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HEMATOBIOCHEMICAL CHANGES IN A KINDLING MODEL OF EPILEPSY

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ABSTRACT

There are many experimental methods of influencing the neuronal excitability and eliciting the convulsions in humans and animals. One of the major procedure or technique for inducing seizures in animals is kindling. In kindling the afterdischarges are elicited that later progress to generalized convulsions in an animal. There are a variety of reports documenting either low or high levels of blood sodium, potassium, calcium, glucose, cholesterol, total protein, blood hemoglobin etc. Blood calcium levels, however, are considered as important in the biochemical fluctuations occurring in epilepsy in humans and experimental animals. But no precise studies have yet been carried out to predict the pathophysiologic role of these factors in epilepsy. In the present work, changes in blood sodium, potassium, cholesterol, total protein, glucose, hemoglobin and other parameters were carried out in pentylenetetrazole (PTZ) induced kindled rabbit. The fluctuations are not significant when compared with the normal controls. But significant decrease in blood calcium levels in PTZ kindled male and female rabbit is evident in the present study. Hence, hypocalcemia might be considered as contributory factor for epilepsy. The results obtained in the present work provide a biochemical and hematological basis of control over the epileptogenic influences/ effects of the biomedical/ environmental agents. Conclusively, the present investigation in the hematobiochemical changes in a kindling model of epilepsy emphasizes to search through different angles the multifaceted role of calcium in epilepsy and other hyperexcitability conditions.

Key words: Epilepsy, kindling, hematobiochemical changes.

INTRODUCTION

It is known that many physiological, biochemical and pathological changes including changes in blood constituents, plasma glucose, electrolytes etc. can effect the occurrence of seizures and vice versa (Hussain *et al.*, 1991; Valencia *et al.,* 2003 ; Castilla-Guerra *et al*., 2006 ; Serrano-Castro *et al.*, 2006). A number of other factors such as fever, hypoxia, alkalosis, hypoglycemia, hypernatremia, hypocalcemia, and hematological changes etc. can influence the excitability of neurons (Futatsugi and Riviello, 1998). There are many experimental methods of influencing the neuronal excitability and eliciting the convulsions in humans and animals (Goddard, 1983). One of the major procedure or technique for inducing seizures in animals is kindling. Kindling is referred to an epilepsy model that has some unique features. The name kindling was proposed by Goddard *et al.* (1969) and much of the early work on kindling was done by Goddard and colleagues.

A large number of animal models are used for the study of epilepsy and epileptic seizures. However, a common feature in kindling is the after discharge that is elicited and later progress to generalized convulsion in an animal. (Hussain and Uddin, 2005). Kindling is the process by which epileptiform activity, perceived as an afterdischarge (AD), can be elicited by applying electrical or chemical stimulation to structures of the brain. Kindling experiments typically involve the surgical implantation of electrodes into the brain region of interest. Often the electrodes are placed within the left and right hemispheres of a structure (if it is bilateral) which allows the researcher to determine whether stimulation of one or the other hemisphere produces different effects. After recovery from surgery, the subject receives low levels of electrical stimulation at spaced intervals. Finally, depending on the nature of the experiment, the researchers observe the various types of behavioural and neurophysiological effects.

A number of factors including electrolytes, glucose, protein metabolites, lipid metabolites, carbohydrate metabolism, temperature etc and more specifically the changes in blood constituents, plasma glucose, calcium and hormonal changes can be studied in the seizure process. Biochemistry of the kindling has been studied in several perspectives. Relationship between electrolyte fluctuations and seizure disorder in human has been studied (Hussain *et al*., 1991). Pathophysiologic role of hormones in epilepsy is fascinating (Qureshi *et al.*, 1988). However, their precise role is still enigmatic.

The present investigation, hence, provides enlarged knowledge in understanding the role of biochemical and hematological changes in kindling/ epilepsy particularly the electrolytes known to be involved in hyperexcitability conditions which may help for future studies related to determination of the efficacy of anticonvulsant substances and drugs.

