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Influence of Hypo-osmolality and Induced Seizures on Blood Brain Barrier Permeability and Brain Concentrations of Trace elements in Rats

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Abstract

Backgrounds: We studied the changes in blood-brain barrier (BBB) permeability and brain trace elements concentration in acute hypo-osmolality and pentylenetetrazol (PTZ)-induced seizures model in rats. Furthermore, the effect of acute hypo-osmolality on seizure activity (patterns) in the PTZ model, the correlation between seizure severity score (SS score) and changes of both of BBB permeability and brain trace elements concentration in rats were also studied. **Materials and Methods:** Four groups (18 animals /group) of adult male albino rats were included. The animal groups are: (1) Control group: received only a vehicle. (2) Hypo-osmolar group: received warm distilled water (DW). (3) Seizure group: injected with PTZ i.p. (4) Hypo-osmolar + seizure group: first injected with DW and then PTZ. During the next 30 minutes, the animals were observed for seizure activity. For evaluation of BBB permeability changes, serum S100ß protein and Evan Blue (EB) dye content of brain tissue were evaluated. Additionally macroscopic and microscopic examinations of brain tissue were completed. Trace elements, magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe), concentration in brain tissue were estimated. **Results:** There were significant increase of EB content of brain tissue, and serum S100ß in hypo-osmolar, seizure and hypo-osmolar + seizure groups, as compared with control animals. These parameters were significantly increased in the hypo-osmolar + seizure group when compared with either hypo-osmolar or seizure groups. These results were confirmed by macroscopic examination of brain tissue. Trace elements concentration of brain tissue, Mg, Zn, Cu and Fe, were significantly decreased in hypo-osmolar, seizure and hypo-osmolar + seizure groups in comparison with control animals. Histological examination of sections from the hilar region of dentate gyrus of the hippocampus revealed degenerative changes in the three groups of animals in comparison with control animals. Morphometric analysis showed a significant decrease in the number of cells in the hilar region of dentate gyrus in the three groups of animals

in comparison with controls. Seizure activity significantly increased in hypo-osmolar + seizure as compared to seizure group. The correlation analysis revealed that significant –ve correlations were found between SS score and trace elements concentration, while significant +ve correlations were observed between SS score, and each of EB content and serum S100ß protein in seizure and hypo-osmolar + seizure groups. **Conclusion:** Our study demonstrated that, hypo-osmolality and PTZ-induced seizures could result in increased BBB permeability with a decrease of brain trace elements concentration. This could play a vital role in the pathophysiology of epileptic seizures. In addition, hypo-osmolality can enhance neuronal excitability and severity of seizures in the PTZ-induced model in rats**.**

Key words: Hypo-osmolality, seizures, Blood brain barrier, Brain trace elements

Introduction

The blood-brain barrier (BBB) is a system of capillary endothelial cells that protects the brain from harmful substances present in the blood stream while supplying the brain with nutrients required for proper function (1). The well-known independence of ion levels in brain extracellular fluid from those in plasma is generally attributed to the BBB function of the cerebral capillary endothelium. Tight junctions of high electrical resistance, providing an effective barrier against the movement of molecules, join these endothelial cells. Furthermore, the glial cells' (astrocytes) foot processes make a substantial contribution to BBB integrity, covering more than 90% of the basement membrane of these capillaries. They synthesize some proteins, such S100ß, and release it next to the capillaries. But these proteins are extravasated into the plasma only when the BBB is damaged. In addition, the passage of peripheral proteins across the BBB into the cerebral parenchyma is almost totally prevented, except if the BBB is damaged (2-4). However, there are several areas of the brain where the BBB is weak and permeable. These areas are known as circumventricular organs through which the brain is able to monitor the makeup of the blood. It includes the following areas: pineal body, posterior pituitary, vomiting center and median eminence (5).

Previous studies found that the changes in BBB permeability could be induced either by changes in plasma osmolality (6) or by epileptic seizures (7). These changes in permeability could result in alteration of the ionic environment of central neurons and glia leading to neuronal damage (8,9). It was observed that the epileptic seizures strongly modified internal conditions within the nervous tissues. There are many changes in the neurotransmitter release, gene activation and elemental composition (10). The balance between trace elements such as Mg, Ca, Fe, Cu, Zn and Se in the nervous tissues is of crucial importance for maintaining human health. In biological system, these metallic elements are usually bound to proteins. Metals in metalloproteins are components of enzymatic systems and fulfill structural and storage function. Hence, these elements are responsible for various metabolic processes including those occurring in the brain (11,12).

