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Electrolyte Therapy Reduces Spike-and-Wave Discharges in the WAG/Rij Rat Model of Absence Epilepsy

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Abstract

Although antiepileptic drugs are often effective in control seizures, some patients show little or no improvement. As alternative treatment, different dietary modifications were shown to be beneficial for patients with poor tolerance for AED. Previous reports have shown that rice-based oral electrolyte hydration therapy is effective in seizure control in patients with refractory absence seizures. In the present study using an animal model of absence epilepsy we show that the occurrence of spike-and-wave discharges significantly decreases upon switching to electrolyte therapy. We also show that consumption of solution with the same osmolarity as rice-based oral electrolyte solution leads to a decrease in number of spike-and-wave discharges per hour. We suggest that the antiepileptic effect of rice-based oral electrolyte hydration therapy can be at least in part due to hyperosmolarity of the ingested solution.

Keywords

spike-and-wave; dietary; hyperosmolarity; electrolytes; epilepsy; seizures; absences WAG/Rij; genetic models of absence epilepsy

Introduction

The primary mode of treatment of epilepsy is antiepileptic drug (AED) therapy. While AED treatment is effective in the majority of patients [1] in many parts of the world AEDs are readily available [2–4]. When AEDs are available they typically consist of older generation drugs such as phenobarbital and phenytoin [2]. While phenobarbital and phenytoin are effective therapies for partial and secondarily generalized seizures, the drugs are not

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effective in absence seizures which respond well to ethosuximide, sodium valproate and lamotrigine [5]. Alternative, cost-effective therapies are needed for absence seizures in regions where third generation AEDs are not available.

It was reported that a rice-based oral electrolyte hydration therapy consisting of rice, sodium potassium and citrate, can control medically refractory absence seizures in patients with familial dysautonomia [6]. Such therapy, which is inexpensive and easy to implement, may provide an alternative to AED therapy in underserved areas of the world.

We hypothesized that hyperosmolarity may be responsible for the anti-seizure effect of the electrolyte solution. In the present study, using a classical animal model of absence epilepsy we attempted to extract the anti-seizure effect of rice-based oral electrolyte solution due to hyperosmolarity from other possible effects of this electrolyte solution.

Methods

All experimental procedures were performed in accordance with the guidelines set by the National Institute of Health for the humane treatment of animals and approved by the Animal Care Committee of Bogomoletz Institute of Physiology.

Adult male WAG/Rij rats ($n = 15$) weighing 260–360 g were used throughout the study. The WAG/Rij model of absence seizures was used since this model has clinical and electroencephalographic signs similar to absence seizures in humans [7, 8]. Adult rats have frequent absences with bursts of spike-and-waves occurring at a frequency of 8–10 Hz. The animals respond to similar anti-absence drugs as humans.

Animals were maintained under 12 h light/dark-cycle with free access to food and water. Each rat was implanted with recording electrodes for the subsequent EEG recordings. At the day of surgery rats were anesthetized with a mixture of ketamine/xylazine (80/8 mg/kg) intramuscularly. Two electrodes were symmetrically placed on the motor cortex (AP = 2 mm, ML = 3.5 mm from bregma), and two stainless steel microscrews driven into the bone above the cerebellum (AP = -11 mm, ML = 2.5 mm from bregma) served as reference and ground electrodes. Connector for the electrodes was located on the surface of the skull.

Animals were allowed at least one week to recover before the recording session started. For recording animals were placed in a special chamber and spontaneous field potentials were recorded using multi-channel extracellular amplifier (AC-916, CWE Inc., Ardmore, PA). Spike 2 (Cambridge Electronic Design, Cambridge, UK) software was used for the acquisition. Control recordings were performed in freely moving WAG/Rij rats for 3 hours per day for 4 days. After the last control recording and thereafter in the intervals between tests water in the cage was substituted with rice based electrolyte oral solution (electrolyte solution) or hyperosmotic solution and recordings were performed using the same protocol for another 6 days.