MATERIALS AND METHODS

Animals and general procedures

The male (N: 73) and female (N: 93) rabbit served for the present study. Age of young male and female animals was in the similar range. Animals were provided water, food and proper medication in separate cages. Two major groups of the animals were the normals (control group) and kindled. Convulsions were produced chemically in rabbits using pentylenetetrazol (PTZ) that induces sustained clonic-tonic convulsions, and the blood samples were collected and divided into aliquates for various determinations. Serum was easily obtained. However, for plasma to be separated, the clotting was prevented by placing blood into a tube containing anticoagulant. The biochemical and hematological parameters mentioned below were determined using standard chemical/ kit methods. The results obtained were statistically analyzed and discussed.

Biochemical and hematological methods

Some of the initial measurements were carried out using chemical methods mentioned below. The purpose was to compare chemical and kit methods and to check the accuracy of results obtained by kit methods. However, both methods did not give significant variations from each other. Hence, the data for total levels of protein, blood glucose and calcium were done also by respective kits. Blood cholesterol levels were measured using cholesterol kits. The data for these parameters included in the present report, however, was the one determined by kit methods. Blood hemoglobin, RBC count and WBC count were carried out using routine clinical/ physiological methods.

The o-toludine method was used for the determination of plasma glucose. This method is essentially free of interferences, simple and widely used. For standardization, stock standard (5g/dl) was prepared by dissolving 5 grams of glucose (dextrose) in 50 ml of benzoic acid solution, and diluted to 100 ml with the same solvent. Working standard (100 mg/dl) was made by diluting 1ml of stock standard to 50 ml with benzoic acid solution and stored in the refrigerator. The 100 mg/dl glucose standard was processed same as the plasma samples. Serum protein was estimated also by the biuret method which is a rapid, simple and accurate technique for protein determination. It depends upon the formation of a violet complex between cupric ions and protein. For the direct measurements for blood sodium and potassium, the sample was diluted quantitatively with water and mixed well. Sample in the form of droplets was then sprayed in to the flame where light emission occurred Flame photometer permitted calibration in concentration and therefore provided direct readout of the concentration of electrolytes in the sample.

In the measurement of blood calcium by chemical method, cresolphthalin complexone (CPC) forms a complex with calcium that is purple in an alkaline medium. Diethylamine buffer is used to produce such a medium of pH 12. Cyanide is included to help stabilize the diethylamine by complexing metal ions that catalyze the oxidation of diethylamine. Dimethyl sulfoxide in CPC reagent increases the solubility of the reagents and stabilizes the system while 8-hydroxyquinoline complexes magnesium and prevents it from reacting with the CPC. All initial pilot experiments were done using chemical methods. However, on the basis of the results obtained in these experiments, the hypotheses were checked by repeating those experiments using kit methods. Once it was sure that the results by two methods did not vary significantly, further evaluations were then carried out. This last part of the data was included in the present manuscript of the investigation.

RESULTS AND DISCUSSION

The mean ± SEM values for blood calcium (mg/100ml) in normal control animals were 6.11 ± 0.62 and 5.92 ± 0.93 respectively for males and females. The levels during various stages in PTZ kindling were measured. The fully kindled male and female rabbit, however, gave the values of 5.08 ± 0.60 and 5.01 ± 0.92 respectively. This showed non-significant variations for control male and female animals. However, significant change ($p < 0.05$) was obtained for both male and female rabbit compared to respective controls. The comparisons are shown in Fig.1.

Blood sodium concentration (mEq/L) was found to be 140.53 ± 2.22 and 142.32 ± 2.83 respectively for male and female controls, whereas kindled male and female rabbit showed values (140.50 ± 2.25 and 141.32 ± 2.79 respectively) in the same range (Fig.2) without any significant variations ($p < 0.05$). Blood potassium (5.72 ± 0.78 and 5.70 ± 0.92 mEq/L respectively for male and female controls; 5.59 ± 0.89 and 5.69 ± 0.88 mEq/L, respectively, for kindled males and females) showed non-significant change ($p < 0.05$; Fig.3).

