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Depletion of Serotonin Synthesis with *p*-CPA Pretreatment Alters EEG in Urethane Anesthetized Rats under Whole Body Hyperthermia

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ABSTRACT

Background: Serotonin is believed as an important factor in brain function. The role of serotonin in cerebral psycho-patho-physiology has already been well established. However, the function of serotonin antagonist in anesthetized subjects under hyperthermia has not been studied properly. **Methods:** Experiments were performed in three groups of urethane-anesthetized rats, such as: (i) control group, (ii) whole body hyperthermia group and (iii) *p*-CPA (para-Chlorophenylalanine) pretreated hyperthermia group. Hyperthermia was produced by subjecting the rats to high ambient temperature of 38 ± 1ºC (relative humidity 45-50%). Each group was divided for EEG (electroencephalogram) study and for determination of edematous swelling in the brain. **Results:** Urethane anesthetized rats under hyperthermia show highly significant reduction in their survival time. The body temperature recorded during the hyperthermia was observed with significant and linear rise with marked increase in brain water content, which was analyzed just after the death of the subjects. The results of the electroencephalographic study in urethane-anesthetized rats recorded before death indicate that brain function varies in systematic manner during hyperthermia as sequential changes in EEG patterns were observed. However, a serotonin antagonist, *p*-CPA pretreatment increases the survival time with significant reduction in edematous swelling in brain but it does not affect the relationship between the core body temperature and the brain cortical potentials as observed in urethane anesthetized subjects exposed to whole body hyperthermia. The core body temperature in *p*-CPA pretreated rats show non-linear relationship with respect to the exposure time as it was observed in drug untreated subjects. **Conclusion:** The findings of the present study indicate that although pretreatment of *p*-CPA in rats has a marked correlation between the extravasations of the blood-brain barrier under hyperthermia but shows minimum effect on the EEG in a model of hyperthermia under irreversible anesthesia. *Iran. Biomed. J. 11 (1): 33-39, 2007*

Keywords: Hyperthermia, Urethane anesthesia, Para-Chlorophenylalanine (*p*-CPA), Electroencephalogram (EEG), Brain edema

INTRODUCTION

Most of the studies on brain electrical activities or EEG (electroencephalogram) under exposure to high ambient temperature are concerned with the conscious subjects [1, 2] in which hypothalamus actively participates in the maintenance of complex homeostatic system of the animal organization. However, it is quite conceivable that a stereotype stress reaction under high environmental heat can be produced through the more diverse means and not only by deranging the homeostasis of the biological systems. Literature suggests that the effect of stress stimuli is not only observed in the conscious moving subjects but also been observed in the anesthetized condition [3]. However, the physiology of the rats under anesthesia is reported to mimic the similar patterns as in unanesthetized rats under light urethane anesthesia, animals were reported to exhibit patterns of cortical activities similar to those seen in wake, drowsiness and slow wave sleep as in unanesthetized animals [4].

Brain serotonin is believed as an important factor for the distribution and regulation of ions in hypothalamus. The functional effects of perfusion of ions in hypothalamus are well known to produce the new set point temperature in response to thermal

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stress [5]. It has been established that dorsal raphe nucleus contains the highest density of the serotonergic neurons and extends serotonin fibers to the lateral hypothalamic area, which suggests that the serotonin has a significant correlation with hypothalamic activities [6, 7]. Aside that, laboratory experiments also demonstrate that dissimilar psychophysiological stress causes a significant increase in the serotonin activity in brain, which is finally responsible for the changes in brain physiology of the subjects [7, 8]. Previous studies established that extravasations in blood-brain barrier are mediated by stimulation of serotonergic systems, followed by secretion of serotonin. Simultaneously, different series of experiments under stressful conditions suggest that the prior administration of para-Chlorophenylalanine (*p*-CPA), a serotonin inhibitor, was found to prevent edema development [9, 10] and changes in brain cortical signals [11, 12].

It is believed that an understanding of physiological system and its association with serotonin controlling centers can provide in-depth knowledge to understand the effects of high environmental heat on the brain function with more physical reality [13]. Although, many neurotransmitters are linked to the brain activity, research studies have widely implicated disturbances in serotonin system and the hypothalamo-pituitary-adrenal axis as neurobiological alterations in these systems are most consistently associated with stress induced pathophysiological changes [14, 15]. But, instead of the fact that changes in brain serotonin level is responsible for the alterations of different physiological functions in brain, no report is available that analyzes the effect of prior administration of *p*-CPA on these parameters in a model of hyperthermia under irreversible anesthesia. Concurrently, the review of literature also reveals that no work has been reported on stepwise EEG changes in urethane anaesthetized and *p*-CPA pretreated subjects under whole body hyperthermia.

