51.4±5.9g, $p<0.05$), but after then there was no difference between two groups (weights at P48 ; SE-KD 181.9±20.0g, Cont-KD 190.7±40.0g, $p>0.05$).

3.3. Ketosis

The level of serum β-OHB (Table 2) was significantly higher in KD groups (Cont-KD 2.936±0.723mM, SE-KD 1.798±0.300mM) than normal diet groups (Cont-ND 0.095±0.028mM, SE-ND 0.198±0.079mM). The prolonged seizure itself didn't influence the level of β-OHB (Fig. 2).

3.4. Water maze test

Mean escape latency (Table 3) was significantly reduced in all four groups by daily training (Fig. 3). In the 1st day, mean escape latency in the Cont-ND group was significantly shorter than that in the other groups. (47.96±14.13sec vs. Cont-KD 72.60±22.76sec, SE-KD 88.11±35.64sec, SE-ND 93.77±35.11sec, all $p<0.05$) in Figure 4. In the 2nd to 4th day, mean escape latency of the SE-ND was longer than that of the Cont-ND (72.92±22.74sec vs. 37.21±16.00sec, 66.04±41.14sec vs. 26.06±10.78sec, 54.23±33.87sec vs. 21.13±7.80sec, all $p<0.05$). In the Cont groups, escape latency of the KD (Cont-KD) was longer than the ND (Cont-ND) at the 1st day but there were no significant differences after the 2nd day. In the KD groups, there were no significant differences in the SE regardless of KD (all $p>0.05$). Mean escape latency of the SE-KD group was significantly longer than that of the Cont-ND in the 1st and 3rd days (88.11±35.64sec vs. 47.96±14.13sec, 58.28±40.83sec vs. 26.06±10.78sec, all $p<0.05$).

The results of the probe test are presented in Table 4. The SE-ND group significantly spent less time in target quadrant than the other groups (24.75±4.62sec vs. SE-KD 34.78±9.30sec, Cont-KD 38.25±11.60sec, Cont-ND 38.00±9.27sec, all $p<0.05$) in Fig. 5.

3.5. The ratio of pCREB/CREB

Western blot analysis revealed that total CREB was more expressed in the Cont-KD, SE-ND, and SE-KD group (Fig. 6). The ratio of pCREB to total CREB compared to Cont-ND was 73% in Cont-KD, 69% in SE-ND, and 29% in SE-KD group, respectively (Fig. 7). After prolonged seizure and also ketogenic diet decreased the ratio of pCREB/CREB ($p<0.05$).

IV. Discussion

The main observations in the present study are as follows. First, KD improved deteriorated spatial learning and memory which were deteriorated by Li/PC induced prolonged seizure in immature rats. Unexpectedly, KD in normal immature rats seems to decrease cognitive function. Second, KD increased the expression of total CREB but decreased the ratio of pCREB to total CREB in the hippocampus of immature rats.

During the ketogenic diet, similar to starvation, lack of available carbohydrate leads to the incomplete mitochondrial oxidization of fatty acids, producing of the ketone bodies, β-OHB and acetoacetate. Consequently, these ketone bodies then become the primary energy source for the brain. The neuroprotective activity of ketone bodies (i.e., β-OHB) has been demonstrated in vivo and in vitro models of neurological injury and disease [10, 20-23]. Elevation of serum β-OHB revealed the ketosis in the KD regardless of prolonged seizure and it was expected from previous studies [10, 24, 25]. Stafstrom et al. [24] have suggested that elevated serum ketone levels seem to be necessary but not sufficient to explain the anticonvulsant effect of KD in their animal model. However, Gilbert et al. [25] had demonstrated a correlation between seizure control and levels of blood β-OHB, and a statistically significant threshold for seizure control was the levels over 4 mmol/L after 3 and 6 months on the diet. The further study maybe needed for its clinical association and usage.

