Convulsive thresholds in mice during the recovery phase from anaesthesia induced by propofol, thiopentone, methohexitone and etomidate

*S. Lowson, †J.P. Gent & *¹C.S. Goodchild

Departments of *Anaesthesia and †Pharmacology, University of Leeds, Leeds LS2 9LN

1 Convulsive thresholds were measured with intravenous pentylenetetrazol in mice during the recovery phase after intravenous anaesthetic doses of propofol (10 and 20 mg kg⁻¹), thiopentone (30 mg kg⁻¹), methohexitone (10 mg kg⁻¹), and etomidate 3 mg kg⁻¹).

2 The convulsive threshold rose after each agent, indicating an anticonvulsant action for all the drugs tested; this declined to control values with initial half times of: 1.56 min (propofol 10 mg kg⁻¹); 1.03 min (propofol 20 mg kg⁻¹); 1.02 min (methohexitone); 3.35 min (etomidate); 13.7 min (thiopentone).

3 At no time during the recovery phase of any agent did the convulsive threshold fall below control values, which might indicate an epileptogenic effect of the drug.

4 The threshold was depressed below control values by intravenous administration of Ro 15-4513, a partial inverse agonist at the benzodiazepine receptor, thus indicating the ability of this pentylenetetrazol test to demonstrate a proconvulsant effect.

5 We conclude that the abnormal movements or convulsions associated with recovery from anaesthesia with short-acting intravenous anaesthetics may not be the result of an intrinsic proconvulsant action of the drugs.

Keywords: Anticonvulsant tests; pentylenetetrazol; intravenous anaesthetics; propofol; thiopentone; methohexitone; etomidate; epilepsy; recovery from anaesthesia

Introduction

Following a series of adverse clinical reports in 1987, the Committee for the Safety of Medicines (1987) issued a warning concerning the possible risk of seizures after anaesthesia induced with propofol. In the same year Hodkinson *et al.* (1987) observed epileptic activity on the electroencephalogram in three patients with temporal lobe epilepsy after the administration of propofol. Since then a number of case findings (Cameron, 1987; Hopkins, 1988; Victory & Magee, 1988; Jones *et al.*, 1988) have reported on the occurrence of epileptic seizures after propofol-induced anaesthesia. In all these cases the seizures occurred during the recovery phase. In contrast, Wood *et al.* (1988) described a case of status epilepticus which was resistant to combined treatment with diazepam, phenytoin, phenobarbitone and chlormethiazole, but which responded rapidly to a single 100 mg bolus of propofol, the seizures subsequently being controlled by a propofol infusion.

In a previous study conducted by the present authors (Lowson *et al.*, 1990) propofol was shown to display marked anticonvulsant properties in mice, as judged by protection against seizures induced by electroshock and the convulsant, pentylenetetrazol (PTZ). Protection was significant even at doses which caused no sedation and was much greater at higher doses. For comparison, the effects of thiopentone were studied; it was found that the anticonvulsant effects of propofol were similar to or greater than those of thiopentone at doses which caused the same degrees of sedation or anaesthesia. However, in that study anticonvulsant activity was examined at the time of peak behavioural effect and effects during the recovery phase of anaesthesia were not investigated.

The present study was undertaken to examine convulsive threshold in mice during the recovery phase of anaesthesia induced with propofol, and to make comparisons with other

intravenous induction agents, viz. thiopentone, methohexitone and etomidate. We chose the intravenous route for administration of anaesthetic agents to reproduce more closely the rapidly changing brain concentrations of these drugs which are encountered in clinical practice when these agents are used for induction of anaesthesia. Under such conditions we looked for a proconvulsive effect during the recovery phase.

Methods

This investigation was conducted on 275 male, Tuck No. 1 mice, weight 25–38 g. They were maintained in cages of five on a 12 h light/dark cycle with free access to water and a standard diet. The mice were divided into groups to receive one of the following intravenous anaesthetics: propofol (ICI Pharmaceuticals: 10 mg ml⁻¹ in 10% soyabean oil emulsion); 20 mg kg⁻¹, *n* = 55; 10 mg kg⁻¹, *n* = 35; thiopentone (May & Baker: 25 mg ml⁻¹) 30 mg kg⁻¹, *n* = 50; methohexitone (Eli Lilly: 10 mg ml⁻¹); 10 mg kg⁻¹, *n* = 40; etomidate (Janssen: 10 mg ml⁻¹); 3 mg kg⁻¹, *n* = 55.