The effects of acute hypo-osmolality and seizures on BBB permeability and trace elements concentration in brain tissue have not previously been thoroughly addressed. The effect of hypo-osmolality on seizure activity (patterns) has not been widely reported. Therefore, the aim of the present work was fourfold. Firstly, we chose to evaluate changes in BBB permeability (by evaluating EB content in brain tissue and serum S100ß protein as a peripheral marker) and brain trace elements concentration (Mg, Zn, Cu and Fe) in acute hypo-osmolality state and PTZ-induced seizures models in rats. Secondly, we chose to study the effect of acute hypo-osmolality on seizure activity (patterns) in PTZ-induced seizures models in rats. Thirdly, we attempted to find the correlation between seizure severity score (SS score) and changes in each of BBB permeability markers and trace elements concentration in brain tissue in rats. Finally, we demonstrated the damaging effects of acute hypo-osmolality and induced seizures on the rat brain by microscopic (histological) examination of brain tissue.

Materials and Methods

Animals

A total of 72 adult male rats (Wistar Albino) weighing 230-250 gm, aged 4-5 months were housed in cages and maintained on a 12 hour (h) light-dark cycle with free access to water and food.

Chemicals

Pentylenetetrazol (PTZ) and Evan-blue (EB) dye were obtained from Sigma Chemical Co. and dissolved in 0.9% NaCl.

Experimental procedures

Four groups of rats were studied (18 animals each). Each group was divided into subgroups (6 animals) for determination of BBB permeability and for trace elements

analysis. The animal groups were as follows:

(1) Control group: which received only the vehicle (0.9% NaCl).

(2) Hypo-osmolar group: This group received warm distilled water (DW) by intraperitoneal injection (i.p.), in a volume of 10% of body weight. Approximately 2 h after injection of D.W., hypo-osmolality was reached when plasma-osmolarity had decreased by 25-30 mosm (13). Rats were then observed 1/2 h for any neurological manifestation of hyponatremia, such as stress, level of consciousness, and any evidence of seizures (14). Two-thirds of the animals (12 rats) were anesthetized with diethyl ether for insertion of a cannula in the femoral vein. For determination of BBB permeability, EB dye was injected intravenously (i.v.) at a dose of 4 ml of 2% solution in saline/kg body wt. (15). Approximately 20 minutes after EB injection, all rats were sacrificed. Brains were removed and weighed. Six were fixed in formalin and sectioned coronally in 2-3 mm thickness slices. They were examined macroscopically for EB extravasations. The extent and intensity of EB staining of brain tissue was used for BBB permeability determination (16). The other six rats were used for quantitative estimation of EB with a spectrophotometer using homogenized brain to release the dye according to methods of Õztas and Kücük (15). Protein content of the supernatants was determined using Lowry's method (17). The final third of the animals (six rats) was used for trace elements analysis (Mg, Zn, Cu and Fe) in brain tissue by Flam Atomic Absorption spectrophotometer (Model AA -630-02) according to Karakoc, et al. (18).

(3) Seizure group: This group of rats was anaesthetized with diethyl ether for insertion of a cannula into the femoral vein. After a 2 h waiting period, the animals regained full consciousness, and EB dye was injected i.v. Five minutes later, PTZ was injected i.p in dose of 55 mg/kg (19). During the next 30 minutes, they were observed for seizure activity: (i) latency in min. "which is the time recorded from start of injection of PTZ until onset of seizures," (ii) duration (minutes) of seizures, and (iii) seizure severity score (SS score) according to Cole, et al., (20) as shown in Table 1. Then animals were sacrificed and BBB permeability and trace elements concentration were evaluated as mentioned previously.

(3) Hypo-osmolar + seizure group: This group was first injected with DW i.p., in a volume of 10% of body wt. Rats were anesthetized with diethyl ether, and then a cannula was inserted into the femoral vein. After 2 h of DW injection (when hypo-osmolality was reached and the rats regained full consciousness, EB dye was injected i.v. Five minutes later, PTZ was injected i.p in a dose of 55mg/kg and the animals were observed for seizure activity for the next 30 min. Then the animals were sacrificed. BBB permeability and trace elements concentration in brain tissue were then evaluated as mentioned above.

Blood samples were obtained from all animal groups before sacrifice to measure serum S100ß protein levels as a peripheral marker of BBB disruption (3) with use of the Nexus Dx S100ß test kit (Nanogen, San Diego, CA) by enzyme–linked immunosorbent assay (ELISA) technique. For histological examination, gallocyanin-chrom alum staining method was used. Steps were completed according to Drury and Wallington examination (21). Morphometric Procedure for estimation of number of cells in the hilar region of dentate gyrus of the hippocampus was performed (22).

