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Diffuse Transcranial Electrical Stimulation (DTES)-induced hypermotility and convulsion: A model for screening drugs with anti-convulsant properties.

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Abstract

Status epilepticus (SE) was induced in male and female Wistar rats by passing low direct current across the brain via steel electrodes clipped to their ear lobes, and the effects of some anti-convulsants on these animals were studied in a motility counter chamber. Sodium valproate was found to significantly attenuate diffuse transcranial electrical stimulation (DTES)-induced hypermotility and protected the animals against DTES-induced convulsions. Higher voltages were needed to induce convulsion in rats pretreated with sodium valproate. Hypermotility induced by DTES was attenuated by carbamazepine, an anti- convulsant. It was also observed that carbamazepine abolished DTES-induced convulsion and higher voltages were needed to induce convulsion in rats pretreated with carbamazepine. Phenytoin inhibited DTES induced convulsion and attenuated DTES-induced hypermotility in Wistar rats. Higher voltages were needed to induce convulsion in pretreated animals than in normal animals. It is therefore suggestive that DTES-induced hypermotility can be used as an animal model for testing drugs that can be of advantage in the management of non convulsive (petit mal) status epilepticus (SE), and DTES induced convulsion as a model for testing drugs with anticonvulsant properties.

Keywords: DTES; Hypermotility; Phenytoin; Cabamazepine; Sodium valproate; Wistar rats.

Introduction

Status epilepticus (SE) is defined generally as prolonged seizure activity. A specific practical definition of SE requires its further division into subtypes based on clinical and EEG characteristics. This classification divides patients into groups with homogeneous characteristics and presumably homogeneous pathophysiology, response to therapy, and prognosis. In practice, SE can be divided into generalized convulsive status epilepticus (GCSE) and non-convulsive status epilepticus (NCSE). GCSE is usually specifically defined as few minutes of convulsive activity or repeated convulsions over the course of time without return of consciousness in between. NCSE has been reported under various names, including absence status, petit mal status, ictal stupor, and complex partial status. There is no accepted classification of NCSE, but one based on pathophysiology has been proposed (Fountain *et al.*, 1995).

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Animal models of SE have given some information on the events that initiate SE and extrapolated from models of acutely induced seizures. Neuronal depolarization begins when glutamate binds to postsynaptic N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors. Initially, only non-NMDA channels are activated and Na^+ and K^+ flow through, depolarizing the cell. Under normal conditions, ions cannot flow through NMDA channels because Mg^{2+} blocks them; gamma-amino butyric acid (GABA)-mediated inhibition and other mechanisms stop depolarization. However, depolarization continues during SE because of, among other things, deterioration of GABA-mediated inhibition. When the cell is depolarized to a sufficient degree and duration, the Mg^{2+} that normally blocks the NMDA channel is removed, allowing Ca^{2+} to flow through these channels, further depolarizing the cell and activating other Ca^{2+} channels. This "use dependence" of the NMDA receptor makes it uniquely suited to perpetuate SE, because the channel is both activated by depolarization and results in calcium entry, which further depolarizes the cell. The subsequent elevated intracellular calcium may be sequestered within the cell or moved outside. However, when it accumulates, it leads directly to cell swelling and necrotic death and activates a cascade of events that result in programmed cell death, or apoptosis. The end result of prolonged depolarization is cell death.

There are various animal models of status epilepticus which include; the pentylenetetrazole induced convulsion, (Okpako, 1994 and Maedonard, 1997,1993.) which induces seizures by blocking Cl^- channel of $GABA_A$ receptors (Corda, *et al.*, 1990), the amphetamine-induced hypermotility model (Vasquez-Freire *et al.*, 1994), and the bicuculline-induced convulsion.

These models are chemical ways of inducing convulsion in an experimental animal. The animals are normally pretreated with the test drug prior to induction of convulsion. The test drug may interfere with the drug used to induce convulsion and give a false result. This may account for the reason some drugs that are effective in animal model of convulsion may not be effective in humans.

The objective of this study was to develop an animal model that is close to epileptic state in man for testing drugs suspected to have anti-epileptic properties.

Experimental

Adult male and female Wister rats (200-250g) inbred in the animal Production Unit of the National Veterinary Research Institute, Vom, were used. The animals had access to tap water and feed (24% protein: Pfizer Products, Lagos, Nigeria) *ad libitum.* The rats were housed at a temperature of 25 ± 2^0C and acclimatized on approximately 12 h light and 12 h dark cycle in the animal house. They were randomly selected from colony of adult male and female rats.

Diffuse transcranial electrical stimulation (DTES) was effected via steel electrodes clipped on the left and right ear lobe of the rat. Electrical stimulation (0-25V, frequency 90Hz, pulse width 1 ms) was delivered for duration of 10 seconds in hypermotility test or 20 seconds in convulsive test using a CFP stimulator model 8048 (C. F. Palmer, London, UK.).