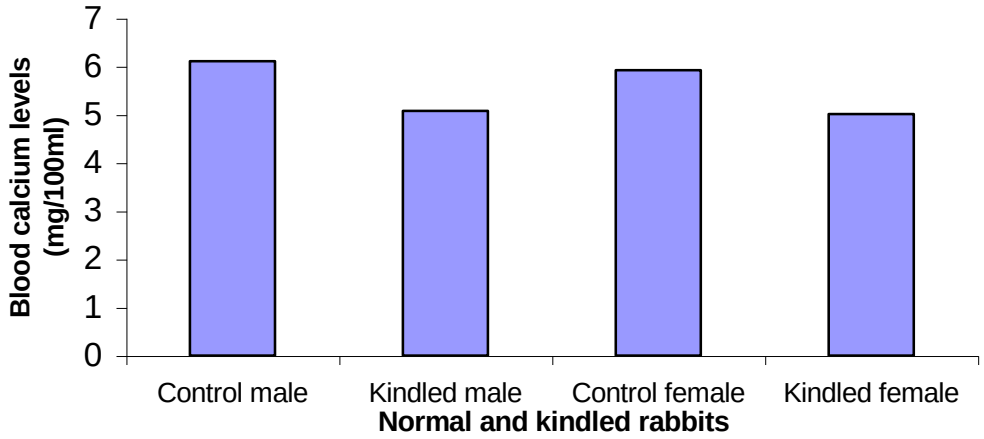

Fig.1. Blood calcium concentration in PTZ kindled rabbit.

Serum protein levels (gm/100ml) (6.57 ± 0.73 and 6.66 ± 0.89, respectively for male and female controls; 6.62 ± 0.84 and 6.69 ± 0.81 respectively for kindled males and females) showed non-significant change ($p > 0.05$; Fig.4). Cholesterol levels (mg/100 ml) in kindled animals (60.54 ± 1.09 and 59.33 ± 1.34 respectively for males and females) were found non-significant ($p > 0.05$; Fig. 5) compared to those respectively in control males and females (63.22 ± 1.56 and 56.18 ± 1.03).

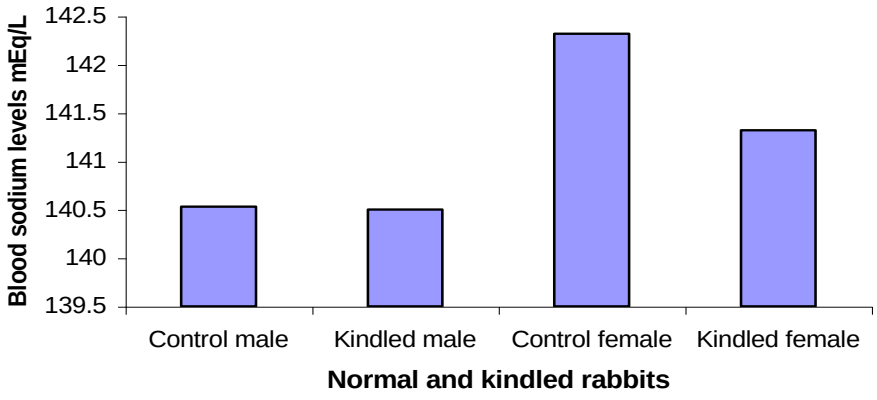

Fig. 2. Blood sodium levels in PTZ kindled rabbit.

Fig.3. Blood potassium in PTZ kindled rabbit.

Fig.4. Serum protein in PTZ kindled rabbit.

Blood glucose concentration (mg/100ml) in kindled animals (110.65 ± 0.68 and 99.44 ± 1.64 respectively for males and females)were also found non-significantly different ($p>0.05$; Fig. 6) compared to those respectively in control males and females (120.13 ± 1.69 and 107.75 ± 2.67). Hemoglobin (g/100ml) in kindled animals (12.42 ± 1.76 and 11.23 ± 1.06 respectively for males and females) were also found non-significantly different ($p>0.05$; Fig. 7) compared to those respectively in control males and females (13.67 ± 2.17 and 12.55 ± 2.00). Various other estimations including blood phospholipids, RBC count, WBC count etc were carried out. However, the initial data did not present significant variations for kindled compared to control rabbits and hence, more comprehensive study was not required for further evaluations.

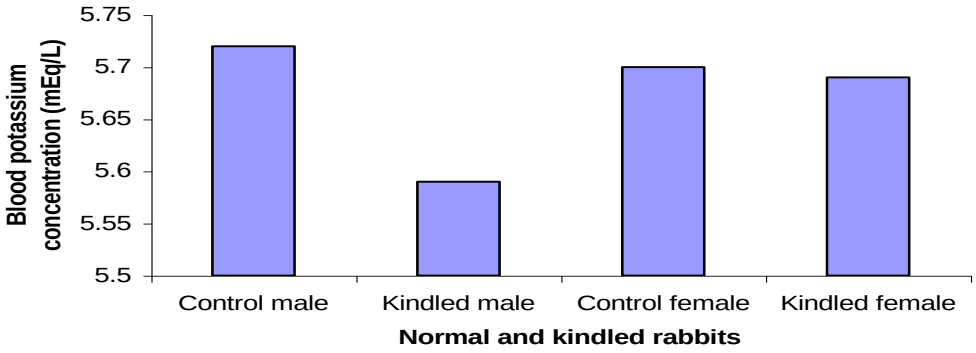

Fig.5. Blood cholesterol in PTZ kindled rabbit.

