Effects of vigabatrin on evoked potentials in dogs

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- 1 The purpose of this study was to evaluate possible changes in brain morphology and evoked potentials associated with daily administration of 300 mg kg⁻¹ vigabatrin in dogs.
- 2 Somatosensory evoked potentials (SEP) and auditory evoked potentials (AEP) were recorded at baseline and weekly for 12 weeks of treatment and every 2 weeks for 17 weeks of recovery. Morphology was assessed immediately after treatment for two treated dogs and after recovery for the remaining five treated and two control dogs.
- 3 Vigabatrin produced a significant slowing of the central transmission measure of the SEP with no alteration in the AEP. Vigabatrin was associated with microvacuolation in select regions of the brain including the fornix, septum, optic tract, hypothalamus, thalamus and cortex. In addition, some microglial proliferation was noted.
- 4 Changes in SEP and the microvacuolation fully recovered after 17 weeks of treatment.
- 5 The study confirms vigabatrin-induced microvacuolation in the dog and suggests these changes are associated with functional slowing of conduction in the somatosensory pathways.

Keywords evoked potentials GABA vacuolation vigabatrin

Introduction

γ -amino butyric acid (GABA) is ubiquitous within the CNS and clearly functions as an important inhibitory neurotransmitter (Iversen *et al.*, 1971; Iversen, 1982). Numerous studies point to the failure of GABAergic inhibition as a key factor in local and global seizures (see Roberts, 1986 for review). Reduction in the efficacy of the GABA system, such as that produced by interference with GABA synthesis or blockage of GABA receptors, has been associated with altered physiology and ultimately with seizures (Roberts, 1976; Tsumoto *et al.*, 1979; Sillito, 1984). Conversely, drugs that interfere with GABA-transaminase (GABA-T), blocking the major catabolic route for GABA, probably would increase available GABA and be efficacious for the treatment of epilepsy. Vigabatrin (γ -vinyl GABA) is an enzyme-activated, irreversible, GABA-T inhibitor that has shown considerable promise in single and double-blind

studies for the treatment seizure disorders in man (Gram *et al.*, 1983; Rimmer & Richens, 1984; Browne *et al.*, 1987). However, the clinical utility of this compound is potentially limited by the observation of microvacuolation within select CNS fibre tracts at high dose levels in the mouse, rat and dog (Yarrington *et al.*, 1985; Butler *et al.*, 1987).

In recent years computer averaged, sensory evoked potentials, have proven effective in detecting clinical and sub-clinical neuropathology (Desmedt & Noel, 1973; Halliday, 1982; Stockard & Iragui, 1984; Cracco & Bodis-Wollner, 1986). Recording from several modalities is particularly valuable in detecting and documenting white matter pathology (e.g. multiple sclerosis, leukodystrophies) affecting the speed of central conduction (Giesser *et al.*, 1987). Evoked potentials from the long tracts of the somatosensory system have demonstrated

early and reliable changes associated with toxic axonopathies and myelinopathies (Arezzo *et al.*, 1982, 1985). The present study was designed to assess the degree to which evoked potentials recorded from the intact scalp are sensitive to vigabatrin-induced pathology, and thus, the ability of these measures to serve as a monitoring tool during the administration of this drug.

Methods

Test drug

Vigabatrin (4-amino-hex-5-enoic acid) was supplied by Merrell Dow Research Institute (Cincinnati, Ohio) in capsular form. The drug was stored at room temperature for a period of no more than 2 weeks prior to administration.

Subjects

Ten pure bred adult beagles (Marshall Farms, North Rose, NY) were used in the study. The dogs weighed between 9.1 and 11.0 kg at the start of the study. They were housed in individual cages, fed a balanced diet (Purina pellets) and had continuous access to water. Throughout the study each dog was exercised for approximately 2 h daily by a primary animal caretaker. Dogs were weighed weekly and examined daily for alterations in behaviour or general health.

