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Persistent changes in the brain of Immature Rats Experiencing febrile seizures

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Abstract

The most prevalent neurological condition in children is febrile seizures (FS), which affects humans. It is possible to explore the underlying processes of FS that are connected to the onset of epilepsy and associated comorbidities using models of hyperthermia for seizure induction. The current study concentrates on examining the changes in the expression of a gap junction protein in two brain areas; the hippocampus and the cortex in young rats who experienced FS. Animals used in experiments were split into two groups, the cage control group, and the hyperthermia-stimulated FS group. Each group of the two groups was further subdivided into young (Y) and Adult (A) groups. Reduction in the expression of Cx43 has been detected in both groups of animals (Y & A) and was suggested to be related to seizure activity. The present study sheds more light on the possible role played by gap junction proteins in FS and epilepsy.

Keywords: Febrile seizure, Hyperthermia, Connexin, Gap junction protein.

1. Introduction

A major characteristic of seizures is synchrony. While the initiation of seizure is driven at least in partially by the individual neurons' burst firing properties, the evolution and spread of seizures often involve amplification and synchronization between neurons within susceptible networks. Seizure amplification is triggered as a result of repetitive excitatory collaterals that create feedback loops, returning the activity of excitatory synaptic to the neurons within the seizure onset zone [1,2]. Interneuronal synchronisation is facilitated by the electrical synapses that are formed by the gap junctions (GJ) between interneurons. [3]. Gap junction intercellular communication (GJIC) is thought to be important for tissue homeostasis as well as the transmission of electric and metabolic signals among cell populations [4]. GJs might reduce seizure activity by redistributing K+ (which would otherwise increase to ictogenic levels) and glutamate away from areas with high activity, according to this theory [5]. Cx43 GJs, on the other hand, may boost seizure production during seizures by promoting glial synchronization (Ca2+ wave propagation) [6]. Increases in astrocytic calcium and propagating Ca2+-waves across astrocytes can activate Ca2+-dependent ion channels and stimulate glutamate release from astrocytes, resulting in seizure production, neuronal synchrony, and ictal activity spread [7].

The strongest evidence connecting GJ and seizures is the seizure-blocking effect of agents that disrupt intercellular GJ connectivity in vitro and in vivo epilepsy models [8]. Therefore, investigation of the GJ channels and their expression is important to understand the propagation mechanism of the seizures in the brain.

Besides the obvious contribution of both monoamines and GJ in the seizures activity and the process of epileptogenesis, these two parameters are linked to each other. A recent study pointed out that the regulation of GJ could be an underlying mechanism for the therapeutic effect of antidepressants [9].

The present work aims to investigate the variations in the expression of GJ protein CX43 in the cortex and hippocampus of an animal model of FS and to investigate the long-lasting and persisting changes in the adult animals that had been subjected to FS at a young age.

2. Materials and Methods

2.1. Ethical approval

All tests were carried out in accordance with international standards for the care and use of animals, and they were all authorised by the regional

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committee for animal care and use (ACUC) under the reference number (CU/I/F/35/18).

2.2. Experimental animals

Wistar rats were provided By the National Cancer Institute in Giza (Egypt). In order to meet the 12-hour light/12-hour dark cycle of the laboratory, the adult rats were maintained for 14 days. There was unlimited access to standard diets and water. At the Faculty of Science at Cairo University, the breeding of animals was done in the center for housing animals. Each rat's neonatal birth date was documented and is regarded as day zero (P0) after birth. Male rats were chosen for the current investigation after the gender of the young rats was identified at P14.

2.3. Experimental design

Cx-43 western blot test was carried out on 6 animals. The diagrammatic display of the experimental design is shown in Fig (1).The total Cx-43 samples were 12 (6 young and 6 adults) and each 6 composed of 4 FS and 2 cage-control samples.