Preliminary experiments were carried out to determine the suitable voltage for brain stimulation. We found that between five to twenty-five volts produced reproducible activity in the rats. Five and ten volts were then chosen for these experiments.

The activity of the rats was recorded using the activity counter (Maurel-Reny, *et al* 1995).

The somatic and behavioral changes, including locomotor activity of Wistar rats pretreated with Carbamazepine, 33.5mg/kg,

sodium valproate, 20mg/kg or Phenytion,15mg/kg, 10s before, during and after electrical stimulation were determined and recorded. The drugs were injected intraperitonially. In the second phase of the experiment, DTES was delivered to 10 animals per group for 20 minutes and the time taken for the animals to go into convulsions were noted and recorded. Effects of Carbamazepine, 33.5mg/kg. Sodium valproate, 20mg/kg or phenytoin, 15mg/kg, on onset of convulsion were measured.

Results were presented as means ± SEM and differences were analyzed using Student's t-test and *p* values less than 0.05 were considered significant (Snedecor and Cochran, 1967).

Results and Discussion

Effect of DTES on motility of male and female Wistar rats. DTES caused a rise in the general activity of the animal. It was observed that motility count 10s before stimulation was 10.0 ± 4.1. During stimulation (10s) the motility count significantly increased to 81.0 ± 13.5 and after stimulation (10 sec.) it fell to 21.6 ± 6.8. The value obtained after stimulation was significantly different from the values before ($P<0.05$) and during ($P< 0.001$) stimulation (Table 1).

Effect of carbamazepine on hypermotility induced by DTES. When carbamazepine (33.5mg/1kg) was administered to rats, there was a marked ($p< 0.05$) attenuation of motility count. The value before administration of carbamazepine was 10.1 ± 4.1 while the values after administration of the drug was 5.5 ±1.31 (Table 1 and Figure 1). It was also observed that the motility count markedly increased ($p<0.001$) during DTES in carbamazepine treated rats. This value was significantly ($p<0.05$) lower than the values obtained in rats that were not pretreated with carbamazepine. The motility count during DTES in carbamazepine treated rats was 26.0 ± 5.37 while that of control (untreated rats) was 79.0 ± 13.5 (Table 1 and Figure 1). Further more, post DTES motility count in carbamazepine treated rats was significantly ($p<0.001$) lower than post DTES motility count of the control rats (table 1 and Figure 1). Figure 1 shows that the motility count for carbamazepine treated rats were 5.5 ± 1.31 (before stimulation), 26.0 ± 5.37 (during stimulation) and 2.1 ±1.0 (after stimulation). These values were significantly deferent ($P<0.05$) from one another when compared.

Effects of Phenytoin on DTES-induced hypermotility. Administration of phenytoin (15mg/1kg) to rats increased motility count from 10.16 ± 4.1 to 14.1 ± 1.75 before DTES was given. Administration of DTES to rats treated with phenytoin, resulted in significant ($p< 0.001$) increase in locomotor activity in comparison with values obtained before DTES (figure 2). After DTES in these rats, locomotor activity also significantly ($p< 0.05$) decreased below that of motility counts for pre –DTES or during DTES.

Effect of sodium valproate on hypermotility induced by DTES. Administration of sodium valproate (20mg/kg) to rats caused a marked ($p<0.05$) fall in locomotor activity from 10.1 ± 4.1 to 6.0 ± 0.36. However, during DTES in rats treated with sodium valproate, there was a significant ($p< 0.001$) increase in locomotor activity (figure 3). After DTES in these rats, locomotor activity did not significantly ($p>0.05$) change when compared with the locomotor activity for pre-DTES, but there was a significant ($p<0.001$) fall when compared with values obtained during DTES. The motility count (30.0 ± 4.31) during DTES in Sodium valproate treated rats was significantly ($P<0.05$) different from that of control, 79.0 ±13.5 (Table 1 and Figure 3).

Effect of anti-epileptic drugs on convulsion induced by DTES. (See Table 2).

The search for an animal model for status epilepticus that is close to what is seen in man is still on. A number of animal models have been in use for screening drugs for possible anti-epileptic properties and for studying the mechanisms underlining status epilepticus. As listed in the introduction most of the models involve the use of drugs to induce convulsion or hypermotility in the animals.

It is possible that the result obtained may be influenced by the drug used in the induction of convulsion or hypermotility in the animals (Corda *et al* 1990). DTES induced convulsion and / or hypermotility in animals does not involve the use of chemicals and thus it is devoid of this disadvantage.

The chemically induced convulsion is due to the actions of these drugs on the nerve endings e.g. amphetamine, (Vasquez-Freire *et al.,* 1994). This is close to what happens during epilepsy but does not show the true picture of what actually takes place in humans. In patients, the whole nerve is involved and not just the nerve endings alone. The results above show that the two types of status epilepsy (GCSE and NCSE) can be used as experimental models for testing for anti-epileptic properties of a drug. At lower voltages, DTES induced hypermotility in male and female Wistar rats, and the animals look confused, stop for a while and then start moving again. This mimics what is seen in a patient with non –convulsive status epilepticus. Varma and Lee (1992) observed that ECT induces nonconvulsive status epilepticus in patients during treatment with ECT. Therefore the epilepsy induced in these experimental animals at low voltages can be said to be the same observed in humans during ECT, which further buttresses the fact that this model may be very close to what is observed in epileptic patients.

