Effect of sodium valproate and flunarizine administered alone and in combination on pentylentetrazole model of absence seizures in rat

Prakash Shantilal, Joy David & Thangam Joseph*
Department of Pharmacology, St. John’s Medical College, Bangalore 560 034, India

Received 13 July 1998, revised 4 January 1999

Sodium valproate (VPA) and flunarizine (FLU) administered individually and together were examined for their effects on behavioural and EEG changes in the pentylentetrazole (PTZ) induced rat model of absence seizures. PTZ, 20 mg/kg, ip, produced behavioural staring and immobility with concomitant, repetitive 7 to 9 Hz spike/wave discharges (SWDs) in EEG, monitored continuously for 1 hr and thereafter, intermittently for 4 hr, post-vehicle/drug. The number and duration (sec) of SWDs/hr were the parameters used for evaluation of vehicle vs. drug effects in normal as well as rats made epileptogenic by repeated cortical stimulation. VPA, 200 mg/kg, ip, produced a significant reduction in the number and duration of SWDs at 20 min only in epileptogenic rats, declining to non-significant levels at 60 min, whereas FLU, 10 mg/kg ip had no effect on either parameter. The combination of VPA and FLU produced a highly significant reduction of the number and duration of SWDs for 60 min in normal and epileptogenic rats. The results provide evidence for a synergistic effect of VPA and FLU in experimental absence seizures and possible potential benefit in pharmaco resistant seizures.

Absence seizures in humans are fundamentally different from any other kind of epileptic manifestations. Classical absence seizures (AS) which start and end abruptly and occur predominantly in children, are characterised by temporary interruption of ongoing activity, staring and unresponsiveness to external stimuli. Concurrently, EEG shows a generalised and bilaterally synchronous 3Hz spike/wave discharge (SWD), indicating that the EEG change is time locked to the behavioural absence. An useful pharmacological animal model displaying the characteristic behavioural and electrical correlates of AS, is induced by the administration of non-convulsive low doses of pentylentetrazole (PTZ), 20 mg/kg, ip, to rats. This model is also pharmacologically unique as SWDs are inhibited by sodium valproate (VPA) and ethosuximide (ESM) and exacerbated by phenytoin (PHT) and carbamazepine (CBZ) as seen clinically.

In previous studies, we have shown that a low dose of flunarizine (FLU), a Ca2+ channel antagonist, enhanced the anticonvulsant effect of an ED50 dose of VPA to 100% in the maximal electroshock seizure test as well as in the PTZ seizure test in mice. We also demonstrated that the combination of VPA and FLU showed a pharmacological synergistic effect in cortical seizure threshold in rats, which is a model of clinical generalized tonic clonic seizures. Our findings suggest that low doses of FLU combined with VPA showed additive effects in experimental anticonvulsant tests. Other workers have also shown that FLU combined with phenytoin and carbamazepine had an anticonvulsant effect in the PTZ seizure incidence/latency test in mice. Hence, in view of the anticonvulsant efficacy and low toxicity of FLU in animal as well as clinical studies, the present study was designed to determine the interaction between FLU and VPA, beneficial or otherwise, on behavioural and electrical correlates on PTZ induced absence seizures in normal and epileptogenic rats.

Materials and Methods

Animals—Male Wistar rats 200 to 220g (n=8 per drug dose) were used for all the experiments and surgical procedures were carried out under pentobarbitone anaesthesia, 30 mg/kg.

Drug Treatment—All drugs were injected intraperitoneally. Pentylentetrazole, (PTZ, Sigma Laboratories), 20 mg/kg was dissolved in saline and Flunarizine (FLU, Torrent Pharmaceuticals), 10 mg/kg was dissolved in 50% polyethylene glycol and 50% distilled water. Sodium valproate (VPA, Reckitt & Colman, India) 200 mg/kg, was dissolved in distilled water. Drugs were administered 10 min after the challenge dose of PTZ. FLU 10 mg/kg and VPA 200

*Correspondent author
mg/kg (hereinafter referred to as the 'combination'), was injected independently in the right and left lower abdominal regions. In view of the long half life of FLU\(^6\) the combination was given after a wash out period of 18 days. The same rats were used for control and drug studies and controls received the corresponding vehicles.