Five further mice were given an intravenous injection of Ro 15-4513; 1 mg kg⁻¹ (Hoffman-La Roche; 0.1 mg ml⁻¹ in a propylene glycol based vehicle, Gent *et al.*, 1988).

In addition 25 control mice did not receive any anaesthetic. These were tested in groups for convulsive thresholds at intervals throughout the whole study. Controls with administration of vehicle alone were not performed because we have previously shown that none of the vehicles used in this study influenced the minimum convulsant dose of PTZ (Gent *et al.*, 1988; Lowson *et al.*, 1990).

Assessment of recovery

After induction of anaesthesia by i.v. injection of anaesthetic into a lateral tail vein recovery was scored as follows: (1)

¹ Author for correspondence.

response to tail pinch elicited by a pair of artery forceps; (2) recovery of the righting reflex; (3) recovery of the ability to balance along the edge of a plastic tray; (4) recovery of full coordination and normal exploratory activity.

The time taken to achieve each of the above responses was recorded in initial groups of five mice which were allowed to make a full recovery and in all other animals until the time of testing with PTZ. The time (mean and range) taken for the mice to achieve each stage of recovery was determined for each test agent.

Measurement of convulsive threshold

A slow infusion (0.3 ml min^{-1}) of a solution of PTZ (Sigma London; 15 mg ml^{-1} in 154 mM saline) was given into the other tail vein of the unrestrained mouse until a clear clonic convulsion was elicited. This was the end-point of the test, and mice were killed immediately after this convulsion had occurred. The minimum convulsant dose of PTZ (MCD of PTZ) was thus obtained for each mouse and the mean ($\pm$ s.e.mean) calculated for each group of 5 mice. The earliest time at which testing was performed was the time of recovery of righting reflex. This gave an initial high value from which the rate of decline of MCD of PTZ with time could be determined. It was not considered necessary to test at earlier times as we had demonstrated in a previous study (Lowson *et al.*, 1990) that for propofol and thiopentone the 'anaesthetized' state was associated with highly significant protection against seizures. Further measurements of MCD of PTZ were made in groups of five mice each at various times after the recovery of the righting reflex: propofol (20 mg kg^{-1}) every 2 min from 4 to 22 min and at 26 min; propofol (10 mg kg^{-1}) every 2 min from 2 to 14 min; thiopentone at 4 min, 10 min and every 10 min thereafter to 80 min; methohexitone every 2 min from 2 to 14 min; etomidate every 2 min from 4 to 14 min and every 4 min thereafter to 30 min.

As we were interested in discovering if any of the anaesthetic agents caused a proconvulsant effect during the recovery phase we felt it was important to show that the method used was capable of revealing a proconvulsant action. The MCD of PTZ was therefore measured in a group of five mice 1 min after they had been given an intravenous bolus dose of Ro 15-4513, a partial inverse agonist at the benzodiazepine receptor (Lister & Nutt, 1988).

Data analysis

Statistical comparisons for recovery data were performed with the Mann-Whitney U-test to avoid any assumptions concerning the distribution of the data. A value of $P < 0.05$ was considered statistically significant. When comparisons of MCD of PTZ were made between experimental and control mice, Student's *t* test was used to compare each group of 5 experimental values with the most recently obtained group of control values. The computer programme CSTRIP (Sedman & Waynes, 1976) was used to analyse the changes in convulsive threshold with time. Although this programme is more usually used to analyse changes in drug levels, to derive phar-

macokinetic parameters, we felt that its use was appropriate to obtain estimates of half-times for the decrease in MCD of PTZ after administration of the different anaesthetic agents.

Results

Behavioural assessment

The times taken for animals to display response to tail pinch, righting reflex, balancing ability and full recovery after each anaesthetic agent are shown in Table 1. The recovery times for the earlier phases were comparable for all groups. However, times to full recovery were significantly longer in etomidate- and thiopentone-treated animals when compared with propofol at both doses and methohexitone ($P < 0.05$; Mann-Whitney U Test).

