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COMPARATIVE PROFILES OF SODIUM VALPROATE AND ETHOSUXIMIDE ON ELECTRO-BEHAVIOURAL CORRELATES IN γ-HYDROXYBUTYRATE AND PENTYLENETETRAZOL INDUCED ABSENCE SEIZURES IN RATS

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Abstract : Sodium valproate (VPA) and ethosuximide (ESM) were compared on behavioural and EEG changes in γ-hydroxybutyrate (GHB) and pentylenetetrazole (PTZ) rat models of Absence Seizures (AS). Both GHB, 100 mg/kg ip and PTZ, 20 mg/kg ip, produced repetitive episodes of staring and immobility with concomitant 6 to 9 Hz spike and wave discharges (SWDs) in the EEG. The parameters used for drug evaluation were the number and duration of SWDs/hour. Though the number of SWDs/hour produced by GHB and PTZ were not significantly different, the duration of SWDs was significantly longer in GHB treated rats ($P<0.001$) VPA and ESM, at 200 mg/kg ip, reduced SWD number and duration in GHB pretreated rats, whereas ESM, 50 mg/kg *ip*, was four times more effective than VPA, 200 mg/kg ip, in the PTZ model. Phenytoin (PHY) 20 and Carbamazepine (CBZ) 10 mg/kg ip, worsened AS, a feature which has also been reported clinically. Both rat models of experimental AS can be used to defect potential anti-absence activity in new chemical entities.

Key words : sodium valproate, EEG, γ-hydroxybutyrate, pentylenetetrazol, ethosuximide, absence seizures, experimental, spike wave discharges

INTRODUCTION

Experimental models of epilepsy are often used to explore seizure mechanisms and delineate pharmacological profiles of activity of antiepileptic drugs. Two well known models are γ-hydroxybutyrate (GHB) and pentylenetetrazole (PTZ) induced absence seizures (AS) in rats, which mimic behavioral and EEG characteristics of human absence seizures (1–4). Well established anti-absence drugs such as sodium valproate (VPA) and ethosuximide (ESM) are effective in these models. However, the effects of phenytoin (PHY) and carbamazepine (CBZ) which are known to aggravate AS both clinically and experimentally (4) have not been clearly defined in the GHB rat model.

The commonly employed screening technique to determine efficacy in AS is the PTZ test in mice (2). In PTZ induced AS in rats, induced by non-convulsive doses of PTZ, the electro-clinical features are specifically inhibited by VPA, as well as

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ESM in a dose dependent fashion (5) and are exacerbated by PHY and CBZ (4).

The objective of this study was to determine: (a) the comparative profiles of VPA and ESM in the above two models (b) if PHY and CBZ exacerbated electro-clinical features of rat AS and (c) to assess the comparative ability of the GHB and PTZ models to predict pharmacological activity in clinically useful as well as candidate anti-absence agents.

METHODS

Experimental animals: Under pentobarbitone 35 mg/kg ip anesthesia, male Wistar rats, 200 to 220 g, were chronically implanted with four extradural fronto-parietal electrodes and one electrode on the frontal sinus serving as reference with the help of the stereotaxic device (INCO Ambala) (5). The co-ordinates were as follows: left and right frontal; 1.5 mm posterior to the bregma and 4 mm lateral to the left (left frontal) and to the right (right frontal). The left and right parietal, 4.5 mm posterior to the bregma and 4 mm lateral to the left (left parietal) and to the right (right parietal). They were fed *ad libitum* with food and water except during experimental sessions. Each experimental group comprising of eight rats were used and experiments were carried out in the forenoon each day. The same rats were used for vehicle control and drug studies.

EEG recordings: Baseline EEG recordings for 15 mins was done with a Grass Model 78 D Polygraph and 14 Channel recording system after a one week post operative period. Animals showing visible jerks or EEG evidence of spikes or spontaneous seizure activity were eliminated from the experiments. The rats were habituated to the EEG recording chamber with subdued background lighting and constant temperature 28 ± 2°C. During EEG recording sessions, naive as well as drug treated rats were placed in a large circular perspex chamber of diameter 30 cms and continuously watched for behavioural changes. Following baseline EEG recording and vehicle/drug treatment, the EEG was recorded continuously at a speed of 25 mm/sec for 60 mins. Thereafter, recording was done intermittently at 15 min intervals for 3 to 4 hours in order to qualitatively observe occurrence as well as cessation of spike/wave discharges (SWDs).