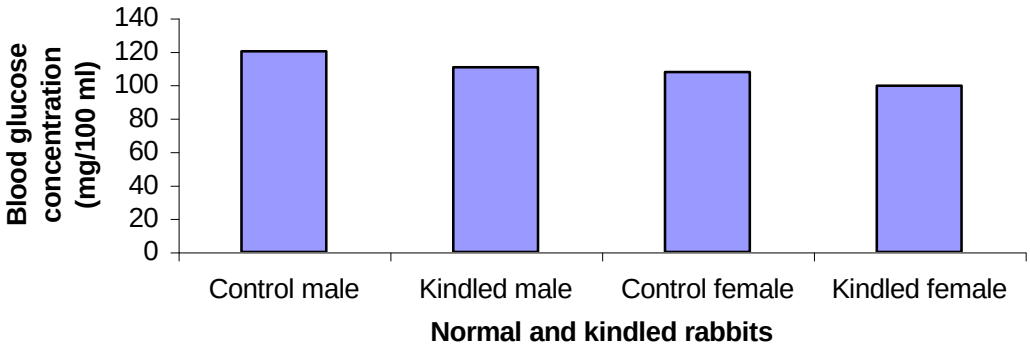

Fig.6. Blood glucose concentration in PTZ kindled rabbit.

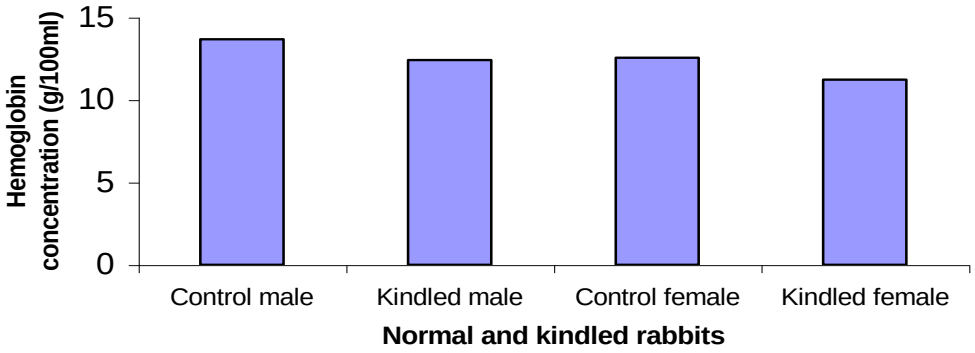

Fig.7. Hemoglobin levels in PTZ kindled rabbit**.**

The present report describes the biochemical and hematological changes occurring in PTZ kindling in male and female rabbits. No significant change in blood sodium in both male and female rabbits found in the present study resemble with some of the previous work where little change or no change and particularly no hypernatremia obtained in seizure disorder. This might have been because of severe hypotonic hyponatremic conditions produced in other studies. The presently obtained results for potassium levels resemble to a report (Borok *et al.*, 1988) , though seizures and hypokalemia are also associated with each other (Vanpee *et al.*, 2001).

The increase in serum cholesterol and high-density lipoprotein cholesterol levels may have clinical relevance with regard to the incidence of atherosclerosis and coronary heart disease in patients with epilepsy receiving carbamazepine medication (Isojärvi *et al.*, 1993). This differs from our results as our study comprises animal model instead of clinical management. However, it has been suggested that hypercholesterolemia may protect against seizures and cerebral ischemia (Devuyst *et al.*, 2003). In our animal experiments, we neither got hypercholesteremia, nor hypocholesterolemia. Our results for kindled animals were in the normal range that predicts the ineffectiveness of presently employed chemical kindling procedure.

