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A simple intravenous infusion method in rodents for determining the potency of anticonvulsants acting through GABAergic mechanisms

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Abstract—A simple method of intravenous infusion of convulsant drugs (pentetrazol and bicuculline) into the tail vein of rats has been used to determine seizure threshold and construct log-dose seizure threshold response curves for several anticonvulsant drugs (diazepam, phenobarbitone, pentobarbitone, chlormethiazole and valproate). It has been shown that an index of the dose required to increase seizure threshold by 50% (TI50) can be obtained using small numbers of animals. The advantages of this method over that of the subcutaneous pentetrazol method are several: small numbers of animals required, speed and reproducibility. The data also demonstrate the concordance between TI50 values obtained using pentetrazol and bicuculline as the convulsant agent. It is suggested that this method can be used routinely in screens of anticonvulsant drugs thought to work through GABAergic mechanisms.

The measurement of the effects on seizure threshold is a part of drug evaluation and the use of intraperitoneal or subcutaneous administration of pentetrazol (pentylenetetrazole) in mice to examine seizure susceptibility remains a major method (Krall et al 1978). A range of doses of the drug under investigation is administered (usually with 8–10 animals for each point) and the number of animals convulsing in the 30 min following injection of pentetrazol (s.c.) determined. The ED50 value for seizure protection is determined by log-probit analysis. Such an approach uses a large number of animals, particularly for a screening program.

Intravenous infusion procedures have been developed (e.g. Hint & Richter 1958) but they have been used mainly to examine changes in the seizure threshold following a procedure or drug administration (Nutt et al 1980, 1981). Killian & Frey (1973) and Loscher (1982) have used pentetrazol infusion to determine doses of drugs that elevate the seizure threshold by 20%, but these reports give few methodological details.

The current study was undertaken to examine further the infusion method to determine its usefulness in the evaluation of anticonvulsant drugs.

Materials and methods

Rats and infusion techniques. Male Wistar derived rats (Olac, Bicester), 140–170 g, were kept in groups of 6 in conditions of controlled temperature and lighting (light period 07·00–19·00 h) with free access to pellets and tap water. Measurement of seizure threshold was essentially by the method of Nutt et al (1980, 1981). Briefly, the rats were lightly restrained and the convulsant drug infused via a 25 gauge 'butterfly' inserted into a tail vein at a rate of 2·6 mL min^{-1}. This rate is similar to that used previously (Nutt et al 1980) and has been shown to give a clear end point, both slower and more rapid rates producing problems. The time of infusion of the convulsant drug required to produce the first myoclonic twitch (which occurs with the first EEG abnormality) was recorded and doses required to produce the seizure calculated. The infused drugs were pentetrazol (10 mg mL^{-1}) and bicuculline (0·1 mg mL^{-1}). Seizure threshold can be calculated from the formula:

$$\frac{\text{Infusion rate (mL min}^{-1}) \times \text{drug concn(mg mL}^{-1}) \times \text{time to twitch (s)}}{60 \times \text{rat weight (kg)}}$$

This gives the threshold in mg kg^{-1}.

Drugs. The drugs used were (sources in brackets); pentetrazol bicuculline, phenobarbitone, pentobarbitone, diazepam (all from Sigma Chemical Co. Poole), chlormethiazole (Astra Research Centre, Sodertalje), sodium valproate (Sanofi, Manchester).

Results

Groups of rats (normally 4–6 per dose) were given by the intraperitoneal route an appropriate range of the anticonvulsant drugs under investigation and the dose of pentetrazol necessary to elicit a seizure determined 15 min later. The percentage rise in seizure threshold above values obtained in a group of control animals examined at the same time was calculated. This allowed log dose versus % increase in threshold curves to be constructed (Fig. 1). Variability about the mean was never more than ± 10% (s.e.mean).