Behavioural aspects and quantitative analysis of SWDs: The behavioural effects seen after drug administration were evaluated (7) and helped to ensure that any sedation occurring during EEG recording sessions would not interfere with the actual number and duration of the SWDs. The quantitative analysis of the SWDs followed the method described (4, 5). Following GHB, 100 mg/kg ip, or PTZ, 20 mg/kg ip, the EEG was recorded at a speed of 25 mm/sec continuously for 60 mins, in order to facilitate recognition and estimation of the number as well as the duration of the SWDs. The number and duration of the SWDs were estimated for each 20 min epoch as well as for the cumulative 60 min period. The following parameters were obtained post drug from the EEG records: (a) latency (in mins) or time to onset of first SWD, and (b) number and duration (in secs) of SWDs/hour.

Drugs: All drugs were given

intrapertoneally. γ-Hyroxybutyrate (GHB, Sigma) 100 mg/kg and pentylenetetrazol (PTZ, Boehringer Knoll, India) 20 mg/kg were dissolved in saline. Two anti-absence drugs, sodium valproate (VPA, Reckitt and Colman, India Ltd.,) 200 mg/kg and ethosuximide (ESM, Parke Davis, India Ltd.,) 50 mg/kg were dissolved in saline. Phenytoin sodium, 20 mg/kg (PHY, Boots, India Ltd.,) was dissolved in distilled water with 1 or 2 drops of 1N NaOH. Carbamazepine, 10 mg/kg (CBZ, Novartis India, Limited) was dissolved in 50% propylene glycol and saline 50%, the mixture being gently warmed in a water bath till a clear solution was obtained. PHY and CBZ are drugs of choice for generalised tonic clonic seizures (GTCS). An interval of 15 mins following GHB and PTZ, was allowed for stabilisation of the SWDs appearing in the EEG, before VPA, ESM, PHY or CBZ were administered.

Rationale for selection of the doses : VPA, 200 mg/kg, ESM, 50 mg/kg, PHY, 20 mg/kg and CBZ 10 mg/kg were the doses employed as these doses were the established effective anticonvulsant doses in our laboratory (6).

Statistical Analysis : The ratio of number and duration of SWDs post drug/number and duration SWDs pre drug for each animal was expressed as a percentage, for all 20 min epochs as well as the cumulative 60 mins period as suggested by previous workers (4). This ensured normalization of the drug effect in each animal against its own baseline number and duration of SWDs and reduced inter-animal variability. The control values were expressed as hundred percent and was not statistically different from baseline PTZ/GHB data as shown in Table I. The percentage conversion of the data was conformed to as described earlier (4). The paired "t" test was used to compare the effects of VPA and ESM with their respective control for each 20 min epoch. The mean cumulative SWD number and duration for the entire 60 min period for each group was statistically compared using One Way ANOVA, followed by Fisher's test of least significance. Statistical significance was validated at $P<0.05$.

TABLE I : Characteristics of EEG parameters of spike wave discharge (SWDs) in GHB & PTZ treated rats.

EEG parameters	*GHB 100 mg/kg*	*PTZ 20 mg/kg*
Latency (mins)	5.2±0.43	5.3±0.74
SWDs		
Epoch 1		
Number	48.25±10.1	36.25±6.11
Duration	2.80±0.88	1.07±0.17
Epoch 2		
Number	41.0±5.07	33.13±4.7
Duration	4.42±1.36	1.05±0.19
Epoch 3		
Number	27.13±6.24	23.38±8.34
Duration	4.6±2.2	0.86±0.4
Cumulative		
Number	116.4±10.2	95.1±11.9
Duration	11.9±3.7	2.7±0.2*

Values are mean ± SEM [n = 8/drug group]. SWD duration in seconds.

GHB = γ-hydroxybutyrate. PTZ = Pentylene tetrazol

Statistical analysis :

GHB Vs PTZ at each epoch by paired 't' test-No significant difference between the 2 groups on any of the parameters at any time point. Cumulative parameters were examined by One Way ANOVA. *P<0.001

RESULTS

Behavioral effects of PTZ, GHB and anti-epileptic drugs in rats: No significant behavioural changes were seen following PTZ or PHY, 20 mg/kg, while CBZ at 10 mg/kg produced mild ataxia. GHB, 100 mg/kg, produced initial excitation, piloerection and increased movement followed by quiescence when compared with saline treated controls. VPA, 200 mg/kg, showed sedation, ataxia, hypotonia and "wet dog shakes" (WDS). Which appeared within 5 min and lasted for about 45 min, while other effects lasted for 1 to 2 hours. ESM, 50 mg/kg, showed no significant behavioural effects.