The total protein level was only slightly reduced as compared to that of controls which is exactly similar to a report showing no change in protein levels (Dastur and Dave, 1987). A significant correlation has been found for both male and female patients between ChE and concentrations of triglycerides, phospholipids, cholesterol, low-density lipoprotein (LDL) phospholipids, LDL-cholesterol, and apolipoprotein B in epilepsy (Tutor-Crespo et al., 2004). Our results for cholesterol and phospholipids are in almost same range for controls and kindled animals. The effect of enzyme-inducing anticonvulsant drugs on the serum concentrations of lipoproteins in patients might had been a reason as it is confirmed in another study showing no change in subjects treated with sodium valproate (Pavone *et al.*, 1982). This shows the involvement of anticonvulsants in the mentioned reports whereas the animals in our experiments did not receive any antiepileptic/ anticonvulsant drugs.

Hypoglycemia is considered as related to seizures (Hennis *et al*., 1992; Groenendaal and Elferink-Stinkens, 2006) in clinical setting, but reports in animal studies are very specific. Convulsions usually stop in human patients once hypoglycemia is under control, but we could not get such results in current experiments. The major reason again might be the experimental and very specific studies instead of patient studies with multitude of conditions.

Anemia is sometimes considered as involved in altering blood hemoglobin (Naveed-ur-Rehman and Billoo, 2005). RBC count (Adigüzel *et al.*, 2006) and other hematological parameters. However, iron deficiency anemia may protect against the development of febrile seizures (Kobrinsky *et al.*, 1995). Presently conducted study found neither causal nor therapeutic effects of hematological parameters. These controversial results require carrying out further controlled studies. The extent and complexity of the epileptic disorder in humans may predict change in peripheral WBCs in seizure disorder (Shah *et al.*, 2001) but the experimental and very specific study like the present one might not obtain any clear evidence of the involvement of leukocytes.

In the present experiments, significant decrease in blood calcium levels has been obtained in PTZ kindled male and female rabbit that is evident in several investigations. Hypocalcemia is important as a precipitant factor in causing epileptic seizures. The experimental studies (Bowdler *et al.*, 1980; Tjellesen and Christiansen, 1982; Fujii *et al.*, 1984; Fagan and Phelan, 2001) show controvercial opinions about the role of calcium (either increase, decrease or no change in serum calcium) in kindling and epilepsy, though our results reside in one particular opinion i.e. a significant decrease in serum calcium in experimentally induced seizures might be considered involved in the process of epileptogenesis.

The cause of hypocalcemia may be a redistribution of calcium into the cells, parallelled by a redistribution of phosphorus from the intra- to the extracellular space. Epilepsy is not rare, yet since many epileptic conditions are considered to be idiopathic, the related seizures are usually considered to be of unknown origin. It does appear that different types of seizures are caused by differing mechanisms. It is hypothesized that calcium levels, which are controlled by various mechanisms in the body, can cause, or at least contribute to, myoclonic (jerk) seizures, as well as to possibly infantile spasms. As these conditions are difficult to treat medically, it has been suggested that nutritional interventions, such as supplemental calcium and vitamin D, might well be considered as an option as a first-line treatment in those with these types of epileptic disorders.

Hypocalcemia can be a contributory factor for epilepsy. From a physiological perspective, it is logical that calcium supplementation may be indicated when myoclonic seizures are encountered. When the calcium ion concentration falls below about one half of normal, a person is likely to experience tetantic contraction of muscles throughout the body because of spontaneous nerve impulses in the peripheral nerves. Since calcitonin and the parathyroid hormone affect serum calcium concentrations, it is possible that problems in the production of either can lead to limited tetantic contractions. Significant changes in important body chemicals such as calcium and magnesium can cause seizures; so can a lack of certain vitamins. These chemical changes may provoke a disturbance in the brain, or a single seizure, by influencing the thresholds for firing. Calcium is a very important mineral for the normal functioning of brain cells, and low levels of calcium can cause seizures. Hypocalcemia can be a consequence of severe kidney disease when too much calcium escapes from the kidney into the urine. It may also, but rarely, be caused by a hormonal problem that has the same effects. A deficiency of magnesium, a mineral that interacts with calcium, may cause low blood calcium and, thus, seizures.

The present report provides us information about the role of major hematological changes, electrolytes and other biochemicals, which in future studies should be helpful in determining the efficacy of certain anticonvulsant substances and drugs.

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