

# Veratridine induced absence like-seizure in the freely moving rats: a study correlating the behavioural findings with the electrophysiological activities

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## Abstract

**OBJECTIVES:** Veratridine was characterized previously as an experimental model of epilepsy *in vitro*. The aim of this preliminary investigation is to identify the pattern of seizure induced by this model *in vivo*.

**MATERIAL AND METHODS:** Veratridine (200 µg/kg) was administered intraperitoneally to male Sprague-Dawley rats and the electrical activity of the brain was recorded as surface electroencephalogram (EEG).

**RESULTS:** The animals developed behavioral effects manifested as grooming, masticatory movements, facial automatism and wet dog shakes (WDSs). There were episodes of complete quiescent periods for 2-5 minutes before the animals resumed activity which were repeated every 15-20 minutes. The seizure activity during this silent activity showed fast frequency signals in the surface EEG correlating with absence seizure. The WDS behaviour was associated with electrical spikes on the EEG. When the rats were pre-treated with 200mg/kg ethosuximide (ETX), EEG recordings did not display the same fast frequency signal as that observed in animals receiving veratridine only. The number and duration of WDSs were not altered by ETX (200-400 mg/kg).

**CONCLUSION:** Veratridine produced an absence like-seizure activity in the surface EEG, sensitive to ETX and correlates with its behavioural effects.

## INTRODUCTION

The characteristics of veratridine as an *in vitro* model of epilepsy was studied by us in rat brain slices using conventional electrophysiological intracellular techniques from hippocampal CA1 pyramidal neurons (Otoom *et al.* 1998; Otoom & Alkadhi 2000a; Otoom & Alkadhi 2000b; Otoom & Nusier 2001; Otoom & Hasan 2004). It was shown that veratridine (0.03-0.3 µM) induced

both evoked or spontaneous rhythmic burstings which were sensitive to membrane potential changes and independent of synaptic transmission (Otoom *et al.* 1998). The veratridine-induced bursting activity in hippocampal CA1 pyramidal neurons was related to accentuation of the depolarizing rectification so that a zero or negative slope appears in the current-voltage curve of untreated

neurons (Tian *et al.* 1995). The seizure activity of veratridine was inhibited *in vitro* by therapeutic concentrations of lamotrigine (Ootom & Nusier 2001), valproic acid (Ootom & Alkadhi 2000a;b), phenytoin (Ootom & Alkadhi 2000b), carbamazepine (Ootom & Alkadhi 1999; Ootom & Alkadhi 2000a) and propofol (Ootom & Hasan 2004) but not by the antiepileptic drug ethosuximide (ETX) even at high concentrations (Ootom & Alkadhi 2000b).

We have also studied the behavioural effects of this convulsant *in vivo*. Veratridine was administered intraperitoneally to male Fisher rats in a dose range of 100–400 µg/kg. The animals had facial automatism, grooming, masticatory jaw movement and profuse salivation. This phenomenon was followed by the development of wet dog shake (WDS) and forelimb clonus. Histopathological studies of the animals 2 weeks after veratridine administration showed evidence of apoptosis in the hippocampus (Ootom *et al.* 2006).

The aim of this preliminary investigation is to test the pattern of seizure activity induced by veratridine in rats using surface electroencephalogram (EEG) and its correlation with the behavioural effects.

## MATERIALS AND METHODS:

### Surgical procedures and EEG recordings

Studies were performed on male Sprague-Dawley rats. Animals were anesthetized with isoflurane, placed in a stereotaxic frame and maintained normothermic by means of a thermostatically controlled heating pad. Burr holes were drilled to accommodate the guide for the three recording electrodes (Plastics One, Roanoke, VA). An electrode pedestal (Plastics One) was subsequently attached to the protruding electrode sockets. Anesthesia was then discontinued and the animal was placed inside a restraining chamber (restraining height: 50.8 mm; length: 150 mm) for full recovery prior to further experimentation. A connector cable was then attached to the electrode pedestal and baseline EEG recordings made for 30 minutes.

### Chemicals

Veratridine and ethosuximide were obtained from Sigma, USA and were dissolved in ethanol 50–100 mg/ml of solution.