Therefore, in the present study, an effort has been made to monitor the effect of pretreatment of *p*-CPA on brain cortical potential in anesthetized subjects exposed to hyperthermia. An attempt has also been made to analyze the changes in EEG signals under continuous exposure of hot environment, throughout the survival of the urethane-anesthetized subjects at a fixed high ambient temperature. Body temperature of the rats was simultaneously recorded to assess the relationship between the EEG signals and the core body temperature.

MATERIALS AND METHODS

All procedures in this study have been conducted in compliance with 'Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA)' (India), as well as with internal institutional policies and guidelines.

Subjects and surgery. Thirty male Charles Foster Albino rats of 90-110 g, age 8-9 weeks, were housed in polypropylene cages (30 cm × 20 cm × 15 cm) on 12L:12D (light during 7.00 A.M. to 19.00 P.M.) cycle at 23 ± 1°C with food and water *ad libitum*. The rats were divided into three groups: (i) control group (n = 10); (ii) whole body hyperthermia group (n = 10) and (iii) *p*-CPA pretreated hyperthermia group (n = 10). Each of three groups was further divided into two equal groups for subsequent electrophysiological study (with electrode implantation) and for determination of edematous swelling in the brain (without electrode implantation). All experiments were performed under Urethane (Sigma Chemicals Co., USA) anesthesia (1.6 g/kg body weight, i.p.). Method of EEG electrode implantation has been used as described by Sinha [16]. In brief, three stainless steel screw electrodes were aseptically implanted on the skull of anesthetized rats. Two bipolar, bilateral screw electrodes were connected 2 mm posterior and 4 mm lateral to bregma under stereotaxic guidance. One grounding electrode was fixed on the anterior most region of the skull. Animals were allowed a post surgery stabilization period of one hour before the onset of first EEG recording.

Hyperthermia model. Four groups of five rats were subjected to the Biological Oxygen Demand incubator at preset constant temperature of 38 ± 1ºC and relative humidity of 45-50% [10]. The base temperature of rats was considered 37ºC. Two groups of rats were used for electrophysiological study and the other two groups were used for determination of edematous swelling of the brain. Two groups of rats (with and without electrodes) were processed and handled similar to the hyperthermia group but at room temperature and then were treated as control rats. The rats subjected to hyperthermia were incubated till death. However, the control rats were subjected in the incubator at room temperature for three hours.

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p-CPA pretreatment. The schedule of *p*-CPA pretreatment and the dose have been used according to the methods described earlier [12, 17]. The *p*-CPA (Sigma Chemical Co., USA) was injected daily 100 mg/kg i.p., for three consecutive days in two groups of ten rats (with and without electrodes). Twenty Four hours after the last injection of *p*-CPA, animals were subjected to the incubator for whole body hyperthermia. This dose schedule inhibits the serotonin synthesis in the brain and induces a long lasting depletion in central nervous system.

EEG recording. The leads from the skull electrodes were connected to the EEG machine (Medicare, India) and to a computer to record single channel EEG data both in analog paper as well as in digital form. Digitized EEG signals were recorded with the help of analog to digital converter (ADLiNK, 8112HG, NuDAQ, Taiwan) and its supporting data acquisition and processing software (VISUAL LAB-M., Version 2.0c, Blue Pearl Laboratory, USA). Digital recording was performed with a sampling frequency of 256 Hz. Selected EEG data at regular interval were stored in computer in separate data files in two-minute segments.

EEG signal analysis. EEG data were fragmented in two-second small epochs. 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 Hz and 30 Hz, as the maximum frequency component of interest in anesthetized animal is less than 25 Hz [18]. Using Power Spectrum under 'Rectangular Window' performed the final processing of the EEG signals for the analysis of dominant frequency. The linear power spectrum was converted into dB scale as it gives better energy distribution pattern. Further, the power spectrum was smoothed (Simple moving average method) to analyze dominant frequency components in the EEG spectra. The processing of EEG signal and power spectrum calculation is presented in Figure 1.

Body temperature. The core body temperature from all rats was recorded continuously through anal route during the full time of incubation using a thermistor probe (Yellow Spring Co., U.S.A.) connected to a telethermometer (Aplab, India).

