ANTICONVULSANT PROPERTIES OF PROPOFOL AND THIOPENTONE: COMPARISON USING TWO TESTS IN LABORATORY MICE

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SUMMARY
Experiments were carried out in mice to assess the protection provided by thiopentone (Intraval, May and Baker) and propofol (Diprivan, I.C.I.) against epileptiform seizures induced by electroshock and pentylenetetrazol. Intraperitoneal administration of propofol 50 mg kg$^{-1}$ and thiopentone 25 mg kg$^{-1}$ produced similar peak behavioural effects of mild sedation and incoordination. However, at these doses propofol afforded a greater degree of protection against pentylenetetrazol than thiopentone and at greater doses both propofol and thiopentone caused marked protection. Both anaesthetics were effective also against electroshock seizures at sedative doses. We conclude that propofol has strong anticonvulsant properties.

KEY WORDS
Anaesthetics i.v.: propofol, thiopentone. Anticonvulsants.

The anticonvulsant properties of thiopentone are well established. However, it is still uncertain if propofol has any influence on seizure activity. The Committee on Safety of Medicines recently issued a warning concerning the possible risk of seizures after administration of propofol [1]. Hodkinson, Frith and Mee reported finding epileptiform activity on the electroencephalogram (EEG) after propofol was administered to patients undergoing temporal lobectomy for intractable temporal epilepsy [2]. In contrast, other studies [3], using the cerebral function analysing monitor, have not demonstrated an epileptogenic effect of propofol.

As part of the original investigations on propofol, Glen and colleagues [4] compared the abilities of propofol and thiopentone to protect against electroshock-induced seizures in mice. Glen found that, whereas thiopentone provided protection against seizures, propofol lacked both anticonvulsant and proconvulsant properties. Other publications [5, 6], have demonstrated that seizures during electroconvulsive therapy are of shorter duration after induction of anaesthesia with propofol compared with methohexitone.

In view of this controversy surrounding the association between propofol and seizures, we have investigated the pro/anticonvulsant properties of propofol in two models of experimentally induced seizures in mice: electroshock and i.v. infusion of pentylenetetrazol (PTZ).

METHODS
The investigation was conducted on Tuck No. 1 mice ($n = 195$; weights 25–38 g) maintained on a 12-h light–dark cycle with free access to water and a standard diet. Handling of mice was kept to a minimum. Propofol was administered as the aqueous emulsion formulation containing 10% soya bean oil (Diprivan, ICI). Control mice for the propofol experiments received an equal volume of 10% intralipid (Kabi-Vitrum). Thiopentone was diluted in water and controls in these experiments received an equal volume of saline. Active and control solutions (2.5 ml kg$^{-1}$) were administered intraperitoneally (i.p.) using a 25-gauge needle. The i.p. route was chosen because...
the pentylenetetrazol was administered by continuous i.v. infusion and the maintenance of two patent cannulae in the tail veins of mice proved difficult and unreliable. Previous investigations of anticonvulsant agents conducted in this laboratory have shown the i.p. route to be a reliable method of drug administration [7]. All animals were killed by a schedule 1 method immediately after a seizure had been induced.

**Assessment of behavioural effects**

In preliminary investigations, it was noted that the doses of propofol and thiopentone required to produce a given behavioural effect and the time course of those effects after i.p. injection were different. The grade of sedation was therefore recorded at the time of peak effect (4.5 min for thiopentone and 5.5 min for propofol) during the subsequent experiments, which assessed anticonvulsant activity on the following scale:

1 = Mice active and grooming with normal exploratory activity. No apparent sedation.
2 = Decreased co-ordination with dragging of hind quarters, still active.
3 = Staggering with inability to walk along the edge of a tray. Easier to handle.
4 = Severe loss of co-ordination, with tendency to slither on the stomach rather than walk.
5 = All mice unable to walk and slithering.
6 = Lying immobile, righting reflex lost in approximately 50% of the mice. Response to tail pinch reduced but present in all animals.
7 = Righting reflex lost in more than 50% of the mice. Response to tail pinch was also markedly reduced or lost in approximately 50% of these animals.

Doses which produced the full range of behavioural effects were used to assess anticonvulsant activity. The two anticonvulsant tests were carried out at the time of peak behavioural effect, as recommended by Swinyard [8].

**Electroshock**

The electroshock test was conducted according to the method of Woodbury and Davenport [9], with two modifications. Instead of Spiegel corneal electrodes, we used platinum ball and clip ear electrodes and the current administered was 15 mA. A 15-mA current of 0.2 s duration has been established in this laboratory as an optimum supramaximal current which produces a 100% incidence of extensor-tonic convulsions in untreated mice. In all mice tested with electroshock, the end-point was the hind limb extensor-tonic component of the convulsion [8]. Mice were tested in groups of 10 for each dose of propofol (control and one group at each sedation level 1–7) or thiopentone (control and groups at sedation levels 1–4 and 6).