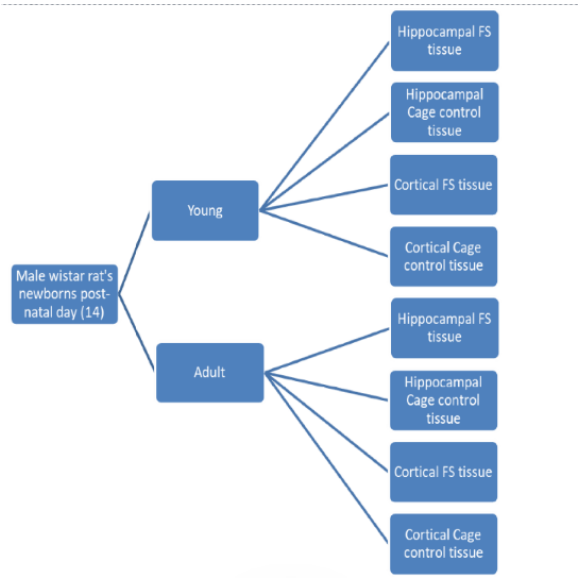

Figure (1): Diagrammatic representation of the experimental design

2.4. Induction of Febrile Seizure

On the 14th postnatal day (P14), FS was generated in newborn rats using the hot air model, which was created by Baram and colleagues [10, 11] and previously reported by Crespo et al. [12,13]. In order to avoid undesirable heating and burning from the glass itself, hyperthermia (HT) was performed in a glass beaker measuring one litre that was covered from the bottom by three layers of paper. Therefore, the only heating was the heated air's variable flow from the warm air source. Every experiment was repeated until the temperature of ambient air reached 50 ± 1˚C by adjusting the length between the animal's position at the bottom of the beaker and the hot air source. Prior to the commencement of the HT, the core temperature (from the rectum) and weight of each animal were measured. Using a temperature sensors thermocouple of a model (TP-02) attached to a digital multimeter model (KYORITSU, KEW1011, Japan), rectal temperature was measured every two minutes. The newborn rats' core body temperatures were measured for 30 minutes or until they reached 40–44˚C. Throughout this HT, the animal's behavior was observed. After 30 minutes of HT exposure or when the animal's core body temperature reaches 40 to 44˚C without a seizure, the HT procedure is halted, and the animal is removed from the group of febrile seizure. The commencement of the seizure was characterized by facial myoclonus activity and toppling over with hind-limb clonus movements Stage 5 on the Racine scale) [14]. Animals were classed as the FS group when convulsions persisted for more than 15 minutes in them, indicating a complex seizure. Cage-control animals were separated from their mothers for the same amount of time as in the heating procedure.

2.5. Tissue sample collection

Animals were abruptly decapitated as sacrifices at the end of the experiment. Gaining access to brain tissues that have not been polluted or functionally altered by gases or anesthesia is a benefit of employing a speedy and quick decapitation. This process is accepted as a merciful euthanasia technique [15]. The cortices and hippocampi were separated from the brain after it had been extracted from the skull and placed on an ice-filled plate. The collected samples were weighed and kept until the assessments in a deep freezer (-30 C).

2.6. Connexin-43 quantification using western blot technique

In the tank buffer, a 12 percent Sodium Dodecyl Sulfate (SDS, cat no.L3771, Sigma Aldrich) Gel was prepared and soaked. 15 µl of the sample buffer was mixed with a sample of 15 µl. The gel comb was carefully extracted, and 20 µl of samples were placed into each well. As an unstained protein marker, the ladder Novex Sharp Protein standard (cat no. LC5800, Hilden, Germany) was used. Samples were run at 80 m Amp and 200 V for 150 minutes. After the samples reached the gel's end and the ladder was fully separated, the electrical current was turned off and the gel was removed from the buffer. The upper glass slide was carefully removed and the gel was released into a Petri dish containing the washing buffer, Phosphate Buffered Saline (PBS). The black side of the transfer sandwich was added to a plate filled with transfer buffer. The nitrocellulose membrane was immersed in the transfer buffer together with two fiber pads, two filter sheets. One

fiber pad was placed on the transfer sandwich's black side, one filter paper was then added, the gel was carefully applied, the membrane was then put to the gel. The other filter paper was then added to the membrane, and the other fiber pad was attached to the filter paper (make sure to remove any air bubbles between each layer).The other side of the transfer sandwich was closed, and a tank containing the transfer buffer was attached to the entire cassette. After the transfer, the membrane was applied to glass Petri dishes, then 10 ml of the blocking buffer was added to the membrane and left on a rocking platform overnight, and the blocking buffer was discarded. After that, 1 ml of 10x wash buffer was combined with 9 ml of distilled water, then 5 µl of the primary antibody "Rabbit anti-Connexin-43 polyclonal antibody with cat no.FNab01861" was applied to the membrane with a concentration of 1:1000 and left on a rocking platform overnight, and then the primary antibody was discarded. Membranes were washed 3 times by 1x washing buffer, and 1 ml of 10x washing buffer was mixed with 9 ml of distilled water, then 5 µl of secondary antibody 'Biotin- XX goat anti-rabbit IgG (H+L) with cat no.1305936 (Thermo Fisher Science, Waltham, MA, USA) was applied to the membrane and left on a rocking platform overnight, then the secondary antibody was discarded. The membranes were washed with a 1x washing buffer 3 times.10 ml of blocking buffer was added, then 5 µl of the substrate 'Qdot R 625 streptavidin conjugate with cat no. W10142' (Thermo Fisher Science, Waltham, MA, USA) was mixed on the membrane and left on a rocking platform overnight, and then the substrate was discarded. In a final step, 1x washing buffer washed membranes 3 times.

2.7. Statistical analysis

The data is presented in percentage difference pd%.The percentage difference was determined using the following formula:

$$\%\ Difference = \frac{Trated\ value - Control\ value}{Control\ value} X\ 100$$

3. Results and Discussion

Quantification of connexin 43 (Cx43)

Protein expression change is related to functional changes manifested in diseased conditions.