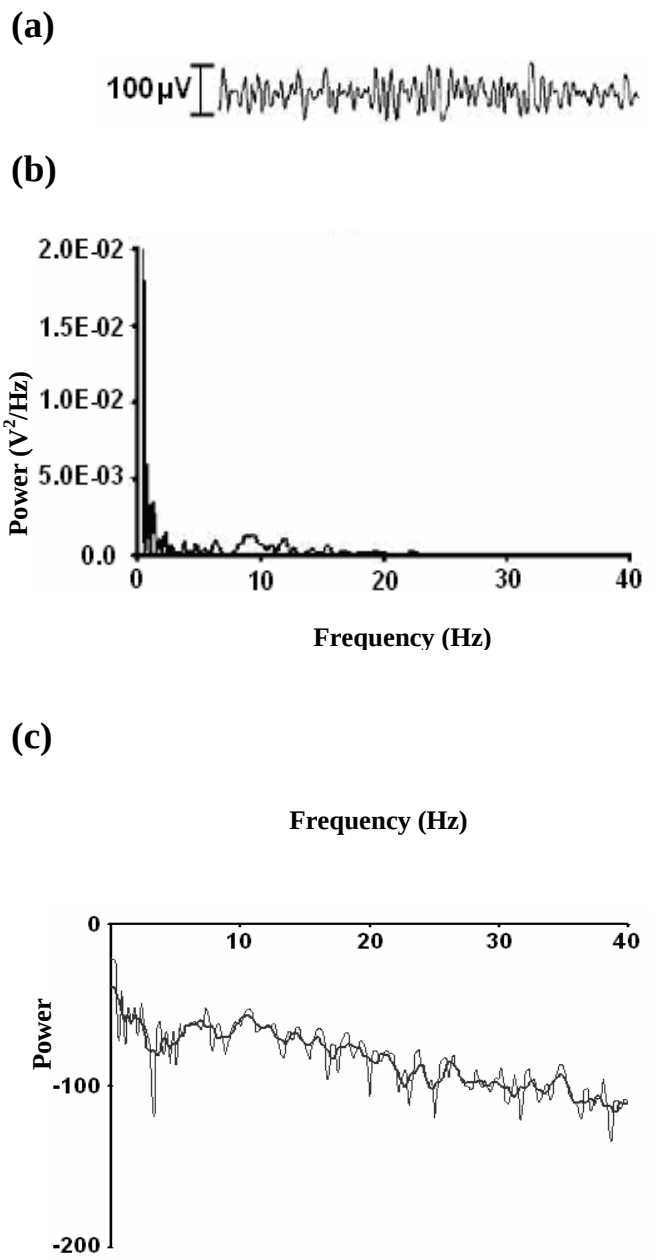

Fig. 1. Steps of EEG signal processing. **(a)** An epoch of EEG signal **(b)** EEG power spectrum in linear scale **(c)** EEG power spectrum in dB scale. Light line shows the power spectrum before filtering and dark line represents after filtering of power spectrum (it was used for final EEG frequency analyses).

Determination of percent water content (edema and edematous swelling). Just after the death, brains of the rats were dissected out. The wet and dry weights of the brains were noted and the percentage of water for each brain was calculated. Dry weights of each brain were determined after repeatedly drying the sample in oven at 80ºC until the weight remains constant.

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The percentage of edematous swelling was calculated by using following formula [19]:

$$\frac{\%\ \text{Water content in control} + f}{100 + f} = \frac{\%\ \text{Water content in experimental animal}}{100}$$

Where f = % of swelling caused by edema.

Statistical analysis. All the statistical analyses were performed in the laboratory with the help of software package (MS EXCEL-98). Student's *t*-test followed by one-way Analysis of Variance (ANOVA-1) was used to compare all the values of percentage water accumulation. For the comparison of EEG changes, the mean values of the EEG power spectra at different intervals were compared with their respective group.

RESULTS

Assessment of edematous swelling in the brain. Due to whole body hyperthermia, a highly significant increase ($P<0.01$) in water content in drug-untreated group was observed (72.808 ± 0.329) in the brain after the death of rats as compared to brain of controlled rats (68.622 ± 0.317). Results show a significant rise in the percentage of brain water content, which reflects severe edematous swelling in the brain of this group of rats. The percentage of water accumulation in the brain of this group of rats shows a significantly high rise (4.186) with respect to control rats, which represents 15.394 % of edematous swelling. However, following *p*-CPA pretreatment followed by whole body hyperthermia did not show much difference (69.806 ± 0.270) in brain water content with respect to the control rats (68.622 ± 0.317). The percentage difference in brain water content in *p*-CPA pretreated rats with respect to control rats was found very low as 1.184, which represents merely 3.92% of edematous swelling.