**Pentylenetetrazol (PTZ) test**

A continuous i.v. infusion of PTZ was given via a tail vein as described previously [7]. Solutions of 1.5% and 3.0% pentylenetetrazol in saline were given at a rate of 0.3 and 0.15 ml min⁻¹, respectively. The minimum dose of PTZ required to elicit a clonic convulsion (the minimum convulsant dose (MCD)) was recorded for each animal. Mice were tested in groups of five for each dose of propofol (control and one group at each sedation level 1–5) or thiopentone (control and one group at sedation levels 1–4). The group mean MCD (SEM) at each dose of propofol and thiopentone was calculated.

Statistical comparisons were performed using the Mann–Whitney U test to avoid assumptions on the distribution of the data. A value of \( P < 0.05 \) was considered statistically significant.

**Assessment of blood concentrations**

In 37 of the 95 animals which had received propofol, blood was withdrawn from the heart immediately post mortem and stored for later measurement of whole blood propofol concentrations by high pressure liquid chromatography.

**RESULTS**

**Behavioural effects**

I.p. administration of both anaesthetics produced a quick onset of behavioural effect which appeared at approximately 3 min after propofol and 2–2.5 min after thiopentone. The maximal effect was reached by approximately 4–5 min after thiopentone and 5–6 min after propofol. The duration of maximal effect was 3–4 min at the lowest doses tested. The depth and the duration of sedation and the recovery time during preliminary experiments carried out before anticonvulsant testing increased with increasing doses of propofol and thiopentone. The behavioural effects produced by the doses of propofol and thiopentone used in the anticonvulsant tests are shown in table 1. Thiopentone was approximately twice as potent (on a weight basis) as propofol in
ANTICONVULSANT ACTIONS

TABLE I. Sedative effects of propofol and thiopentone at different doses, with corresponding blood concentrations for propofol

<table>
<thead>
<tr>
<th>Blood propofol concentration (mean (SEM)) (µg ml⁻¹)</th>
<th>Propofol (mg kg⁻¹)</th>
<th>Grade of sedation</th>
<th>Thiopentone (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil detected (n = 5)</td>
<td>12.5</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td>Nil detected (n = 5)</td>
<td>25.0</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td>3.83 (0.72) (n = 6)</td>
<td>50.0</td>
<td>3</td>
<td>25.0</td>
</tr>
<tr>
<td>6.87 (1.39) (n = 5)</td>
<td>75.0</td>
<td>4</td>
<td>37.5</td>
</tr>
<tr>
<td>7.76 (0.58) (n = 6)</td>
<td>100.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>16.87 (5.52) (n = 5)</td>
<td>125.0</td>
<td>6</td>
<td>50.0</td>
</tr>
<tr>
<td>18.6 (2.35) (n = 5)</td>
<td>150.0</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

producing sedation of each grade. The doses of thiopentone used did not produce levels of sedation which could be classified as grades 5 or 7. The dose range of the two drugs which produced the range of behavioural effects was 12.5–150 mg kg⁻¹ for propofol and 6.25–75 mg kg⁻¹ for thiopentone. Neither intralipid nor saline produced sedation in control animals.

Electroshock

Propofol and thiopentone protected against electroshock-induced seizures at doses producing heavy sedation and marked loss of co-ordination (propofol > 50 mg kg⁻¹; thiopentone > 25 mg kg⁻¹). At levels of sedation greater than grade 3, thiopentone provided greater protection against electroshock-induced seizures than did propofol (fig. 1).

Pentylenetetrazol

At those doses of propofol and thiopentone producing loss of the righting reflex (propofol 125 and 150 mg kg⁻¹; thiopentone 50 mg kg⁻¹) the maximum dose of PTZ which we could administer (15 mg) failed to elicit a seizure (fig. 2). As profound protection against chemically induced seizures had been demonstrated at these doses, it was unnecessary, and also contrary to procedures used previously in this laboratory, to increase the concentration of PTZ further in order to demonstrate a convulsive threshold. At doses less than those producing loss of the righting reflex (propofol < 125 mg kg⁻¹, thiopentone < 50 mg kg⁻¹), both drugs markedly increased the MCD of PTZ and even at doses producing minimal behavioural effects (grade 1 sedation), both agents significantly increased the MCD of PTZ (P < 0.05). Propofol produced an increase in MCD of PTZ which was significantly (P < 0.05) greater than that caused by thiopentone; this was seen at doses causing grades of sedation 2, 3 and 4.

Assessment of blood concentrations

The mean blood concentration of propofol increased from 3.83 (SEM 0.72) µg ml⁻¹ after 50 mg

![Fig. 1. Bar chart showing number of mice protected from electroshock-induced seizures at seven grades of sedation caused by i.p. thiopentone (■) and propofol (□). Mice were tested in groups of 10; the number of mice protected is the number that did not exhibit extensor movements of the hind limbs following passage of 15 mA for 0.2 s. All control animals and all those in both groups at sedation levels 1–3 had such a seizure; none was protected.](http://bja.oxfordjournals.org/)
**DISCUSSION**

The ability of an agent to provide protection against experimentally induced seizures depends on a number of factors: the method of seizure induction used, the relationship between the mechanisms of action of the convulsant and anticonvulsant agents, the concentration of drug in the brain at time of testing, the dose administered, the route of administration, and the pharmacokinetic properties of the agent. i.p. administration is a reliable method of administering anticonvulsants in this preparation, and it was chosen for practical reasons described above. In addition, i.p. administration of propofol caused greater sedation at all grades of sedation which correlated well with the subsequent measurements of blood concentrations of the drug. Our experiments produced a range of blood concentrations of propofol of 0.72–35.6 μg ml⁻¹, which is the range found in man to produce sedation and anaesthesia [10].