EEG Changes following GHB and PTZ challenge: GHB, 100 mg/kg and PTZ, 20 mg/kg (n = 8/ drug group) produced bilaterally synchronous spike/wave discharges (SWDs) in the EEG as shown in Fig. 1. The conformation of the spike and wave in SWD discharges are better seen in GHB treated rats (Fig. 1 A) with morphological similarity to the human EEG in clinical AS, whereas Fig 1 B (PTZ treated rats) shows that the spikes are more prominent, indicating that the excitatory phase (represented by the spike) is more dominant as compared to the GHB rats, in which the inhibitory phase (represented by the wave) was more prominent. The SWDs of both GHB and PTZ treated rats had a frequency of 6 to 9 Hz and amplitude ranging from 50 to 200 μV. (These SWDs were always accompanied by behavioural signs of "absence" which were characterized by arrest of ongoing activity, staring and unresponsiveness to external stimuli).

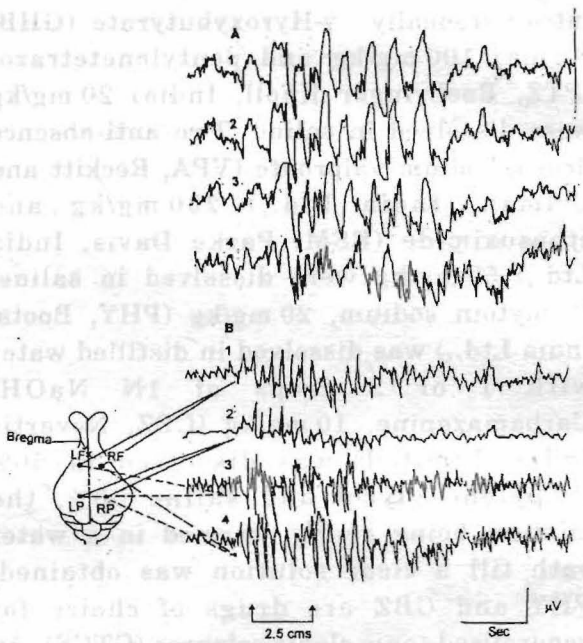

Fig. 1: γ-Hydroxybutyrate (GHB), 100 mg/kg ip, (A) and Pentylenetetrazol (PTZ), 20 mg/kg ip (B) induced spike-wave discharges (SWD) in cortical EEG's of rats. On the left in Fig. 1 B, is shown the skull montage (not to scale) diagram, indicating the areas of placement of cortical leads with reference to Bregma. The EEG recordings in A and B are from leads 1: left frontal (LF) to right frontal (RF); 2: left parietal (LP) to right parietal (RP); 3: left frontal to left parietal and 4: right frontal to right parietal. The calibration signal, shown at the bottom of B, is the same for both the GHB and PTZ EEG recordings.

The latency period (time to onset of the first SWD), and the number and duration of SWDs in GHB and PTZ treated rats over 20 min epochs and cumulatively for one hour are given in Table I. The latency period after GHB and PTZ were almost similar and were 5.2 ± 0.43 and 5.3 ± 0.74 mins, respectively None of the antiepleptic drugs employed influenced the SWD latency in a significant manner, in either of the two groups.

SWD number: Table I also shows that with GHB alone, the number of SWDs gradually declined from 20 to 60 mins (all epoch times mentioned hereafter are in sequential order viz., 20, 40 and 60 mins, 48.25 ± 10.1; 41.0 ± 5.07 and 27.13 ± 6.24, respectively). A similar trend in time course was also shown by PTZ (36.25 ± 6.11; 33.13 ± 4.7 and 23.38 ± 8.34). Although the number of SWDs produced by PTZ was lower than that produced by GHB, there were no statistically significant differences at any time point. Though the cumulative number of SWDs/hour in the GHB rats, 116.4 ± 10.2, was numerically greater than in PTZ rats, 95.1 ± 11.9, the difference was not statistically significant as tested by One Way ANOVA ($F = 1.59$ df 1, 14; $P<0.5$).