Convulsive thresholds

There were no significant differences in MCD of PTZ between any of the control groups, which were tested at different times throughout the study. The overall mean MCD of PTZ in control mice was $40.7 \pm 0.74 \text{ mg kg}^{-1}$ (mean $\pm$ s.e.mean).

At no time during the course of these investigations did the group mean MCD of PTZ in animals given any of the anaesthetic agents fall significantly below that of the corresponding control group. However, the MCD of PTZ for the group given Ro 15-4513 was $24.8 \pm 0.81 \text{ mg kg}^{-1}$, a value significantly ($P < 0.001$) below that of the control group.

The MCD of PTZ declined with time after all anaesthetic agents (Figure 1). With propofol (20 mg kg^{-1}), thiopentone and etomidate, the initial MCD of PTZ obtained at 4 min post induction was the highest value recorded in the study for each agent; thereafter the MCD of PTZ fell towards control values. Values at 4 min were significantly higher after propofol (20 mg kg^{-1}) than those after thiopentone which in turn were significantly higher than the 4 min values recorded following etomidate ($P < 0.05$). The time taken for the group mean MCD of PTZ to decline to a value not significantly different from controls was 18 min for propofol (20 mg kg^{-1}) and 26 min for etomidate. With thiopentone, values had not fallen to control levels at 80 min post induction, indicating a prolonged anticonvulsant effect during and after the recovery phase of thiopentone induced anaesthesia. With propofol 10 mg kg^{-1} and methohexitone the highest MCD of PTZ values recorded in the study were at 2 min, at which time animals had recovered their righting reflex. The 2 min values after propofol (10 mg kg^{-1}) were significantly greater than those after methohexitone. The MCD of PTZ had returned to control levels by 10 min after propofol (10 mg kg^{-1}) and 12 min after methohexitone.

Computer analysis of the values for MCD of PTZ to obtain polyexponential parameter estimates showed that, for each set of data, the best fit was with two exponentials; the correlation coefficient was greater than 0.98 in all cases. For the initial, rapid phase of the decreasing anticonvulsant effect, the half times were as follows: for propofol (10 mg kg^{-1}), 1.56 min; for

Table 1 The times taken to reach four stages of recovery after intravenous anaesthesia

	Recovery time: mean (range) min			
	Tail pinch	Righting reflex	Balancing ability	Coordination and exploratory activity
Propofol 20 mg kg^{-1}	2.60 (2-4)	4.09 (3-5)	7.90 (6-10)	13.90 (12-16)
Propofol 10 mg kg^{-1}	1.03 (1-2)	2.00 (1-3)	3.50 (3-4)	6.80 (6-8)
Methohexitone	1.26 (1-2)	2.62 (2-3)	4.83 (4-6)	9.33 (8-10)
Thiopentone	2.12 (1-3)	3.84 (2-5)	11.76 (8-16)	22.84 (18-25)
Etomidate	1.84 (1-3)	3.78 (2-5)	9.56 (8-12)	19.78 (18-22)

Figure 1 Decrease in anticonvulsant effect (MCD of PTZ = minimum convulsive dose of pentylenetetrazol) after intravenous injection of anaesthetic agents. Points represent means ($n = 5$), bars show standard error of the mean. (a) (■) propofol (20 mg kg⁻¹); (□) propofol (10 mg kg⁻¹); (○) etomidate (3 mg kg⁻¹); (◆) methohexitone (10 mg kg⁻¹); (b) (■) propofol (20 mg kg⁻¹); (●) thiopentone (30 mg kg⁻¹).

propofol (20 mg kg⁻¹), 1.03 min; for methohexitone, 1.02 min; for etomidate, 3.35 min; and for thiopentone 13.67 min. Thus the initial decline was fast after propofol and methohexitone, intermediate after etomidate and relatively slow after thiopentone. A similar pattern was seen in the half times for the second, slower phase; that for propofol (20 mg kg⁻¹) and methohexitone being similar (19 min), with etomidate longer at 40 min but thiopentone much longer at 170 min.