**Normal rats**—Rats were chronically implanted with four extradural fronto-parietal electrodes and one electrode on the frontal sinus serving as reference\(^7\). Baseline EEG recordings for 20 min, were done in conscious rats, one week after surgery in order to detect EEG evidence of abnormal spikes, sharp waves or spontaneous seizure activity. Rats with abnormal EEG activity were excluded from the study. During EEG recording sessions, the rats were placed in a large circular perspex chamber of diameter 30 cm and continuously watched for behavioural changes.

**Epileptogenic rats**—A parallel group of rats, already chronically implanted with extradural electrodes (vide supra), were stimulated twice daily for 30 days via cortical leads, until severe and forceful clonic jerks of the forelimbs progressing to vigorous clonic activity occurred\(^8\). These rats were potentially epileptogenic and prior to PTZ challenge, showed abnormal baseline EEG patterns, with spikes, polyspikes, dysrhythmia and hypersynchronous discharges. However, they had no visible spontaneous seizures.

**Non-convulsive PTZ dosing and EEG recording**—Normal (n=8) and epileptogenic rats (n=7) were habituated to the EEG recording chamber, with subdued background lighting and constant temperature 28\(^\circ\)C ± 2\(^\circ\)C. A baseline EEG was run for about 20 min at a speed of 25 mm/sec. After administration of PTZ 20 mg/kg (as a dose of 10 mg/kg as suggested by Marescaux et al.\(^2\)), was insufficient to produce reproducible spike/wave discharges, SWDs, in EEG, in our experiments). Behavioural changes were continuously monitored while the EEG was scanned for the occurrence of concurrent SWDs for 1 hr for quantitative assessment. Thereafter, recording was done intermittently at 15 min intervals for 3 to 4 hr, in order to qualitatively observe occurrence as well as cessation of SWDs in control and drug treated groups.

**Behavioural and EEG effects**—The behavioural effects seen after drugging were also evaluated\(^9\) and the quantitative analysis of the SWDs, described below followed the method described\(^1\), with some modifications. The latency to the onset of the first SWD (in min), the number and duration of SWDs/h encountered after PTZ, as well as morphological aspects of the SWD patterns, was determined from the EEG recording.

**Statistical Analysis—SWD Number and Duration**—The ratio of SWDs post drug/SWDs pre drug for each animal was expressed as a percentage. The ratios were calculated for epochs of 20 min (1st, 2nd and 3rd), and for the overall 60 min period. This ensured normalization of the drug effect in each animal against its own baseline SWD number and reduced inter-animal variability. The effect of VPA, FLU and the combination of VPA and FLU over each epoch was compared using the paired \(t\)-test with Bonferroni correction\(^5\). The mean cumulative SWDs of the total 60 min period for each group was calculated and were statistically compared using the one way analysis of variance (ANOVA) followed by Fisher’s test of least significance. Statistical significance was validated at \(P < 0.05\)\(^6\).

The duration of SWD for each animal during each 20 min epoch was measured from the EEG record and the cumulative duration for each epoch and for the total of 60 min was calculated. Normalization and statistical analyses were carried out as explained for analysis of number of SWDs, described above.

**Results**

**Behavioural effects of VPA, FLU and the combination in normal and epileptogenic rats**—In normal and epileptogenic rats, FLU 10 mg/kg showed no behavioural changes when compared with vehicle treated controls. VPA 200 mg/kg showed mild sedation, ataxia, hypotonia and "wet dog shakes" (WDS). The WDS appeared within 5 min and lasted for about 45 min, while the other effects lasted for 1 to 2 hr. The combination produced mild sedation, reduced motor activity without ataxia. No specific behavioural changes occurred when PTZ was co-administered with the test drugs in both groups of rats.

**EEG Changes in normal and epileptogenic rats following PTZ challenge**—In normal rats (n=8), PTZ, 20 mg/kg, produced bilaterally synchronous spike/wave discharges (SWDs) in the EEG as shown in Fig 1. The latency (time to onset of first SWD) was 3±0.36 min. The number of SWDs in the first hour was 95.12±11.9. The SWDs occurred 1.6±0.2 times a minute, with a frequency of 7 to 9 Hz, 50 to 150 \(\mu\)V amplitude, and a mean duration of 1.9 ± 0.22 sec. The SWDs were always accompanied by behavioural signs of "absence" characterized by arrest of ongoing
activity, staring and unresponsiveness to external stimuli. No visible or EEG evidence of clonic seizures were seen pre and post PTZ.

