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SEVOFLURANE: ITS ACTION ON HEME METABOLISM AND PHASE I DRUG METABOLIZING SYSTEM

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Dedicated to the memory of our dear colleague and friend Dr. Susana Afonso

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Abstract – Acute attacks of porphyria are most commonly precipitated by events that decrease heme concentrations. Enzyme inducing-drugs are the most important triggering factors, particularly in relation to anaesthesia. We have reported previously that Enflurane and Isoflurane produced significant heme metabolism alterations, indicating that the use of these anaesthetics in porphyric patients should be avoided. The aim of this work was to evaluate the effect of the anaesthetic Sevoflurane on heme pathway and drug metabolizing Phase I system in mice. To this end, animals received different doses of the anaesthetic (1-2 ml/kg) and were sacrificed at different times (5-60 min). Data revealed important alterations in the enzymes involved in Acute Intermittent Porphyria, such as an induction in hepatic 5-Aminolevulinic acid synthetase activity and a diminished Porphobilinogen deaminase activity in liver and blood 20 minutes after Sevoflurane administration to mice in a dose of 1.5 ml/kg. Heme oxygenase activity was also induced, indicating the onset of oxidative stress. Total CYP levels and CYP2E1 expression were enhanced. As a consequence of these events, heme free pool would be depleted. In conclusion, our results in mice would suggest that Sevoflurane should be used with caution and very careful control in porphyric patients.

Key words: Sevoflurane, Heme metabolism, 5-Aminolevulinic acid synthetase, PBG deaminase Heme oxygenase, Cytochrome P450

INTRODUCTION

The porphyrias can be classified according to the organ where clinical expression of the biochemical defect is maximum in either hepatic or erythropoietic tissues, or according to the main clinical symptom in either acute or cutaneous porphyrias (1). The latter classification is particularly useful for anaesthetic practice because only the acute forms of porphyrias are of major anaesthetic relevance, since these are the conditions that may result in life-threatening reactions to drugs (16).

Acute attacks of porphyria are most commonly precipitated by events that decrease heme concentrations, thus increasing the activity of 5-Aminolevulinic acid synthetase (ALA-S) and so stimulating the production of precursors and porphyrinogens (23, 26). Acute exacerbation may be precipitated by a number of factors, including physiological hormonal functions, fasting, stress and infection (11, 34). Enzyme inducing-drugs are by far, the most important triggering factors, particularly in relation to anaesthesia (14).

Sevoflurane (polyfluorinated methyl-isopropyl compound, $(CF_3)_2CHOCH_2F$) is an inhalation anaesthetic agent, first described in 1972, that was released for clinical use first in Japan in 1990 and then, in 1995-1996 in Germany and United States (3, 10, 25, 28, 36, 38). In comparison with other inhalational agents such as Isoflurane or Halothane, the most important property of Sevoflurane is its low solubility in blood (3, 13, 31). This results in a

Abbreviations: ALA-S, 5-aminolevulinic acid synthetase; **AIP**, Acute Intermittent Porphyria; **CPR**, NADPH Cytochrome P450 reductase; **CYP**, cytochrome P450; **HO**, heme oxygenase; **i.p.**, intraperitoneal; **PBG-ase**, Porphobilinogenase; **PBG-D**, porphobilinogen deaminase

more rapid uptake and induction, than other anaesthetics of the same family, and also faster elimination and recovery. In humans, 2 to 5% of the absorbed dose of Sevoflurane is metabolised; leading to the formation of inorganic fluoride and the organic fluoride metabolite hexafluoroisopropanol which is conjugated with glucuronic acid and rapidly excreted by the kidneys (18). Cytochrome P4502E1 (CYP2E1) is predominantly responsible for the biotransformation of Sevoflurane (17, 37).

We have reported previously that Enflurane and Isoflurane produced important heme metabolism alterations, indicating that the use of these anaesthetics in porphyric patients should be avoided (5-9).

The aim of this work was to evaluate the effect of Sevoflurane on heme pathway in mice with special attention to the enzymes altered in Acute Intermittent Porphyria (AIP). Drug metabolizing Phase I system was also investigated.

MATERIALS AND METHODS

Chemicals

Sevoflurane was from Abbott Laboratories S.A. Antibodies were from Stressgen Bioreagents or Santa Cruz Biotechnology and ECL detection system was from GE Healthcare. All other chemicals used were reagent grade obtained from Sigma Chem. Co., St. Louis, USA.

Animals

Albino male *CF1* mice (4-6 animals/group) weighing 25-30 g (6 weeks old) were maintained in controlled conditions and allowed free access to food (Purina 3, Asociación de Cooperativas Argentinas, San Nicolás, Buenos Aires, Argentina) and water. Animals received human 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).

Experimental design

- Dose-response studies: Animals received a single dose of Sevoflurane: 1, 1.5 or 2 ml/kg (0.3:3; 0.45:3; 0.6:3, v/v in corn oil, i.p.) and were sacrificed 20 minutes after injection.
- Time-response studies: Animals received a single dose of Sevoflurane: 1.5 ml/kg (i.p.) and were sacrificed at different times (10-60 minutes) after injection.

Control animals were injected with corn oil used as vehicle, and were sacrificed at the same time of anaesthetised group. Animals were starved 16 hours prior to the treatments all performed at the same time of the day.

Homogenate preparation

Liver, previously perfused with saline solution, was scissored and immediately processed. Homogenates were prepared using a manual glass homogenizer or pestle Teflon Ultraturrax at 4º C. Blood was collected in heparinized tubes through cardiac puncture.

