Clinical and Experimental Pharmacology and Physiology (2000) **27**, 796–800

RELEVANCE OF CYTOCHROME P450 LEVELS IN THE ACTIONS OF ENFLURANE AND ISOFLURANE IN MICE: STUDIES ON THE HAEM PATHWAY

Ana Maria Buzaleh, Maria del Carmen Martinez and Alcira Maria del Carmen Batlle

Centro de Investigaciones sobre Porfirinas y Porfirias, Ciudad Universitaria, Pabellón II, Buenos Aires, Argentina

SUMMARY

1. The effect of the fluorinated ether anaesthetics enflurane and isoflurane in mice on haem metabolism and regulation in different metabolic states, such as depression and induction of cytochrome P450 produced by allylisopropylacetamide (AIA) and imidazole, respectively, was investigated.

2. Mice previously treated with AIA (350 mg/kg, i.p.) or imidazole (400 mg/kg, i.p.) received a single dose (1 mL/kg, i.p.) of enflurane or isoflurane and were killed 20 min after anaesthetic administration.

3. Induction of δ-aminolevulinic acid synthetase (ALA-S) activity was found, as expected, in animals receiving AIA and also in animals treated with AIA plus anaesthesia, but no change in the activity of either porphobilinogenase (PBGase) or porphobilinogen deaminase (PBG-D) activities was detected in these two groups of animals. An additional increase in haem destruction was observed in the AIA plus isoflurane-treated group. When mice were injected with imidazol alone or in combination with the anaesthetics, ALA-S activity was increased 50–90% in all groups, but again no change in PBGase or PBG-D activity was observed. Haem oxygenase was diminished in mice receiving imidazole and anaesthesia.

4. In conclusion, neither enflurane nor isoflurane caused additional disturbances in haem metabolism to those produced by AIA or imidazole alone.

Key words: allylisopropylacetamide, cytochrome P450, enflurane, haem metabolism, imidazole, isoflurane, volatile anaesthetics.

INTRODUCTION

The porphyrias are genetically transmitted disorders of haem metabolism. Their clinical expression may be influenced by external factors as well as by endogenous processes. Thus, in the acute types of porphyria, neuropsychiatric symptoms are precipitated by exposure to cytochrome P450 (CYP)-inducing agents and by conditions such as hormonal changes, infection and low caloric intake.[1,2]

A common denominator for the acute porphyrias during the attack is the overproduction of the porphyrin precursor δ-aminolevulinic acid (ALA). Therefore, it seems plausible to assume that the reason for the varying susceptibility to clinical manifestations of the disorder may be found in differences in metabolic processes determining the rate of ALA generation at given degrees of induction of the rate-limiting enzyme in haem synthesis, namely δ-aminolevulinic acid synthetase (ALA-S).[3]

The same drugs that induce ALA-S also induce the synthesis of several members of the microsomal CYP family.[4] Considering that the concentration of CYP in hepatocytes is relatively high, the induction of this mixed-function oxidase will deplete the free haem pool and, so, increase the activity of ALA-S.[5]

Porphyrias present special anaesthetic challenges, including pre-operative assessment of a patient with acute abdominal pain, intra-operative management of a known porphyria and respiratory and cardiovascular management of acute porphyric crisis.[6]

Enflurane (Ethrane; Abbott Laboratories Argentine SA, Sarmiento, Argentina; 2-chloro-1,1,2-trifluorethyldifluormethylether) and isoflurane (Forane; Abbott Laboratories Argentine SA, 1-chloro-2,2,2-trifluormethyldifluormethylether) are volatile anaesthetics commonly used in medical practice. The toxicity of these anaesthetics is closely related to their metabolism catalysed by hepatic CYP.[7]

Allylisopropylacetamide (AIA), a known porphyrinogenic xenobiotic, causes destruction of the cytochrome haem group, thus requiring new haem synthesis with consequent ALA-S induction.[8,9]

Imidazol is a powerful CYP inductor; pretreatment of rabbits with this xenobiotic resulted in a two-fold increase in hepatic microsomal CYP content.[10,11] Hoffman *et al*[12] observed a 250% increase in enflurane metabolism in rabbits treated with imidazole.

