PORPHYRINOGENICITY OF ETOMIDATE AND KETAMINE AS CONTINUOUS INFUSIONS
Screening in the DDC-primed Rat Model
G. G. HARRISON, M. R. MOORE AND P. N. MEISSNER

The acute porphyrias are a group of inherited metabolic diseases, acute attacks of which are often triggered by the use of certain drugs (Moore and Disler, 1983). Prevention of life-threatening crises of acute porphyria in genetically susceptible individuals depends on identification of those individuals, and identification of porphyrinogenic drugs. In the past, information on the latter was largely anecdotal, but additional objective evidence can now be obtained by observing, in liver homogenates of experimental animal models, the effects of the drug in question on 5-aminolaevulinate synthase (ALAs) activity and the production of the haem biosynthetic porphyrin intermediates (Parikh and Moore, 1978; Blekkenhorst et al., 1980).

Prominent among known porphyrinogenic drugs are the barbiturates most commonly used for induction of general anaesthesia. Of the recently introduced i.v. anaesthetic induction agents, two (Althesin and flunitrazepam (Rohypnol)) have now also been incriminated, while animal screening of a third (etomidate) has produced results that are suggestive of porphyrinogenicity (Disler et al., 1982). Parikh and Moore (1978) also had concluded that this drug was porphyrinogenic, following their demonstration in the rat that repeated i.p. doses of etomidate caused an increase in hepatic ALAs activity. However, in the study of Blekkenhorst and colleagues (1980), in the 3,5-dicarboxy-1,4-dihydrocolloidine (DDC)-primed rat model which simulates latent variegate porphyria, the mean increase in hepatic ALAs activity that followed single dose i.p. administration of etomidate just failed to achieve statistical significance. In humans, no corroborative examples of acute porphyria precipitated by etomidate have yet been reported.

The short disposal half-life of etomidate makes it particularly suitable for use not only for i.v. induction of general anaesthesia when administered as a single dose, but also for total i.v. anaesthesia when administered as a continuous i.v. infusion. In view of this and the above findings, it seemed to us important to extend the screening of etomidate for porphyrinogenicity from single dose administration to continuous i.v. infusion in the DDC-primed rat model.

Control conditions of total i.v. anaesthesia were produced by continuous infusion of ketamine, a drug generally regarded as non-porphyrinogenic
PORPHYRINOGENICITY OF ETOMIDATE AND KETAMINE IN RATS

(Disler et al., 1982).

METHODS

Male Wistar rats (weight 200–300 g) were submitted to i.v. anaesthesia for 6 h by continuous infusion of the test drug to a cannulated femoral vein by means of a mechanically driven syringe pump.

Twelve rats were anaesthetized with etomidate and 12 with ketamine, the dose of anaesthetic having been established empirically as that which maintained adequate depth of anaesthesia without causing anaesthetic death. With etomidate, 4 mg i.p. was adequate for anaesthesia for femoral vein cannulation, whereafter anaesthesia was maintained by continuous i.v. infusion of 10–15 mg kg\(^{-1}\) h\(^{-1}\). With ketamine the doses were 100 mg kg\(^{-1}\) i.p. and 10–15 mg kg\(^{-1}\) h\(^{-1}\), respectively. Both drugs were diluted so that the volume of fluid administered approximated 2 ml h\(^{-1}\).

Spontaneous respiration of air was maintained throughout anaesthesia. Experiments were conducted using pairs of rats one from each group anaesthetized simultaneously. Six animals in each group were treated for 3 days before anaesthesia with DDC administered orally at a dose of 50 mg kg\(^{-1}\) dissolved in 1 ml of corn oil. All animals were starved for 24 h before anaesthesia, but were permitted water ad libitum.

After 6 h of anaesthesia, animals were sacrificed and their livers removed, blotted free of blood and washed with ice-cold saline. Hepatic ALAs activity was assayed and full porphyrin analysis of hepatic homogenates carried out.

Assays

ALAs activity was measured by the method of Moore and others (1980), in which the production of \(^{14}\)C-labelled ALA from \(^{14}\)C-labelled glycine is measured after separation of ALA by high voltage electrophoresis. Incubations were carried out using liver homogenate at 37 °C for 1 h.

Porphyrin analysis. Porphyrins were measured, using HPLC, by the method of Seubert and Seubert (1982). The methyl esters of porphyrins used as standards were obtained from Porphyrin Products (Logan, Utah) and dissolved in chloroform. The separations were performed on a Waters liquid chromatograph with a Model 6000-A solvent delivery system, a Model 710-B WISP automatic injector, a Model RCM-100 module and Radial-Pak silica cartridges, 10-μm particle size and a Model 420-AC fluorescence detector (Waters Associates, Milford, Mass.). The chromatograms were evaluated with a Waters Model 730 Data Module.

Liver samples for porphyrin analysis were prepared as follows: known weights of liver were esterified overnight, in the dark at room temperature in 10 volumes of 5% sulphuric acid in methanol solution. After centrifugation the supernatant was neutralized with 5% ammonia solution in a separating funnel where the porphyrin esters were extracted into chloroform, washed with distilled water and dried by draining over anhydrous sodium sulphate. Before injection to the chromatograph, each sample was evaporated to dryness in a Buchi rotary evaporator and redissolved in exactly 1 ml of chloroform.