The Morris water maze is the most popular test of hippocampus dependent visuospatial learning and memory in the past two decades [19, 26-29, 39]. In present water maze test, escape latency of the KD group was irrespectively extended on the 1st day of the water maze test. However, the learning function of the Cont-KD and SE-KD group was time-dependently improved like that of the Cont-ND group. The KD groups regardless of SE showed a memory improvement in comparison with that of SE-ND group on probe test. There are some evidences supporting the view that KD improves the long-term outcome in children with refractory epilepsy [30-33]. In contrast with the present data, Zhao et al. [28] have reported young rats that were treated with KD for 1 month had deficiency in spatial learning and memory as tested with the Morris water maze, regardless of whether they experienced Li/PC-induced SE. Similarly, Su et al. [34] have showed that rats treated with KD after kainic acid (KA)-induced SE had more severe deficits in spatial memory than rats with regular rat chow. They found that the impaired water maze performance occurred despite a reduction in seizures in animals with KD. Taken together, these findings raise the safety problems of KD on the long-term cognitive function in immature rat brain, even possibly in humans. The KD effects on long-term cognitive function still remain controversial topic, so it is necessary to reveal the cellular and molecular mechanisms.

There are several reports that prolonged seizure in immature brain resulted in hippocampal cell loss and altered neurogenesis [15, 18, 19, 35]. The best characterized long-term consequences of early-life seizures are those affecting the hippocampal plasticity, e.g. sprouting, and cognitive functions, specifically

those affecting learning and memory [35]. Ser-133 phosphorylation of CREB in the hippocampus is considered to be a critical event that mediates the initiation of transcription regarded for learning and memory [29]. Several studies on underlying molecular mechanism indicate that both behavioral long-term memory and its neural representation require gene expression triggered by $pCREB^{Ser\text{-}133}$ that consequently leads to the learning-related synaptic plasticity [29, 36, 37]. Faverjon et al. [19] demonstrated that decreased $pCREB^{Ser\text{-}133}$ in the hippocampus of the rats subjected to Li/PC-induced status epilepticus at P20. Huang et al. [29] have also reported that impaired phosphorylation of $CREB^{Ser\text{-}133}$ in the hippocampus played an important role in cognitive deficits resulted from recurrent seizures in the developing brain. Like the previous studies, the present study showed that the levels of pCREB/CREB in the hippocampus were decreased after the experimental Li/PC-induced prolonged seizure. Then does KD affects to levels of pCREB/CREB? Unexpectedly, KD decreased levels of phosphorylation of $CREB^{Ser\text{-}133}$ seemed to play a major role in the cognitive function. The relationship between KD and phosphorylation of $CREB^{Ser\text{-}133}$ have not yet reported to our knowledge. Early-life malnutrition was reported to result in a reduced level of $pCREB^{Ser\text{-}133}$ of hippocampal CA1 region in the absence of long-term cognitive deficit or hippocampal neuronal loss [38].

An understanding of how KD has an effect on gene expression may be helpful for the therapeutic modification for long-term neuronal plasticities. For instance, SE-induced alterations in the $GABA_A$ receptor α1 subunit mRNA and protein expression preceded the onset of epilepsy [39]. Alterations in the

expression of subunits, and their altered incorporation pattern in the $GABA_A$ receptor complex can lead to long-term functional changes in the $GABA_A$ receptor-mediated inhibition. [40]. Ketogenic diet is anticipated to modify the tricarboxcylic acid cycle to increase GABA synthesis in brain, limit reactive oxygen species (ROS) generation, and regulate mitochondrial biogenesis [41]. The recent studies have demonstrated that seizures activate multiple cell death pathways involving Bcl-2 family comprised of proapoptotic (e.g., Bad, Bid, Bax, Bak) proteins [42, 43]. Bad resides in an inactive state and complexes with the chaperone proteins of the 14-3-3 family. 14-3-3 forms a very stable complex with phosphorylated Bad and regulates Bad function. Akt and its downstream target Bad play an important role in KA-induced cell death. Noh et al. [44] suggested that KD protected brain cell death by prevention of p-Akt down-regulation and blockage of releasing Bad from 14-3-3. Further studies are needed to identify the factors of cellular and molecular mechanisms associated with KD.

V. Conclusion

It is concluded that (1) The prolonged seizure during immature brain deteriorated the spatial learning and memory. (2) Ketogenic diet after prolonged seizure in immature brain improved the function of the spatial learning and memory, even though it decreased the ratio of pCREB/CREB. (3) The further studies remain to reveal the mechanisms of ketogenic diet to improve the spatial learning and memory after prolonged seizure during immature brain.