Statistical Analysis

Data were presented as means ± standard error (SEM). Differences between groups were determined by unpaired Student's Newman–Keuls "t" test. The level of significance was accepted with $p < 0.05$. Pearson's correlation coefficients were used to assess the correlation between parameters. Prism computer program (graph pad version 3.0) was used for statistical analysis (23).

Results

Markers of BBB permeability

a- Evan blue content

Figure 1. shows EB content (mg%) of whole brain tissue (means ± SEM), which represents an index for BBB permeability. There were significant increases of the EB content in hypo-osmolar ($p<0.05$), seizure ($p<0.001$) and hypo-osmolar + seizure groups ($p<0.001$) as compared to control animals. Also, EB content in hypo-osmolar + seizure group was significantly increased when compared with either hypo-osmolar or seizure groups ($p<0.01$ and $p<0.05$ respectively). EB content in control animals represented the extravasation of EB dye in regions where capillaries are known to be leaky.

Table 1. Seizure Severity Score for PTZ according to Cole et al.,(20)	
Score	**Behavioral response**
0	No response.
1	Staring, unresponsive.
2	Focal clonic convulsion (e:g head nod, twitch, myoclonic Jerk, backing).
3	Fore limb hyperextension (tonic, clonic seizures).
4	Hind limb hyperextension.
5	Loss of posture (e:g Jumping , rearing and falling).
6	Status epilepticus / death.

Table 2. Trace elements concentration of brain tissue in the studied groups of animals.				
	Mg (mg/gm)	Zn (μg/gm)	Cu (μg/gm)	Fe (μg /gm)
Control	3.97 ± 0.63	8.96 ± 1. 1	5.98 ±0.93	30.7 ± 4. 6
Hypo-osmolar	2.22 ± 0.42 *a*	5.21 ± 0.98 *a*	3.31 ±0.87 *a*	17.6 ± 3. 3 *a*
Seizure	1.0 ± 0.33 *b,d*	2.8± 0.*4 c,d*	1.2±0.13 c*,d*	9.0 ± 2. 0 *b,d*
Hypo-osmolar + seizure	1.04± 0.31 *b,d,g*	1.5 ± 0.*22 c,e,f*	1.30 ± 0. *2 c,d,g*	8.8 ± 2. 1 *b,d,g*
Data are the mean ± SEM. a: P<0.05, b: P<0.01, c: P<0.001 as compared to control group. d: P<0.05 and e: P<0.01 as compared to hypo-osmolar group .f: P<0.05 and g: non significant as compared to seizures group. Each group is 6 animals.				

Table 3. Correlations coefficients between SS score and each of trace elements concentration, EB content of brain tissue and serum S100ß protein in seizure and hypo-osmolar + seizure groups.						
SS score	Mg(mg/gm)	Zn(μg/gm)	Cu(μg/gm)	Fe(μg/gm)	EB content mg%	Serum S100ß
Seizure group	r = - 0.69 P<0.001	r = - 0.75 P<0.001	r = - 0.58 P<0.01	r = - 0.63 P< 0.01	r = 0.56 P<0.01	r = 0.75 P<0.001
Hypo-osmolar +seizure group	r = - 0.84 P<0.001	r = - 0.88 P<0.001	r = - 0.76 P<0.001	r = - 0.74 P< 0.001	r = 0.78 P<0.001	r = 0.8 P<0.001

b- Serum S100ß protein level

Figure 2. shows serum S100ß protein (means ± SEM), which represents a peripheral marker of BBB permeability. The level in the control group was a nearly negligible value so; comparison was done between the other groups. It was found that S100ß protein level was significantly higher in the hypo-osmolar+ seizure group as compared to hypo-osmolar ($p<0.001$) or seizure ($p<0.05$) groups. Also, the level was significantly elevated in the seizure group as compared to the hypo-osmolar group with p value < 0.01.

c-Macroscopic examination

Figure 3. "A, B, and C" shows macroscopic examination of coronal sections of the brain of hypo-osmolar, seizure and hypo-osmolar+seizure groups respectively. In the hypo-osmolar group, EB extravasations (leakage) was limited and lightly stained multiple brain areas such as thalamus, hypothalamus, hippocampus, and arcuate nucleus. In the seizure group, EB extravasation was diffuse and most frequent noted in the same previously mentioned areas. Hypo-osmolar +seizure group showed more intense and diffuse EB extravasation in the same previous areas as