Acute and chronic administration of enflurane and isoflurane has been shown to produce marked alterations in haem metabolism, confirming the porphyrinogenic properties of these drugs.[13]

We have demonstrated that enflurane[14] and isoflurane[15] alter haem metabolism in Swiss mice when the anaesthetics were administered in a single dose of 2 mL/kg and the results tested 20 min later. Strain and sex differences in the effects of enflurane and isoflurane have also been reported.[16]

Alterations in haem metabolism produced by chronic enflurane anaesthesia may be directly related to increased CYP levels, which eventually lead to ALA-S induction. In contrast, chronic isoflurane anaesthesia may induce ALA-S through an alternative mechanism, which appears either not to involve CYP or to be through specific variations in CYP2E1.[17]

The aim of the present study was to investigate the effects of enflurane and isoflurane on haem metabolism and its regulation in mice in different metabolic states, such as induction and depression

Correspondence: Professor Alcira Batlle, Viamonte 188L 10° 'A', C1056ABA, Buenos Aires, Argentina. Email: batlle@mail.retina.ar

Received 12 November 1999; revision 1 June 2000; accepted 8 June 2000.

of CYP produced by imidazole and AIA, respectively. The focus of the present study points out the possibility that raising or lowering CYP levels in the liver may change the metabolism of volatile anaesthetics and, thus, influence the effects of these agents on haem metabolism.

In the present study, we have measured the activity of ALA-S and the enzyme system porphobilinogenase (PBGase) and porphobilinogen deaminase (PBG-D) to evaluate any possible alteration in haem biosynthesis, as well as the activity of haem oxygenase (haem-ox), the first enzyme involved in the breakdown of haem, and rhodanese, the enzyme responsible for the degradation of cystine trisulphide, which has been proposed as an activator of ALA-S.[18]

METHODS

Enflurane and isoflurane were obtained from Abbott Laboratories (Sarmiento, Argentina). All other chemicals used were reagent grade and were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Animals

Albino male adult CF1 mice (six animals/group), weighing 25–30 g, were maintained under controlled conditions and were allowed free access to food (Purina 3; Asociación de Cooperativas Argentinas, San Nicolás, Buenos Aires, Argentina) and water. Animals received humane care and were treated in accordance with the guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC).

Treatments

Enflurane or isoflurane

Animals received a single dose of either anaesthetic of 1 mL/kg, i.p. (0.03 : 0.3 mL oil, v:v) and were killed 20 min later.

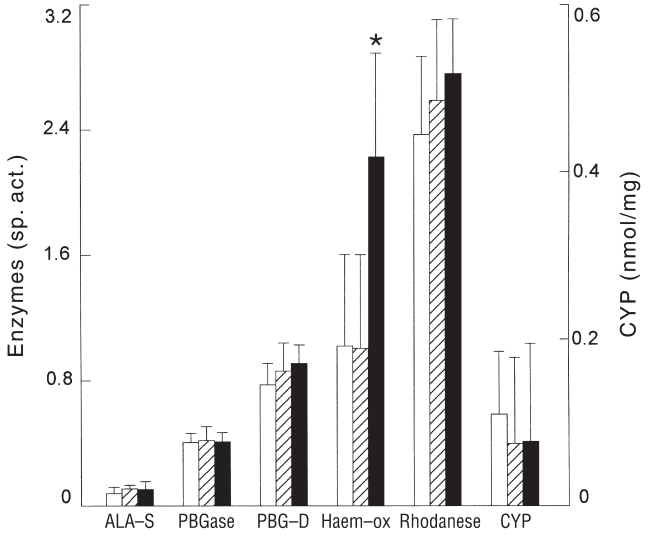

Fig. 1 Effects of the administration of enflurane and isoflurane on liver haem metabolism and its regulation. (□), control; (▨), enflurane; (■), isoflurane. Mice received one dose of 1 mL/kg enflurane or isoflurane and were killed 20 min later. Data are the mean±SD of at least six animals. *$P < 0.01$ for comparisons between treated and control groups. Other experimental details are as described in the text. ALA-S, δ-aminolevulinic acid synthetase; PBGase, porphobilinogenase; PGB-D, porphobilinogen deaminase; haem-ox; heam oxygenase; CYP, cytochrome P450; sp. act., specific activity.