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Table 1. Composition of Normal Diet and Ketogenic Diet

Ketogenic diet	Ketogenic diet Ingredient (%)	Normal diet	Normal diet Ingredient (%)
Casein	9.24	Saturated fat	1.5
DL-Methionine	0.16	Unsaturated fat	8.5
Primex	78.67	Protein	23.4
Corn Oil	11.57	Fiber	5.3
Vitamin mix* #310035	0.36	Ash	6.9
		Carbohydrates	49.0

* Vitamin Mix #310035(grams/kg): Vitamin A Palmitate (500,000 IU/g) 3.96, Vitamin D2 (500,000 IU/g) 0.44, Vitamin E Acetate (500 IU/g) 24.23, Ascorbic Acid 101.66, Inositol 11.01, Choline Bitartrate 300.17, Medadione Sodium Bisulfite 7.95, P-Aminobenzoic Acid 11.01, Niacin 9.91, Riboflavin 2.2, Thiamine HCl 2.2, Pyridoxine HCl 2.2, Calcium Pantothenate 6.61, Biotin 0.044, Folic Acid 0.2, Vitamin B12 2.97, Dextrose 513.24.

Table 2. The Levels of Serum β-OH Butyrate

β-OHB (mM)	SE-ND (n=5)	SE-KD (n=5)	Cont-KD (n=5)	Cont-ND (n=5)
Mean	0.198	1.798	2.936	0.095
SD	0.177	0.672	1.618	0.057
Standard error	0.079	0.300	0.723	0.028

Table 3. Comparisons of Escape Latencies in The Water Maze Test for Four Days

	1st day (mean±SD, sec)	2nd day (mean±SD, sec)	3rd day (mean±SD, sec)	4th day (mean±SD, sec)	P value
SE-ND (n=8)	93.77±35.11	72.92±22.74	66.04±41.14	54.23±33.87	0<0.05
SE-KD (n=9)	88.11±35.64	61.50±39.86	58.28±40.83	47.82±36.57	0<0.05
Cont-ND (n=8)	72.60±22.76	45.00±32.03	40.46±32.30	28.69±37.99	0<0.05
Cont-KD (n=8)	47.96±14.13	37.21±16.00	26.06±10.78	21.13±7.80	0<0.05

Table 4. The Times in The Probe Test

	SE-ND (n=8)	SE-KD (n=9)	Cont-KD (n=8)	Cont-ND (n=8)
mean±SD, sec	24.75±4.62	34.78±9.30	38.25±11.60	38.00±9.27

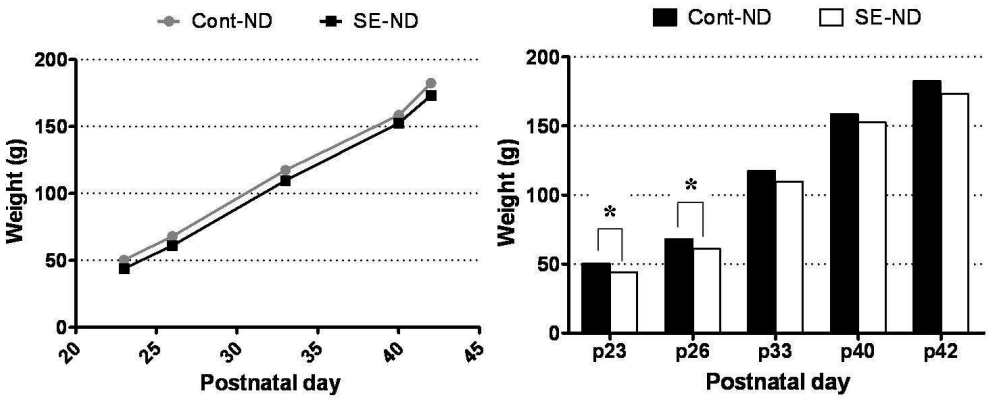

Figure 1A. The comparison of weight gain. At the P23 and P26, the mean weights of SE-ND group was significantly lighter than Cont-ND, but after then there was no weight difference between two groups (*; $p<0.05$).

