

Effect of p-CPA Pretreatment on EEG Power Spectra in Experimental Open Brain Injury in Rats

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ABSTRACT

Continuous four hours EEG (electroencephalogram) recordings and its power spectrum analysis using fast fourier transform (FFT) in urethane anesthetized male Charles Foster rats were performed in two groups: open brain injury and p-CPA (para-Chlorophenylalanine) pretreated before brain injury, respectively, and compared with the EEG power spectrum of control rats. The EEG power spectrum analysis showed that there was a faster recovery in p-CPA pretreated group than the injury group of rats. The results showed that the p-CPA (a 5-HT inhibitor) prevents pathological changes following brain injury. Simultaneously, the inference can also be drawn that EEG power spectrum analysis is a useful technique for monitoring the brain injury and its recovery following pharmacological treatments. *Iran. Biomed. J. 7 (3): 119-126, 2003*

Keywords: Brain injury, p-CPA, EEG power spectrum

INTRODUCTION

Brain edema is considered as a untoward sequelae following traumatic, infective or metabolic insult to the central nervous system (CNS) [1] and if untreated, a progressive brain edema will lead to the compression of cerebral compartment resulting in an increased intracranial pressure and brain tissue softening. Excessive swelling of the brain in a closed cranial compartment will result in the depression of vital centers leading to death [2]. Therefore, in order to develop suitable therapeutic measures for such a common serious complication of brain disease occurring under a wide variety of noxious insults to the CNS, detailed study of progression, persistence and resolution of brain edema is very important.

Brain injury results in vasogenic edema due to the injury in the vascular elements and characterized by breakdown of blood-brain barrier (BBB) [3] that can be considered as one of the main factor contributing to morbidity and mortality of patients following brain trauma [4]. Various investigations have tried to explain the edema development following trauma in terms of specific alteration in the metabolism of particular neurotransmitters. It is suggested that 5-HT (5-Hydroxytryptamine), noradrenaline, adrenaline, dopamine, prostaglandins, histamine and glycine all play major role in the edema development following brain trauma [1]. Based on data obtained from different laboratories, a working hypothesis proposed according to which the widespread changes observed in focally traumatized brain has been mediated through a neurotransmitter system(s). According to the scheme of postulated inter-relationships between the release of substances by the lesion and alteration of synaptic function, it has been envisaged that prostaglandins and/or serotonin and/or catechol-amines derived from injured brain tissue, induce changes in ionic channels in membranes causing functional depression [3].

Hypothalamic 5-HT is believed to be an important factor in regulation of ion distribution. The dorsal raphe nucleus contains the highest density of the serotonergic neurons and extends 5-HT fibers to the LHA (lateral hypothalamic area) [5]. High concentrations of immunoreactivity of serotonin positive fibers and varicosities

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from the dorsal raphe nucleus were found in some areas of the hypothalamus including LHA axons ascending through the medial forebrain bundle [6]. Further, biochemical studies have shown that traumatic conditions cause a significant increase in the serotonergic activity in the brain and the destruction of the serotonin fiber system led to extravasations of fluid in the brain [2, 7-10]. Also, it has been suggested that in general, the drugs that are able to diminish 5-HT level in brain, thwart the edema development. In some series of experiment, it has been observed that edema development following stab injury have been remarkably reduced by prior administration of para-chlorophenylalanine (p-CPA), a 5-HT synthesis inhibitor that prevents edema development in experimental brain injury in rats [1, 10].

Monitoring and analyzing the electro-encephalograms (EEG) has been used in a variety of clinical situations including minor head injury [11, 12], acute hyperventilation [13], cerebrovascular surgery [14] and as a measure of anesthetic depth [15]. Among several signal-processing techniques applied for the EEG analysis, power spectrum using fast fourier transform (FFT) is the most popular approach to estimate frequency and amplitude changes [16] in different pathological and psychological states. The power spectrum analysis of EEG signals has been shown to be adequate in most quantitative procedures [17] as it conveys more information that usually not present in conventional analog EEG records [12, 18]. Previous laboratory experiments show increased amount of higher frequency immediately after head injury [19] and its persistence for nearly two hours with significant reduction in power. In another experiment, p-CPA pretreatment was found prevented the flattening of EEG activity induced by acute immobilization stress [20]. Studies show that 5-HT plays a critical role in producing brain edema following brain injury that changes the EEG power spectrum. However, in p-CPA pretreated subjects followed by experimental brain injury, a long-term EEG power spectrum analysis has not been studied in a systematic manner so far.

In the present study, by considering EEG as a diagnostic tool, it has been tried to monitor that whether p-CPA pretreatment helps in stabilizing the EEG following brain injury or not.