Allylisopropylacetamide

Mice received a single dose of 350 mg/kg, i.p. (1 : 3 ethanol:water) AIA 16 h prior to the injection of the anaesthetics and were killed 20 min after administration of anaesthetic.

Imidazole

Animals received 400 mg/kg, i.p., imidazole over 4 days. Twenty-four hours after the last injection of imidazole, mice were injected with the anaesthetics and were killed 20 min later.

Control animals and animals receiving AIA or imidazole alone were injected with corn oil, used as vehicle for the anaesthetics, and were killed at the same time as the group receiving enflurane or isoflurane only.

Methods of analysis

Control and treated mice were always killed at the same time of the day, under ether anaesthesia by cardiac puncture and bled. Tissue (liver and whole blood) preparations were obtained and enzyme (ALA-S, PBGase, PBG-D, haem-ox and rhodanese) measurements were performed as described previously.[14] Liver assays were performed in whole homogenates for ALA-S or in the cytosolic fraction obtained after centrifugation at 18 000 *g* for other enzyme activities. The levels of CYP were determined in liver microsomes using the method of Omura and Sato.[19] Protein concentrations were estimated by the method of Lowry *et al*.[20] One enzyme unit was defined as the amount of enzyme that catalysed the formation of 1 nmol product under the standard incubation conditions. Specific activity was expressed as enzymic units per mg protein.

Statistical analysis

Data are shown as the mean±SD for each variable for each group and values were compared using the Newman–Keuls' test. The significance of differences between groups was analysed using analysis of variance (ANOVA) and $P < 0.05$ was regarded as significant.

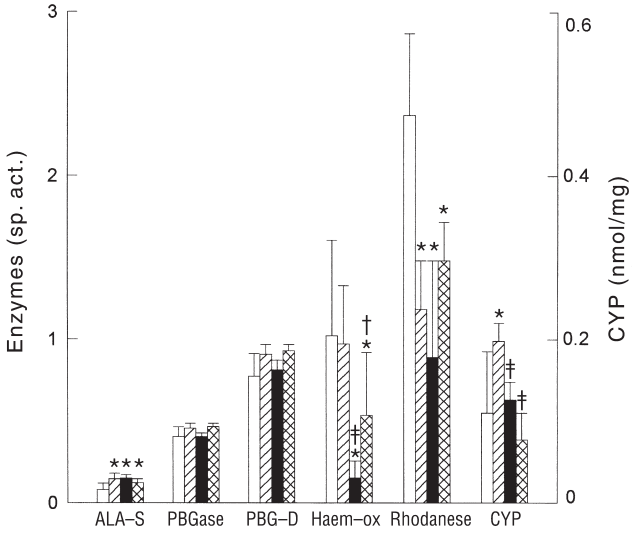

Fig. 2 Effects of the administration of enflurane and isoflurane to imidazole-treated animals on liver haem metabolism and its regulation. (□), control; (▨), imidazole; (■), imidazole + enflurane; (▩), imidazole + isoflurane. Mice received 400 mg/kg, i.p., imidazole over 4 days; 24 h after the last injection of imidazole, they were injected with the anaesthetics and were killed 20 min after the administration of the anaesthetic. *$P < 0.01$ for comparisons between treated and control groups; †$P < 0.05$, ‡$P < 0.01$ for comparisons between mice treated with imidazole and imidazole plus anaesthetic. Other experimental details or conditions are as given in Fig. 1 or the text. ALA-S, δ-aminolevulinic acid synthetase; PBGase, porphobilinogenase; PGB-D, porphobilinogen deaminase; haem-ox; heam oxygenase; CYP, cytochrome P450; sp. act., specific activity.