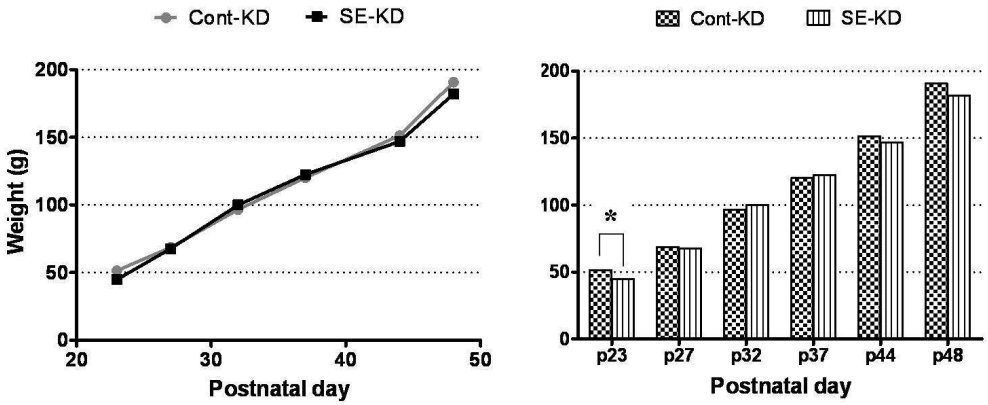

Figure 1B. The comparison of weight gain. At the P23, the mean weights of SE-KD group was significantly lighter than Cont-KD, but after then there was no weight difference between two groups (*; $p<0.05$).

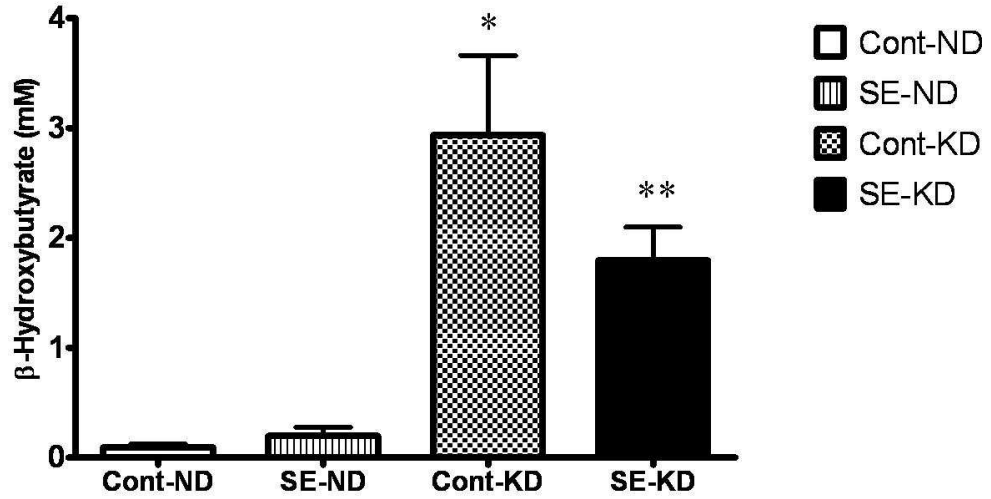

Figure 2. The level of serum ß-hydroxybutyrate was significantly higher in KD groups (Cont-KD, SE-KD) than normal diet groups (Cont-ND, SE-ND). *; $p<0.05$, **; $p<0.01$.