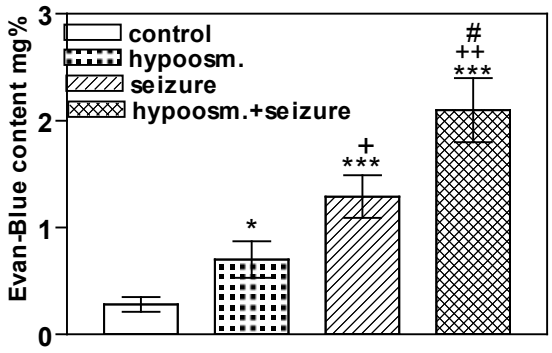


Figure 1. Evan-blue content (mg%) of brain tissue in the studied groups of animals.*: P<0.05 and ***: P< 0.001 as compared to control group. +:P<0.05 and ++ : P<0.01 as compared to hypo-osmolar group. #:P<0.05 as compared to seizure group. Each group is 6 animals. Data are means ±SEM.

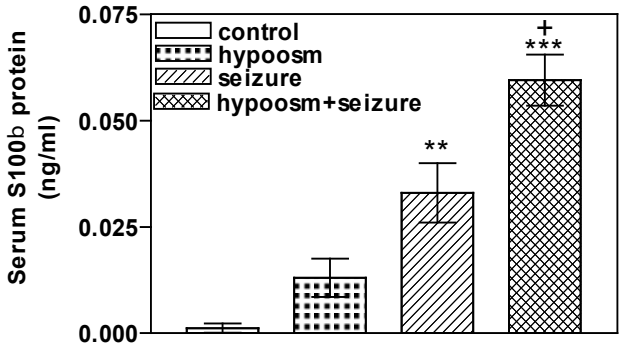


Figure 2. Serum S100ß protein (ng/ml) in the studied groups of animals. **: P<0.01 and ***: P< 0.001 as compared to hypo-osmolar group. +:P<0.05 as compared to seizure group. Each group is 18 animals. Data are means ±SEM.

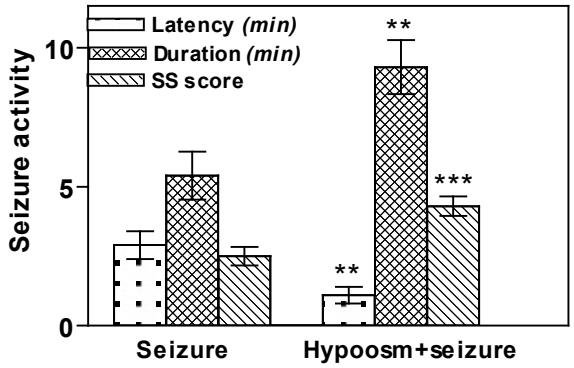


Figure 4. Seizure activity (latency, duration and SS score) in seizure and hypo-osmolar +seizure groups of animals. **: P<0.01 and ***: P< 0.001 as compared to seizure group. Each group is 18 animals. Data are means ±SEM.

