EFFECT OF PROPOFOL, THIOPENTONE AND ETOMIDATE ON ADRENAL STEROIDOGENESIS IN VITRO

A. LAMBERT, R. MITCHELL AND W. R. ROBERTSON

Etomidate, a carboxylated derivative of imidazole, is a rapidly acting hypnotic drug which is administered i.v. either as a bolus or by continuous infusion, to produce anaesthesia. It is known to inhibit adrenal steroidogenesis in vivo (Allolio et al., 1983; Fellows, Bryne and Allison, 1983) and in vitro (Lambert et al., 1983; Lambert, Frost et al., 1984; Lee Wagner et al., 1984), and has been implicated in the increase in mortality found in multiply-injured patients (Ledingham and Watt, 1983). As a result, etomidate has been the object of considerable attention in the past year (Owen and Spence, 1984). The association between etomidate and adrenocortical suppression prompted us to investigate two further i.v. anaesthetic agents for possible anti-adrenal effects. The first, thiopentone, a short-acting barbiturate, has been used frequently for the induction and, on occasion, the maintenance of anaesthesia since its introduction in 1934 (Dundee and Wyant, 1974). The second drug, propofol (diisopropylphenol, Diprivan), is a relatively new anaesthetic first described in 1977 (Kay and Rolly, 1977). It is a rapidly acting agent which is suitable for anaesthesia of short duration or for prolonged anaesthesia when administered as a continuous infusion.

MATERIALS AND METHODS

The method used for the preparation of isolated guineapig adrenal cells has been described in detail elsewhere (Lambert and Robertson, 1982; Lambert, Garner et al., 1984). Briefly, the adrenals from two male guineapigs were chopped into 1-mm cubes using a McIlwain tissue chopper, the tissue pieces washed with Eagle's minimum essential medium (EMEM) and dispersed by mechanical agitation in EMEM containing collagenase 2 mg ml⁻¹. The cells were collected by centrifugation (300 g for 5 min) and washed twice with Eagle's medium containing 0.5% bovine serum albumin (BSA), calcium 8 mmol litre⁻¹ and ascorbate 2 mmol litre⁻¹. The above mixture served as incubation medium. Finally, the cell suspension was filtered through nylon 100-µm mesh. The cells (1 x 10⁶ cells ml⁻¹) were then pre-incubated for 2 h at 37 °C in an atmosphere of 100% oxygen. After preincubation, the cell suspension was centrifuged to remove any secreted cortisol and resuspended in fresh incubation medium.

In all experiments, 40-µl aliquots of cell suspension were dispensed into a 96-well tissue culture plate and stimulated for 90 min at 37 °C with ACTH (1-24) 50 ng litre⁻¹ either alone or in combination with increasing concentrations of the anaesthetic. The anaesthetics were incorporated into cell suspension in dimethylsulphoxide (DMSO) such that the final concentration of solvent was 2.5%. In some experiments thiopentone was incorporated into the

SUMMARY

The i.v. anaesthetic agents propofol, thiopentone and etomidate inhibited ACTH-stimulated production of cortisol by guineapig dispersed adrenal cells in a dose-related manner. For two of the drugs, propofol and thiopentone, inhibition occurred over a similar concentration range: 2 x 10⁻⁶ – 5 x 10⁻⁴ mol litre⁻¹. With etomidate, inhibition occurred over a much lower concentration range (5 x 10⁻⁸ – 5 x 10⁻⁶ mol litre⁻¹). The concentrations of anaesthetic which induced 50% inhibition of cortisol secretion were propofol 1.7 x 10⁻⁴, thiopentone 1.6 x 10⁻⁴, and etomidate 1.0 x 10⁻⁷ mol litre⁻¹.
cell suspension in aqueous medium (EMEM). When appropriate, 2.5% DMSO was also included in the basal (no added ACTH) and ACTH 50 ng litre\(^{-1}\)-stimulated wells. The total volume of incubate in each well was 80 \(\mu\)litre and the final cell concentration was \(0.75 \times 10^6\) cells ml\(^{-1}\). After 90 min incubation, duplicate 10-\(\mu\)litre samples were taken and assayed for cortisol by specific radioimmunoassay (Amerlex kit, Amersham International, U.K.). None of the anaesthetic agents interfered with the radioimmunoassay at the concentrations used.

**Statistical analysis of results**

In each experiment, duplicate wells were set up containing no added ACTH (basal) and ACTH 50 ng litre\(^{-1}\) in the absence of drug. Single wells were set up at each drug concentration. Duplicate samples were taken from each well. Intra- and inter-well coefficients of variation for this system are <6 and <10%, respectively (\(n > 100\) in both cases) (Lambert, Garner et al., 1984). Levels of significance were estimated by Student's \(t\) test.

**Source of chemicals**

ACTH (1–24) (Synacthen) was a gift from Dr C. McMartin (Ciba-Geigy, Horsham, West Sussex). Propofol (Diprivan) was supplied by ICI Pharmaceuticals, Macclesfield, Cheshire and thiopentone by May and Baker Ltd, Dagenham. Etomidate was obtained from Janssen Pharmaceuticals, Marlow, Bucks. DMSO was purchased from Fisons Ltd, Loughborough.