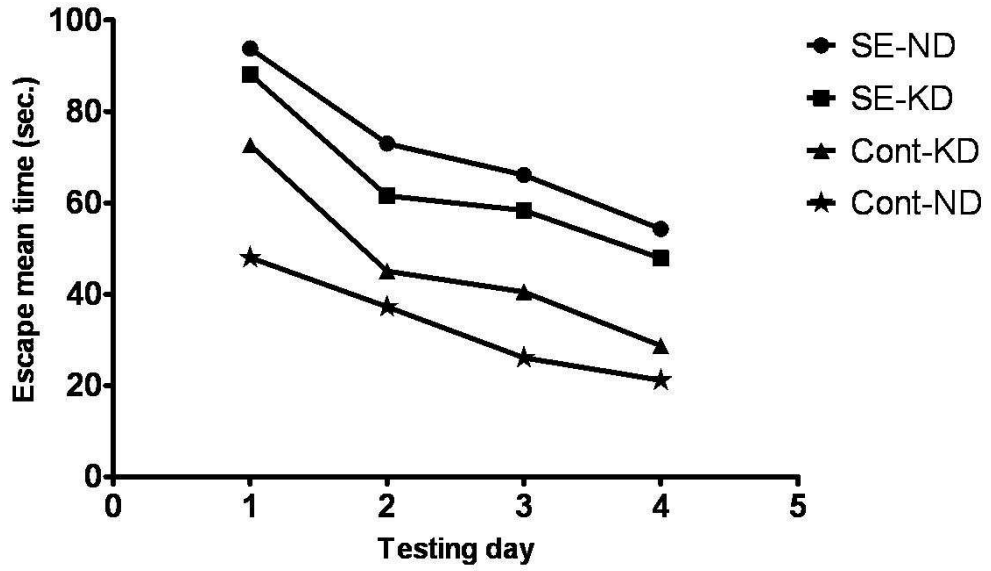

Figure 3. Comparison of escape latencies to platform in the water maze in each group. All groups improved their performance during testing day.

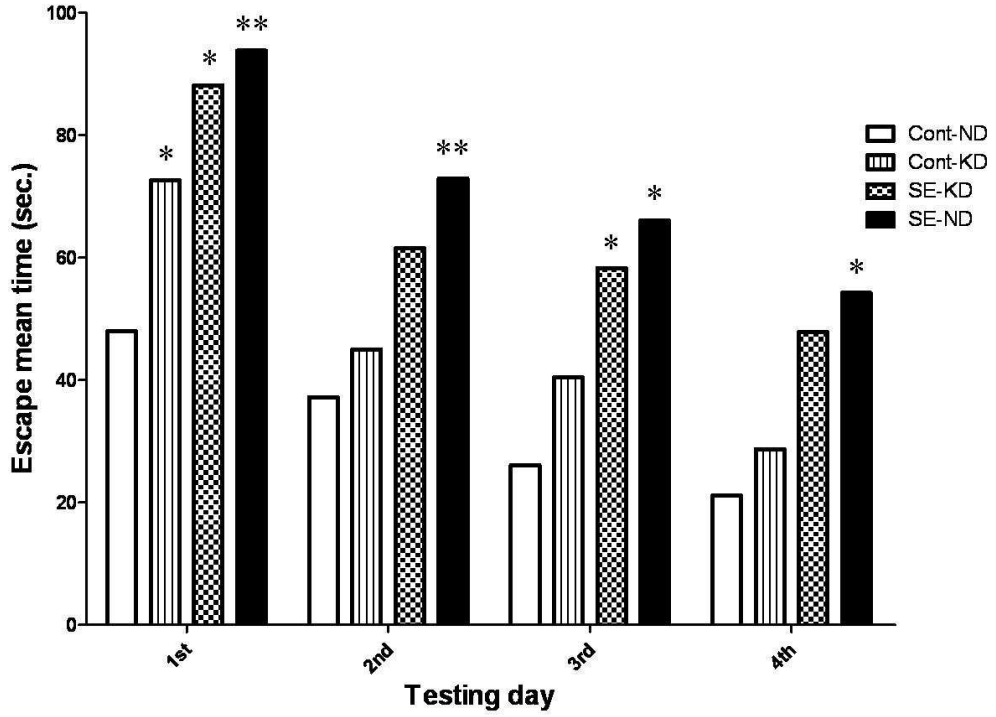

Figure 4. Comparison of escape latencies to platform in the water maze in each day. At the 1st day of testing, Cont-ND group was shorter latency than the other groups, but there was no difference between Cont-KD, SE-KD and SE-ND group. The 4th day of testing, Cont-ND group was shorter latency than the only SE-ND group (* $p < 0.05$; ** $p < 0.01$).