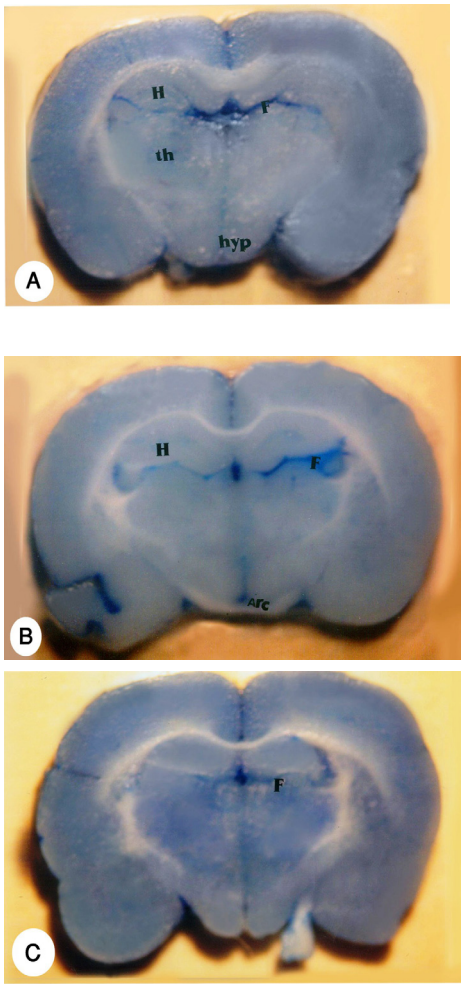
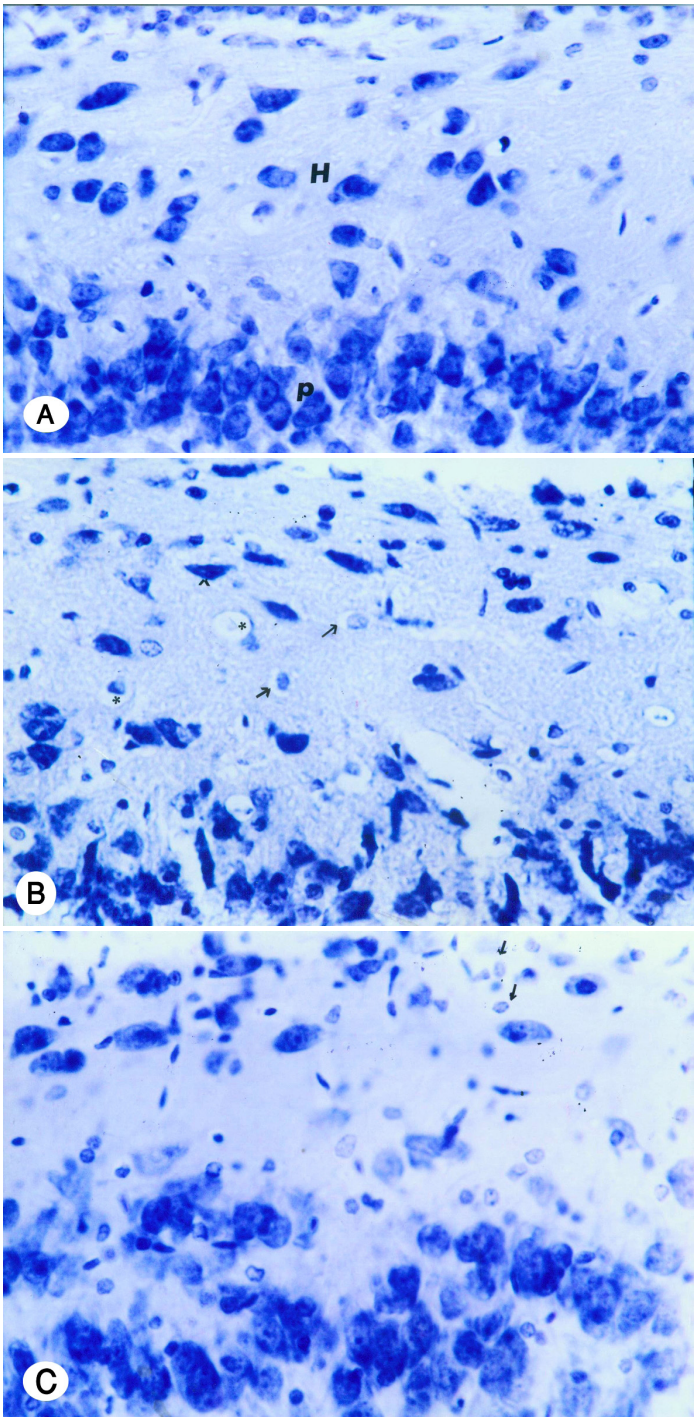


Figure 3. "A, B, C" Photomacrography of coronal section of the brain of hypo-smolar, seizure and hypo-osmolar+seizure groups respectively. In hypo-osmolar group (A) EB extravasations (leakage) was limited and lightly stained multiple brain areas. In seizure group (B) EB extravasations was diffuse and most frequent nearly in same areas. Hypo-osmolar+seizure group (C) showed more intense and diffuse EB extravasations in the same previous areas as compared with the two other groups. Brain areas are hippocampus(H), thalamus(th), hypothalamus(hyp), arcuate nucleus (Arc) and fimbria of the hippocampus (F)



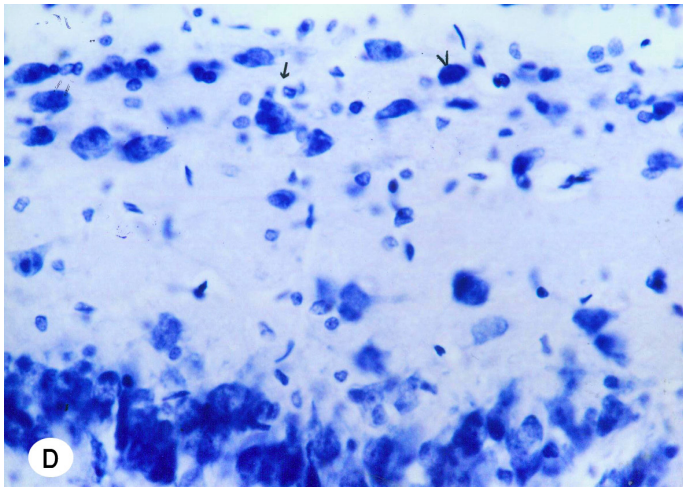


Figure 5. "A, B, C, D" showed histological structures of the hilar region of dentate gyrus of the hippocampus in the studied groups of animals (control, hypo-osmolar, seizure and hypo-osmolar+seizure groups respectively), stained with Gallocyanin stain. Degenerative changes were observed in the three groups of animals in comparison with control. These changes were in the form of marked swelling of some cells (especially in hypo-osmolar group *), vacuolated and pale cytoplasm (arrows) and pyknotic nuclei (arrow head).