SWD duration: Table I indicates that the duration of SWDs (secs) in GHB treated rats showed a different trend as compared to the number, as it increased over a one hour period. In GHB rats, SWD durations were 2.8 ± 0.88; 4.42 ± 1.36 and 4.6 ± 2.2, while in PTZ rats the durations were 1.07 ± 0.17; 1.05 ± 0.19 and 0.86 ± 0.4 secs. Although PTZ produced SWDs of lesser durations compared to GHB at all time points, the differences were not statistically significant. When the cumulative duration of SWDs for one hour was compared, the duration was significantly lower in the PTZ group when compared to the GHB group, as shown by One Way ANOVA, 11.9 ± 3.7 (GHB) and 2.7 ± 0.2 secs (PTZ) respectively ($F = 5.61$ df 1, 14, $P<0.001$).

Effect of antiepileptic drugs on SWD number and duration induced by GHB and PTZ: In GHB pretreated rats neither PHY 20 mg/kg nor CBZ 10 mg/kg significantly altered the cumulative SWD number or duration/hour. PHY and CBZ, induced changes in cumulative SWD number from a baseline value of 116.4 ± 10.2 to 112.7 ± 11.2 and 87.7 ± 13.9 respectively. Likewise, SWD durations for PHY or CBZ were 8.4 ± 0.58 secs and 8.2 ± 0.05 secs respectively, as compared to the GHB control value of 11.9 ± 3.7. In rats treated with PTZ, and subsequently with the same doses of either PHY or CBZ, repeated clonic seizures were seen and some rats developed absence seizure status, impeding quantitative evaluation.

In contrast to the results obtained with specific drugs, PHY or CBZ, for generalised tonic clonic seizures, the standard anti-absence drugs of choice, VPA or ESM were highly effective in reducing the number and duration of SWDs in GHB or PTZ induced absence seizures in the EEG.

In Table II are shown the comparative effects of VPA and ESM on the SWD number and duration as a function of time, as well as cumulatively, in GHB and PTZ challenged rats, expressed as percentages of control values.

VPA showed significant reduction in SWD number at all epochs in GHB treated animals (61.7 ± 14.9 $P<0.05$; 51 ± 13.1 $P<0.01$ and 61.9 ± 15.1 $P<0.05$). In contrast, no significant reduction (NS) was seen at any epoch in PTZ treated animals (52.9 ± 18.5 NS; 82.2 ± 20 NS and 95.8 ± 17 NS). On the other hand, ESM showed a highly significant reduction in SWD number both GHB and PTZ treated rats at all epochs. In GHB treated rats (41.2 ± 7.6 $P<0.001$; 32.6 ± 5.7 $P<0.001$ and

TABLE II: Effect of VPA & ESM on SWD number and duration as a function of time and cumulative over a 60 min period (All values are percentages Mean ± SEM, compared with baseline GBH/PTZ as 100%).

Period	*SWD*	*GHB 100 mg/kg*		*PTZ 20 mg/kg*	
		VPA 200 mg/kg	*ESM 200 mg/kg*	*VPA 200 mg/kg*	*ESM 50 mg/kg*
Epoch 1	Number	61.7±14.9*	41.2±7.6***	52.9±18.5	13.9±6.0**
	Duration	63.5±17.9*	35.7±11.7***	47.9±16.4**	10.5±3.0**
Epoch 2	Number	51.0±13.1**	32.6±5.7***	82.2±20.0	13.3±1.6**
	Duration	24.5±7.9***	15.7±5.6***	74.3±27.0	1.33±0.04**••
Epoch 3	Number	61.9±15.1*	45.8±8.3***	95.8±17.0	0.0±0.00*••
	Duration	31.3±13.5***	31.1±12.1***	86.8±2.1	5.24±5.3**
Cumulative	Number	50.9±7.6***	34.2±3.1***•	62.6±8.4***	7.4±2.5***•
	Duration	30.5±8.7***	16.6±4.4***•	50.8±4.6***	6.0±2.0***•

Each epoch indicates 20 min duration

[i] The stars indicate statistical analysis by paired 't' test at each epoch and by One Way ANOVA for cumulative against controls as 100%

*P<0.05 **P<0.01 ***P<0.001

[ii] The dots indicate significant differences between the effects of ESM & VPA in the particular model analysed by-paired 't' test at each epoch and One Way ANOVA for cumulative, followed by Fisher's test of least significance

•P<0.05 ••P<0.01 •••P<0.001

45.8 ± 8.3 P<0.001) and in PTZ rats 13.9 ± 6 P<0.01; 13.3 ± 1.6 P<0.01 at 20 and 40 mins respectively. At 60 mins, no SWDs were seen following ESM in PTZ treated rats.