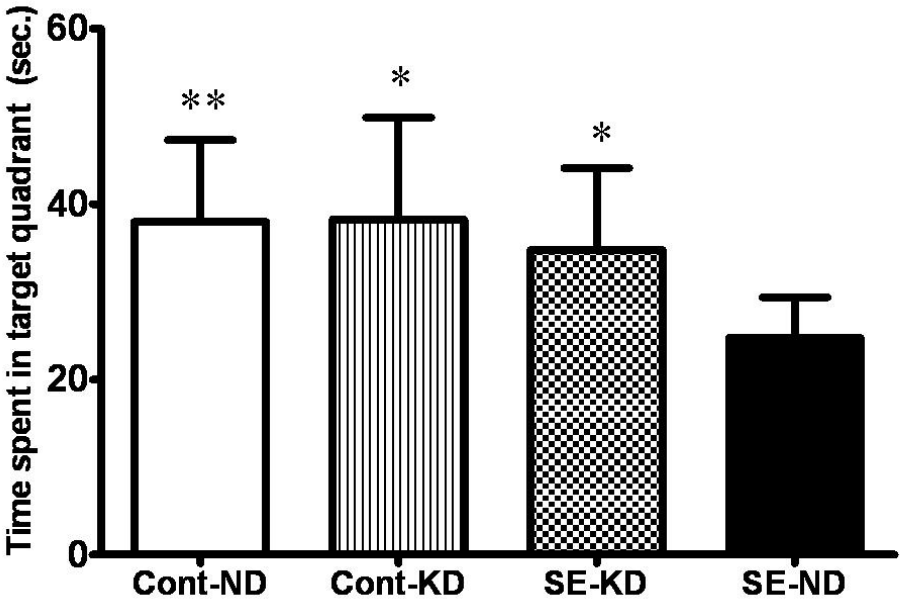

Figure 5. Time in the probe test; the Cont-ND, Cont-KD, and SE-KD groups spent more time in the quadrant where the platform had previously been located than SE-ND groups (*; $p < 0.05$, **;$p<0.01$).

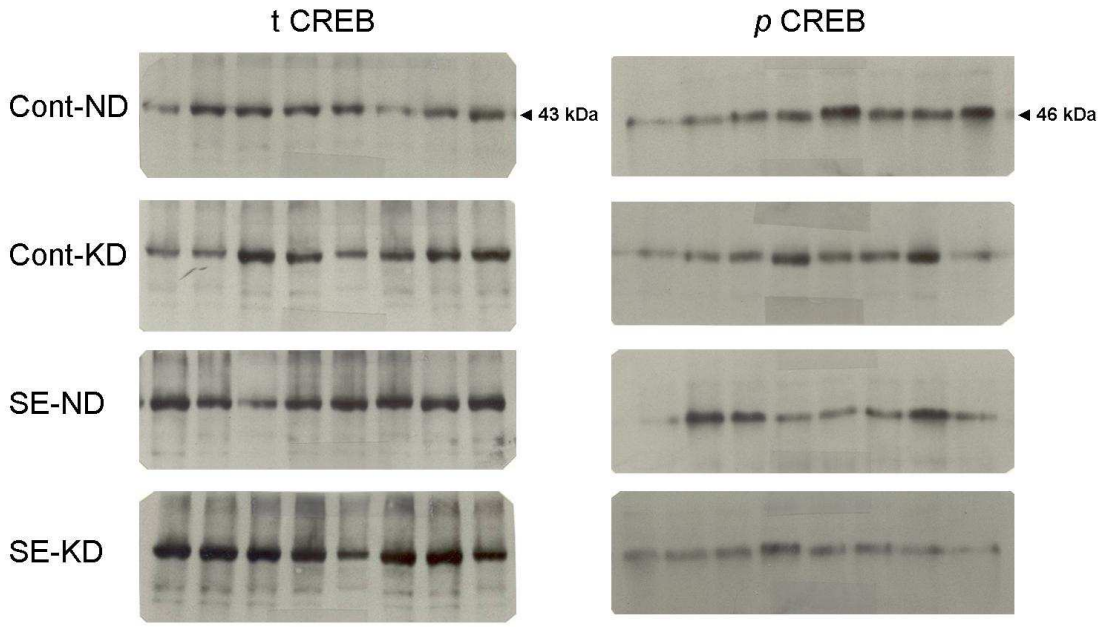

Figure 6. The Western blotting for CREB. The total CREB was higher in the SE groups (SE-ND, SE-KD) than control groups (Cont-ND, Cont-KD), and also increased by ketogenic diet. But the pCREB/CREB was higher in the control groups than SE groups. Ketogenic diet decreased the pCREB/CREB.

