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Electroencephalogram Power Spectra in Thioacetamide-induced Hepatic Encephalopathy

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ABSTRACT

During the development of hepatic encephalopathy after thioacetamide (TAA) injection to rat, EEG was recorded at two different states: without or with tactile stimulation of tail at regular intervals. Calculations based on the spectral and band analysis were used. The changes in the power spectra and bands were examined in 3 different behavioral stages: normal, mild ataxia and severe ataxia. In normal rats, the stimulation produced the increase in the power of the theta (3.5~8 Hz) and the gamma (30~50 Hz) bands. These changes could not be produced in rats with the mild and severe ataxia. The changes in the power of the theta band occurred earlier than those of the beta3 and the gamma bands in the stimulated state. Gradual decreases in the spectral power of the beta3 (21~30 Hz) and the gamma bands were correlated with the progress of the stages from normal condition to mild to severe ataxia in both unstimulated and stimulated states. The results indicate that the spectral and band analysis used in this study can quantify the severity of the neurological malfunction during HE.

Key Words: Thioacetamide, Hepatic encephalopathy, Electroencephalogram, Power spectrum

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome characterized by a global depression of central nervous system function, of which manifestaions vary from a slight mental disturbance to deep coma, occurring during acute or chronic liver failure.

The cause of this metabolic disorder is thought to be a group of toxic substances at least part of gut derived, such as ammonia and other neurotoxins, branched-chain amino acids, aromatic amino acids, mercaptans, fatty acids and "false neurotransmitters" (Basile *et al.*, 1991) normally cleared from blood by healthy liver. Recently, it was suggested that a benzodiazepine enhancement of GABAergic neurotransmission may contribute to the neural manifestation of HE (Mullen *et al.*, 1988). This hypothesis was supported by early reports of a striking arousal effect of the benzodiazepine antagonist flumazenil in HE in man (Scollo-Levizzaire & Steinmann, 1985) and in animal models (Baraldi *et al.*, 1984; Bassett *et al.*, 1987) and by the detection of endogenous benzodiazepine like substance in the cerebrospinal fluid (Mullen *et al.*,

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1989) and brain extracts (Basile *et al.*, 1989) of animal models and also in the cerebrospinal fluid (Mullen *et al.*, 1990; Olasmaa *et al.*, 1989) and plasma (Mullen *et al.*, 1990) of patients with HE. But conflicting results, such as the report of no beneficial effect of flumazenil in the rat hepatic ischemic model (Zieve *et al.*, 1987) and low response in patients (Pomier *et al.*, 1990; Van der Rijt *et al.*, 1989), give the question whether the benzodiazepine-like activity in HE is both necessary and sufficient to explain the neural manifestation of HE.

A problem in the clinical and behavioral study in HE model is a lack of adequate quantitative measurement of brain malfunction. Since electroencephalogram changes during development of HE can give indices of quantitative changes in the brain function, we tried to examine the quantitative electroencephalogram for evaluating the severity of HE. Also, since the reports showed that the specific increase of the power in the beta frequency band of the EEG is relevant measure of the intensity of the pharmacological effect of benzodiazepines (Friedman *et al.*, 1992; Mandema *et al.*, 1992a; 1992b), we examined by the quantitative electroencephalogram analysis whether the benzodiazepine-like activity during the development of thioacetamide-induced HE in rats may present.

MATERIALS AND METHODS

Animal model

Five Sprague-Dawley rats (body weight 300~400 g) were used for all experiments. They were housed in standard facilities with free access to food and water and a 12-hour day and night cycle. The rat model of HE used in this study was due to acute fulminant liver failure, that was induced with intraperitoneal injection of thioacetamide (TAA, 500 mg/kg/day in 4 ml/kg 0.9% NaCl) for 3 consecutive days (Gammal *et al.*, 1990). To prevent hypoglycemia and dehydration after the first dose of TAA, we gave 12.5 ml/kg 5% glucose in 0.45% NaCl containing 20 mEq/L KCl every day before and after the recording session by subcutaneous injection (Zimmermann *et al.* 1989).

