SCREENING OF CERTAIN ANAESTHETIC AGENTS FOR THEIR ABILITY TO ELICIT ACUTE PORPHYRIC PHASES IN SUSCEPTIBLE PATIENTS

G. H. BLEKKENHORST, G. G. HARRISON, E. S. COOK AND L. EALES

SUMMARY

The activity of δ-aminolaevulinic acid synthetase (E.C. 2.3.1.37) (ALA-S) was measured in rat liver after the simultaneous administration of various anaesthetic agents and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) in vivo. Flunitrazepam, Althesin and phenobarbitone caused a significant increase in the activity of the enzyme which was not observed with propanidid, etomidate and minaxolone. It is suggested that DDC-treated rat, which resembles latent human variegate porphyria, may be a more valid method of testing drugs for their ability to elicit acute porphyric phases in susceptible individuals. The anaesthetic agents which induced the activity of hepatic ALA-S in this model are not recommended in patients with genetic hepatic porphyria.

The porphyrias are a group of disorders of haem metabolism characterized by specific patterns of haem precursor overproduction, accumulation and excretion, each pattern defining a particular form of porphyria. Variegate, or South African genetic porphyria, is one of the forms particularly common in southern Africa. It has been estimated that there are more than 8000 susceptible individuals (Dean, 1971) with a frequency of 1 in 250 in one region. The acute phase of this condition and of two other forms of the hereditary "hepatic" porphyrias, acute intermittent porphyria and hereditary coproporphyria, may be life-threatening. It is usually caused by exposure of the susceptible individual to drugs, particularly barbiturates (Eales, 1971). The frequency of acute porphyrinic attacks in South Africa has been reduced dramatically by effective family surveys, when asymptomatic but biochemically positive members of families have been warned to avoid precipitating agents (Eales, 1971). Identification of drugs which are porphyrogenic is also necessary to prevent life-threatening acute porphyrinic crises. Previously this has been largely a process of trial and error or anecdote, but it is now possible to identify the porphyrogenicity of drugs in the laboratory.

Rats given a relatively low dose of 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) exhibit a condition which resembles latent human variegate porphyria. The animals become sensitive to drugs which can precipitate the metabolic disorder such that, biochemically, the reaction is typical of the human attack (De Matteis, 1973). We have used the DDC-primed rat to test the porphyrogenicity of phenobarbitone and the i.v. anaesthetic agents Althesin, etomidate, flunitrazepam, propanidid and minaxolone by determination of the activity of hepatic δ-aminolaevulinic acid synthetase (E.C. 2.3.1.37) (ALA-S). This is the initial and rate-limiting enzyme of haem biosynthesis (Granick and Urata, 1963) and is increased in the three hereditary "hepatic" porphyrias (Elder, Gray and Nicholson, 1972; Meyer and Schmid, 1973).

MATERIALS AND METHODS

Male Wistar rats (weight 180–220 g) were starved for 24 h but water was freely allowed. In each experiment the five animals in the control group each received arachis oil 1 ml; the second group of five received the drug under study; a third group of five received DDC 100 mg kg⁻¹ suspended in arachis oil and the fourth group received DDC 100 mg kg⁻¹ simultaneously with the drug under study. The drugs were administered i.p. in the doses: phenobarbitone (Gardenal, May and Baker, Port Elizabeth, South Africa) 50 mg kg⁻¹; propanidid (Fabantol, Bayer, Leverkusen, Germany) 100 mg kg⁻¹; etomidate (Ethnor, Halfway House, South Africa) 5 mg kg⁻¹; flunitrazepam (Rohypnol, Roche, Isando, South Africa) 0.5 mg kg⁻¹; Althesin (Glaxo–Allenbury, Waddeville, South Africa) 12 mg kg⁻¹ and minaxolone (Glaxo Group Research, Greenford, Middlesex, England)

© Macmillan Publishers Ltd 1980
The volume of the drug injected was usually 1 ml and drugs were diluted if necessary in sodium chloride solution (0.15 mmol litre; saline).