The electrolyte solution contained: sodium chloride of 3.4 g/L, potassium chloride 1.3 g/L, trisodium citrate, 2.9 g/l; rice-carbohydrate 40 g/L, which provided sodium 90 mEq/L; potassium 20 mEq/L (270 milliosmoles). The hyperosmotic solution contained: sodium chloride 90 mM, potassium chloride 20 mM, calcium chloride 5 mM, magnesium chloride 5 mM, glucose 20 mM, sorbitol 10 mM (270 milliosmoles). The electrolyte solution was purchased from Cera Products Inc. (Cera Lyte 90, Hilton Head, SC, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA). The osmolarity of solutions were measured by freezing-point depression with an Osmomat 030 (Gonotec, Berlin, Germany).

Spike and wave discharges (SWD) were counted manually by an investigator blinded to treatment group for per blocks of one hour. Statistical analysis was performed using GraphPad Prism (La Jolla, CA, USA) and Origin 7.5 (Microcal Software, Northampton, MA). SWD occurrence, mean SWD frequency and duration between groups before and after treatment were compared using a Wilcoxon matched-pairs signed rank test. The differences between the effect of the electrolyte and hyperosmotic solution was analyzed using the repeated measures ANOVA. Results are expressed as the mean $\pm$ SEM. The number of animals was designated as “n”.

Results

Absence epilepsy is characterized by spontaneously occurring bursts of bilateral synchronous spike and wave activity accompanied by a brief alteration of consciousness. Generalized spike and wave discharges, even when brief, are associated with subtle changes in awareness [9]. To assess anti-seizure effect of electrolyte/hyperosmotic solution we estimated the changes in occurrence, peak frequency and duration of SWD before and after introduction of WAG/Rij animals to electrolyte/hyperosmotic solution therapy (Fig. 1).

During control recording session the number of SWD varied considerably from animal to animal ranging from 12 to 54 per hour with a mean value 24.7 ± 1.2 events per hour ($n = 15$), however the occurrence, peak frequency and duration of SWD for each individual rat was stable during 4 days of control recording. The mean peak frequency of SWD during the recording session was 6.9 ± 0.6 Hz and mean duration 7.6 ± 2.6 s. In the first set of experiments ($n = 8$) rats were provided with electrolyte solution for a duration of six days after the control recordings. Analysis of EEG recordings showed a gradual decrease of number of SWD per hour during the time of electrolyte solution consumption ($33.0\pm2.9\%$ of baseline levels, $p < 0.01$). There were no changes in peak frequency (7.2 ± 0.4 Hz) and duration (6.4 ± 1.5 s) of SWD compare to control recordings. None of the experimental rats showed signs of dehydration or behavioral abnormalities. After six days of electrolyte consumption rats were turn back to the tap water supply. After one week of tap water drinking the EEG was recorded again. The occurrence of SWD recovered and was close to the control level ($88.7\pm3.7\%$ of baseline levels, Fig. 2).

It is well established that osmolarity is one of the important factors in regulating neuronal excitability [10]. To elucidate the hypothesis that anti-seizure effect of electrolyte solution was due to its hyperosmolarity, in the second set of experiments rats ($n = 7$) underwent the same procedure as in the first set, but was provided with physiological solution with the same osmolarity as the electrolyte solution (hyperosmotic solution). As in the first group of animals, we saw a gradual decrease in occurrence of SWD per hour, but this change was less steep than in the first group ($p < 0.05$). Comparison of the effect of electrolyte and hyperosmotic therapy on the occurrence of SWD per hour during six days of solution consumption show a small but significant difference in the effect of these two therapies ($p=0.01$, repeated measures ANOVA). Frequency (7.9 ± 0.3 Hz) and duration (5.6 ± 0.8 s) of SWD were not significantly changed during consumption of the hyperosmotic solution. As in the first set of experiments occurrence of SWD restored to the control level one week after discontinuance of consuming of hyperosmotic solutions ($91.4\pm3.7\%$ of control, Fig 2).

Discussion

The main finding in the present study can be summarized as follow: i) electrolyte/hyperosmotic therapy in a genetic rat model of absence epilepsy resulted in quick onset and gradual suppression of SWD occurrence; ii) electrolyte/hyperosmotic treatment caused no

changes in peak frequency and duration of SWD compare to control recordings; and iii) discontinuation of either therapy resulted in a quick return to SWD frequency and duration.