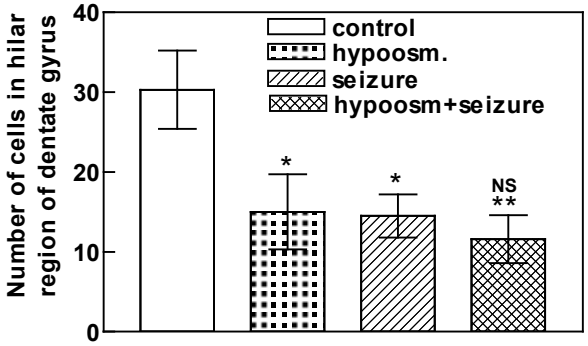


Figure 6. Number of cells in hilar region of dentate gyrus in the studied groups of animals. *: P<0.05 and **: P< 0.01 as compared to control group. NS: non significant as compared to hypo-osmolar or seizure groups. Each group is 6 animals. Data are means ±SEM.

compared with the two other groups. No EB extravasation was seen in the previous areas of brain tissue from control animals.

Trace elements concentration

Table 2 shows the concentration (means ± SEM) of trace elements in brain tissue of the studied groups of animals. It was found that Mg, Zn, Cu and Fe were significantly decreased in hypo-osmolar ($p<0.05$ for each element), seizure ($p<0.01$ for Mg and Fe, $p<0.001$ for Zn and Cu) and hypo-osmolar + seizure groups ($p< 0.01$ for Mg and Fe, $p<0.001$ for Zn and Cu) in comparison to control animals. In seizure and hypo-osmolar + seizure groups, Mg, Zn, Cu and Fe concentrations were significantly decreased as compared to hypo-osmolar groups ($p<0.05$ for each element in seizure group) and ($p< 0.05$ for Mg, Cu, Fe, $p< 0.01$ for Zn in hypo-osmolar + seizure group).

Patterns of seizure activity

Figure 4. shows the patterns of seizure activity (latency, duration in minutes, and SS score) in seizure and hypo-osmolar + seizure groups. It was observed that latency was significantly decreased ($p<0.01$) while the duration and SS scoring were significantly increased ($p<0.01$ and $p<0.001$ respectively) in hypo-osmolar + seizure as compared to the seizure group. In the hypo-osmolar group, no seizures were observed, but six animals were stressed.

Correlation analysis

In Table 3, significant –ve correlations were found in seizure and hypo-osmolar + seizure groups between SS score and each of Mg ($r = -0.69$, $p<0.001$ and $r = - 0.84$, $p<0.001$ respectively), Zn ($r = - 0.75$, $p<0.001$ and $r = - 0.88$, $p<0.001$ respectively), Cu ($r = -0.58$, $p<0.01$ and $r = - 0.76$, $p<0.001$ respectively) and Fe concentrations ($r = - 0.63$, $p< 0.01$ and $r = - 0.74$, $p< 0.001$ respectively). On the other hand significant +ve correlations were observed between SS scores, and each of EB content and serum S100ß protein ($r = 0.56$, $p<0.01$ and $r =0.75$, $p<0.001$ respectively in the seizure group and $r = 0.78$, $p<0.001$ and $r=0.8$, $p<0.001$ respectively in hypo-osmolar + seizure group).

Histological examination

Fig. 5 "A, B, C, and D" shows histological structure of the hilar region of dentate gyrus of the hippocampus in control, hypo-osmolar, seizure and hypo-osmolar+ seizure groups respectively, stained with gallocyanin stain. Degenerative changes were found in the form of marked swelling of some neurons (especially in the hypo-osmolar group), vacuolated pale cytoplasm and pyknotic nuclei.

Morphometric or stereological analysis: In figure 6, in comparison to controls, a significant decrease in the number of cells of the hilar region of dentate gyrus was observed in hypo-osmolar ($p<0.05$), seizure ($p<0.05$) and hypo-osmolar+ seizure groups ($p<0.01$).