Animal preparation

All animals were anesthetized with pentobarbital (25 mg/kg, i.p.) and urethane (0.5 g/kg i.p.), and were operated in a stereotaxic apparatus. Four or two cortical electrodes (gold-plated screws, tip diameter 1 mm) were implanted over bilateral frontal (AP 2.5 mm, ML 2.5 mm) and parietal (AP −2.5 mm, ML 2.5 mm) or bilateral frontal cortex. Two screws driven into the bone above cerebellum served as indifferent and ground electrodes. The electrodes and screws with connecting pins were installed over the skull with dental acrylic altogether. The animal was allowed to recover for at least 2 days prior to start the first recording session.

EEG recording and analysis

The EEG from the two or four screw electrodes over bilateral frontal (and parietal) cortices and from bilateral hippocampal CA1 regions was recorded monopolarly with respect to the indifferent screw electrode via a bioelectric amplifier (A-M Systems, Inc., USA). The signals were amplified with 1000x and filtered with the range of 1 to 500 Hz. They were sampled by the A/D converter (DigiData 1200A, Axon Instrument, Inc., USA) at a sampling frequency of 1 KHz. The 1 or 2-min EEG recording was carried out before and 1 hr after TAA administration. For 3 consecutive days, At every recording time, we recorded EEG under two different behavioral states: without ("unstimulated") and with tactile stimulation of tail ("stimulated"). These were conducted every day for 3 consecutive days. The EEG was analyzed by a modified methods that were described in our earlier reports (Lee *et al.*, 1992; Park & Lee, 1991). The 1-min segment of EEG data from every recording electrodes at every time was divided into 4.096-sec epochs. Each epoch was converted to the amplitude spectrum of frequency domain by Fast Fourier Tranform algorithm and then calculated to the power spectrum. A absolute spectrum at one state and one condition of the animal was averaged from noise-free 12 epochs. A standardized spectrum was calculated from division of the absolute spectrum by the normalized total power (total

power/number of frequency points) of the spectrum at unstimulated and normal condition. Smoothing procedure (2 Hz window), that reduces the normalized standard error (Bendat & Piersol, 1971), was applied to the standardized spectrum. Each animal had its own control spectrum derived from unstimulated normal condition. Also, we calculated power of the frequency bands (delta 1~3.5; theta 3.5~8; alpha 8~13; beta1-2 13~21; beta3 21~30; and gamma 30~50 Hz). Using paired t-test, significant differences in the power of each frequency (range 1 to 50 Hz) of spectrum between pre-(normal) and post-TAA (mild to severe ataxia) or between unstimulated and stimulated state were evaluated with p-value ($p<0.05$).

Bhavioral recordings

The behavioral manifestation of HE of the animals were staged by means of observation of the spontaneous motor and exploratory activity and by testing of pain and righting reflexes: normal; mild ataxia (lack of movement during stimulation but intact righting reflex); and severe ataxia (no righting reflex) including coma (Jones *et al.*, 1986; Bassett *et al.*, 1987; Schafer *et al.*, 1984).

Drugs

Thioacetamide (Sigma Chem. Co., USA), pentobarbital (Hanlim Pharm. Inc., Korea) and urethane (Wako Chemical Co., Japan) were used in this study.