RESULTS

Effect of anaesthetics

When animals received the anaesthetics alone, no significant changes in any of the liver parameters assayed were observed, except a 118% ($P < 0.01$) induction of haem-ox activity after isoflurane administration (Fig. 1). No alterations were detected in blood parameters (data not shown).

Effects of anaesthetics in imidazole-treated mice

Results obtained in the livers of mice with CYP induction produced by imidazole administration are shown in Fig. 2.

The activity of ALA-S was increased 84% ($P < 0.01$) in animals treated with imidazole. Enzyme activity was induced by approximately 90 and 53% (both $P < 0.01$) by imidazole + enflurane or imidazole + isoflurane, respectively. The magnitude of the induction was not statistically different between the different groups.

The activity of PBGase and PBG-D was not modified in any group.

Imidazole administration alone produced no changes in haem-ox activity. However, mice treated with a combination of this xenobiotic and an anaesthetic showed a 50–85% ($P < 0.01$) inhibition of enzyme activity. Statistical studies showed a statistically significant difference ($P < 0.01$) between the effects of imidazole alone and in combination with the anaesthetics.

Rhodanese activity was reduced 50–60% ($P < 0.01$) in animals treated with imidazole or imidazole + enflurane and reduced 40% ($P < 0.01$) when isoflurane was the anaesthetic injected instead of enflurane.

The induction of CYP levels caused by imidazole (80%; $P < 0.01$) was abolished by the anaesthesia and the difference in CYP levels in imidazole- and imidazole + anaesthetic-treated groups was statistically significant ($P < 0.01$).

Blood activity of PBGase, PBG-D and rhodanese (data not shown) was unchanged in all groups studied.

Effects of anaesthetics in AIA-treated mice

Results obtained in mice with CYP depletion caused by AIA administration are shown in Fig. 3.

Liver ALA-S activity was increased approximately 190% ($P < 0.01$) in animals treated with AIA or AIA + enflurane and was increased 120% ($P < 0.05$) in mice receiving AIA + isoflurane (Fig. 3a). The differences between the level of induction in the different groups were not statistically significant.

Both liver (Fig. 3a) and blood (Fig. 3b) PBGase and PBG-D activities were unchanged in all groups.

Liver haem-ox activity was enhanced 240% ($P < 0.05$) by AIA administration. When animals received AIA plus enflurane or isoflurane, the induction of haem-ox activity was 200 ($P < 0.05$) and 430% (both $P < 0.01$), respectively (Fig. 3a). There was a significant difference observed between the AIA- and AIA plus isoflurane-treated groups.

Liver rhodanese activity (Fig. 3a) was reduced by 40 and 83% (both $P < 0.05$) in mice treated with AIA or AIA + isoflurane, respectively, while the administration of AIA + enflurane had no effect on the activity of this enzyme. A similar profile was found for the activity of the enzyme in the blood (Fig. 3b) in mice receiving AIA or AIA + enflurane, without any changes in enzyme activity seen after the administration of AIA + isoflurane. Statistical studies of differences between groups treated with AIA and AIA + isoflurane in the liver or AIA and AIA + enflurane in the blood revealed no differences, but when groups in which rhodanese activity was unchanged were compared with groups