Discussion

Epilepsy is a neurological condition characterized by recurrent seizures caused by neuronal hyper-excitability regulated by a complex interaction of excitatory and inhibitory neurotransmitter systems, ionic environment and brain capillary endothelial structures (8). Clinical studies and animal models of epilepsy proved that even a short episode of epileptic convulsions could produce local or remote degenerative changes in brain tissue (24,25). Also, epileptic seizures could affect the metabolism and distribution of trace elements in nervous tissue, and these elements are responsible for various brain functions (11,12). Likewise, hypo-osmolality is one of the clinical problems that can cause a variety of neurological symptoms and induce brain damage (26). This background was the basis for the present study.

Results of the present study reported that the EB extravasation (EB content) in brain tissue was significantly greater in hypo-osmolar, seizure and hypo-osmolar + seizure groups in comparison to control animals. EB content was significantly increased in seizure in comparison to the hypo-osmolar groups. Also, hypo-osmolar + seizure group showed an increased content as compared to hypo-osmolar or seizure groups. This was a coincidence with the macroscopic findings, which revealed that in hypo-osmolar group, EB extravasation (leakage) was limited, and lightly stained multiple brain areas as thalamus, hypothalamus and hippocampus. In the seizure group, EB extravasation was diffuse and most frequently seen in the same previously mentioned areas. Hypo-osmolar+seizure group showed more intense and diffuse EB extravasation in the same previous areas as compared with the two other groups.

Similar to these results, Sahin, et al. (19), noticed that an enhanced BBB permeability with bilateral extravasation of EB dye in brain tissue was greater in single dose PTZ-induced seizures than in repeated doses in rats. They proved that PTZ-induced seizures caused BBB breakdown in most of the brain stem and diencephalic structures as seizures can cause lessening of cerebral endothelial tightness. Ziylan

and Ates (27) stated that in adult rats, certain brain areas are more vulnerable to PTZ, or the BBB has an increased fragility in particular to seizure activity. Also, the present study was supported by the findings of Õztas et al. (6), who found that EB extravasation was greater in the brain of water-intoxicated animals (hypo-osmolar) than in non water-intoxicated rats after PTZ injection. An explanation for BBB breakdown and increase its permeability in hypo-osmolality is that with acute decrease in plasma ECF osmolality, brain cells behave as osmometers and swell as a result of movement of water into the cells along osmotic gradients with development of cerebral edema. Such effects are present in the capillary endothelial cells as well. The gradually developed osmotic swelling of endothelial cells causes the tight junctions between these cells to be opened and lets the EB dye, which is normally excluded, to enter the brain parenchyma (28).

The present results revealed degenerative changes in the hilar region of dentate gyrus of the hippocampus in hypo-osmolar, seizure and hypo-osmolar + seizure groups. In addition, stereological analysis of the number of cells in the hilar region of dentate gyrus recorded a significant decrease in hypo-osmolar, seizure and hypo-osmolar+ seizure groups compared with control animals. These findings are in agreement with Arieff (29), who observed that dilutional hyponatremia could result in permanent brain damage. In previous studies it was reported that morphological alterations (cell loss) in the hilus of the dentate gyrus were observed in animals receiving PTZ, and concluded that neurons were destroyed by PTZ insult (30,31). In addition, it was found that in adult rats, the epileptic seizures caused overt neuronal cell death (32-34). In the current study, the increased BBB permeability observed in the seizures group could be attributed to this damaging effect of seizures on brain tissue, which may involve the capillary endothelial cells, and induce breakdown of BBB. Also, this study revealed that seizures combined with hypo-osmolality produced a major increase in BBB permeability. This could be attributed to the combination of the mechanisms involved in BBB damage in both hypo-osmolarity and seizures.

In the present study, serum S100ß protein as another putative marker of BBB permeability was evaluated. The level in the control group was a nearly negligible value so comparison was done between the other groups. It was found that S100ß protein levels were significantly higher in hypo-osmolar+ seizure group as compared to either hypo-osmolar or to seizure groups. The level was significantly elevated in the seizure group as compared to the hypo-osmolar group. Abbott et al, (3,4) stated that certain proteins such S100ß are synthesized by brain astrocytes and released next to the cerebral capillaries, but these proteins are extravasated into the plasma only when the BBB is damaged. Marchi et al. (35) reported that elevation of serum S100ß protein had been cited as evidence for BBB disruption in seizures. Therefore, in our study the observed elevation of S100ß protein could be seen as the result of BBB opening and increased BBB permeability following neuronal damage either in hypo-osmolar or seizure groups. Thus, testing serum levels of S100ß protein which is exclusively present in brain astrocytes represents a non-invasive means for evaluating BBB integrity that may be a reliable way to diagnose the prognosis of neurological diseases.