In epileptogenic rats (n=7), PTZ, 20 mg/kg, produced 2.7 ± 0.4 SWDs/min [a 68.8% increase compared to normal rats (P<0.05)], while the latency, 2.8±0.18, mean duration of 1.4±0.08 min, frequency and amplitude were not significantly different from normal rats. The major difference compared to normal rats, was that although no visible seizures occurred prior to PTZ, several myoclonic jerks and mild to severe clonic seizures were observed in all the rats after PTZ, suggesting that the epileptogenic rats had a lowered seizure threshold to an equivalent dose of PTZ.

Effect of VPA, FLU and the combination on SWD after PTZ challenge in normal and epileptogenic rats — SWD Number as a function of time — The effects of VPA, FLU and the combination as a function of time (20 min epochs) as compared to controls (SWD number post drug / SWD number pre drug), expressed as a percentage in normal and epileptogenic rats is shown in Fig 2. VPA reduced the % SWD number in both normal and epileptogenic rats. The maximum reduction occurred in the first 20 min epoch in both groups, however this suppressant effect was significant only in the epileptogenic rats (P<0.05). The suppressant effect of VPA declined in both groups and reached pre drug values at 60 min.

In the case of FLU, the SWD number remained above controls throughout the 60 min test period in both normal and epileptogenic rats, the effects on the latter group being more prominent. However, statistical significance could not be obtained for any of these differences as the rats exhibited variability in their responses, resulting in higher standard errors.

When VPA and FLU were given together a significant (P<0.05) reduction in the number of SWDs was seen for the entire 60 min period, with respect to controls in both normal and epileptogenic rats, the maximum effect occurring at 60 min.

SWD number cumulative — The cumulative SWDs/hr over the 60 min test period in normal and epileptogenic rats are shown in Fig. 2 (bar diagrams). One way ANOVA revealed that the effects were significantly different between the three treatment groups F(df 3,31)=10.38, and F(df 3,27)=31.73, in normal and epileptogenic rats respectively, (P<0.001). While VPA reduced cumulative SWD number (64.3±8.6 and 63.4±9.4 % of controls in normal and epileptogenic rats respectively), FLU did not significantly influence the SWD number (121.7±24.7 %) in normal rats, as well as for epileptogenic rats (167.2±20.2 %). When FLU and VPA were given
Fig. 2—Number of spike/wave discharges (SWDs) as a percentage is represented as a function of time. On the left, the line diagrams represent the number of SWDs at 20, 40 and 60 min and on the right, is the cumulative SWD number for 60 min.* P < 0.05. Abbreviations: VPA: sodium valproate, FLU: flunarizine.

Fig. 3—Duration of spike/wave discharges (SWDs) as a percentage is represented as a function of time. On the left, the line diagrams represent the duration of SWDs at 20, 40 and 60 min and on the right, is the cumulative SWD duration for 60 min.* P < 0.05. Abbreviations are same as Fig. 2.
together, FLU potentiated the seizure suppressant effect of VPA, reducing the seizure number to 3.9±1.6 and 16.3±3.7 % in normal and epileptogenic rats respectively (P < 0.05).

**SWD duration as a function of time**—As observed with SWD number, VPA likewise reduced SWD duration in both normal and epileptogenic rats, maximum effect being in the first 20 min (Fig 3). This effect was statistically significant only in epileptogenic rats (P < 0.05). The seizure duration declined thereafter, reaching pre drug levels at 60 min.

In normal rats, FLU marginally reduced SWD duration at 20 min, increased at 40 min and the duration tended to return to predrug values at 60 min. However, in epileptogenic rats, SWD duration was increased throughout 60 min, although statistical significance could not be obtained due to high variabilities in responses.

The combination of VPA and FLU produced marked reductions in SWD duration at 20,40 and 60 min, in both groups, all differences being statistically significant (P < 0.05) with respect to controls.

**SWD duration cumulative**—The cumulative SWD duration over the 60 min period in normal and epileptogenic rats are shown in Fig. 3 (bar diagrams). The SWD duration was significantly different between the treatment groups as revealed by one way ANOVA (F df 3,31=12.68; and F df 3,27=30.09; for normal and epileptogenic rats respectively, (P < 0.001). While VPA reduced cumulative SWD duration in normal and epileptogenic rats respectively (P < 0.05 with respect to controls), FLU did not significantly influence the SWD duration (105.9±26 %) in normal rats but the reverse was true for epileptogenic rats (190.4±25.2 %). When VPA and FLU were given together, FLU potentiated the seizure suppressant effect of VPA in normal and epileptogenic rats (P < 0.05).