Study design

Eight dogs, comprising the treatment group, received $300 \text{ mg kg}^{-1} \text{ day}^{-1}$ of vigabatrin on a 7 day a week regimen for 12 weeks. Two dogs, comprising the control group, received a placebo capsule administered on the same regimen for the same period. Dogs were randomly assigned to treatment or control groups and there was no cross-over. Both treatment and control groups were housed, handled and tested in an identical manner. After 12 weeks, two of the treated dogs (selected randomly) were sacrificed for histological examination. The remaining treated and control dogs were allowed to recover for an additional 17 weeks, at which time they were sacrificed for histological examination.

Electrophysiological data were obtained from all dogs weekly during the treatment phase and every 2 weeks during the recovery phase. For technical reasons, one recovery assessment was spaced at 3 weeks. In addition, electrophysiological data were obtained for each dog prior to initiation of treatment (Baseline period). One treatment dog (#576492) died of pneumonia

within 3 weeks of commencing drug therapy and the electrophysiological data from this dog was not included in the study. An autopsy revealed no gross CNS pathology.

Electrophysiological procedures

During all recording sessions animals were tranquilized using a combination of ketamine-HCL (25 mg kg^{-1}) and xylazine (22 mg kg^{-1}) and placed in a support sling. Temperature and respiration were maintained within normal physiological limits throughout the recording sessions. Surface evoked potentials were recorded using sub-dermal needle electrodes (platinum-iridium). Recording locations were determined with reference to bony landmarks and were indelibly marked to facilitate constant electrode placement during subsequent recording sessions.

Neuroelectric signals were amplified with a gain of 5000 and a band pass (down 3dB) of 3 Hz to 3 kHz. EEG, along with a synchronizing pulse, was stored on analog FM magnetic tape (band pass 0–5 kHz). In parallel, the signals were digitized at a rate exceeding 5000 Hz and averaged on-line ($N = 200\text{--}500$) using a Nicolet Med 80 computer. The averaging programs provided for a pre-trigger epoch, artifact rejection and baseline correction. Latencies were scored from stimulus onset and amplitudes from a pre-stimulus baseline using computer cursors.

Auditory evoked potentials (AEP) were recorded to binaural, high intensity (80 db/SPL), 50 μs compression clicks delivered through headphones at a rate of 10 s^{-1} . The recording montage consisted of vertex to right mastoid and vertex to mid-inion configurations.

Somatosensory evoked potentials (SEP) were evoked by a 200 μs , constant current, electrical square wave delivered to the right forelimb (median nerve) at a rate of 2 s^{-1} . Stimulating electrodes were positioned with the anode 1.0 cm distal to the cathode, and current was adjusted to supra-maximal levels for the cervical spinal response (i.e. between 3.0 and 6.0 mA). The recording montage consisted of cervical spinal cord and contralateral sensorimotor cortex referenced to ipsilateral ear.

Histological procedures

At the conclusion of the experiment, animals were anaesthetized with sodium pentobarbitone and perfused through the heart with para-formaldehyde for 5 min and with 2.5% gluteraldehyde for 20 min. The brains were removed, sectioned into blocks, embedded in paraffin and

stained with H and E. Areas sampled for light microscopy included optic tract, optic chiasm, mesencephalon, fornix, cerebellum, cerebral cortex and cervical spinal cord. Additional sections were taken through the fornix, dehydrated and embedded in epoxy resin. One-micron sections were stained with toluidine blue. Thin sections were cut and stained with uranyl acetate and lead citrate and examined with the electron microscope.

Results

The study design does not allow simple data comparisons between the treated and control groups due to the small number of subjects ($n = 2$) in the control population. Assessment of longitudinal changes in the treated group are of critical importance in this study.

Treatment period

Shortly after initiating treatment, one dog became anorexic and died. Although pneumonia was the cause of death, drug induced anorexia (Yarrington, *et al.*, 1985) was probably contributory. There were no obvious changes in behaviour or general health for any of the dogs studied. Weight remained stable in both control and treated populations (Table 1). No overt changes in EEG were observed and there was no evidence of seizure.