Gap junction protein Cx43 expression quantification determined by western blot is considered a straightforward and direct method of protein expression determination (Fig2). The alteration obtained in the Cx43 expression is linked to the mechanisms underlying the gap junction disruption. Quantification of the Western blots revealed lower Cx43 expression in hyperthermic FS (HT-FS) treated rats than in the cage-control group. Cortical and hippocampal Cx43 concentrations per gram tissue in ng/gram in young (14 days) and adult (3 months) rats were determined. There was a decrease in cortical Cx43 concentration of treated animals by 28.43%with respect to the cage-control in young animals but this decrease was turned back to 13.80% in the adult group. Otherwise, for the hippocampus area, there was a decrease in Cx43 concentration in young treated animals with respect to their cage-control by 6.06% and this percentage turned to 9.30% in the adult group.

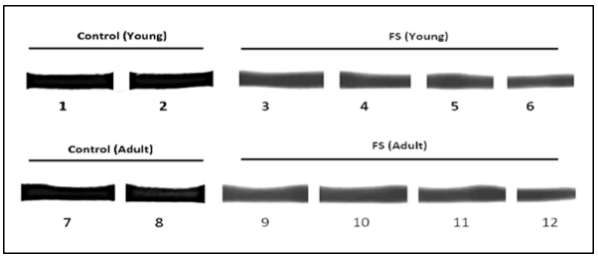

Figure (2): Illustrates the SDS gel (12 %) electrophoresis of Cx 43 for young animals (upper trace) and adult animals (lower trace). 1, 2, 7, and 8 are control samples of the cortex and hippocampus of young and adult animals, respectively. 3, 5, 9, and 11 are FS samples from the cortex of young and adult animals, respectively. 4, 6, 10, and 12 are FS samples from the hippocampus of young and adult animals, respectively. Quantitative values of Cx 43 in ng/gm of tissue are shown in the table. %D is the percentage difference.

A connection between protein expression and epileptic seizures was discovered at the molecular level [16]. Connexin (Cx) expression and coupling were different in the tissue of epileptic patients [17, 18].

Table 1: Represents the concentration of Cx43 in (ng/gm) in young and adult animals, including cortical and hippocampal tissue. The percentage difference is calculated and tabulated.

Hippocampus				**Cortex**			
Adult Animals		Young Animals		Adult Animals		Young Animals	
control (ng/gm)	FS (ng/gm)	control (ng/gm)	FS (ng/gm)	control (ng/gm)	FS (ng/gm)	control (ng/gm)	FS (ng/gm)
168.37	152.69	243	228.26	113.27	97.63	168.08	120.28
%D = - 9.31 %		%D = - 6.06 %		%D = - 13.80 %		%D = - 28.43 %	

The cortical and hippocampal tissues of the FS group of rats in the current investigation included lower levels of Cx43. This result is consistent with the findings of Khan et al. [19], who claimed that FS, resulted by hyperthermia, caused a more than 50% decrease in the astrocyte gap junctional coupling in the hippocampus. This astrocyte dysfunction has been linked to this decrease in Cx43 expression [20].

Furthermore, it has been observed that the surviving glial cells in the hippocampi of individuals with mesial temporal lobe epilepsy (MTLE) have a considerable decrease of GJ coupling. Moreover, it has been demonstrated that the uncoupling ofastrocytes causes neuronal death and hyperactivity[50]. The uncoupling that took place in the last stage of the illness can be blamed for the varied Cx43 expressions in young and adult animals in the current investigation [20]. Like epileptogenesis, the process of uncoupling takes time to mature.

Many factors and circumstances, including neurotransmitters, might disrupt astrocyte GJ coupling [21]. In striatal astrocyte cultures, alpha-1 adrenergic stimulation was shown to inhibit coupling through the actions of NE and pharmaceutical agonists and antagonists [22]. The fact that young FS animals in the current research had higher cortical NE than the cage-control group and lower cortical Cx43 concentrations may be attributable to the inhibitory effect of NE on the GJ coupling.

One of the drawbacks of the current investigation is the small sample size for the measurement of GJ proteins. The other restriction is that just one kind of GJ protein has been identified, leaving the other varieties unidentified.

In future studies and due to the importance of these functional proteins in epilepsy initiating and progression, we are planning to target different types of GJ proteins and seeking for their functional coupling with seizure activity. Additionally, lack of the studies that targets the relation between GJ protein expression and monoamines and links this relation to epileptic seizures and epilepsy is considered an open question that needs further investigations.

4. Conclusion

The current study draws the conclusion that FS can lead to persistent changes in the cortical and hippocampal expression of the gap junction protein Cx43 at an embryonic stage of development. The current results provide further clarity on the relationship between FS and the change in expression of gap junction protein Cx43 that may show effects in adults who experienced FS when they were younger.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

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