In the current study regarding trace elements concentration of brain tissue, it was found that Mg, Zn, Cu and Fe were significantly decreased in hypo-osmolar, seizure and hypo-osmolar + seizure groups compared to control animals. Compared to hypo-osmolar group, Mg, Zn, Cu and Fe concentrations were significantly decreased in seizure and hypo-osmolar + seizure groups. Our findings are nearly in line with Sahin et al. (19), who found significant decreased Cu and Fe concentrations of brain tissue in seizure groups compared with controls. However, Zn concentration did not show any significant difference between groups. In studies conducted by Chwiej et al. (36), and Chwiej and Janeczko (37), in rat brains of pilocarpine-induced epilepsy, it was observed that Fe, Cu, and Zn were decreased in certain brain areas which underwent neurodegenerative changes as a result of epileptic seizures.

The decrement of brain trace elements concentration in seizure groups in our study might be related to the state of BBB permeability. This could be explained by the post epileptic changes (increases) in BBB permeability, which caused massive outflow of these elements from the brain tissues affected by seizures related to the leaky blood vessels (19).

In the same manner, the decrease of these elements in hypo-osmolar group in our study may be due to the outflow of these elements from brain tissue as a result of the breakdown of the BBB. Sarin (38) observed that the BBB endothelium could efficiently regulate cerebrovascular transport mechanisms of trace elements.

The decrease of these elements in brain tissue with increased BBB permeability may be involved in the pathophysiology of epileptic seizures. Prasad et al. (39), and

Bohic et al. (40), reported that the trace elements participate in a wide range of processes such as neurotransmitter synthesis, synaptic transmission, and prevention of neurological disorders. Seizure susceptibility has been detected in zinc deficient conditions (41). Takeda et al. (42), hypothesized that decreased zinc could lead to an inhibition of gamma-amino-butyric acid (GABA), an inhibitory neurotransmitter and facilitator of N-Methyl-D-Aspartate (NMDA) receptors. This is one of the glutamate (excitatory neurotransmitter) family receptors, which may contribute to prolonged neuronal discharge and epileptiform activity. In another work, Kulkarni et al. (43), reported that at the neuronal level, vesicular zinc acts as a neurotransmitter in glutaminergic and GABAergic transmission by decreasing extracellular glutamate concentration and increasing extracellular GABA concentration.

Copper (Cu) inhibits Mg^{++} adenosine triphosphatase (ATpase) and Na-K ATpase enzymes. The decrease of Cu disturbs Na^{+} and K^{+} homeostasis, which results in the genesis of epileptiform discharges (12). Lowering Mg concentrations resulted in increased nervous system irritability, and induced spontaneous epileptiform activity in various cortical structures. This could be secondary to the facilitatory effect on NMDA receptors. It also increased the facilitatory effects of calcium on synaptic transmission (39).

Regarding the patterns of seizure activity, the present study demonstrated that the latency was significantly decreased while the duration of seizures and SS score were significantly increased in hypo-osmolar +seizure group, compared to seizure group. It was apparent from this study that hypo-osmolality could increase neuronal excitability and severity of seizures in the PTZ-induced model in rats. The means hypo-osmolality enhanced neuronal hyper-excitability and seizures may be partially due to the decrease of brain tissue trace elements as mentioned previously. In addition, hypo-osmolality results in transiently increased local brain extracellular fluid concentrations of osmolytes, especially amino acid glutamate during volume regulatory decreasing mechanisms. This could produce significant effects on the neuronal membrane potential and could increase neuronal excitability (44).

Finally, in this study an attempt was made to discover the correlation between SS score and each of trace elements concentration, EB content of brain tissue, and serum S100ß protein. Our results demonstrated significant –ve correlations found either in seizure or in hypo-osmolar + seizure groups, between SS score and each of Mg, Zn, Cu and Fe concentrations. On the other hand, significant +ve correlations were observed between SS score and each of EB content and serum S100ß protein in both groups of animals. This meant that a decrease of trace elements concentration of brain tissue and increased BBB permeability (increased EB content of brain tissue and serum S100ß protein) are associated with increased SS score. It is noteworthy that the strongest correlations observed in our study were in hypo-osmolar + seizure group confirms our observation that hypo-osmolality is a contributing factor that can enhance neuronal excitability and severity of seizures in the PTZ-induced model in rats.

In conclusion, our study demonstrated that, hypo-osmolality and PTZ- induced seizures could result in increased BBB permeability with a decrease of brain trace elements concentration. This could play a vital role in the pathophysiology of epileptic seizures. In addition, hypo-osmolality can enhance neuronal excitability and severity of seizures in the PTZ-induced model in rats.

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