EEG changes under hyperthermia in p-CPA untreated anesthetized rats. The EEG recordings in urethane anesthetized rats following whole body hyperthermia demonstrated characteristic changes in both rhythmicity and amplitude indicating a significant and diffuse alteration in the function of electrical activity of cerebral cortex during the exposure period. The temperature elevation rate was recorded as 0.045 ± 0.003ºC/min. The rectal temperature before warming was observed as 36.5 ± 0.24ºC and the 7-8 Hz activities were found dominant in EEG power spectra. Nearly 1ºC increase in the body temperature induced high amplitude theta waves. As the body temperature increased (38ºC), there was usually an increase in fast wave (18-25 Hz) EEG activity, which was observed irregular and disrhythmic with low amplitude. Further increase in body temperature by whole body hyperthermia sometimes induces either regular or intermittent small spikes in EEG recording. When body temperature approached 40ºC, the frequency response of EEG recording was slowed (8-12 Hz). The slowed EEG signals (theta waves) with lower amplitude were recorded with increase in body temperature up to 42ºC. At body temperature above 43ºC, intermittent high voltage irregular activities were observed followed by flattening of EEG waves up to the time of death, where the mean rectal temperature at the time of death was recorded as 43.4 ± 0.43ºC. The relationship between the rectal temperature and the duration of incubation was found linear (Fig. 2a). The mean duration of heating until death was observed as 152 ± 10.7 minutes.

EEG changes under hyperthermia in p-CPA treated anesthetized rats. In *p*-CPA pretreated rats, the temperature elevation rate was found less than *p*-CPA untreated rats (with average of 0.036 ± 0.011°C/min). The relationship between the rectal temperature and the duration of incubation was found non-linear but significant ($P<0.05$) (Fig. 2b). The mean duration of heating until death or the survival time of the *p*-CPA pretreated subjects was found significantly increased with respect to *p*-CPA untreated rats ($P<0.05$) (188 ± 6.8 min). However, the changes in EEG frequencies with respect to changes in body temperature in this group of rats were found comparatively similar to the *p*-CPA untreated group of rats. The core body temperature before incubation in this group was found as 37.1 ± 0.13°C and the mean rectal temperature at the time of death was observed as 43.9 ± 0.26°C.

DISCUSSION

Similar to the reported finding [10], the results of this study on urethane anesthetized rats also

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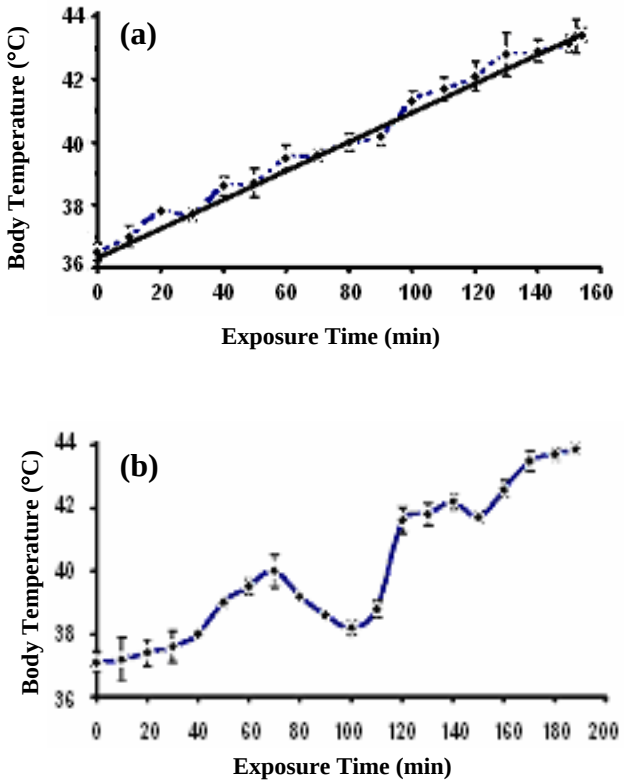