Differences in the pharmacokinetics of propofol and thiopentone given i.p. have been demonstrated in our study by the observation of a more rapid onset and earlier peak effects of thiopentone. It is evident that pharmacokinetic differences between the two anaesthetic drugs are important when their anticonvulsant activities are compared. In previous experiments, using i.v. administration, Glen found that the hypnotic potency (mg for mg) of propofol was 1.8 times greater than that of thiopentone [11]. This contrasts with our own findings with i.p. administration where twice as much propofol as thiopentone was required to produce equivalent sedation. However, there was a more rapid onset to the peak sedative effects of thiopentone. It may be that more rapid absorption of thiopentone from the peritoneal cavity led to higher peak concentrations and the slower absorption of propofol from the peritoneal cavity into the circulation could allow a substantial proportion of the administered dose to be metabolized. In the mouse, Glen demonstrated a high “utilization rate” of propofol (2.22 mg kg⁻¹ min⁻¹) [11]. To avoid the complications of possible pharmacokinetic differences, comparisons of the anticonvulsant properties of propofol and thiopentone were made at doses producing equivalent behavioural effects.

Electroshock- and PTZ-induced seizures are recognized screening tests for potential anticonvulsant properties of drugs. It is known that anticonvulsant drugs do not protect against all forms of experimentally induced seizures [8]. Results obtained in different tests may correlate with clinical effectiveness in man; protection against the application of a supramaximal electroshock predicts activity against grand mal [8] and partial seizures [12]. Drugs active against primary generalized seizures of the absence type may not demonstrate protection against maximal electroshock but are effective against PTZ-induced seizures [12]. Used together, these two complementary tests detect the anticonvulsant actions of all the major antiepileptic drugs in clinical use [12].

Clinically useful anticonvulsants demonstrate differing abilities to protect against various forms of experimentally-induced seizures. These differences probably reflect the ability of some agents to prevent spread of epileptic activity in the CNS (detected by electroshock), while other drugs increase the threshold of discharge of an epileptic focus (detected by sub-maximal/threshold doses of chemical convulsants) [8]. In the current
investigation, propofol and thiopentone protected against electroshock-induced seizures at doses which produced heavy sedation and marked loss of co-ordination. Statistically significant ($P < 0.05$) increase in the PTZ-induced seizure threshold was demonstrated for both agents at doses that produced minimal behavioural effects. The difference in the relative anticonvulsant activity of the anaesthetic agents demonstrated in the two tests probably results from the fact that our version of the PTZ test measures convulsive threshold rather than overall protection.

It is interesting to compare the values of MCD of PTZ obtained using the compounds with those produced by benzodiazepines, as the latter are particularly effective at antagonizing the convulsant actions of PTZ [13]. In this laboratory we have found that the maximum MCD of PTZ which can be produced by diazepam is approximately 180 mg kg$^{-1}$ (unpublished results)—considerably less than the effects of both thiopentone and propofol reported here, although at grade 2 sedation, the anticonvulsant effect of diazepam is similar to that of propofol and rather greater than that of thiopentone.

The results obtained in this study with electroshock contrast with those of Glen and colleagues [4], who demonstrated the anticonvulsant properties of thiopentone 40 mg kg$^{-1}$ i.v., but failed to show any anticonvulsant activity for propofol 20 mg kg$^{-1}$ i.v. The electroshock experiment was performed 15 min after administration of each agent. However, Glen [11] showed that mice given propofol 26 mg kg$^{-1}$ i.v. had regained their righting reflex and co-ordination and were able to walk by this time. In contrast, mice given thiopentone 40 mg kg$^{-1}$ i.v. did not regain co-ordination for approximately 40 min after the time of testing, which indicates the persistent CNS effects of thiopentone. In the same study, Glen demonstrated that the EEG changes produced by propofol in rats were similar to those produced by rapidly acting barbiturates [11]: propofol produced burst suppression at an i.v. dose close to its median hypnotic dose, but the barbiturates only produced burst suppression at doses in excess of twice the median hypnotic dose for these agents.

In conclusion, our investigation has demonstrated anticonvulsant properties of propofol similar to those of thiopentone. These laboratory observations in the mouse support the apparent anti-epileptic activity of propofol in man [3, 5, 6]. These results indicate the necessity to re-evaluate the evidence for convulsions associated with the use of this drug for anaesthesia in man [1].

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**REFERENCES**