The clearest finding was an increase in SEP central transmission time (CTT) for the vigabatrin group. CTT is defined as the difference in timing of the depolarization overlying the dorsal mid-cervical spinal cord and the peak of the negativity recorded overlying the contralateral sensorimotor cortex (Figure 1a). In the beagle, CTT is approximately 5.0 ms and reflects propagation of the fastest afferent activity within the

Table 1 Weight of dogs, SEP and AEP data, during treatment and control periods

Week	Treatment period					
	Vigabatrin group ($n = 7$)			Control group ($n = 2$)		
	Weight (kg)	SEP CTT (ms)	AEP V-I (ms)	Weight (kg)	SEP CTT (ms)	AEP V-I (ms)
0	10.4	4.48 (0.51)*	2.54 (0.08)	10.3	4.86 (0.41)	2.20 (0.16)
1	10.2	4.55 (0.69)	2.56 (0.10)	10.5	5.35 (0.56)	2.26 (0.09)
2	10.0	4.75 (0.42)	2.57 (0.08)	11.0	4.58 (0.72)	2.19 (0.16)
3	9.6	4.55 (0.50)	2.59 (0.13)	10.5	4.93 (0.79)	2.21 (0.09)
4	10.1	4.58 (0.47)	2.58 (0.08)	10.5	5.02 (0.64)	2.27 (0.05)
5	10.2	4.62 (0.50)	2.53 (0.12)	10.6	4.69 (0.80)	2.17 (0.01)
6	10.3	5.38 (0.82)	2.62 (0.03)	10.5	4.80 (0.69)	2.22 (0.23)
7	10.0	5.38 (0.80)	2.59 (0.07)	10.5	4.83 (0.84)	2.23 (0.21)
8	10.1	5.48 (0.86)	2.56 (0.12)	10.5	4.72 (0.39)	2.17 (0.23)
9	10.1	5.32 (0.69)	2.60 (0.07)	10.3	4.87 (0.05)	2.31 (0.10)
10	10.2	5.82 (0.74)	2.63 (0.16)	10.7	4.94 (0.29)	2.41 (0.10)
11	10.2	5.66 (0.42)	2.61 (0.14)	10.3	4.80 (0.29)	2.36 (0.19)
12	10.3	5.88 (0.76)	2.59 (0.13)	10.5	4.79 (0.17)	2.38 (0.15)

Week	Recovery period					
	Vigabatrin group ($n = 5$)			Control group ($n = 2$)		
	Weight (kg)	SEP CTT (ms)	AEP V-I (ms)	Weight (kg)	SEP CTT (ms)	AEP V-I (ms)
14	11.3	5.19 (0.65)	2.57 (0.10)	10.4	4.99 (0.34)	2.32 (0.22)
16	11.0	5.12 (0.51)	2.54 (0.06)	10.4	4.81 (0.34)	2.32 (0.25)
19	10.9	4.97 (0.59)	2.54 (0.06)	11.0	4.88 (0.30)	2.26 (0.18)
21	11.3	5.00 (0.52)	2.60 (0.05)	10.9	4.69 (0.52)	2.38 (0.23)
23	11.5	4.60 (0.40)	2.60 (0.11)	10.9	4.63 (0.39)	2.37 (0.17)
25	11.5	4.61 (0.31)	2.58 (0.06)	10.7	4.81 (0.52)	2.46 —
27	11.3	4.61 (0.43)	2.63 (0.06)	10.6	4.71 (0.27)	2.38 (0.20)
29	11.4	4.58 (0.47)	2.59 (0.07)	11.2	4.78 (0.40)	2.31 (0.12)

* numbers in brackets represent 1 s.d.