**RESULTS**

The effects of increasing concentrations of propofol, thiopentone and etomidate on ACTH 50 ng litre\(^{-1}\)-stimulated cortisol secretion are shown in figure 1. For two of the anaesthetics (etomidate and propofol) the composition of the medium was 97.5% EMEM–2.5% DMSO, and in this medium ACTH 50 ng litre\(^{-1}\) provoked a 21 ± 4-fold (mean ± SD, \(n = 12\)) increase in cortisol secretion over the control. Inhibition of cortisol secretion by etomidate and propofol occurred over the ranges \(5 \times 10^{-6}\) to \(5 \times 10^{-4}\) mol litre\(^{-1}\) and \(2 \times 10^{-3}\) to \(5 \times 10^{-2}\) mol litre\(^{-1}\), respectively. In the case of thiopentone, the maximum solubility of this drug in a 97.5% EMEM–2.5% DMSO mixture is \(1.25 \times 10^{-4}\) mol litre\(^{-1}\) and this concentration gave rise to only 34% inhibition of cortisol secretion. In 100% EMEM the limit of solubility was

![Fig. 1. Inhibition of ACTH 50 ng litre\(^{-1}\)-stimulated cortisol secretion by etomidate (○), propofol (☆) and thiopentone (◇). Propofol and etomidate were incorporated into the cell suspension in DMSO such that the final concentration of solvent was 2.5%; thiopentone was incorporated in aqueous medium. When appropriate, 2.5% DMSO was also included in the basal (no added ACTH) and ACTH 50 ng litre\(^{-1}\)-stimulated wells. Results are mean ± SD (\(n = 6\); each experimental point was performed in duplicate and the experiment was repeated three times).](image-url)
TABLE I. Plasma concentrations 1 min after injection of an induction dose of etomidate, thiopentone or propofol.

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Induction dose (mg kg(^{-1}))</th>
<th>Plasma concentration† 1 min after injection (mol litre(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etomidate</td>
<td>0.3</td>
<td>4.6-5.7×10(^{-6})</td>
<td>Doenicke and others (1972)</td>
</tr>
<tr>
<td>Thiopentone</td>
<td>6.0</td>
<td>1.3×10(^{-4})</td>
<td>Burch and Stanski (1983)</td>
</tr>
<tr>
<td>Propofol</td>
<td>1.3</td>
<td>2.2-4.5×10(^{-5})</td>
<td>Adam, Kay and Douglas (1982)</td>
</tr>
</tbody>
</table>

increased to 5×10\(^{-4}\) mol litre\(^{-1}\) and this concentration resulted in 87% inhibition. Over the thiopentone concentrations where comparison could be made (5×10\(^{-6}\) to 1.25×10\(^{-4}\) mol litre\(^{-1}\)) there were no significant differences in the degrees of inhibition observed in the two media. For example, with thiopentone 1.25×10\(^{-4}\) mol litre\(^{-1}\) the degrees of inhibition in 97.5% EMEM-2.5% DMSO and 100% EMEM were 37±3 and 42±6% (mean ± SD, n = 6), respectively, and with 5×10\(^{-5}\) mol litre\(^{-1}\), inhibitions were 20±8 and 20±12% (n = 6), respectively. The inhibition plot for thiopentone shown in figure 1 is over the extended concentration range up to 5×10\(^{-4}\) mol litre\(^{-1}\) in 100% EMEM. The concentration of anaesthetic which provoked 50% inhibition of cortisol secretion was (mean ± SD, n = 3 experiments) 1.6±0.3×10\(^{-4}\), 1.7±0.3×10\(^{-4}\) and 1.0±0.1×10\(^{-7}\) mol litre\(^{-1}\) for thiopentone, propofol and etomidate, respectively.

**DISCUSSION**

Direct anti-adrenal steroidogenic effects have been demonstrated for the first time for the anaesthetic agents propofol and thiopentone using an in vitro approach based upon dispersed guineapig adrenal cells (Lambert et al., 1983; Lambert, Ratcliffe and Robertson, 1984; Lambert et al., 1985). The ED\(_{50}\) values recorded for inhibition of ACTH-stimulated cortisol secretion by these two drugs were similar (1.7 and 1.6×10\(^{-4}\) mol litre\(^{-1}\), respectively). In contrast, etomidate, an anaesthetic now known to block adrenal steroidogenesis in vitro (Allolio et al., 1983; Fellows, Byrne and Allison, 1983; Lee Wagner et al., 1984) was approximately 1500-1600 times more potent as an adrenal inhibitor in vitro, having an ED\(_{50}\) of 1.0×10\(^{-7}\) mol litre\(^{-1}\), a figure in excellent agreement with our earlier reports (Lambert et al., 1983; Lambert, Ratcliffe and Robertson, 1984).

In general, the relevance of the relative anti-adrenal steroidogenic potency measured in any in vitro system to its pharmacological effects in vivo must be viewed with some caution. However, this does not mean they give no indication as to possible in vivo activity and it can be seen (table I) that the concentration of etomidate found in plasma 1 min after administration of an induction dose to human subjects greatly exceeds (>50 times) its in vitro ED\(_{50}\) value. In contrast, thiopentone, an anaesthetic for which there is no clinical evidence of adrenal inhibition, may achieve plasma concentrations which can only approach its ED\(_{50}\). Moreover, an induction dose of propofol leads to a 1-min plasma concentration of 4.5×10\(^{-5}\) mol litre\(^{-1}\), a concentration which would lead to <20% inhibition of adrenal steroidogenesis in our in vitro assay. On this basis, and in the absence of any clinical or other evidence from animal studies, it is unlikely that these two drugs have any short-term deleterious effects on the steroidogenic function of the adrenal gland in vivo.

In conclusion, we have demonstrated that propofol and thiopentone are relatively weak inhibitors of adrenal steroidogenesis in vitro. Furthermore, we have confirmed that etomidate is a potent inhibitor of adrenal function, a characteristic it shares with ketoconazole, another structurally related substituted imidazole drug (Pont et al., 1982; Loose et al., 1983).

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**REFERENCES**