## RESULTS

### Behavioural and EEG assessment of seizure activity

The typical baseline EEG recording is shown in Figure 1 (Control). Veratridine 200 µg/kg (n=4) was given intraperitoneally. Twenty five–30 minutes post injection, all animals developed grooming, masticatory movements, facial automatism and wet dog shake. The animals went into a quiescent period for 2–5 minutes before they resumed activity. This quiescent period recurred every 15–20 minutes. The seizure activity during this

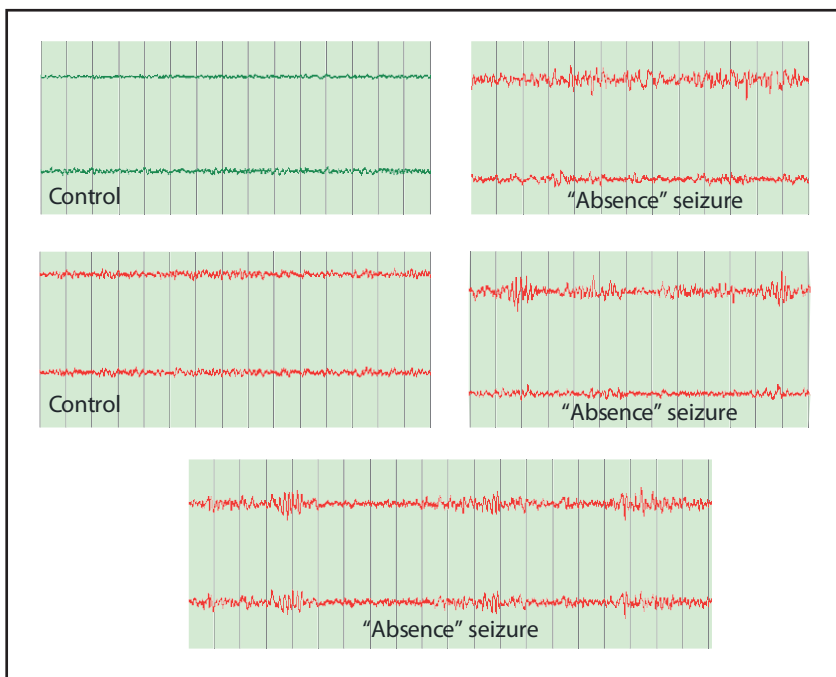
silent period, in one of the animals, is shown (Figure 1, right hand side panel). The WDS (a stereotype behaviour) was associated with electrical spikes on the EEG (Figure 2). When the rats were pre-treated 30 minutes earlier with 200 mg/kg ethosuximide (n=4), EEG recordings did not display the same pattern of fast frequency signal observed in animals receiving veratridine only (Figure 3). The number and duration of WDS were not altered by ETX (200–400 mg/kg).

## DISCUSSION

Chemical-induced seizures in animals have been used as the experimental models for evaluating drugs, particularly those effective in absence seizures. Non-specific stimulants (pentylenetetrazole, picrotoxin), GABA antagonist (bicuculline), glutamic acid decarboxylase inhibitors (isonicotinic hydrazide, 3-mercaptopropionic acid), excitatory amino acid – related substances (kainic acid, quisqualic acid, N-methylaspartic acid, homocysteic acid), cholinergics (pilocarpine, sarin), adenosine antagonist (theophylline), inhalants (flurothyl) are some such drugs / chemicals that have been used for producing seizure in animals (Pitkanen *et al.* 2006; Peterson & Albertson 1998), although there are few detailed descriptions of seizures as well as associated EEG patterns. It is certain, however, that the use of *in vivo* animal models has contributed enormously to our understanding of the mechanisms of seizures, as ultimately, they point to a dysfunction in the thalamo-cortical system (Gilman 2007).

Veratridine is commonly used and well characterized pharmacological tool in various electrochemical studies. This alkaloid can activate Na<sup>+</sup> influx causing depolarization and increase in [Ca<sup>2+</sup>]<sub>i</sub> in the cells. Previous studies *in vitro* using brain slices (Ootom & Alkadhi 1998) and *in vivo* in rats (Ootom *et al.* 2006) have suggested veratridine as a potential tool for screening antiepileptic drugs. The present study has confirmed that the EEG pattern after veratridine is comparable to that seen in absence seizures; moreover, the effectiveness of ethosuximide in controlling veratridine-induced seizure further supports the view that veratridine-induced seizure in rats is a useful model for evaluating drugs with potential antiepileptic activity, particularly those likely to be effective against absence seizures.

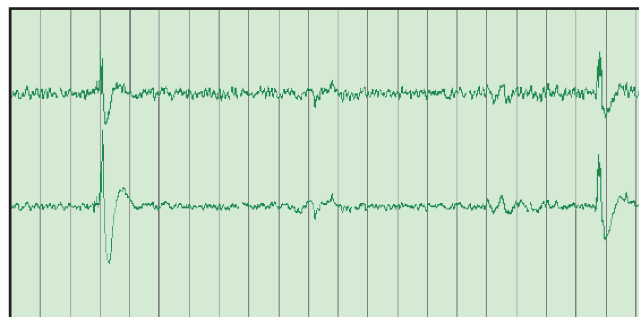
Our *in vivo* results regarding the sensitivity of the electrical activity induced by veratridine to ETX are at variance with those from *in vitro* studies. The antiepileptic drug ETX did not suppress the seizure-like activity induced by veratridine evoked in brain slices (Ootom & Alkadhi 2000b). This may be explained by the fact that seizure-like activity evoked *in vitro* is related to activation of sodium channels that are ETX insensitive (Ootom *et al.* 1998). However, the seizure activity induced by veratridine *in vivo* may involve other channels, in particular, calcium channels which are ETX sensitive. A broad range of pharmacological