Figure 1a Somatosensory evoked potentials (SEP) recorded from surface electrodes overlying the mid-cervical spinal cord (upper trace) and the contralateral sensorimotor cortex (lower trace). Central transmission time (CTT) represents the latency difference between the points indicated by arrows. **b**) Auditory evoked potentials recorded from a surface electrode overlying the vertex referenced to the ipsilateral mastoid. The V-I latency was measured as the difference between the point indicated by arrows.

dorsal column-medial lemniscal pathway. Increases in CTT began for four of the seven treated dogs between the 5th and 6th week after vigabatrin administration and for an additional dog between the 6th and 7th week after the onset of treatment (Table 1). By the 12th week all treated dogs showed a prolongation in CTT, relative to baseline, which ranged from 22% to 45% (Figure 2). At this time point, the mean CTT of the treated group was approximately 30% longer than the mean of the baseline values; a difference significant at the 0.05 level (Figure 3). In contrast, the control dogs showed no consistent alteration in central latencies throughout the study, with most values within 5% of the baseline mean. The increase in CTT in the treated dogs was entirely attributable to

prolongation of the cortical response; there was no significant change in the absolute latency of the response overlying the cervical cord. The morphology of the initial cortical component remained consistent, with both the onset and peak delayed (Figure 4). The amplitude of the cortical response varied greatly, reflecting the use of surface needle electrodes, and did not show a consistent relationship to latency.

AEP data failed to demonstrate a consistent alteration in peripheral or central transmission latency throughout the course of the study. Peripheral auditory transmission times were assessed as the peak of initial component of the brainstem response (Wave 1), while an estimate of central transmission was obtained by subtracting the latency of Wave 1 from that of Wave V, defined as the last positivity in the brainstem complex (Figure 1b). Values for central transmission generally remained within 10% of the baseline for all dogs and there was no consistent AEP amplitude effect associated with vigabatrin (Figure 5).

After dosing for 12 weeks two dogs were killed for histological evaluation. These dogs were representative of the treated group in that their mean somatosensory CTT was elevated 27.2% from baseline with no associated change in AEP. Each of these dogs had extensive abnormal vacuolation in the fornix, septum, optic tract and hypothalamus and to a lesser extent in the mid-brain and thalamus. Vacuolation was also pronounced in the hippocampal endplates. Mild to minimal vacuolation was noted symmetrically within the cerebral cortex. Some vacuolated regions, such as endplates, hypothalamus, thalamus and midbrain, were associated with microglial proliferation. The cervical spinal cord was normal. The electron micrographs taken from the fornix of one dog showed regions of extreme intramyelinic oedema with axonal sparing.

Recovery period

The prolongation of CTT latency began to reverse for each treated dog immediately upon withdrawal of vigabatrin. The mean prolongation for the five remaining dogs in the treated group was reduced by approximately half within 2 weeks of the onset of the recovery period (Figure 3). A consistent but slower decrease in CTT continued to be evident until week 23 (11th recovery week) at which time central SEP latencies were stable and apparently fully recovered to baseline values. Values at 29 weeks (17 weeks of recovery) did not significantly differ from baseline for any measure tested.

Figure 2 CCT expressed as a percent of baseline values for both treated and control dogs. Note that after 12 weeks of vigabatrin the CTT was prolonged for each treated dog; values returned to normal during the recovery period. ▨ week 12, ■ recovery.

Figure 3 Mean CTT for both treated (●) and control (▲, $n=2$) groups expressed as a % change from baseline.

Figure 4 Cortical SEP waveform demonstrating delay in response onset and peak.

Figure 5 Auditory evoked potential (AEP) expressed as a percent of baseline values for both treated and control dogs. No significant change was detected between baseline and 12 week values or between treated and control groups. ▨ week 12, ■ recovery.

Histological evaluation at the conclusion of the study revealed no significant difference in the degree of vacuolation between the control and treated dogs. Some degree of vacuolation was present in the centrum semiovale region of both groups, apparently due to fixation artifact; however, no vacuoles were noted in the fornix, optic tract, hippocampal formation or cerebral cortex in the recovered animals. Swollen Purkinje cell dendrites were seen in both control and dosed animals, but treated animals displayed a mild degree of hippocampal endplate

microgliosis that was not found in the control dogs.