After 17 h, the animals were sacrificed by decapitation, exsanguinated and the livers removed, blotted free of blood and washed with ice-cold saline. For measurement of ALA-S, the liver was weighed, chopped into small pieces, washed twice with ice-cold saline and homogenized in Tris : HCl buffer 0.01 mol litre⁻¹, pH 7.4 containing sodium chloride 0.15 mmol litre⁻¹ and EDTA 0.5 mmol litre⁻¹ using a motor-driven Teflon pestle in a glass mortar to give a 10% (w/v) homogenate. The assays for ALA-S were performed as described previously (Pimstone, Blekkenhorst and Eales, 1973) using a modification of the method of Strand and others (1972b).

The significance of difference between mean values was assessed by Student's t test.

RESULTS

A preliminary experiment established that a dose of DDC 100 mg kg⁻¹ gave a maximal increase in ALA-S activity when administered with phenobarbitone, a known porphyrogen (Eales, 1971). When administered alone, this dose of DDC gave only a modest increase in ALA-S activity.

The activity of hepatic ALA-S in rats treated with the anaesthetic agents together with DDC is shown in figure 1. Phenobarbitone and DDC caused an increase in the activity of the enzyme but no change was observed in the activity when propa- nidid and DDC were administered compared with the activity of ALA-S when DDC alone was given. No significant difference in hepatic ALA-S activity was observed when etomidate and minaxolone were given together with DDC, but flunitrazepam and Althesin given with DDC caused an increase in the activity of hepatic ALA-S (P<0.05).

The activities of the enzyme in rats treated with only the drug under test were in all cases found to be not significantly different from those in rats receiving oil only.

DISCUSSION

The results of this study demonstrate that phenobarbitone and the anaesthetic agents flunitrazepam and Althesin, when given simultaneously with DDC to rats cause a significant increase in the activity of hepatic ALA-S compared with the activity of the enzyme when DDC is given alone. On this basis, these drugs must be regarded as porphyrogenic and contra-indicated in patients with genetic porphyria.

A similar investigation (Parikh and Moore, 1978) showed that repeated doses of various anaesthetic agents, including Althesin and etomidate, increased hepatic ALA-S activity. The experimental approaches adopted by these workers may not be correct to screen drugs for their porphyrogenicity, since it appears that increased activity of hepatic ALA-S results from the coexistence of two requirements, each of which alone may have little or no effect on the enzyme. These are: first, an effect on haem synthesis and, second, exposure to lipid-soluble inducers of haemoprotein synthesis (Maxwell and Meyer, 1978). Thus when rats are primed with small doses of DDC which cause a partial block at the ferrochelatase level (De Matteis, 1973), and a lipid-soluble inducer of haemoprotein synthesis such as phenobarbitone is administered, a potentiation of ALA-S activity is observed. However, single doses of
the inducing drugs administered to animals with an intact haem biosynthetic pathway have little effect on hepatic ALA-S activity (De Matteis, 1978; Maxwell and Meyer, 1978).

Similarly, inducing drugs can superimpose their effects on ALA-S in the hereditary hepatic porphyrias. The basis genetic defects in the haem biosynthetic pathway have been shown to be decreased uroporphyrinogen synthetase in acute intermittent porphyria (Strand et al., 1972a), decreased coproporphyrinogen oxidase in hereditary coproporphyria (Elder et al., 1977) and a decreased ferrochelatase activity (Becker et al., 1977) and decreased protoporphyrinogen oxidase, or both (Brenner and Bloomer, 1979), in variegate porphyria. When these drugs are administered to patients with the metabolic defect, the acute porphyrin phase becomes manifest, with an inappropriate increase in hepatic ALA-S activity (Meyer and Schmidt, 1973).