RESULTS

Thioacetamide was injected into rats and EEG recording was conducted for 3 consecutive days. Provided there was a standardized behavioral state and a standard electrode implantation, the normal EEG power spectra are proved to be fairly constant (Popkin *et al.*, 1983). The power spectra recorded during the state controlled with or without the tactile stimulation of tail were fairly constant between animals as well as within animal. We used 2 controlled behavioral states: unstimulated or stimulated state. There was a characteristic difference of power spectra between two different states in normal rat. The stimulation induced the increase in the power of the theta and gamma band ranges (Fig. 1A; Fig. 2A). The difference between the states disappeared with the progression of neurological severity of HE (Fig. 1 & Fig. 2). In rat with severe ataxia, the stimulation, which cause the change of vigilance in normal animal, did not produce significant

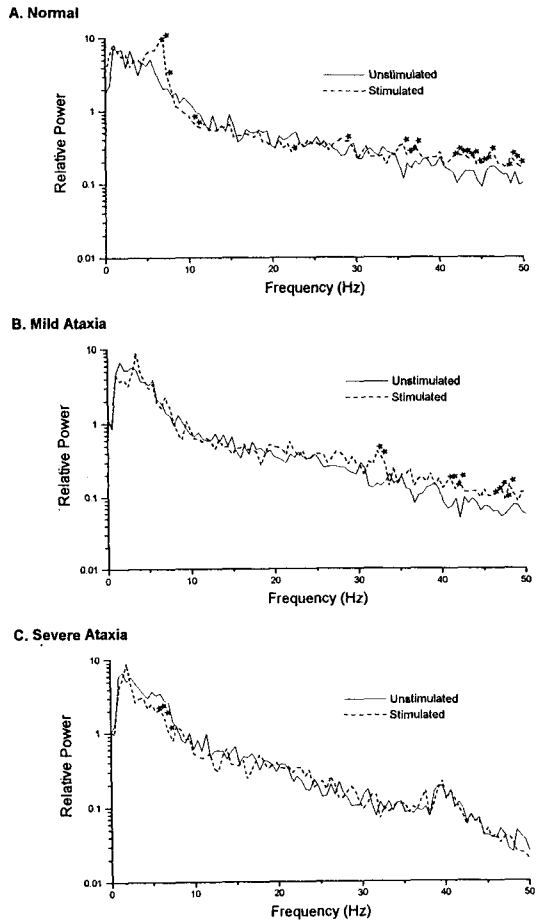

Fig. 1. The average of the standardized power spectrum of the frontal EEG at unstimulated (continuous line) and stimulated (dashed line) with tactile stimuli to tail. A. normal condition; B. mild ataxia; and C. severe ataxia. The asterisk (*) indicates significant difference between states ($p<0.05$).

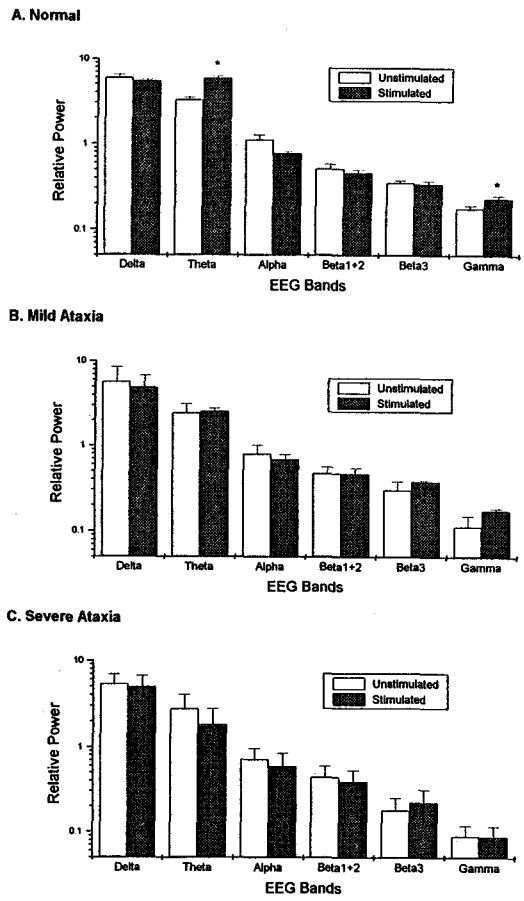

Fig. 2. The standardized power of each band of the frontal EEG in rat at unstimulated (open column) and stimulated (gray column). A. normal condition; B. mild ataxia; and C. severe ataxia. The asterisk (*) indicates significant difference between states ($p<0.05$).