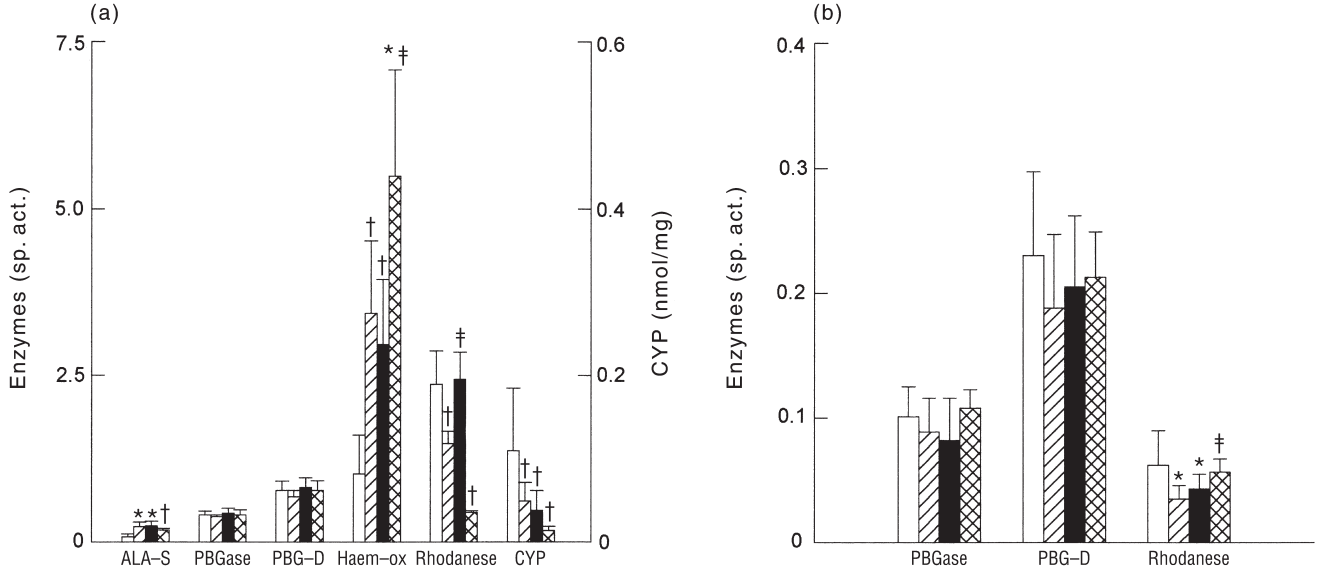

Fig. 3 Effects of administration of enflurane and isoflurane to allylisopropylacetamide (AIA)-treated animals on (a) liver and (b) blood haem metabolism and its regulation. (□), control; (▨), AIA; (■), AIA + enflurane; (▩), AIA + isoflurane. Mice received a single dose of 350 mg/kg (1 : 3 ethanol:water; i.p.) AIA 16 h prior to the injection of the anaesthetics and were killed 20 min after administration of the anaesthetic. $^{\dagger}P < 0.05$, $^{*}P < 0.01$ for comparisons between treated and control groups; $^{\ddagger}P < 0.05$ for comparisons between animals treated with AIA alone and AIA plus anaesthetics. Other experimental details or conditions are as given in Fig. 1 or the text. ALA-S, δ-aminolevulinic acid synthetase; PBGase, porphobilinogenase; PGB-D, porphobilinogen deaminase; haem-ox; heam oxygenase; CYP, cytochrome P450; sp. act., specific activity.

receiving only AIA the differences were statistically significant ($P < 0.05$).

The marked reduction in CYP levels (55%; $P < 0.05$) caused by AIA alone (Fig. 3a) was also observed after the administration of enflurane (64%; $P < 0.05$) and isoflurane (90%; $P < 0.05$). These differences are not of statistical significance.

DISCUSSION

The effect of fluorinated ether anaesthetics, such as enflurane and isoflurane, in mice on haem metabolism and regulation was investigated in the following different metabolic states: (i) induction of CYP by imidazole; and (ii) depression of CYP by AIA.

Oxidative metabolism represents a major route of elimination of many drugs and, because many drugs can compete for the same enzyme, inhibition of CYP enzymes is one of the main reasons for drug interactions.[21]

Allylisopropylacetamide, an olefinic derivative, is a classic suicide substrate of CYP. This compound is now recognized as an effective haem-alkylating inactivator of rat CYP2B1 and CYP3A1, while CYP2C6 and CYP2CII are less susceptible.[22]

In AIA-treated animals, the known marked induction of ALA-S was observed in animals receiving this drug and anaesthesia, but no changes were found for either PBGase and PBG-D activity, the enzymes specifically altered in acute intermittent porphyria. The activity of haem-ox was induced above 100% in animals treated with AIA and the anaesthetics, indicating increased haem destruction.