It is likely that the effects reported here are largely mediated by osmotic changes in the extracellular environment. With an increase in salt concentration of the ingested solution, the osmotic pressure increases and cells are not able to extract water as easily as they can from a hypo-osmotic solution. Even if a rat consumes the same amount of water in control and experimental condition, the water becomes less available for absorption due to an increased salt concentration.

The relationship between osmotic pressure and susceptibility to seizures has been the emphasis in prior scientific reports. Common clinical examples of increased seizure susceptibility due to over-hydration include conditions such as rapid rehydration therapy, the dialysis disequilibrium syndrome, compulsive polydipsia and the syndrome of inappropriate antidiuretic hormone [11]. Conversely, treatments designed to dehydrate or reduce the fluid levels within the body have been shown to protect against unprovoked seizures [11–13].

Animal studies have demonstrated that decrease in extracellular osmolarity increased cellular excitability and induced epileptiform discharges [14–16] whereas increases of extracellular osmotic pressure abolished high $[K^+]_o$ -induced spontaneous seizures in hippocampal slices [17] and blocked action potentials in rat myelinated sensory fibers [18]. There are several possible mechanisms which could explain the osmolarity effect on neuronal activity. One of them is that hyperosmolarity causes cell shrinking and as a result decreases ephaptic transmission e.g. conduction of currents through the extracellular space [19]. Changes in osmolarity could alter the mechanical activity of the membrane ion channels and these effects have been explained by stretch-sensitive mechanisms of neuronal ion channels [20–23].

Both solutions tested decreased SWD number in the WAG/Rij rats, but the electrolyte solution showed a more prominent effect. This was surprising as the osmolarity and concentration of Na^+ and K^+ , the main ions that take part in action potential was similar in both solutions. Moreover in the “hyperosmotic” solutions we added 5 mM of Ca^{2+} and 5 mM Mg^{2+} - divalent cations reported to have anti-seizure and inhibitory effects on neuronal net activity [24]. The discrepancy between the effect of electrolyte and the hyperosmotic solution could relate to the rice-based carbohydrates and trisodium citrate being more hypertonic than a solution containing glucose and sorbitol. Membranes with high permeability to glucose and sorbitol will result in molecules of water entering the cell down their diffusion gradient causing an effect opposite to hyperosmolar effects.

Other dietary therapies have been used in the treatment of epilepsy including the ketogenic diet and low glycemic index diet [25–28]. Both of these diets are quite efficacious and are used to treat a variety of seizures in addition to absence seizures. Unfortunately, the mechanisms of action of these dietary forms of therapy are currently unknown.

In summary, the present study shows that using electrolyte base therapy can be effective as add on therapy to treat absence epilepsy. The observation reported here show that the effect of electrolyte therapy shown on epileptic patients [6] is reproducible on animal model of absence seizures and provides a basis for studying its mechanism on the cellular level. Our results support the finding of Ochoa [6] who used the electrolyte therapy in humans with absence epilepsy. Treatment of refractory absence seizures with rice-based formulas may provide a cost effective, efficacious treatment of absence seizures. However, it should be noted that neither solution totally suppressed SWD. Also it is not known whether such dietary treatment will have efficacy in other seizure types.

Acknowledgments

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Figure 1.

Electroencephalographic presentation of spike-wave discharges (SWD) in left motor cortex of WAG/Rij rats. SWD recordings before (A) and after four days of electrolyte consumption (B). Arrows show identified discharges. Fewer SWD were seen in the group receiving the electrolyte solution. Examples of SWD outlined with boxes are shown on expanded scales below each trace. Note that the SWD are more rapid (8–10 Hz) than seen in humans. Only differences in occurrence of discharges per hour (SWD per hour) were found. The morphology, duration and interdischarge frequency (peak frequency) were unaltered by the electrolyte solution.

Figure 2.

Electrolyte/hyperosmotic therapy on WAG/Rij rat model of absence epilepsy resulted in suppression of SWD occurrence. Summary plot shows the effect of electrolyte (white) and hyperosmotic (grey) solution on SWD occurrence recorded from the motor cortex of WAG/Rij rats. All data and statistics are shown relatively to the mean control obtained from four day recordings before electrolyte therapy. Data is presented as mean $\pm$ SD. Statistics were done using Wilcoxon matched-pairs signed rank test, * $p < 0.05$, ** $p < 0.01$ versus baseline levels.