MATERIALS AND METHODS

Subjects. All experiments were carried out on male Charles Foster rats (above six months old, weighing 200-250 grams) obtained from Central Animal House, Institute of Medical Science, Banaras Hindu University, Varanasi (India). The rats were individually housed in polypropylene cages (30 cm × 20 cm × 15 cm) with drinking water and commercial laboratory food pellets (Hindustan Liver Limited, India) ad lib. Ambient room temperature was maintained at $24 \pm 1^\circ\text{C}$. The experiments were performed under urethane (Sigma Chemical Co., USA) anesthesia (1.8 gm/kg i.p.). The rats were divided into three groups: Control group (n = 10): Anesthetized rats with about 12mm^2 of parietal cortex of right hemisphere exposed without trauma. Injured group (n = 10): Anesthetized rats in which about 12mm^2 of parietal cortex of right hemisphere was exposed and injured (3 mm × 3 mm) using a sharp scalpel.

p-CPA pretreated group (n = 10). p-CPA (Sigma Chemical Co., USA) was injected i.p. (100 mg/kg) daily into the rats for three consecutive days [10]. Twenty four hours after the last injection of p-CPA, the animals were anesthetized. About 12mm^2 of parietal cortex of the right hemisphere was carefully exposed and a stab injury like in injury group was made. The dose schedule inhibits the 5-HT synthesis in brain and induces a long lasting depletion in CNS [10].

Surgery and electrode implantation. Detailed method of electrode implantation was described by Sarbadhikari *et al.* [18] in brief, three stainless steel screw electrodes were aseptically implanted on the skull of the anesthetized rats. The coordinates for the parietal cortex were 2.0 mm lateral to the mid line and 1.0 mm anterior to lambda for one electrode and 1.0 mm posterior to bregma in right parietal region for another electrode. The reference electrode was placed at anterior most region of the skull. All animals were allowed a post surgery stabilization period of one hour before the onset of first recording. Injury was done between the recording electrodes after the stabilization.

Equipment. Cortical EEG had been recorded through a 8-channel electroencephalograph (Medicare, India). The electroencephalograph was also connected through a 12-bit ADC (Analog to Digital Converter) (ADLiNK, 8112HG, NuDAQ, Taiwan) with its supporting software (VISUAL LAB-M, Version 2.0c, Blue Pearl laboratory, USA) to record digitized EEG signals. For all recordings, processing and analysis purpose, IBM-PC (Pentium-III, 700 MHz) under WINDOWS-95 environment was used.

Data collection and preprocessing. A single-channel bipolar EEG was recorded continuously from injury period to the first 4 hour of recovery. Recording was done with the sensitivity of $10\ \mu\text{V/mm}$. The analog

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cutoff frequencies were set at 0.3 and 70 Hz for high and low pass filters, respectively. Power line (50 Hz.) notch filter was used for entire recording for the rejection of AC line frequency. A sampling frequency of 256 Hz was used to acquire all components of EEG signals in digital form and to avoid aliasing. The digital data were stored on the computer hard disk in 2-minute data files at regular intervals. The time points have been used as discussed by Goel *et al.* [12] with slight modification of base line (the control period before subjecting the rats to any injury), just after the injury, 5, 10, 15, 30, 60, 120, 180 and 240 minutes after injury.

Data from each episode were fragmented in two-second epochs. Ten epochs from each episode were considered for spectral analysis. Each epoch was preprocessed for noise reduction before final power spectrum analysis. At first, the DC value was subtracted from the data and then the base line movement was reduced. In the final step of preprocessing, the data were band pass filtered with cutoff frequencies of 0.25 and 30 Hz, as the maximum frequency component of interest in anesthetized animal is less than 25 Hz [12]. The entire frequency spectrum of the filtered and processed data were subdivided in three bands to analyze three dominant frequencies (Fig. 1) that fall in the range of 1.0-5.5 Hz for low frequency, 9.0-14.0 Hz for medium frequency and 18-21 Hz for high frequency [12].

Spectral Analysis. The final processing of the EEG signals for the analysis of dominant frequency was performed by using power spectrum, which was developed to calculate discrete fourier transform (DFT) efficiently and rapidly. Recorded EEG responses consisting of short realizations of voltage against time were converted to the frequency domain by determining the discrete values of voltage at regular sampling intervals [21]. Using the DFT that transforms N discrete values to N discrete complex DFT amplitude and phase values carried out the conversion. Multiplication of the DFT components by T_s/N (where T_s is sampling interval) converts them to fourier transform components, the amplitude of which has dimensions of volts per hertz. A plot of the square of these amplitudes against frequency is referred as 'power spectrum'. For the present work, the power spectrum analysis was performed using 'Rectangular Window'.

Changes in BBB permeability. Evans blue dye (2% in 0.9% saline, 0.3 ml/100 g body weight) was injected through femoral vein before rats were sacrificed and dye extravasations on the brain parenchyma was noted through naked eye [2, 9].

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Fig. 1. A typical power spectrum of a two-seconds epoch of EEG signal and classification of EEG frequencies in three bands. EEG frequencies were classified as low frequency (-1.0 to 5.5 Hz), medium frequency (-9.0 to 14.0 Hz) and high frequency (-18.0 to 21 Hz).