The determination of hepatic ALA-S in the DDC-primed rat, which biochemically resembles latent variegate porphyria, appears to be a reliable technique in screening drugs for their potential porphyrogenicity. Phenobarbitone is the classic porphyrogenic agent, while propanidid is known from clinical use to be free of this disadvantage. The inclusion of these drugs among those tested serves to an extent as a control of the method. This method appears more sensitive than determination of hepatic haem precursors after administration of DDC and drugs (Eales and Blekkenhorst, 1978), when large variations in the individual responses of rats to the drugs were observed. It should be pointed out, however, that induction of ALA-S by drugs is a dose-related phenomenon, and a large species variation in the therapeutic or toxic effects of chemical substances exists. Furthermore, there is an interspecies variation in drug metabolism and pharmacological response especially to liposoluble drugs (Brodie and Reid, 1971), all of which must be considered when extrapolating these findings to man. In the present experiments, large doses were used, but less than the known lethal doses of the anaesthetic agents. The in vivo screening technique presented here is more valid than the exquisitely sensitive chick embryo liver cell culture (Granick, 1966) and chick embryo in vivo (Anderson, 1978) screening systems, in which metabolites which may augment synthesis of ALA-S cannot be readily excreted. It is conceivable that some drugs classified as porphyrogenic using these methods might be safe to use in the hereditary hepatic porphyrias.

In this investigation of the porphyrogenicity of newer i.v. anaesthetic agents, we conclude that etomidate and minaxolone are probably non-porphyrogenic in susceptible patients, whereas Althesin and flunitrazepam are likely to be dangerous. Nevertheless, the ultimate test of porphyrogenicity of these anaesthetics can only be the response of individuals who suffer genetic porphyria to administration of these agents.

ACKNOWLEDGEMENT

We thank the South African Medical Research Council for financial support.

REFERENCES


DEPISTAGE DE CERTAINS AGENTS ANESTHESIANTS SUSCEPTIBLES DE PROVOQUER DES PHASES PORPHYRIQUES AIGUES SUR DES MALADES SUJETS A CE GENRE DE REACTION

RESUME
On a mesuré l'activité de la symthétase de δ-aminolévulinique (E.C. 2.3.1.37) (ALA-S) dans le foie d'un rat après l'administration simultanée de divers agents anesthésiants et de 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) in vivo. Le flunitrazépam, l'Althesine et le phénobarbitone ont provoqué une augmentation significative de l'activité de l'enzyme, ce que l'on n'a pas observé avec le propanidide, l'éтомidate et le minaxolone. Ceci laisse penser que le rat traité au DDC, lequel ressemble à la porphyrie humaine diversicolore latente, peut constituer une méthode plus valable d'essayer les médicaments susceptibles de provoquer des phases porphyrigiques aiguës sur les individus sujets à ce type de réaction. Les agents anesthésiants qui ont provoqué l'activité de l'ALA-S hépatique dans ce modèle ne sont pas recommandés pour les malades souffrant de porphyrie hépatique génétique.

PRÜFUNG GEWISSEER NARKOSEMITTEL AUF IHRE EIGENSCHAFT, AKUTE PORPHYRIEPHASEN IN ANFÄLLIGEN PATIENTEN HERVORZURUFEN

ZUSAMMENFASSUNG

Die Narkosemittel, die in diesem Modell die Aktivität von hepatischer ALA-S hervorriefen, werden für Patienten mit genetischer hepatischer Porphyrie nicht empfohlen.

SELECCION DE CIERTOS AGENTES ANESTESICOS CON ARREGLO A SU HABILIDAD PARA ELUCIDAR LAS FASES PORFIRICAS EN PACIENTES SUSCEPTIBLES

SUMARIO
Se midió la actividad de la sintetasa de ácido δ-aminolevulínico (E.C. 2.3.1.37) (ALA-S) en el hígado de ratas después de la administración simultánea de varios agentes anestésicos y de 3,5-diétoxicarbonil-1,4-dihidrocollidina (DDC) in vivo. El flunitrazepam, la Altesina y la fenobarbitona ocasionaron un incremento significativo en la actividad de la enzima, que no se observó con el propanidida, etomidate ni con la minaxolona. Parece ser que el tratamiento de la rata con DDC, que se asemeja a la variedad de porfiria latente en el ser humano, es un método de mayor validez para la verificación de drogas respecto a su habilidad para elucidar las fases porfíricas agudas en individuos susceptibles. Los agentes anestésicos que indujeron la actividad del ALA-S hepático en este modelo, no se recomiendan para pacientes con porfiria hepática de tipo genético.