Discussion

The results of the present study confirm the occurrence of microvacuolation within the CNS of dogs treated with high doses of vigabatrin and further demonstrate that this pathology is associated with functional changes in afferent conduction within the somatosensory fibre tracts. The sites of vacuolation are focused and similar

to those previously reported in rat and dog (Yarrington *et al.*, 1985; Butler *et al.*, 1987). However, our results also suggest mild vacuolation within the cerebral cortex and microglial proliferation in some affected regions. Electron-micrographs suggest that vacuolation is the result of separation of the outer lamellae of the myelin sheath which may be secondary to focal accumulation of fluid between the myelin layers. Oedematous changes in myelin have been demonstrated to play a major role in a number of neurotoxic injuries (Powell *et al.*, 1980), including those associated with exposure to lead (Lampert & Schochet, 1968), isoniazid (Blakemore *et al.*, 1972) and hexachlorophene (Lampert *et al.*, 1973; Towfigh *et al.*, 1974).

A principal goal of this research was to evaluate evoked potentials as an index of vigabatrin-induced pathology. SEP latencies were clearly and progressively altered by vigabatrin. The study design did not permit evaluation of the morphological correlates of the earliest SEP changes, however, the timing of these changes (5–6 weeks in some dogs) suggests that SEP indeed provide a sensitive measure of onset of pathology. A cardinal feature of the somatosensory system is the long length and heavy myelination of its pathways. Changes in SEP latency most likely reflect the observed myelinopathies within the midbrain, thalamus and cortex. The lack of consistent amplitude effects, and the preserved response morphology, suggests functional slowing of a broad band of fibres with little of the conduction block characteristic of demyelination (McDonald & Sears, 1970; Sears & Barstock, 1981; Raine, 1985). Short latency AEP were not altered during the 12 weeks of treatment, consistent with the lack of vigabatrin-induced vacuolation within the lower brainstem.

A major feature of our data is the degree of functional and morphological recovery evident; these data are more striking considering that the dose level was several times higher than proposed for clinical studies. SEP latencies returned to normal even in those dogs demonstrating the

most severe slowing (an approximate 50% change from baseline). The speed of functional recovery was also surprising, with much of the effect achieved within 2 weeks of the end of treatment. It is clear that these results cannot be generalized to longer treatment periods. Although there is some suggestion in our data that changes in the SEP had become asymptotic by the end of treatment, this cannot be confirmed without a longer study. Moreover, the presence of microglial proliferation in both the dogs sacrificed after treatment and in those sacrificed after recovery suggests that some degree of neuronal pathology cannot be ruled out. These changes may be evident later than the vacuolation and may not recover to the same degree. Consistent with this possibility, Yarrington *et al.* (1985), report enlarged, eosinophilic sherdoid-appearing axons and focal mineralized bodies in addition to vacuoles in long term vigabatrin-treated rats, but not in long term vigabatrin-treated dogs.

Finally, our results must be interpreted with caution regarding the use of evoked potentials to monitor patients treated with vigabatrin. We recorded responses using scalp electrodes to approximate more closely the situation in man and selected measurements that are standard clinical procedures in many laboratories. In man, visual evoked potentials are easy to record and robust so that they could be added to the evaluation. However, sensory evoked potentials monitor only a portion of the system demonstrating vigabatrin-induced pathology. Thus, sites such as the fornix and hippocampus are not adequately evaluated by these measures. Moreover, patients treated with vigabatrin are likely to have functional disorders with possible electrophysiological manifestations that could severely hamper the detection of altered evoked responses. In spite of these potential limitations, initial studies measuring serial evoked potentials in 15 epilepsy patients treated with vigabatrin found no significant evidence of drug induced changes in response morphology or latency (Hammond *et al.*, 1988).

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