Like wise, VPA showed significant reduction in SWD duration in GHB treated rats, but not in PTZ treated rats. In GHB rats, (63.5 ± 17.9 P<0.05; 24.5 ± 7.9 P<0.001 and 31.3 ± 13.5 P<0.001 at 20, 40 and 60 mins.) In PTZ treated rats, it was significant only at 20 mins (47.9 ± 16.4 P<0.01) and not significant at 40 and 60 mins (74.3 ± 27 and 86.8 ± 2.1, respectively). On the other hand, ESM showed a highly significant reduction in SWD duration in both GHB and PTZ treated rats. In GHB rats, (35.7 ± 11.7 P<0.001; 15.7 ± 5.6 P<0.001 and 31.1 ± 12.1 P<0.001 at 20, 40 and 60 mins). Likewise in PTZ treated rats the values were for the same time periods, 10.5 ± 3.0 P<0.01; 1.33 ± 0.04 P<0.01 and 5.24 ± 5.3 P<0.01.

When the cumulative effects of VPA and ESM on SWD number and duration for the entire hour were compared, One Way ANOVA revealed a significant difference between the means of the two groups for both number ($F = 39.33$ df 2, 16; $P<0.001$) and for duration ($F = 150.09$ df 2, 16; $P<0.001$). Fisher's test of least significance revealed that the inhibitory effect of SWD parameters was significantly greater for ESM rather than VPA, in rat models, GHB and PTZ of absence seizures ($P<0.05$).

In the PTZ model of absence seizures, ESM at one-fourth the dose of VPA, was highly significantly effective on both the cumulative number and duration of SWDs.

DISCUSSION

GHB and PTZ produce a predictable sequence of behavioural and EEG events when administered to rats, showing electro-clinical analogies to human Absence Seizures (AS), or non-convulsive forms of generalized epilepsy (3, 4). The results obtained in this study show that their pharmacological reactivity, to both VPA and ESM, is the same as that obtained in humans (8, 12). However, differences are apparent when EEG characteristics of animal and human are compared. In humans, spike and wave frequencies are usually 3Hz, but in rats, both types of chemically induced seizures evoked 6 to 9 Hz spike/wave activity. A second difference is that clinical manifestations of AS accompanied by EEG 3 Hz spike/wave activity, appear predominantly in children, whereas in our experiments similar features were elicited in adult rats.

AS remain as one of the most enigmatic of neurological disorders and there is no widely accepted theory regarding its aetiology. Its cause is increasingly regarded as genetic (8, 13). The SWD's seem to be provoked by an abnormal oscillatory pattern of discharges that involve a thalamo-cortical loop (10, 11). They are also considered to be pharmacologically unique, as VPA, ESM and trimethadione (the last is not used now) are solely effective in AS. Reciprocally, antiepileptic drugs (AEDs) which are effective in generalised tonic clonic convulsions, such as PHY and CBZ, are known to make AS worse (9).

VPA and ESM were effective in reducing the number and duration of SWD's in both rats models of AS in these experiments. A double blind crossover study compared the efficacy of VPA and ESM in patients with AS (13, 14). VPA was as effective as ESM in reducing the number and duration of SWDs in patients. Likewise, our results in rats also demonstrated similar findings with VPA and ESM. In GHB induced experimental AS, VPA and ESM at 200 mg/kg and 50 mg/kg respectively, inhibited the cumulative SWD number and duration/hour to a highly significant extent. On the other hand, it was clear that ESM was at least four times more active than VPA in PTZ induced AS in rats. There is no easy explanation for the superior efficacy of ESM. The pharmacodynamic effect that could contribute to this difference is that ESM exerts its anticonvulsant effect by specific inhibition of T and L type Ca^{2+} channels in thalamo-cortical pathways, whereas VPA is not known to possess this activity (12). Moreover, pharmacokinetic aspects of VPA show that it has a very short half life in humans and animals (12). The utility of these models for detecting potential anti-absence activity has been substantiated in these experiments. Another important aspect of the above two rat models that are predictive of human responses, was that both PHY and CBZ failed to influence SWD number and duration, while PHY caused "absence status" in some rats.