**Fig. 1.** Left hand side panels show control activity before veratridine (200  $\mu\text{g}/\text{kg}$ ) injection. Panels on the right show some different types of EEG activity correlating with "absence" behaviour. Each interval represents 1s and all traces are at the same scale.

and biochemical effects including the release of various neurotransmitters such as acetylcholine, norepinephrine, dopamine and gamma aminobutyric acid (GABA) were shown to be triggered by veratridine-induced increase in  $\text{Na}^+$  permeability of neural cells *in vitro* (Cunningham & Neal 1981; Tapia 1985). Veratridine can activate  $\text{Na}^+$  influx causing depolarization and an increase in  $[\text{Ca}^{2+}]_i$  in the cells. Therefore, it can also be used to simulate ischemic attack in brain cells (Zelles *et al.* 2001). The cell death evoked by veratridine in rat cerebrocortical cell culture could be blocked by submicromolar concentration of vinpocetine more effectively than the  $\text{Na}^+$  channel blocker anticonvulsant phenytoin.

Moreover, the calcium channel blocker flunarizine also protects against neural damage induced by veratridine (Pauwels *et al.* 1989). In rat cortical synaptosomes lamotrigine, phenytoin, carbamazepine, riluzole, but not phenobarbital, inhibit glutamate release by blocking preferentially presynaptic  $\text{Na}^+$  channels, evoked by 20  $\mu\text{M}$  veratridine (which requires both  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channel activation) or 30  $\mu\text{M}$  KCl (which require  $\text{Ca}^{2+}$  channel but not  $\text{Na}^+$  channel activation). These results suggest that presynaptic  $\text{Na}^+$  channel blockade with inhibition of the release of glutamate, and possibly other neurotransmitters, may contribute to their anticonvulsant and neuroprotective effect (Lingamaneni & Hemmings 1999). Also, the inhibitory effect of felbamate on veratridine induced release of glutamate may be due to inactivation of voltage-sensitive  $\text{Na}^+$  channels (Srinivasan *et al.* 1996). Additionally, there is evidence that the preservation of mitochondrial integrity and energy metabolism during experimental seizures leads to neuronal apoptosis in the hippocampus in rats



**Fig. 2.** Spike activity associated with Wet Dog Shake behaviour in one of the rats.



**Fig. 3.** EEG recordings in one rat pre-treated with ETX (200 mg/kg).

(Chuang *et al.* 2009), and that antiepileptic drugs such as valproic acid counteract such an effect (Brandt *et al.* 2006). Further studies should focus on veratridine induced seizures in the contexts of these findings.

Our results indicated that the number of WDS was not altered by ethosuximide. Both gamma-hydroxybutyrate and pentylenetetrazole-induced models of absence seizure in rats are characterized by repetitive episodes of staring and immobility with concomitant 6 to 9 Hz spike and wave discharges (SWDs) in the EEG.

In gamma-hydroxybutyrate model both valproate and ethosuximide reduced SWDs number and duration, whereas, in pentylenetetrazole model, ethosuximide was more effective (Kumaresan *et al.* 2000). Furthermore, combination of flunarizine either with valproate or ethosuximide showed significant further reduction in SWD number and duration, suggesting an additive effect of these drugs with the calcium channel blocker on experimental absence seizure in rats (Subramanyan *et al.* 2001).

The effect of ETX on SWDs was also studied in genetic model of absence epilepsy (GAERS) which have confirmed that the SWDs have characteristic EEG patterns that respond to drugs such as ethosuximide. The primary motor cortex also has been shown to be involved in the SWD activity and GABA-mediated neurotransmission in the frontal cortex is more critical than that of ventrolateral thalamus in terms of ethosuximide mediated neurotransmitter effect (Terzioglu *et al.* 2006). Also, in WAG/Rij rats, the incidence, duration and peak frequency of the SWDs was blocked by levetiracetam, another drug effective in absence seizures (Bouwman & Van Rijn 2004). The blockade of adenosine receptors by theophylline decreases the occurrence of SWDs, probably by augmenting the excitatory neurotransmission and increasing vigilance and arousal level (Ates *et al.* 2004).

In conclusion, this preliminary study has characterized the pattern of veratridine induced seizures *in vivo* in rat model. Veratridine produced an absence like-seizure activity in the surface EEG, sensitive to ETX and correlated with its behavioural effects.

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