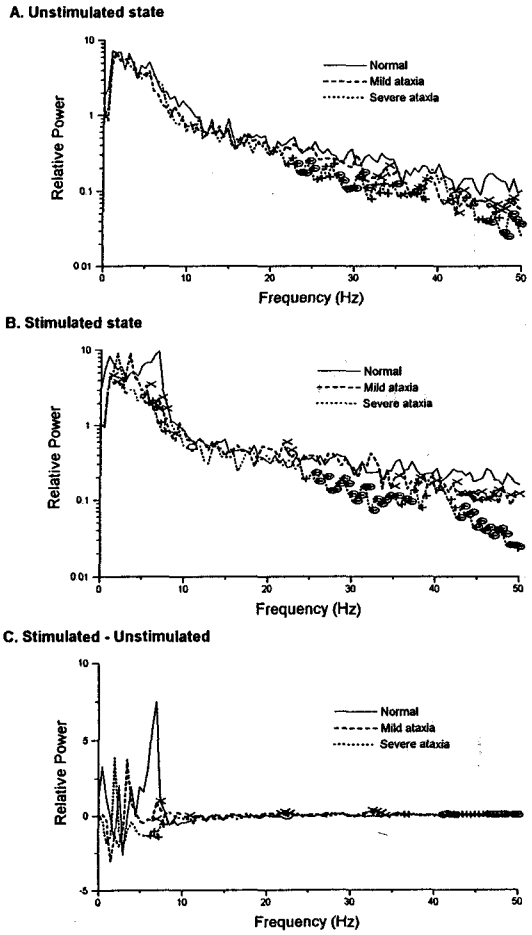

Fig. 3. The average of the standardized power spectrum of the frontal EEG in three conditions: normal (continous line); mild ataxia (dashed line); and severe ataxia (dotted line). A. unstimulated; B. stimulated state; and C. difference between stimulated and unstimulated state. The significant difference ($p<0.05$) was indicated by cross (normal vs. mild ataxia), plus (normal vs. severe ataxia) and circle (mild vs. severe ataxia).

change in the power spectra (Fig. 1C), except small significant decrease in the power of some theta frequencies.

The progress of neurological manifestation in rat from normal, mild ataxia to severe ataxia, could be correlated to the changes of the spectral pattern at each vigilance state such as unstimulated or stimulated state (Fig. 3A, B). At unstimulated state, the decrease in the power of the beta3 and the gamma bands was related to the severity of HE (Fig. 4A). At stimulated state, the decrease in the power of the theta band as well as the beta3 and the gamma bands was related to the severity of HE (Fig. 4B). The changes of the power of the theta band started earlier than that of the beta3 and