The induction of CYP is a slow regulatory process that can reduce drug concentrations in plasma and may compromise the efficacy of the drug in a time-dependent manner.[21]

In drug therapy, there are two major concerns related to CYP induction. First, induction will result in a reduction of the pharmacological effects caused by increased drug metabolism. Second, induction may create an undesirable imbalance between 'toxification' and 'detoxification'. Depending on the delicate balance between detoxification and activation, induction can be a beneficial or harmful response.[21]

In the present study, in animals treated with imidazole, ALA-S activity remained high when mice also received anaesthesia. No changes in activity were detected in liver and blood PBGase and PBG-D. The activity of haem-ox was diminished by the combination of imidazole and the anaesthetics.

No explanation can be given about the different effects of isoflurane on haem-ox when CYP levels were either unchanged or decreased or increased. Animal data have demonstrated that there are considerable differences among the anaesthetics in their responses to enzyme induction following pretreatment with specific drugs.[23] To this end, we have previously observed that induction of haem-ox provoked by cobalt treatment was reversed by isoflurane administration without further alterations in CYP levels.[24] In contrast, haem-ox enhancement found in chronically alcoholized animals persisted after isoflurane anaesthesia, with a concomitant increase in CYP levels.[25,26]

In conclusion, neither enflurane nor isoflurane produced further disturbances in haem metabolism than those due to the sole action of AIA or imidazole. However, in animals treated with AIA, its combination with isoflurane led to a significant induction in haem-ox activity, indicating enhanced degradation of haem. In contrast, anaesthetic administration to the imidazole-treated group significantly reduced this enzyme activity. The induction of ALA-S by AIA or imidazole and also after anaesthetic administration has both fundamental and clinical relevance because ALA-S induction is not only responsible for adapting haem synthesis to rapid changes in haem demand but also because it plays a key role in the genetic disorders leading to the hepatic porphyrias.[27]

The inverse relationship observed between the activity of rhodanese and ALA-S confirms, once more, the involvement of this sulphur metabolism enzyme in the regulation of haem synthesis in mammals.[28]

ACKNOWLEDGEMENTS

AMB and AMdelCB are members of the Career of Scientific Researcher at the Argentine National Research Council (CONICET). MdelCM is a fellow of the Agencia de Promoción Científica y Tecnológica (UBATEC). This work has been supported by grants from the CONICET and the University of Buenos Aires, Argentina.

REFERENCES

1. Batlle AM del C. Porphyrins, porphyrias, cancer and photodynamic therapy: A model for carcinogenesis. *J. Photochem. Photobiol. B Biol.* 1993; **20**: 5–22.
2. Ratnaike S, Blake D. The diagnosis and follow-up of porphyria. *Pathology* 1995; **27**: 142–53.
3. Thunnel S, Floderus Y, Harper P, Henrichson A, Lindh U, Marklund S. Pathogenic mechanisms of the acute porphyric attack: Speculative roles of manganese associated enzymes. *Cell. Mol. Biol.* 1997; **43**: 1–8.
4. Nelson DR, Koymans L, Kamataki T *et al.* P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 1996; **6**: 1–42.
5. Moore MR, McColl KEL, Rimington C, Goldberg A. Porphyrins and enzymes of the heme biosynthetic pathway. In: *Disorders of Porphyrin Metabolism.* Plenum, New York. 1987; 21–116.
6. Murphy PC. Acute intermittent porphyria. The anaesthetic problem and its background. *Br. J. Anaesth.* 1964; **36**: 801–12.
7. Quail AW. Modern inhalational anaesthetic agents. A review of halothane, isoflurane and enflurane. *Med. J. Aust.* 1989; **150**: 95–102.
8. De Matteis F. Rapid loss of cytochrome P-450 caused in the liver microsomes by the porphyrinogenic agent 2-allyl-2-isopropylacetamide. *FEBS Lett.* 1970; **6**: 343–5.
9. Polo CF, Navone NM, Afonso SG *et al.* Allyl-isopropylacetamide and glycerine reproduce clinical and biochemical manifestations of acute intermittent porphyria in mice. *Med. Sci. Res.* 1987; **15**: 1335–6.
10. Hajek KK, Novak RF. Spectral and metabolic properties of liver microsomes from imidazole-pretreated rabbits. *Biochem. Biophys. Res. Commun.* 1982; **108**: 664–72.
11. Hostetler KA, Wrighton SA, Molowa DT, Thomas PE, Levin W, Guzelian PS. Coinduction of multiple hepatic cytochrome P-450 proteins and their mRNAs in rats treated with imidazole antimycotic agents. *Mol. Pharmacol.* 1989; **35**: 279–85.
12. Hoffman J, Konopka K, Buckhorn C, Koop DR, Waskell L. Ethanol-inducible cytochrome P450 in rabbits metabolizes enflurane. *Br. J. Anaesth.* 1989; **63**: 103–8.
13. Buzaleh AM, Vázquez ES, Enríquez de Salamanca R, Batlle AM del C. An overview of anaesthesia and porphyria. *Endocrinol. Metab.* 1997; **4**: 267–79.
14. Buzaleh AM, Enríquez de Salamanca R, Batlle AM del C. Porphyrinogenic properties of the anesthetic enflurane. *Gen. Pharmacol.* 1992; **23**: 665–9.
15. Buzaleh AM, Enríquez de Salamanca R, Batlle AM del C. Administration of the anesthetic isoflurane to mice: A model for acute intermittent porphyria? *J. Pharmacol. Toxicol. Methods* 1992; **28**: 191–7.