**Discussion**

Many genetic and pharmacological models of Absence Seizures(AS) have been described. Each model has unique advantages and disadvantages for studying the diverse mechanisms that appear to underlie typical AS. Our objective in this study was to provide supporting evidence for newer pharmacological approaches in the management of AS. Another goal was to address the problem of pharmacoresistant epilepsy, as a part of our continuing endeavor in experimental epilepsy. The pentylenetetrazole (PTZ) rat model of AS used in our study, shows electro-clinical analogies with human AS, and the dose/efficacy pharmacological responsiveness mimics the clinical situation.

The results of this study showed that PTZ, 20 mg/kg in normal rats, produced SWDs, the number and duration/hour being comparable to that obtained previously, while the behavioural aspects were also identical. There was a 68% increase (P < 0.05 ) in the number of SWDs/hour occurring in rats made epileptogenic by repeated direct cortical stimulation compared to normal, suggesting that the seizure threshold was lowered in epileptic rats. The pharmacological responses to VPA, 200 mg/kg in both normal and epileptic rats, were comparable to that previously obtained by Marescaux et al., 1984.

The Ca2+ channel antagonist, flunarizine (FLU), readily crosses the blood brain barrier and is thought to inhibit the entry of Ca2+ into neurones primarily under pathophysiological conditions, such as ischaemia or seizure activity, but has no effect on normal Ca2+ homeostasis. Hence, it has been proposed as a promising candidate, particularly as add-on therapy for pharmacoresistant epilepsy. In several controlled clinical trials, FLU has been shown to decrease seizure frequency in therapy resistant patients, being treated with established antiepileptic drugs. FLU has been shown to have anticonvulsant activity in the maximal electroshock and metrazol induced seizures in rats. FLU 20 mg/kg with VPA 200 mg/kg did show an inhibitory effect on SWD discharges, but we have previously shown that this dose combination has side effects of ataxia and hypotonia. For this reason we reported only the lower dose of FLU, 10mg/kg in combination with VPA, as this showed potentiation in the absence of adverse effects, an aspect which is deemed important for clinical extrapolation of pharmacological data.

A combination of FLU with VPA yielded promising results in experimental models of generalized seizures. The natural outcome of our earlier studies was to determine whether a combination of VPA and FLU would demonstrate similar/superior efficacy in the PTZ model of AS. Using this model, VPA combined with FLU showed a marked and highly significant reduction in the number and duration of SWDs in normal as well as epileptogenic rats, suggesting that this drug interaction also has a synergistic effect in AS.

There is a possibility that FLU may exert its anticonvulsant effect by interacting with voltage
dependent Na\(^+\) channels (like VPA) in addition to the selective blockade of Ca\(^2+\) channels. Moreover, the dose of FLU employed in this study produced its anticonvulsant effects with no adverse effects. Nuglisch et al.\(^{20}\) have shown that an oral dose of FLU, 40 mg/kg produced no changes on mean arterial blood pressure in rats.

Two general hypotheses have been proposed to explain the mechanism of action of VPA viz. blocking of voltage dependent Na\(^+\) channels and enhancing GABA mediated inhibition. FLU possesses a similar anticonvulsant profile to carbamazepine and phenytoin and this suggests the possibility of an analogous mechanism of action on Na\(^+\) channels. FLU does not increase GABAergic inhibition, but it readily crosses the blood brain barrier and also blocks T type calcium channels. However, it is not known which of these actions contributes to its anticonvulsant actions. For the present, the pharmacological mechanism underlying the enhanced effect of these two drugs must await further elucidation.

The pharmacological rationale of combining FLU with VPA, was to take advantage of their differing mechanisms of action to produce a supra-additive effect and converge to a common CNS target in a synergistic manner. However, these are speculative explanations and definitive evidence must await further elucidation.

In conclusion, FLU has been shown to potentiate the activity of VPA against PTZ induced absence seizures in normal and epileptogenic rats, at well tolerated dose combinations employed in this study.

References: