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The evaluation of different types of anti-convulsant drug activity against leptazol-induced epileptogenic activity in the anaesthetized rat

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1 The effects of various anticonvulsant drugs were evaluated quantitatively on the development of the epileptogenic EEG, induced by the intravenous infusion of leptazol in rats anaesthetized with urethane.

2 Leptazol alone produced five distinct phases of EEG activity developing from early wave and small spike and wave activity to larger spikes which later grouped and led to full body convulsion (FBC).

3 Drugs effective in petit mal such as clonazepam (0.1 and 0.25 mg kg^{-1}) and ethosuximide (100 and 200 mg kg^{-1}), significantly delayed the time to FBC by prolonging the early phases of the epileptogenic EEG and delaying the appearance of spiking.

4 Drugs effective in grand mal such as sodium valproate (60 mg kg^{-1}) and phenytoin (5 mg kg^{-1}) significantly prolonged the time to FBC by extending the later phases of the EEG and the development and grouping of spikes. Higher doses of these compounds were without effect. Carbamazepine and phenobarbitone produced mixed effects but were generally not markedly anticonvulsant.

5 The model is sensitive to drugs effective in both petit mal and grand mal, and appears able to differentiate usefully between them.

Introduction

Subconvulsant doses of leptazol in animals produce marked changes in electrocortical activity characterized by the appearance of slow negative waves and spike-wave complexes similar to those seen in petit mal epilepsy (Libet, Fazekas & Himwich, 1940; Ajmone-Marsan & Morossero, 1950; Rodin, Rodin & Lavine, 1979). However, it is known that repeated subconvulsant doses of leptazol can 'kindle' rats to give grand mal type seizures and that these seizures are sensitive to drugs effective in the control of grand mal (Ito, Hori, Yoshida & Shimizu, 1977; Rodin *et al.*, 1979). Antagonism of leptazol-induced seizures in mice is also considered a useful screening model for drugs of value in petit mal (Desmedt, Niemegeers, Lewi & Janssen, 1978) despite the fact that the convulsions produced might be considered more characteristic of grand mal.

In an attempt to produce one model with leptazol which might be used to determine the potential activity of drugs in both petit mal and grand mal, we have used a slow intravenous infusion of leptazol in rats to produce a developing epileptogenic EEG with distinct phases of activity. In order to determine whether the early phases with spike-wave activity may be related to petit mal and the late phases with large grouped spikes to grand mal, we tested a range of anti-convulsant drugs with proven efficacy in the two conditions to see if they preferentially affected a particular phase of EEG.

Methods

Male Sprague-Dawley rats (300–350 g) were anaesthetized throughout the experiment with urethane (6 ml kg^{-1}, 25% w/v, intraperitoneally) and a femoral vein was then cannulated. The skull was exposed and two small EEG recording electrodes screwed in above the parietal cortex.

EEG activity was amplified (Devices 3160, time constant 0.2 s) before display on an oscilloscope and a pen recorder (Washington, 400 MD/2). A digital index of total EEG activity per min was obtained by voltage-integration of the oscilloscope output, and a

separate index of spiking activity per min was obtained by integration of the spike voltage above a set threshold of 20 mV, controlled by Devices signal processor 4010.

After recording a period of control activity, the anticonvulsant to be tested was injected intravenously and a further 6 min of EEG recorded before the start of the infusion of a leptazol solution (0.5 M) at a rate of 30 μl min^{-1} from a Palmer injection apparatus 6130 (except in the case of carbamazepine which was injected intraperitoneally 30 min before the start of the infusion). Immediately a full body convulsion (FBC) occurred, the animal was killed by a rapid intravenous injection of excess urethane.

Drugs

Leptazol, ethosuximide, sodium valproate and phenobarbitone were prepared in normal saline.

Phenytoin was dissolved in a minimum volume of absolute ethanol and diluted with saline.

Clonazepam (Rivotril, Roche) was supplied, 1 mg ml^{-1}, in proprietary solvent and diluted with saline.

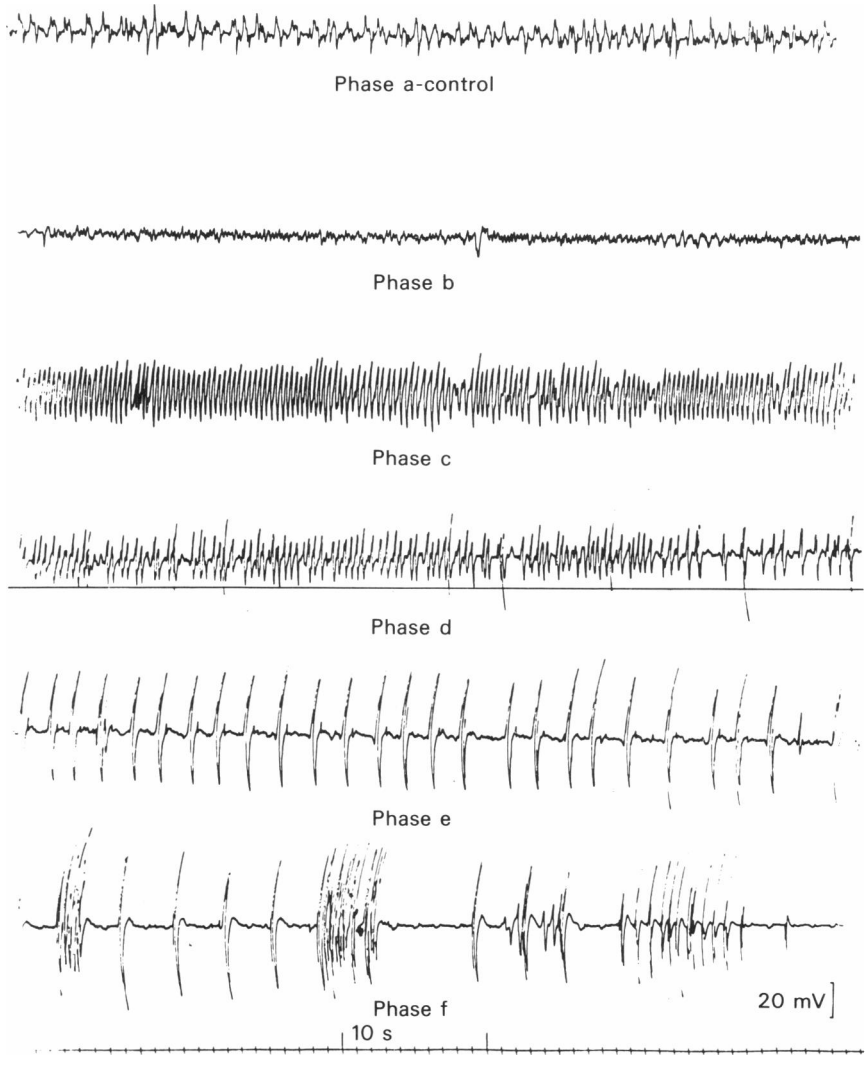

Figure 1 Different phases (a–f) of EEG activity recorded from a rat anaesthetized with urethane during the intravenous infusion of leptazol (0.5 M) at a rate of 30 μl min^{-1}. Time constant 0.2 s. The voltage level above which activity was regarded as a spike is shown as a horizontal line in (d). These recording were made at the following times after the start of infusion: (b) 5 min; (c) 15 min; (d) 22 min; (e) 28 min and (f) 33 min.

Carbamazepine was suspended in 0.5% Tween-80 solution for intraperitoneal injection.

All anticonvulsants were injected in a volume of 1 ml kg^{-1} except carbamazepine (50 mg kg^{-1}) which was injected in a volume of 2 ml kg^{-1}.

None of the vehicles modified the effect of leptazol.

Results

Development of leptazol-induced epileptogenic EEG activity; control studies

Up to five phases of EEG activity may be distinguished during the continued intravenous infusion of leptazol (Figure 1). In the control (no drug) period (a), total EEG activity is normally low and spike activity zero under urethane anaesthesia. Phase (b) follows within 2 min of the start of the leptazol infusion and is associated with a small degree of EEG arousal shown by high frequency, low amplitude activity. Phase (c) is characterized by waves of steadily increasing amplitude but fairly low frequency (2 Hz). Phase (d) signals the first appearance of spikes on the background of high amplitude, low frequency waves. In phase (e) spiking becomes the predominant feature, with a loss of wave activity, and in (f) the spikes become grouped (associated with slight body movements) and increasing periods of interspike 'silence' appear. A full body convulsion (FBC) inevitably follows.

Although it is possible to estimate the duration of these phases by visual inspection of the EEG trace, we obtained a more quantitative analysis by the use of voltage integration of the EEG. Counts of integrated total voltage activity (T_v) and integrated spike activity (i.e. that above a 20 mV threshold, S_v) were obtained. Figure 2 shows the mean time course of both activities (T_v and S_v) for leptazol infusion in control animals. Thus, in phase (b), arousal is shown by a small decrease in T_v; in phase (c), T_v rises steeply and in phase (d) counts start in S_v with the appearance of spikes. In phase (e), S_v is still rising, but T_v has reached a peak, while in phase (f) both T_v and S_v start to fall as the spikes begin to group and interspike

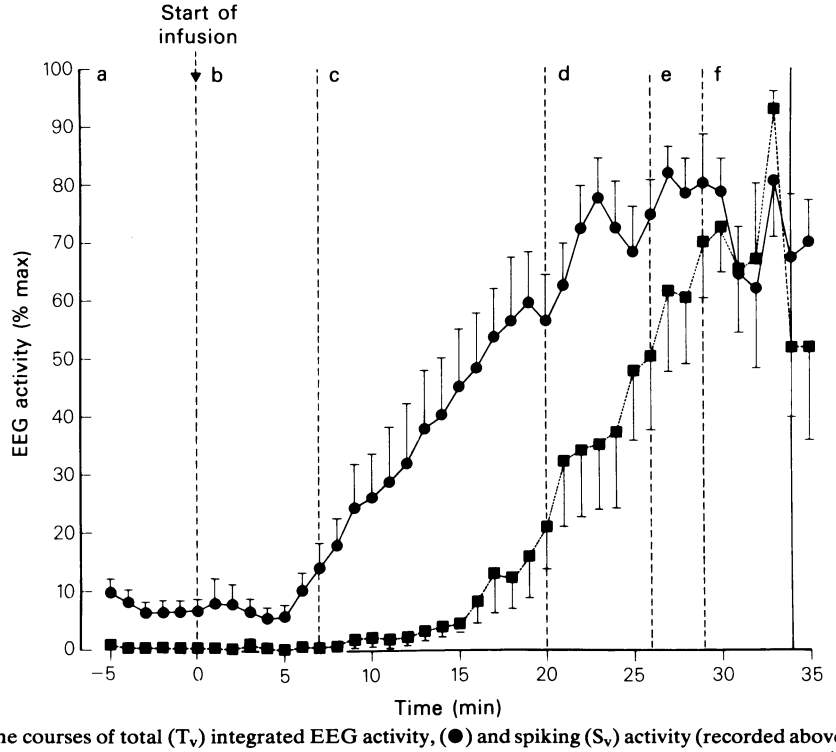

Figure 2 Time courses of total (T_v) integrated EEG activity, (●) and spiking (S_v) activity (recorded above a 20 mV threshold) (■), seen during the continuous intravenous infusion of leptazol (0.5 M) at a rate of 30 μl min^{-1} in the rat anaesthetized with urethane. Activity in both cases is expressed as a percentage of the maximum for that parameter in each animal at each minute and then averaged to give mean values per min for all animals ($n = 9$); vertical lines indicate s.e.mean. The mean times at which one phase of activity (as seen in Figure 1) changes to another are indicated by the broken vertical lines.

Table 1 Effects of various anticonvulsants on the duration (in min) of the different phases of EEG activity produced during the intravenous infusion of leptazol in the rat anaesthetized with urethane

Drug	*Dose* ($mg\,kg^{-1}$)	*Duration of phase (min)* *b*	*c*	*d*	*e*	*f*	*Time to FBC* (min)
Control		7.22 ± 0.99	12.6 ± 1.26	5.77 ± 1.02	3.67 ± 0.79	5.22 ± 1.11	34.2 ± 1.97
Clonazepam	0.1	10.60 ± 1.33	20.5 ± 2.85*	4.75 ± 0.84	5.00 ± 0.80	4.25 ± 0.98	45.1 ± 2.82*
Clonazepam	0.25	9.25 ± 1.89	20.5 ± 1.29*	11.10 ± 1.89*	7.63 ± 1.57*	4.50 ± 0.82	53.0 ± 2.84*
Ethosuximide	100	9.37 ± 1.10	14.4 ± 0.88	8.25 ± 1.05	5.25 ± 0.84	4.75 ± 0.86	42.0 ± 2.24*
Ethosuximide	200	5.37 ± 0.75	30.5 ± 0.46*	8.75 ± 1.75	4.57 ± 1.36	4.71 ± 0.99	54.4 ± 3.77*
Valproate	60	7.63 ± 1.38	12.0 ± 1.00	13.8 ± 1.56*	6.13 ± 0.97	4.13 ± 0.35	43.4 ± 1.87*
Phenytoin	5	8.50 ± 1.02	17.3 ± 2.25	7.25 ± 0.97	9.00 ± 2.00*	5.88 ± 0.89	47.8 ± 2.90*
Carbamazepine	10	12.50 ± 0.78*	14.1 ± 1.94	6.38 ± 1.01	3.75 ± 0.84	3.75 ± 0.59	40.5 ± 1.78*
Valproate	150	7.00 ± 0.94	12.9 ± 1.69	6.25 ± 1.03	4.38 ± 0.59	3.63 ± 1.16	34.1 ± 3.38
Phenytoin	25	7.13 ± 0.91	13.5 ± 2.20	6.25 ± 1.03	5.00 ± 1.02	2.75 ± 0.41	34.5 ± 2.22
Carbamazepine	50	5.25 ± 0.72	8.37 ± 1.46*	5.87 ± 0.87	4.00 ± 1.36	1.50 ± 0.27*	25.0 ± 2.15*
Phenobarbitone	5	7.00 ± 0.68	8.25 ± 1.63*	7.37 ± 0.86	6.50 ± 1.05*	4.37 ± 0.50	33.5 ± 2.16

The phases are defined in the text and illustrated in Figure 1.
All drugs were given i.v. except carbamazepine, which was given i.p.
FBC = Full body convulsion.
Results expressed as means ± s.e. of 8 experiments for each treatment.
Only those treatments above the horizontal line showed significant anticonvulsant effects.
*Results significant ($P < 0.05$) compared to control by Student's *t* test (one tailed).

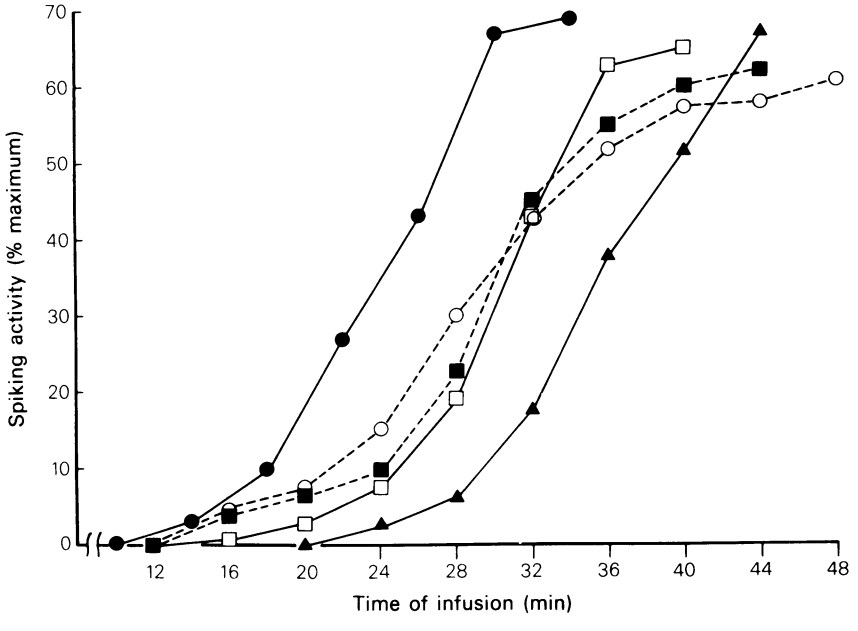

Figure 3 Mean time course ($n = 8$) of the development of spiking activity, plotted as the % maximum of integrated spike voltage during successive 4 min recording periods of the EEG, in the rat anaesthetized with urethane during the intravenous infusion of leptazol (0.5 M) at a rate of $30\,\mu l\,min^{-1}$, alone (●), or after pretreatment with clonazepam, $0.1\,mg\,kg^{-1}$ (▲); phenytoin, $5\,mg\,kg^{-1}$, (○); sodium valproate, $60\,mg\,kg^{-1}$ (■); or ethosuximide, $100\,mg\,kg^{-1}$ (□), given intravenously 6 min before the start of the infusion. The first 10 min of the time course whilst spiking activity was zero, is omitted.

silence increases. The mean durations of the various phases are also shown on Figure 2, but these do not correspond exactly to the times that might be expected from inspection of the graph, since they are obtained by averaging the duration of the phases in individual animals, whereas the graph is plotted from the mean counts for all animals at each time interval. Since the animals were of similar weights, the time of infusion was considered to be a sufficiently accurate indication of the actual amount of leptazol infused per kilogram, rather than calculating the precise quantity at each stage in terms of mg kg^{-1}.

Effects of anticonvulsants on the development of the leptazol-induced epileptogenic EEG

The anticonvulsants were tested at dose levels related, as far as possible, to those used clinically. The dose range used acutely in these experiments approximates to the human daily doses per kg (assuming 70 kg body weight) of phenytoin (8.5 mg kg^{-1} d^{-1}) carbamazepine (17 mg kg^{-1} d^{-1}), clonazepam (0.15 mg kg^{-1} d^{-1}) and phenobarbitone (8.5 mg kg^{-1} d^{-1}). The doses of ethosuximide and sodium valproate tested were about four times the human daily dose but lower doses were inactive in either reducing or augmenting the convulsant activity of leptazol.

The effects of the anticonvulsants were both drug- and dose-dependent. All except phenobarbitone significantly prolonged the time to FBC at at least one dose level, but increasing the dose of phenytoin and sodium valproate diminished anticonvulsant activity whilst carbamazepine actually reduced the time to FBC. Higher doses of phenobarbitone were lethal when given to animals already anaesthetized with urethane.

The results are summarised in Table 1. Clonazepam was the most effective compound, it significantly prolonged the time to FBC at both dose levels, the lower dose (0.10 mg kg^{-1}) prolonged phase (c) whilst the higher dose (0.25 mg kg^{-1}) also prolonged phases (d) and (e). Ethosuximide showed similar activity, both doses prolonged the time to FBC, but the lower dose (100 mg kg^{-1}) had no specific effect on any phase whilst the high dose (200 mg kg^{-1}) significantly prolonged phase (c).

Phenytoin (5 mg kg^{-1}) and sodium valproate (60 mg kg^{-1}) both prolonged the time to FBC, but higher doses, 25 and 150 mg kg^{-1} respectively, were inactive. In both cases the effect of the lower dose was more marked in the later phases than with clonazepam. The low dose of phenytoin significantly prolonged phase (e) and sodium valproate phase (d). Carbamazepine (10 mg kg^{-1}) prolonged the time to FBC with most effect in phase (b), that is, it retarded the appearance of wave-like activity. At a higher dose (50 mg kg^{-1}) it significantly reduced the time to FBC, mainly by shortening phase (c). Phenobarbitone was inactive overall and although it significantly prolonged phase (e) it also shortened phase (c).

Thus, overall, the drugs normally used for the treatment of petit mal, that is, clonazepam and ethosuximide, not only extended the time to FBC but preferentially prolonged the early phases (c and d) and so delayed the appearance of spiking. This is shown clearly in Figure 3, in which the level of spiking is plotted against time so that the appearance of spiking, the rate of its development and the time taken for it to reach a maximum, can be distinguished. Thus drugs which prolong the early phases (c and d) and delay the onset of spiking (e.g. clonazepam and ethosuximide) shift the position of the curve to the right along the time axis. Drugs which do not affect the appearance of spiking (phases c and d) but retard its development (phases d and e) (e.g. phenytoin and valproate) reduce the slope of the curve without markedly shifting its starting point. No drug prolonged phase (f), i.e. once spikes became grouped, FBC followed within 5 min.

None of the drugs had any marked effect of their own on the EEG, although phenytoin (25 mg kg^{-1}), clonazepam (0.25 mg kg^{-1}) and ethosuximide (200 mg kg^{-1}) produced a slight decrease in amplitude.

Effects of clonazepam, ethosuximide and phenytoin on the established leptazol-induced epileptogenic EEG

Since clonazepam is the drug of choice in the treatment of status epilepticus, we carried out experiments to see if, in addition to delaying the appearance of spikes, it could actually abolish spiking once it had become established in phase (e) despite the continued infusion of leptazol.

Figure 4 shows that spiking was substantially reduced by the injection of clonazepam (0.25 mg kg^{-1}) and the EEG reverted to a pattern of activity more characteristic of phase (c) or (d) for 30 min before spiking returned and FBC rapidly followed. In contrast, phenytoin (50 mg kg^{-1}) produced only a transient reduction in spiking with no significant effect on the time to FBC, and ethosuximide (200 mg kg^{-1}), which had a profound effect on the development of spiking when given before the start of infusion, produced a much shorter abolition of established spiking than clonazepam.

Discussion

The different phases of EEG activity which were seen

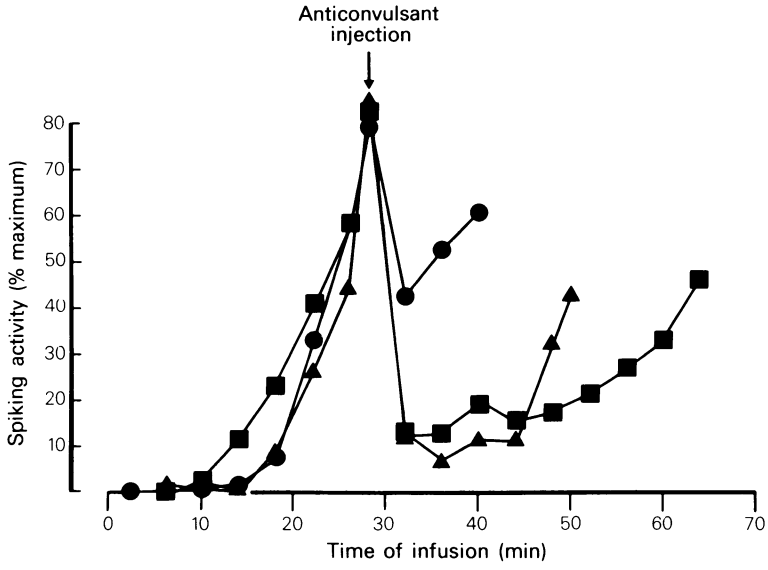

Figure 4 Effect of clonazepam, 0.25 mg kg^{-1}, (■), ($n = 8$); ethosuximide, 200 mg kg^{-1}, (▲), ($n = 5$) and phenytoin, 50 mg kg^{-1}, (●), ($n = 5$) on the mean time course of spiking when injected intravenously after spiking had developed during an established phase (e) of the developing EEG produced by the continuous intravenous infusion of 0.5 M leptazol (30 μl min^{-1}) in the rat anaesthetized with urethane. Spiking activity is plotted as the mean % maximum of integrated spike voltage during successive 4 min recording periods.

during the intravenous infusion of leptazol in the anaesthetized rat were reproducible in respect of their characteristics and duration. They were still present after pretreatment with anticonvulsants and, although the duration of the individual phases could be markedly altered, their characteristics changed only slightly.

The effects of the anticonvulsants fell broadly into three groups. The first group includes those which prolonged the time to FBC, mainly by extending the early phases (b and, especially, c) and thus delaying the appearance of spikes. Clonazepam and ethosuximide clearly come in this group. They also showed an increase in anticonvulsant activity with increased dosage. This is, perhaps, to be expected, since these drugs are effective in petit mal and leptazol is an established agent for use in screening tests for drugs useful in petit mal (Desmedt *et al.*, 1978). Clonazepam is also the drug of choice in status epilepticus and it is interesting in this respect, that it effectively reduced the spiking when injected during an established (e) phase, whereas ethosuximide, which is not used in this condition, had much less effect on established spiking.

The second group includes phenytoin and sodium valproate which prolonged the time to FBC in a low dose but lost this effect at higher doses. With these compounds, the prolongation of the time to FBC is mainly apparent in the late phases (d and e), that is, there is little effect on the actual appearance of spiking compared with clonazepam and ethosuximide, but its grouping is delayed. Thus, sodium valproate, which is used in petit mal and, to some extent in grand mal, acts in phase(d) somewhat later than group 1 drugs. It delays the development more than actual appearance of spiking. Phenytoin, which is used primarily in grand mal, prolonged the time to FBC by mainly delaying the grouping of spikes (phase e) before body movements developed (phase f). Both sodium valproate and phenytoin lost their activity at higher dose levels and, whilst phenytoin is known to augment convulsions in overdose (Woodbury, 1980 b), we know of no such reports for sodium valproate. Phenytoin did not significantly affect spiking when given during an established (e) phase, and is not a drug of choice in status epilepticus.

Phenobarbitone probably comes in a third group and had no effect on the time to FBC at the dose used. Larger doses were too depressant generally, and lower doses inactive. Nevertheless, phenobarbitone did modify the EEG and, although it actually reduced phase (c) and speeded up the appearance of spiking, it also prolonged the grouping of spikes (phase e), like phenytoin. This is, perhaps, in keeping with its known ability to exacerbate petit mal (Prichard, 1980).

Carbamazepine is more difficult to classify; the low dose was anticonvulsant and prolonged the time to FBC, but, alone of the drugs tested, it actually prolonged the arousal phase (b) and thus delayed the

development of waves (phase c). Higher doses significantly reduced the time to FBC. Carbamazepine, although sometimes used in grand mal, is the drug of choice in partial seizures. There is no clinical correlate for its apparent ability to reduce the threshold for convulsions as detected with the high dose in this test.

At the cellular level, the actions of leptazol are diverse (see Woodbury, 1980a) and this probably reflects the diversity of the electroencephalographic events seen during its intravenous infusion. In a recent review, Woodbury (1980a) suggests it may act by blocking postsynaptic inhibition whilst, at the same time increasing excitability by a direct effect on neuronal membranes; it may increase cholinergic function by blocking acetylcholinesterase, and it may block γ-aminobutyric acid (GABA) mediated inhibition either by a direct receptor effect or by an action on the chloride ion channel. An action involving GABA antagonism is borne out by the fact that two of the most effective drugs in these experiments, clonazepam and sodium valproate, act in part through enhancement of GABA function.

From these experiments it appears that the developing EEG produced by the infusion of leptazol correlates well with the changes seen in the EEG in clinical epilepsy. Certainly, petit mal is associated with wave and spiking activity of the type seen in phases (c) and (d) of the developing EEG, and it is development of this feature that was delayed by drugs used in petit mal. In grand mal, spiking in the EEG is associated with body movements, as in phases (e) and (f) of the developing leptazol EEG in the rat, and it is the development of these features that was affected by drugs used in grand mal. In addition, this procedure can obviously demonstrate direct effects of the anticonvulsants on the EEG and will show the possible danger of actually augmenting convulsant effects in high doses.

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