The motility counter recorded an increase in motility, which was attenuated by Phenytoin, Sodium Valproate and carbamazepine. This can be used as an animal model for non-convulsive status epilepsy.

The second part of the experiment showed that these drugs also inhibited DTES induced convulsion at voltages between 12V and 15V. It was also observed that higher voltages were needed to induce convulsion in animals pretreated with these drugs. This model can be used as a model for convulsive status epilepticus. Devisnky and Duchowny (1983), observed Seizures after convulsive therapy (ECT), and he described it as a form of convulsive epilepsy.

These models are at advantage over the other models because the convulsion observed are not drug induced and the likelihood of drug interaction and interference with the test result is reduced, if not completely eliminated.

Table 1. Effect of DTES on locomotor activity of normal rats (n=10).

Duration of exposure	No. of rats	Motility count (mean ± SE)	% increase in motility
Before DTES (10 s)	10	10 ± 4.1*	-
During DTES (10 s)	10	79.0 ±13.5*	709.9
After DTES (10 s)	10	21.6 ±6.8*	114

* Values significantly different from one another

Table 2. Effect of anti-epileptic drugs on convulsion induced by DTES.

Stimulation voltage (V)	0	5	10	12	15	20	25
Drugs	Responses						
Control	N	N	N	N	Y	Y	Y
Phenytoin-pretreated rats	N	N	N	N	N	Y	Y
Carbamazepine-pretreated rats	N	N	N	N	N	N	Y
Sodium valproate-pretreated rats	N	N	N	N	N	N	Y

N = no convulsion; Y = convulsion

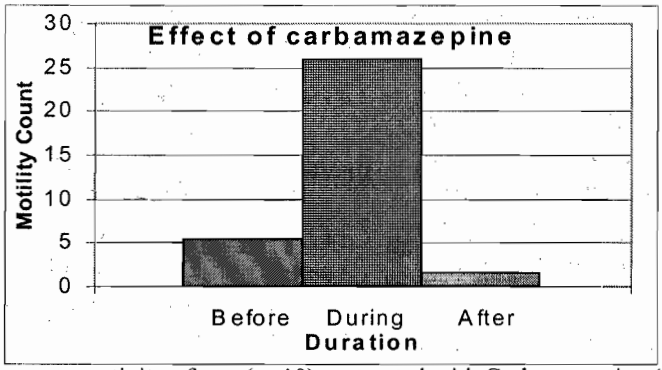

Figure 1: Effect of DTES on locomotor activity of rats (n=10) pretreated with Carbamazepine (3.35mg/100g)

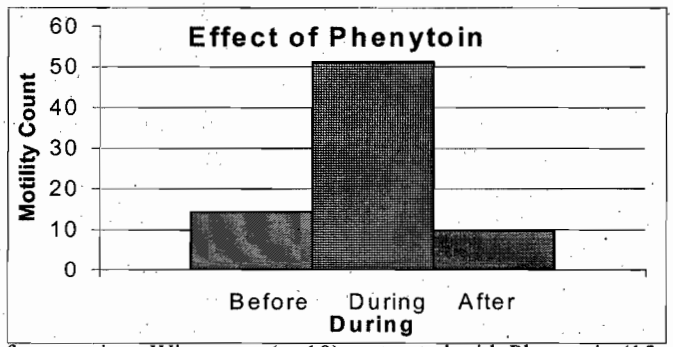

Figure 2: Motility count for conscious Wistar rats (n=10) pretreated with Phenytoin (15mg/1kg)

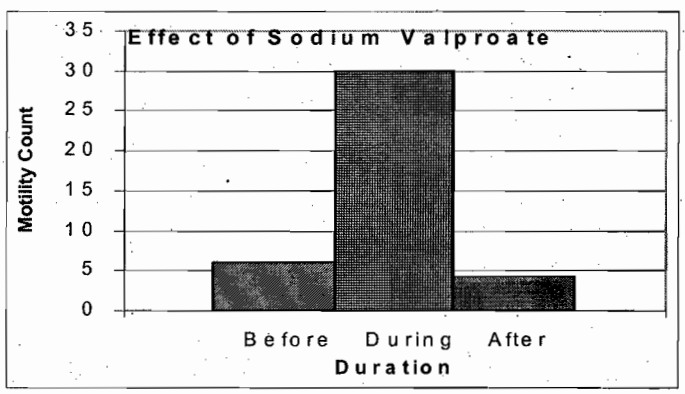

Figure 3: Motility count for conscious rats (n=10) pretreated with sodium valproate (20mg/1kg)

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