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Optimizing the pentetrazol infusion test for seizure threshold measurement

D. J. NUTT*, S. C. TAYLOR, H. J. LITTLE, *University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX and University Department of Pharmacology, South Parks Road, Oxford, UK

Seizure thresholds in mice were determined using the pentetrazol infusion method. Concentration of infusate and rate of infusion were varied to assess the optimal parameters for seizure threshold detection. Seizure threshold elevations were produced by flurazepam and threshold decreases by FG 7142. An infusion rate of 1·1 ml min^{-1} was best for detecting both increases and decreases in threshold. However a concentration of 2·5 mg ml^{-1} gave optimal measurement of elevations in threshold whereas decreased thresholds were best detected with a concentration of 10 mg ml^{-1}.

Pentetrazol (leptazol, metrazol) is probably the most widely used convulsant for evaluating both the anticonvulsant and proconvulsant effects of drugs. After its initial introduction into clinical practice as a substitute for camphor in the therapeutic production of seizures (Meduna & Friedman 1939) it was widely used in animal experiments (see Hahn 1960; Stone 1972). For many years the 'metrazol' test which used either i.p. or s.c. pentetrazol to produce seizures has been the primary screen for anticonvulsant activity in new pharmaceutical compounds. In addition, as the benzodiazepines were so effective against pentetrazol effects, and the relative potency of these effects followed closely their clinical dosage (Randall & Kappell 1973), the test was also used to screen for novel anti-anxiety compounds. Unfortunately the i.p. or s.c. routes of administration pose problems when pentetrazol is used in a more precise or quantitative fashion than is generally the case with screening tests. For instance, we have shown that the variability of both seizure latency and incidence with i.p. pentetrazol made it difficult to use in evaluating the effects of electroconvulsive treatment on seizure susceptibility in rats (Nutt et al 1980). In preference we developed an intravenous infusion method similar to that used previously by others (Orloff et al 1949; Chen et al 1954; Hint & Richter 1958). This gave consistent and reliable values of seizure threshold, and was able to detect drug effects using many fewer animals than the earlier ED50 or CD50 tests (see Stone 1972). We have now calibrated the i.v. pentetrazol infusion in mice in an attempt to determine the optimal conditions of drug concentration and infusion rate for the detection of both elevations and reductions in seizure susceptibility.

* Correspondence to: National Institute on Alcohol Abuse and Alcoholism, Building 10, Room 3 B19, 9000 Rockville Pike, Bethesda, MD 20205, USA.

Methods

Male Charles River CD_1 mice (30–35 g) were used. Pentetrazol was dissolved in saline at concentrations of either 1, 2·5 or 10 mg ml^{-1} and infused at different rates. Infusion rates used ranged from 0·138 to 1·1 ml min^{-1}. Seizure threshold elevations (anticonvulsant effects) were produced by pretreatment with the water-soluble benzodiazepine, flurazepam, 10 mg kg^{-1} given i.p. (10 ml kg^{-1} in saline). Seizure threshold reductions (proconvulsant effects) were produced by treatment with the β-carboline benzodiazepine receptor ligand FG 1742 (ethyl β-carboline-3-carboxylate methylamide; see

Table 1. The effect of changing pentetrazol concentration and infusion rate on seizure thresholds.

Rate of infusion (ml min^{-1})	Concentration of pentetrazol 10 mg ml^{-1}			2·5 mg ml^{-1}			1 mg ml^{-1}		
	C	FZ	FG	C	FZ	FG	C	FZ	FG
1·1	70 ± 8	141 ± 40	34 ± 8	37 ± 5	115 ± 11	24 ± 4	42 ± 9	81 ± 6	n.d.
0·55	73 ± 9	142 ± 24	n.d.	46 ± 7	138 ± 17	33 ± 4	55 ± 4	n.d.	n.d.
0·275	44 ± 4	150 ± 30	25 ± 7	38 ± 4	n.d.	n.d.		n.d.	
0·138	50 ± 1	n.d.	n.d.		n.d.			n.d.	

Numbers are mean ± s.d. pentetrazol seizure thresholds (mg kg^{-1}), n = 7–8; n.d. = not determined. Treatments given 30 min before infusion of pentetrazol.
C = Control; FZ = flurazepam; FG = FG 7142.
All drug treatment seizure thresholds are significantly different from controls $P < 0.01$ or greater.

Little et al 1984), 40 mg kg^{-1} i.p. (10 mg kg^{-1} in a vehicle of distilled water with one drop of polysorbate 80 per 10 ml). To save animals, and because we have previously shown that the polysorbate vehicle has no effect on seizure thresholds (Nutt 1982), only 1 group of controls was used and these were treated with saline. Both flurazepam and FG 7142 were given 30 min before the infusion of pentetrazol which was performed as described by Nutt et al (1980). Mice were restrained in a transparent Perspex box with ventilatory holes and a 25 g 'butterfly' (Abbot Labs) was inserted into a tail vein that had been made more apparent by dipping the tail of the mouse into warm water.

The end point of the infusion was taken as the onset of repeated, clonic contractions of forelimbs and neck muscles. Mice were killed immediately this end point was reached. From the time of infusion, weight of mouse and concentration of drug, the seizure threshold in mg kg^{-1} pentetrazol was calculated.

Results and discussion

The first observation was that the rate of delivery of pentetrazol had a large effect on seizure thresholds in the control group. Highest thresholds were obtained at the fastest rates (1·1 and 0·55 ml min^{-1} and 10 mg ml^{-1}). All other rates of delivery produced roughly similar control thresholds (Table 1). The lowest rates of delivery (10 mg ml^{-1} at a rate of 0·138 ml min^{-1} and 1 mg ml^{-1} at 0·55 ml min^{-1}) produced end points which were difficult to assess. Similarly, the next slowest rates also had relatively unclear end points, and full data were not collected. In general, when two different concentrations of infusate were used to produce equivalent rates of pentetrazol delivery, the faster infusion rate produced the clearest end points.

An index of sensitivity to drug effects was taken as being the ratio of threshold with drug treatment to that of control. This showed that a rate of pentetrazol delivery of 2·75 mg min^{-1} (1·1 ml min^{-1} and 2·5 mg ml^{-1} or 0·275 ml min^{-1} and 10 mg ml^{-1}) were optimal for detecting the threshold elevating effects of flurazepam with the 1·1 ml min^{-1} infusion giving the clearest end point. In contrast, the rate of delivery of 11 mg min^{-1} (10 mg ml^{-1} at 1·1 ml min^{-1}) produced the best discrimination of proconvulsant effect of FG 7142.

These experiments show that seizure susceptibility as determined by the infusion of pentetrazol is a variable that depends on the rate of its delivery. From this study it would appear that a rate of 11 mg min^{-1} gave the best discrimination for the detection of decreases in threshold. However, for detecting elevations in threshold, a rate of delivery of about 2·7 ml min^{-1} was optimal and could be obtained by two concentrations of infusate, but that of 2·5 mg ml^{-1} (1·1 ml min^{-1}) gave the most definite end points. These findings may help improve the value of the pentetrazol infusion method for screening new anticonvulsant anxiolytic drugs and for estimating changes in brain excitability.

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