Thus, pharmacological validation of clinical findings with established anti-absence agents, ensures that these tests offer distinct advantages for the following reasons: (a) detecting anti-absence properties of new chemical entities (b) prediction of side effects, viz. sedation, ataxia, hypotonia, stereotype etc., is possible

as conscious, trained, minimally restrained animals are used (c) since single doses of drugs are examined, the frequency of drug testing of a chemical series is accelerated following adequate wash-out periods (d) from the results obtained with all the anti-epileptic drugs employed in this study, it would appear that a good correlation exists between their pharmacological ability to control/exacerbate behavioral and electrographic features of AS.

In conclusion, both the GHB and PTZ rat models of generalised AS meet all criteria proposed (to date) for experimental AS and are useful models for the neurophysiological and pharmacological study of bilaterally synchronous SWD production.

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REFERENCES

1. Rodger C, Pleuvry BJ. Protective effect of flunarizine and nifedipine alone and in combination with anticonvulsant drugs against PTZ induced seizures in mice. *Neuropharm* 1993; 32: 257–263.
2. Joseph S, David J, Joseph T. Additive anticonvulsant effect of flunarizine and sodium valproate on electroshock and chemoshock induced seizures in mice. *Indian J Physiol Pharmacol* 1998; 42 (3): 383–388.
3. Snead OC. γ-Hydroxybutyrate Model of Generalized Absence seizures: Further Characterization and Comparison with other Absence Models. *Epilepsia* 1988; 29: 361–368.
4. Hosford DA, Wang Y, Liu CC, Snead OC. Characterization of the anti-absence effects of SCH 50911, a GABA$_B$ receptor antagonist, in the lethargic mouse, γ-hydroxybutyrate, and pentylenetetrazole models. *J Pharmacol & Exp Therap* 1995; 274: 1399–1403.
5. Prakash S, David J, Joseph T. The effect of sodium valproate and flunarizine administered alone and in combination on the pentylenetetrazole model of absence seizures in the rat. *Ind J Exp Biol* 1999; 37: 228–233.
6. Kulkarni C, Joseph T, David J, influence of adenosine receptor antagonist, aminophylline and caffeine, on seizure protective ability of antiepileptic drugs in rats. *Ind J Exp Biol* 1991; 29: 751–754.
7. Desmedt LKC, Niemegeers CJE, Janssen PAJ. Anticonvulsant properties of Cinnarizine and Flunarizine in rats and mice. *Arzneimittel Forsch* 1975; 25 (9): 1408–1413.
8. Marescaux C, Vergnes M. Animal models of absence seizures and absence epilepsies, in Typical Absences and related epileptic syndromes, edited by Duncan JS and Panayiotopoulos CP (B. I. Churchill Livingstone Pvt. Ltd. New Delhi) 1996, pp 8–18.
9. Loiseau P. Childhood absence epilepsy in Roger J, Bureau M, Dravet Ch, Dreifuss FE, Perret A, Wolf P (eds) Epileptic syndromes in infancy, childhood and adolescence John Libbey, London 1992 pp 135–150.
10. David J, Marathe SBM, Patil SD, Grewal RS. Behavioural and electrical correlates of absence seizures induced by thalamic stimulation in juvenile Rhesus monkeys with frontal aluminium hydroxide implants: a pharmacological evaluation. *J Pharmacol Methods* 1982; 7: 219–229.
11. Gloor P. Generalised epilepsy with spike-and wave discharges : a reinterpretation of its electrographic and clinical manifestations. *Epilepsia* 1979; 20: 1571–1588.
12. Rogawski MA, Porter RJ Antiepileptic drugs pharmacological mechanisms and clinical efficacy with consideration of developmental stage compounds. *Pharmacol Reviews* 1990; 42: 223–286.
13. Sato S, White BC, Penry JK, Dreifuss FE, Sackellares JC, Kupferberg HJ. Valproic acid versus ethosuximide in the treatment of absence seizures. *Neurology* 1982; 32: 157–163.
14. Redenbaugh JE, Sato S, Penry JK. Sodium valproate pharmacokinetic and effectiveness in treating intractable seizures. *Neurology* 1980; 30: 1–6.