<table>
<thead>
<tr>
<th></th>
<th>ALAs (mmol h(^{-1})/g protein)</th>
<th>Uroporphyrin (mmol/g liver) (wet)</th>
<th>Coproporphyrin (mmol/g liver) (wet)</th>
<th>Protoporphyrin (mmol/g liver) (wet)</th>
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<tbody>
<tr>
<td>Unanaesthetized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
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<td>0.8046</td>
</tr>
<tr>
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<td>21.0</td>
<td>0.0044</td>
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<tr>
<td>Ketamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
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<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
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<td>0.2193</td>
<td>0.7613</td>
</tr>
<tr>
<td>SD</td>
<td>22.0</td>
<td>0.0450</td>
<td>0.1293</td>
<td>0.1936</td>
</tr>
<tr>
<td>Etomidate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mean</td>
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<td>0.2975</td>
<td>0.9115</td>
</tr>
<tr>
<td>SD</td>
<td>12.0</td>
<td>0.0584</td>
<td>0.1000</td>
<td>0.2936</td>
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TABLE II. DDC-primed rats anaesthetized for 6 h with ketamine or etomidate. *Significant difference from control (unanaesthetized)

<table>
<thead>
<tr>
<th></th>
<th>ALAs (mmol h⁻¹ g protein)</th>
<th>Uroporphyrin (mmol/g liver) (wet)</th>
<th>Coproporphyrin (mmol/g liver) (wet)</th>
<th>Protoporphyrin (mmol/g liver) (wet)</th>
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<tbody>
<tr>
<td>Unanaesthetized</td>
<td>6</td>
<td>426.1</td>
<td>0.0271</td>
<td>0.679</td>
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<tr>
<td>Mean</td>
<td>122.3</td>
<td>0.0238</td>
<td>0.1709</td>
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<tr>
<td>Ketamine</td>
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<td>473.0</td>
<td>0.0544</td>
<td>0.6950</td>
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<tr>
<td>Mean</td>
<td>77.0</td>
<td>0.7123</td>
<td>0.2799</td>
<td></td>
</tr>
<tr>
<td>Etomidate</td>
<td>6</td>
<td>625.0</td>
<td>0.0712</td>
<td>0.8790</td>
</tr>
<tr>
<td>Mean</td>
<td>105.0</td>
<td>0.2666</td>
<td>0.4452</td>
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</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.02</td>
<td>ns</td>
<td>&lt;0.05</td>
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Control data were obtained from similar assays undertaken on six unanaesthetized rats, half of which were pretreated with DDC. The significance of differences between sample means was established by means of Student's t-test.

RESULTS
Anaesthesia with ketamine caused no significant change when compared with unanaesthetized controls in either untreated (table I) or DDC-primed (table II) rats. Hepatic ALAs activity and porphyrin content were the same in both groups.

By contrast, 6 h of etomidate anaesthesia, which produced no change from control in untreated animals (table I), caused a marked increase in all these parameters in DDC-primed rats (table II, fig. 1). The 47% increase in hepatic ALAs activity and corresponding 85% increase in coproporphyrin and 40% increase in protoporphyrin content were statistically significant. The almost 10-fold increase in uroporphyrin failed to achieve statistical significance because of the large variance in the values.

DISCUSSION
The essential criterion for judging the porphyrinogenicity of a drug in the DDC-primed rat model of latent variegate porphyria is a demonstrable increase in hepatic ALAs activity in response to administration of the drug (Blekkenhorst et al., 1980). On these grounds we must infer from our experiments that etomidate is potentially porphyrinogenic. The 47% increase in ALAs activity observed in response to etomidate was accompanied by a corroborative increase in resultant haem precursors. The failure of the observed increase in uroporphyrin to achieve statistical significance reflects the large variation in haem precursor levels.

![Fig 1. Effect of a 6-h infusion of etomidate on 5-aminolaevulinate synthase (ALAs) activity and haem intermediates in DDC-primed rats. 0 = control. URO = uroporphyrin; COPRO = coproporphyrin; PROTO = protoporphyrin. *Significantly different from control. Vertical bar = standard error of difference.](https://academic.oup.com/bja/article-abstract/57/4/420/275101)
response to drug administration, previously observed by Eales and Blekkenhorst (1978), and the small numbers of animals used in the present trial.

Identification of a porphyrinogenic drug in an animal model is far removed from proof that it is potentially dangerous to the human sufferer from genetic hepatic porphyria. We identify only that, in this particular animal model, the drug in question displays a property known to characterize those drugs which do precipitate crises in susceptible humans. Drug metabolism differs profoundly between species, and even animals so apparently similar as rats and mice display marked variation in the response of their haem biosynthetic pathway to the effects of various drugs.

Final proof of the porphyrinogenicity of a drug should come from a clinical report of the initiation of an acute crisis in a susceptible individual following exposure to the drug. Regrettably, even this premise may be false—there are well documented inconsistencies.

Disler and colleagues (1982) devised the following simple classification of drugs to guide clinicians in the assessment of porphyrinogenicity:

- **Category A**
  - Drugs reported in terms of clinical experience as dangerous or safe by three or more authorities.
  - Associated with corroborative experimental animal data.

- **Category B**
  - As above but only two or fewer authorities.

- **Category C**
  - Drugs evaluated only in the experimental rat model.
  - No corroborative reports of human cases.

- **Category D**
  - Drugs evaluated in chick embryo liver cell culture or "in ovo".
  - Associated with corroborative experimental animal data.

On the basis of our present observations and those of Parikh and Moore (1978), etomidate qualifies for a "C" rating. From this we infer that, although its use in susceptible individuals cannot be definitely contraindicated, it should be so used only when no convenient alternative is available. Thereafter, the patient should be observed and appropriately screened for a period, using biochemical tests, in order to detect any nascent crisis in good time.

Although ketamine has been implicated as porphyrinogenic in a single case report (Wetterberg, 1976), it is considered safe by many authorities (Rizk, Jacobson and Silvay, 1977; Parikh and Moore 1978; Silvay, Miller and Tausk, 1979). Our present study supports this belief in its safety for use in the patient with porphyria.

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**REFERENCES**