Involuntary movements

We observed involuntary movements in some mice in all groups. Following administration of etomidate all mice exhibited myoclonus, of varying severity during the early recovery phase (3–8 min). Differentiation between myoclonus and clonic seizures induced with PTZ at times proved difficult and may partly account for the low MCD of PTZ recorded at 4 min for etomidate. The myoclonus was made substantially worse when the animals were touched. Opisthotonus was a frequent occurrence following methohexitone; it was observed early in the recovery phase (1–2 min).

After both propofol and thiopentone a fine tremor of the mouse's head and limbs was occasionally observed in the early recovery phase. More frequently we observed rapid, repetitive movements of the limbs (more often of the hind limbs) at around the time of recovery of the righting reflex. The latter movements were more common following propofol anaesthesia. They were exacerbated if mice were laid supine, reduced if mice were laid prone (the normal position) and largely stopped by the application of a strong sensory stimulus, e.g., tail pinch. Such movements superficially resembled myoclonus. The convulsion following PTZ was easily distinguished from these and the fact that sensory stimulation stopped the abnormal movements rather than triggering or exacerbating them (as was the case after etomidate) led us to believe that these movements represented semi-purposeful actions made by animals as they were awakening from anaesthesia and regaining limited coordination.

Discussion

In these experiments we sought to study convulsive threshold during the recovery phase following anaesthesia induced with different intravenous anaesthetic agents. We were interested to discover if a proconvulsant effect could be demonstrated at this time and in the light of reports of epileptic seizures occurring during the recovery phase of anaesthesia induced with propofol, we were particularly interested in this agent. Propofol was therefore tested at two doses, a dose of 20 mg kg⁻¹ as used by Glen *et al.* (1985) and a lower dose of 10 mg kg⁻¹ which produced a more rapid recovery. However, at neither dose could we detect a proconvulsant effect during the recovery phase. We did not continue our studies beyond 26 min for propofol 20 mg kg⁻¹ and 14 min for propofol 10 mg kg⁻¹. In both groups the group mean MCD of PTZ had been shown to be not significantly different from control values at three preceding test times and in both cases these times were well beyond the time for full recovery, the longest recovery times for propofol being 16 min (20 mg kg⁻¹) and 8 min (10 mg kg⁻¹). After 8 min of recovery the MCD of PTZ was significantly higher with thiopentone than with propofol. This is in contrast to our previous findings when the two drugs were given intraperitoneally; at equivalent levels of sedation in the previous study the MCD of PTZ was much higher in propofol-treated animals (Lowson *et al.*, 1990). This difference may be due to differences in the route of administration of the anaesthetics and the fact that the convulsant testing in the former study was performed at the time of peak behavioural effect for different doses. This study has provided no evidence that either propofol, thiopentone, methohexitone or etomidate produce proconvulsant activity during the recovery phases from anaesthesia induced with single bolus doses of these agents. However, the method used in the present investigation was well able to detect proconvulsant activity, as was demonstrated by the significant reduction in convulsive threshold induced by the partial inverse agonist at the benzodiazepine receptor, Ro 15-4513. One must be mindful of the fact, however, that it is possible for a drug to be proconvulsant yet not interact with PTZ.

These studies were conducted on a pure bred strain of

laboratory animals. The advantage of using such animals is that responses produced by a pharmacological agent will be consistent and will be minimally affected by individual pharmacokinetic or pharmacodynamic differences. However, a disadvantage arising from this consistency of response is that a pure bred strain of animals does not accurately mimic the likely range of responses obtained from a heterogeneous population such as human patients. Individuals differ in their convulsive thresholds and therefore in their susceptibility to seizures, whatever their cause. It is of interest that in three out of the four case reports describing epileptic seizures in patients recovering from a propofol-induced anaesthetic there was a personal or close family history of epilepsy.