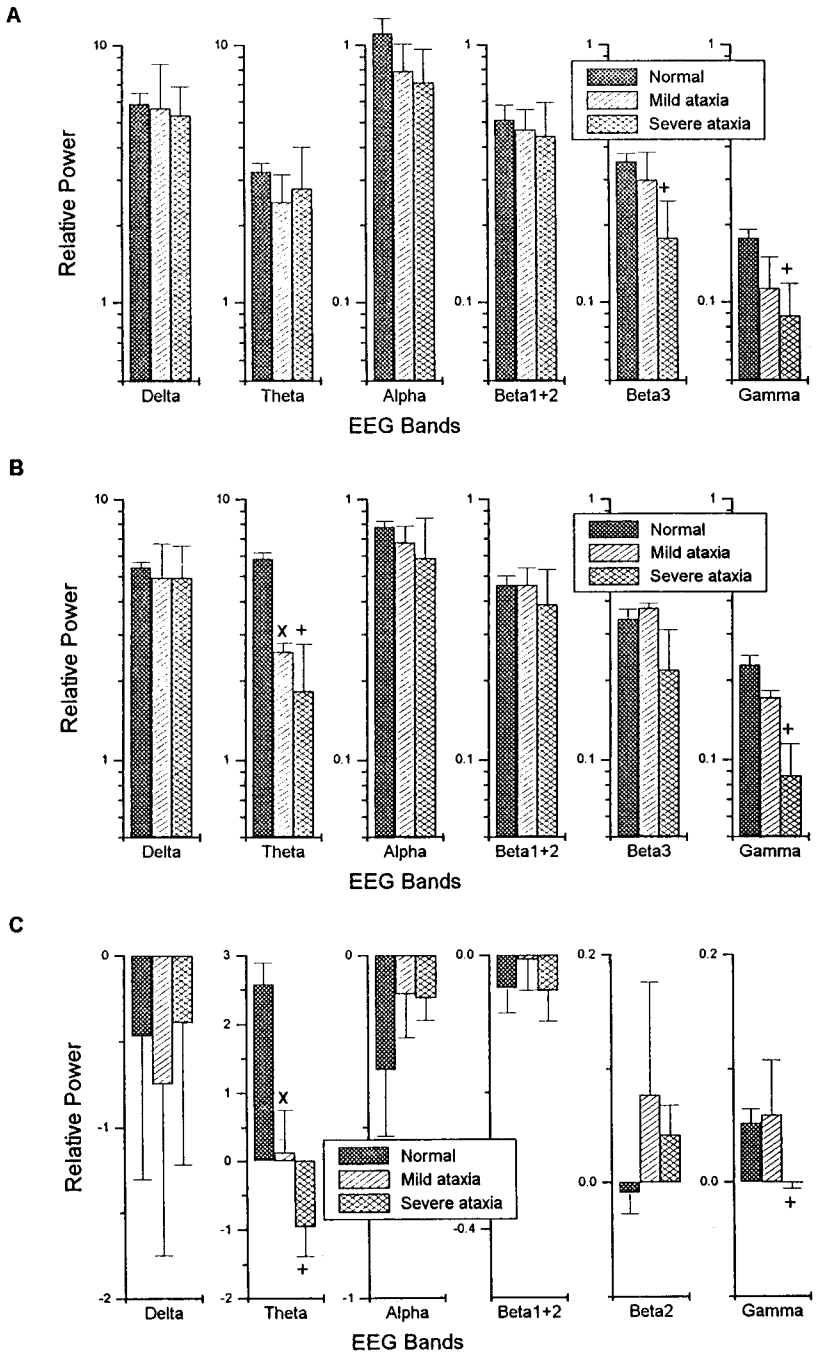

Fig. 4. The standardized power of each band of the frontal EEG in three conditions: normal (gray column); mild ataxia (hatched column); and severe ataxia (cross-hatched column). A. unstimulated state; B. stimulated state; and C. difference between stimulated and unstimulated states. The significant difference ($p<0.05$) was indicated by cross (normal vs. mild ataxia) and plus (normal vs. severe ataxia).

gamma bands (Fig. 4B).

DISCUSSION

A few study had been conducted to relate the clinical manifestation of HE through electrophysiological methods (Basile *et al.*, 1988; Popken *et al.*, 1983). Calculations based on the spectrum and band analysis were applied in this study to quantify the changes during progress of clinical manifestation of HE. The spectral pattern showed some gradual changes related to the severity of the neurological manifestation of HE. Especially, the power of the high beta and the gamma frequencies gradually decreased with the severity of HE. Interestingly we observed the interaction between the severity of HE and the external sensory stimulation, which we used tactile stimulation of tail. Stimulation in normal rat produced the increase in power of the theta band, which is believed to occur with the movement and sensory processing (Vanderwolf, 1969). Then, the disappear of the stimulation related changes may be an indication of the severity of the behavioral and mental disturbance during development of HE. The spectral pattern did not change with the presence of stimulation after full development of HE. This result suggest that the normal sensory processing may not occur during HE.