16. Buzaleh AM, Batlle AM del C. Strain and sex differences in the effect of enflurane and isoflurane on heme metabolism in mice. *Gen. Pharmacol.* 1992; **27**: 1009–12.
17. Buzaleh AM, Vázquez ES, Nuñez G, Batlle AM del C. Effect of chronic anaesthesia on drug metabolizing enzyme system and heme pathway regulation. *Gen. Pharmacol.* 1997; **28**: 577–82.
18. Vázquez E, Buzaleh AM, Wider E, Batlle A. Reduced thiosulphate affinity for erythrocyte rhodanese: An explanation for increased δ-aminolevulinic synthetase activity in some porphyrias? *IRCS Med. Sci.* 1986; **14**: 999–1000.
19. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.* 1964; **239**: 2370–8.
20. Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951; **193**: 265–75.
21. Lin JH, Lu AYH. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin. Pharmacokinet.* 1998; **35**: 361–90.
22. Bornheim LM, Underwood MC, Caldera P *et al.* Inactivation of multiple hepatic cytochrome P450 isozymes in rats by allylisopropyl-acetamide: Mechanistics implications. *Mol. Pharmacol.* 1987; **32**: 299–308.
23. Halsey MJ. Drug interactions in anaesthesia. *Br. J. Anaesth.* 1987; **59**: 112–23.
24. Buzaleh AM, Batlle AM del C. Administration of volatile anaesthetics to animals treated with cobal as inductor of heme oxygenase. *Pharmacol. Rev. Commun.* 2000 (in press).
25. Buzaleh AM, Vazquez ES, Batlle AM del C. Alterations in fluorinated ether anesthetics effects on heme metabolism following chronic ethanol consumption. *Gen. Pharmacol.* 1995; **26**: 1425–9.
26. Buzaleh AM, Vazquez ES, Batlle AM del C. The involvement of microsomal drug metabolizing system during isoflurane anaesthesia in alcoholized animals. *Pharmacol. Commun.* 1996; **7**: 101–6.
27. Jover R, Hoffmann K, Meyer UA. Induction of 5-aminole vulinate synthase by drugs is independent of increased apocytochrome P450 synthesis. *Biochem. Biophys. Res. Commun.* 1996; **226**: 152–7.
28. Vazquez E, Buzaleh AM, Wider E, Batlle A. Red blood cell rhodanese: Its possible role in modulating δ-aminolaevulinate synthetase activity in mammals. *Int. J. Biochem.* 1987; **19**: 217–19.