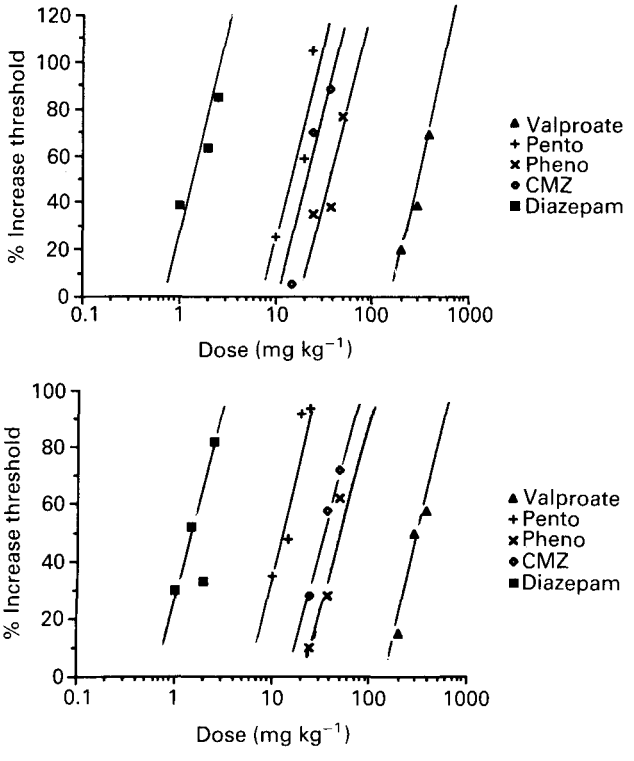

FIG. 1. The effect of various doses of anticonvulsant drugs on the increase in seizure threshold measured by infusion of pentetrazol (top) or bicuculline (bottom). Each point shows the mean percentage increase in threshold of a group of 4–6 animals over a group of controls (vehicle injected animals) measured at the same time. Variability about the mean was always less than ± 10%. Doses necessary to increase threshold by 50% given in Table 1.

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Table 1. Calculated doses of anticonvulsant drugs necessary to increase seizure threshold by 50% (TI50) following infusion of pentetrazol or bicuculline.

	TI50 values (mg kg^{-1})	
Injected	Pentetrazol	Bicuculline
Diazepam	1·3	1·6
Pentobarbitone	16	14
Chlormethiazole	22	34
Phenobarbitone	36	48
Valproate	320	330

Data calculated from results shown in Fig. 1.

Similar curves were constructed using bicuculline as the convulsant agent (Fig. 1). From these data threshold increase 50% or TI50 values (the dose required to raise threshold by 50%) were obtained (Table 1). Regression analysis revealed concordance between the dose of anticonvulsant required to raise the seizure threshold to either pentetrazol or bicuculline ($R = 0{\cdot}99$).

Discussion

The infusion method of determining seizure threshold produces accurate measures of doses of convulsant drug required to elicit a seizure with little variability about the mean (see Nutt et al 1981). Perfectly adequate dose response curves could therefore be constructed using 3–4 points and 4–6 animals per point (Fig. 1). This contrasts with log-probit analysis following pentetrazol (s.c.) which usually requires 6 points and 8–10 animals per point. The saving in animals is therefore considerable and additionally it was found that rapid termination of the infusion at the time of the first myoclonic jerk prevented the onset on the full tonic seizure.

Bicuculline has been used much less than pentetrazol as a convulsant agent but it is clear that essentially identical results are obtained to those using pentetrazol. There seems therefore little justification in using both agents.

Obviously the method can only be used to examine drugs thought to act by increasing GABA function, for example at the benzodiazepine/GABA/chloride ionophore complex. Phenytoin and carbamazepine do not raise seizure threshold to pentetrazol (see Nutt et al 1981). Nevertheless many drugs are encompassed in this grouping and it is interesting that chlormethiazole is one, since information on its mechanism of action are scant at present, but do suggest a GABAergic mechanism (Harrison & Simmonds 1983; Ogren 1986; Cross et al 1989). Even though the drugs examined (benzodiazepine, barbiturates, chlormethiazole and valproate) almost certainly act through several diverse mechanisms to enhance GABA function, they nevertheless produced parallel dose-response curves. This suggests that TI50 estimates should be possible in a group of related compounds from a single point.

While drugs acting to enhance GABA function are also sedative the relative potency of the drugs in this seizure test corresponds with their effect on GABA function but not their sedative action (see Ogren 1986), indicating that the current test should be indicative of the clinical usefulness of the drugs as anticonvulsants.

In summary therefore a simple, rapid (a complete curve for an anticonvulsant drug can be constructed in 15 min) method for examining anticonvulsant potency has been reported in this study. The reproducibility is also good and perhaps best illustrated by the fact that the study by Nutt et al (1981) on the effect of single doses of diazepam and valproate on pentetrazol threshold produced data which lie exactly on the curves shown in Fig. 1. The problems due to slight variations occurring in absolute threshold values due, for example, to variability in the weight of groups of animals are removed by calculating the threshold rise as a percentage of the values of control animals examined at the same time.

Finally this method should be applicable to the infusion method which has been reported in mice (Nutt et al 1986).

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