At the unstimulated state, the gradual changes of the power which can be related to the severity of HE were observed in the high frequency ranges such as the beta3 and the gamma bands. But, the power of the theta band at the unstimulated state was independent to the neurological manifestation of HE. It has demonstrated that gamma oscillations in the mammalian brain is not a property of single neurons, but rather depends on feedback interactions in pools of neurons (Freeman, 1975). This oscillation of field potential is a common instantaneous frequency across the surface of the cortex (Bressler, 1984) and is an integrating activity among widely distributed neocotex related event input (Bressler, 1990). Then, the decreases in the power of the gamma band (here 30~50 Hz though the frequencies of gamma band (20~80 Hz) vary according to persons) during the development of HE may indicate that the coordinated information processing over the neocortex is impaired and that the global activity of the brain is depressed.

Interest in the role of benzodiazepines in hepatic encephalopathy first developed because of the close association of the benzodiazepine and GABA receptors in the brain and the find that binding sites were increased in galactosamine induced acute liver failure (Schafer *et al.*, 1983; Baraldi *et al.*, 1984). Much greater intereset followed early reports of a striking arousal effect of the benzodiazepine antagonist flumazenil in hepatic encephalopathy in man (Scollo-Levizzaire & Steinmann, 1985). Subsequent reports showed beneficial results in animal models (Baraldi *et al.*, 1984; Bassett *et al.*, 1987). We could not detected the specific changes in the beta ranges, which can be produced by benzodiazepine injection into rats (Friedman *et al.*, 1992; Mandema *et al.*, 1992a; 1992b), from the spectrum during TAA-induced coma. Also, the early enthusiastic reports have not been sustained. In the thioacetamide model of acute liver failure, flumazenil does not reverse the motor deficit, that usuallly persists after drug administration (Gammal *et al.*, 1990). No effect could be shown in the rat hepatic ischaemia model (portacaval shunt plus hepatic artery ligation) despite administration of a dose sufficient to reverse benzodiazepine induced coma (Zieve *et al.*, 1987). In man, the results are even more controversial some authors claiming beneficial results (Bansky *et al.*, 1989) in 60% of patients (Grimm *et al.*, 1988) while other (Van der Rijt *et al.*, 1989), including a placebo controlled trial, found only a 27% response rate (Pomier *et al.*, 1990). Then, clinical manifestation of HE may not be related to the endogenous benzodiazepine.

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=국문초록=

Thioacetamide 유발 간성뇌장애에서 뇌파 Power Spectra

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흰쥐에 thioacetamide(TAA)를 투여하면 간성뇌장애가 유발된다. 두가지 행동 상태(꼬리 접촉 자극 및 미자극)와 세 단계의 신경학적 이상의 단계(정상, 약한 운동실조, 심한 운동실조)별로 뇌파를 기록하였고, 뇌파 스펙트럼 및 대역 분석을 통하여 간성뇌장애 진행동안 뇌파의 정량적인 변동을 관찰하였다. 정상 쥐에서 자극은 theta(3.5~8 Hz) 및 gamma(30~50 Hz) 대역의 power을 증가시켰다. 운동실조가 있는 쥐에서 이러한 변화가 일어나지 못하였다. 꼬리 자극을 하는 상태에서 theta 대역의 변화는 gamma 대역의 변화 보다 더욱 일찍 관찰되었다. 신경학적 행동 단계가 심해짐에 따라서 beta3(21~30 Hz) 및 gamma 대역에서 power가 점차적으로 감소하였다. 또한 간성뇌장애의 신경학적 증상은 benzodiazepine계 물질에 의하여 야기된다는 가설이 있으므로 beta power의 증가가 관찰될 것으로 기대하였으나 이 연구에서는 관찰하지 못하였다.

이 결과들로 미루어 볼때 이 연구에 사용된 스펙트럼 및 대역 분석이 간성뇌장애동안 신경학적 증상의 정도를 정량화 할 수 있다고 생각된다.