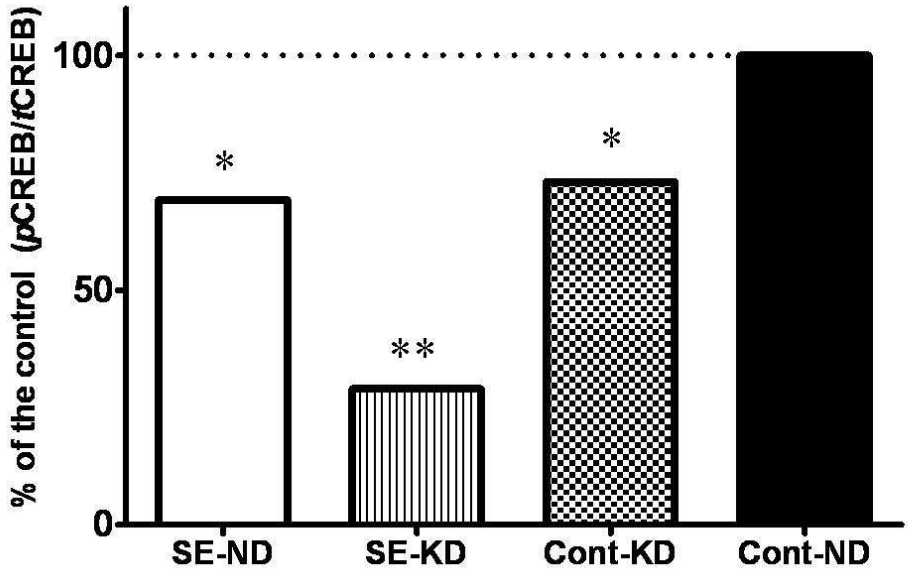

Figure 7. The ratio of $pCREB^{ser-133}$ immunoreactivity to Cont-ND. The graph shows decreased ratio of $pCREB^{Ser-133}$ to total CREB in the rats of the SE-ND, SE-KD or Cont-KD groups (*; $p < 0.05$, **;$p<0.01$).

Abstract in Korean

미성숙 백서에서 지속적인 간질발작 유발 후 해마의 Phosphorylated CREB/CREB에 대한 케톤생성 식이요법의 효과

The Effect of Ketogenic Diet on Phosphorylated CREB/CREB in the Hippocampus after Prolonged Seizure in Immature Rat Brain

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홍 주 희

목적: 미성숙 백서에서 지속적인 간질발작 유발한 후에 해마의 Phosphorylated CREB/CREB을 측정하여 케톤생성 식이요법의 인지기능에 대한 효과를 알아보고자 하였다.

대상 및 방법: Lithium-pilocarpine(Li-PC) 모델을 실험에 이용하였다. 생후 20일(P20)에 24마리에 Pilocarpine 피하 주사를 하여 지속적인 간질발작을

일으켰으며, 7마리(29%)는 죽었다. 살아남은 17마리를 정상식이(SE-ND, n=8)와 케톤생성식이(SE-KD, n=9) 2개 군으로 나누었다. Pilocarpine 대신 생리식염수를 준 대조군 16마리도 정상식이(Cont-ND, n=8)와 케톤생성식이(Cont-KD, n=8)로 나누었다. 생후 51일(P51)에서 55일(P55) 동안 Morris water maze test를 시행하였다. serum ß-OHB 을 spectrophotometry를 이용하여 측정하였다. 해마는 적출해서 phosphorylated $CREB^{Ser-133}$를 분석하였다.

결과: Morris water maze 검사에서 날이 갈수록 모든 군에서 의미 있는 향상을 보였다. 첫날에는 Cont-ND가 다른 세 군에 비해 플랫폼을 찾는 시간이 유의하게 짧았으며, 세 군 간에는 차이가 없었다. 그러나 4일째에는 Cont-ND와 SE-ND만이 유의한 차이를 보였다. Probe test에서 Cont-ND, Cont-KD, SE-KD 세 군은 SE-ND에 비해 플랫폼이 있던 사분구획에서 유영하는 시간이 유의하게 길었다. 혈청 β-OH butyrate(β-OHB) level은 간질과 상관없이 정상군보다 케톤군에서 유의하게 높았다. $CREB^{Ser-133}$ phosphorylation은 prolonged seizure를 한 경우와 케톤생성 식이를 한 경우에 감소를 보였다.

결론: 미성숙 백서에서 지속적인 간질발작은 visuospatial learning and memory를 악화시켰으며, 케톤생성 식이로 Phosphorylated CREB/CREB은 감소하였으나 learning and memory function은 향상되었다.

핵심되는 말: 케톤생성 식이요법; 필로카르핀; 지속적인 간질발작; 인지기능; CREB