Fig. 2. (a) Body temperature response with respect to exposure time for hyperthermia without *p*-CPA pretreatment' group of rats; **(b)** Body temperature response with respect to exposure time for hyperthermia with *p*-CPA pretreatment' group of rats.

illustrate the hyperthermia induces edematous swelling in brain. The survival time of these rats was significantly reduced as compared to those reported by Menon and Dandiya [20] in unanesthetized rats. Panjwani *et al.* [21] have also reported reduced survival time in a hyperthermia model of anesthetized (Ketamine Hydrochloride) rats, which supports the findings of the present study. Pharmacological studies clearly indicated that an increased level of serotonin under hot environment is a major contributing factor for increased blood-brain barrier permeability either directly or through some indirect mechanism [10] and if the synthesis of the serotonin is blocked with the help of a serotonin synthesis inhibitor such as *p*-CPA, it prevents the edema formation in the brain [12].

In the present study, experimental rats have shown sequential EEG changes during whole body hyperthermia, which suggested that the brain function varies in stepwise manner during the exposure time. Reports published earlier on the effects of artificially induced heat on brain electrical activity have already demonstrated that the overall pattern changes in EEG activity was a function of ambient temperature [22] and if the elevation of temperature maintained for long duration at higher ambient temperature, a major transient reduction in EEG activity was observed [23]. The sequential EEG changes in conscious rats have also been reported earlier. In their report, Morimoto *et al.* [24] showed that hyperthermia initially induces theta burst, and after some it is time accompanied by small spikes and wave burst in freely moving rats.

Although in *p*-CPA pretreated rats, the rate of body temperature changed was significantly low in comparison to the *p*-CPA untreated group of rats, the changes in brain cortical potential follows the same pattern as it was in drug untreated rats with respect to change in body temperature. The study of dose dependency of the *p*-CPA was not the area of interest in this particular work. However, there is a dose dependent relationship between the *p*-CPA pretreatment and the serotonin action. It has been shown that *p*-CPA selectively decreases the concentration of serotonin without altering any other neurotransmitter and the drug by itself has no pharmacological action [7]. Similar to the present report, the evidences put forward that administration of serotonin antagonist blocked the temperature response, which ultimately results in non-linear increase in body temperature with respect to the exposure time [5]. The reduction in survival time of rats and sequential changes in EEG activities may have occurred due to disruption of thermoregulatory action that is primarily controlled by the hypothalamus, which is also responsible for heat exchange with the environment by changing different physiological activities [25]. The results of the present study suggest that although pretreatment of *p*-CPA in rats has a marked correlation among the extravasations of the blood-brain barrier under hyperthermia but shows minimum effect on the EEG.

The exact cause of the sequential changes in EEG signal as observed in our experiments is not clear. However, there are several studies indicating that the changes in EEG frequency components due to hyperthermia may have occurred due to changes in CNS functions, which can attributed to anorexia, dehydration, metabolic imbalance, energy failure, changes in brain neurotransmitter level, abnormalities in enzymatic process, neuronal and non-neuronal changes [1, 10, 26]. Reports from

various laboratories also indicate that the neuroglial cells are highly activated by hot environment [26], which controls the ion environment and neurotransmitter metabolism in the brain and helps in the genesis of cortical EEG waves. Thus, the contribution of glial cells in altering the EEG frequency in hot environment cannot be also ruled out. Therefore, there may be several factors contributing the changes in cortical electrical activity as observed in our studies.

In conclusion, the hyperthermia produces stepwise sequential changes in EEG activity. These changes in EEG signals may have occurred due to change in the function of CNS as well as other physiological processes. Hyperthermia reduces the survival time of the subjects under anesthesia and pretreatment of a serotonin antagonist prevents this reduction in survival time. This condition may have a vital clinical importance in surgical procedures, where a need of reduction in metabolic process is required to get a greater operating time, which is usually achieved by lowering the body temperature. At the same time, *p*-CPA pretreatment also prevented the opening of blood-brain barrier, which ultimately prevented the edematous swelling in the subjects. However, *p*-CPA pretreatment did not alter the relationship between body temperature and EEG, which indicates that there is moderately low interference of pretreated *p*-CPA in brain cortical potential under hot environment. Although the neuronal and non-neuronal functions in rats are highly dependent on alterations in brain neurochemicals, it is extremely difficult to indicate only one specific neurotransmitter responsible for the change in a particular parameter. Thus, to extract out more information regarding the involvements of different types of neurochemicals in alterations in brain functions, an extensive, detailed and systematic experimental investigation is needed.

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