We are therefore driven to speculate on the mechanism which might be responsible for these involuntary, often epileptiform movements which we have observed in mice during the recovery phase of short-acting anaesthetics. One possibility, as suggested above, is that they are semi-purposeful, uncoordinated grooming/righting reflexes. Another possibility is that the depressant effect of an anaesthetic may be removed from different parts of the central nervous system at different rates, perhaps as a result of perfusion differences. This could conceivably lead to an imbalance between excitatory and inhibitory influences on a potential epileptic focus, or areas involved in the control of movement. Such an effect might be most clearly seen in those drugs which are removed most rapidly; our data for behavioural recovery and loss of anticonvulsant activity (as measured by MCD of PTZ) show that this is the case for propofol (at both doses) and methohexitone. The shorter half time for the effect of the higher dose of propofol compared with the lower dose of the same agent seems incon-

gruous; this is probably artifactual and due to the initial very high level of MCD of PTZ after 20 mg kg⁻¹ propofol.

The occurrence of seizures after propofol is obviously a rare event considering the widespread use of this agent. However, in the light of the case reports described (Cameron, 1987; Hopkins, 1988; Victory & Magee, 1988; Jones *et al.*, 1988) and the results of the present study we feel that when anaesthesia is to be induced with a single bolus dose of an intravenous anaesthetic agent it would be prudent to select thiopentone in individuals with epilepsy or a strong family history of this disease. In the experiments described here it is clear that, whereas at the time of recovery from the anaesthetic effects of propofol, methohexitone and etomidate, there is still some residual protection against seizures, this is far more marked, and lasts for much longer, with thiopentone. The prolonged anticonvulsant action exhibited by thiopentone during the recovery phase will provide a greater degree of protection against seizures during this susceptible period than will propofol or any other of the intravenous anaesthetic agents commonly used.

Our results, however, would not preclude the use of propofol in epileptic patients; its powerful anticonvulsant effects may be useful in the control of drug resistant seizures in intensive care (Wood *et al.*, 1988), but prior to cessation of propofol administration a longer acting anticonvulsant must be administered and well-controlled, known epileptics are usually taking such medication.

The authors would like to thank Mrs S. Barlow and Mrs M.E. Bruce for secretarial assistance and ICI Pharmaceuticals for financial support and supplies of propofol.

References

- CAMERON, A.E. (1987). Opisthotonus again. *Anaesthesia*, **42**, 1124.
- COMMITTEE FOR SAFETY OF MEDICINES (1987). *Propofol*. *Current Problems*. No. 20.
- GENT, J.P., BENTLEY, M., FEELY, M. & HAIGH, J.R.M. (1988). Benzodiazepine cross-tolerance in mice extends to sodium valproate. *Eur. J. Pharmacol.*, **128**, 9–15.
- GLEN, J.B., HUNTER, S.C., BLACKBURN, T.C. & WOOD, P. (1985). Interaction studies and other investigations of the pharmacology of propofol ('Diprivan'). *Postgrad. Med. J.*, **61** (Suppl. 3), 7–14.
- HODKINSON, B.P., FRITH, R.W. & MEE, E.W. (1987). Propofol and the electroencephalogram. *Lancet*, **ii**, 1518.
- HOPKINS, C.S. (1988). Recurrent opisthotonus associated with anaesthesia. *Anaesthesia*, **43**, 904.
- JONES, G.W., BOYKETT, M.H. & KLOK, M. (1988). Propofol, opisthotonus and epilepsy. *Anaesthesia*, **43**, 905.
- LISTER, R.G. & NUTT, D.S. (1988). Interactions of the imidazodiazepine Ro 15-4513 with chemical convulsants. *Br. J. Pharmacol.*, **93**, 210–214.
- LOWSON, S.M., GENT, J.P. & GOODCHILD, C.S. (1990). The anticonvulsant properties of propofol and thiopentone: a comparison using two tests in laboratory mice. *Br. J. Anaesth.*, **64**, 59–63.
- SEDMAN, A.J. & WAYNES, J.G. (1976). CSTRIP, a Fortran IV computer program for obtaining initial polyexponential parameter estimates. *J. Pharmacol. Sci.*, **65**, 1006–1010.
- VICTORY, R.A.P. & MAGEE, D. (1988). A case of convulsion after propofol anaesthesia. *Anaesthesia*, **43**, 904.
- WOOD, P.R., BROWNE, G.P.R. & PUGH, S. (1988). Propofol infusion for the treatment of status epilepticus. *Lancet*, **i**, 480–481.

(Received August 20, 1990)

Revised December 4, 1990

Accepted December 20, 1990)