A fraction of non perfused liver was excised and homogenised in NaCl (0.9%) containing EDTA (0.5 mM) and Tris-HCl buffer (pH 7.4; 10 mM) (1:3, w/v) and was used to measure ALA-S activity. Afterwards, the remainder liver was perfused with sterile ice cold saline and removed. A fraction was homogenised (1:3, w/v) in ice cold sucrose (0.25 M). After differential centrifugation of the homogenate, the 18,000xg supernatant was employed to determine the activities of Porphobilinogenase (PBG-ase), PBG-deaminase (PBG-D) and Heme oxygenase (HO); the pellet obtained after centrifugation at 105,000xg for 90 minutes was used for measuring total cytochrome P450 (CYP) and CYP2E1 activity. For blood PBG-ase and PBG-D activity determinations, whole blood was hemolyzed with Triton X-100 (5%) and diluted in Tris-HCl buffer (pH 7.4; 0.05 M) (1:5, v/v).

For immunoblotting, liver tissue was homogenized (1:5, w/v) in 10 mM TRIS-HCl pH 7.4, containing 20% glycerol (v/v), 1.14% KCl (w/v), 0.2 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulphonyl fluoride, 10 µg/ml leupeptin and 1 µg/ml pepstatin A. Homogenates were centrifuged with the same protocol as described above and the pellet obtained after centrifugation at 105,000xg for 90 minutes was used for measuring CYP2E1 and NADPH Cytochrome P450 reductase (CPR) expression.

Assays

Liver ALA-S activity was measured by the method of Marver et al. (21). Liver and blood PBG-ase and PBG-D enzymes were determined by the method of Batlle et al. (2) and liver HO according to Tenhunen et al. (33). Total CYP content was determined in the microsomal fraction as described Omura and Sato (22) and CYP2E1 activity was measured using the method of Reinke and Moyer (27). Enzyme units were defined as the amount of enzyme forming 1 nmol of product under standard incubation conditions. Specific activity was expressed as units/mg protein.

Expression of CYP2E1 and CPR proteins was determined by Western Blot analysis. Protein (3 µg) were separated on a 7.5% SDS-PAGE and transferred to nitrocellulose membranes. After blocking overnight with 5% bovine seroalbumine (BSA) in Tris-buffered saline containing 1% Tween 20 (TBS-T 1%), the blots were incubated for 1 hour at room temperature with the specified primary antibody *antiCYP2E1* (1:2,500 in TBS-T 0,1% with 0.5% BSA; Stressgen) or *antiCPR* (75 µg/ml in TBS-T 0.1% with 0.5% BSA; Stressgen). After several washings, blots were incubated for 1 h with a secondary goat anti-rabbit antibody conjugated with horseradish peroxidase (HRP) (1:8,000 v/v in TBS-T 1%, Santa Cruz Biotechnology). Blots were detected by chemiluminescence using ECL detection system and exposed to X-ray film (AGFA). Quantification of bands was performed using the Scion Image software.

Protein concentration was estimated by the procedure of Lowry et al. (19) or according to Bradford method (4) for immunoblotting technique.

Statistical analysis

Data were expressed as mean values ± s.d. Differences in mean values between treated and control groups were evaluated using the analysis of variance (ANOVA) and $p<0.05$ was considered statistically significant.

RESULTS

The effects of Sevoflurane on heme metabolism were evaluated through the enzymes ALA-S in liver and, PBG-ase and PBG-D in liver and blood. Results are shown in Figures 1 and 2.

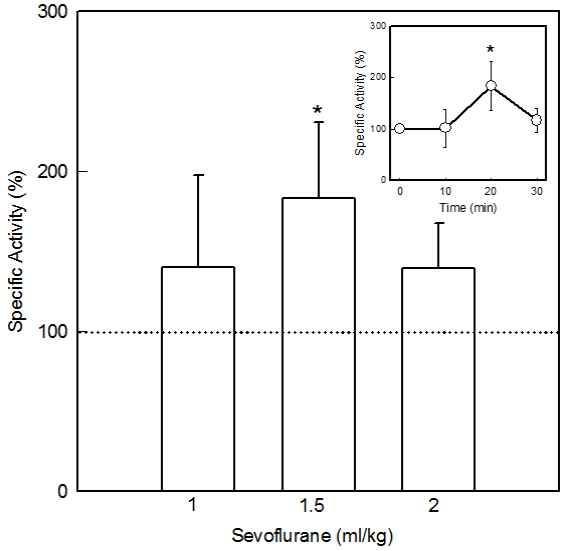

FIGURE 1. Effect of Sevoflurane on hepatic ALA-S activity: Dose and time-response.
Animals received different doses of the anaesthetic (1 ml/kg, 1.5 ml/kg and 2 ml/kg, i.p.) and were sacrificed 20 minutes later. Inset: Animals received one dose of anaesthetic (1.5 ml/kg, i.p.) and were sacrificed at different times after injection (10, 20 and 30 minutes). Data represents mean value ± s.d. of at least 4-6 animals and are expressed relative to specific activity of control (·····) value. Mean control value (nmol/mg): 0.079±0.040 (n=16). (✱) p<0.05, significance of differences between treated and control groups. Other experimental details are described in Material and Methods.

ALA-S activity was 80% (p<0.05) induced when the dose of the anaesthetic was 1.5 ml/kg (Figure 1). Hepatic PBG-ase and PBG-D were 30% (p<0.05) diminished when animals received a dose of 1.5 mg/kg (Figure 2 A); while no alterations of these enzyme activities were observed as a function of the different doses assayed in blood (Figure 2 B).

Taking into account the results obtained, the dose of 1.5 ml/kg was chosen and the effects of Sevoflurane were evaluated at different times (10, 20 and 30 minutes) after anaesthetic administration. Results are shown in the insets of Figures 1 and 2. A maximum in ALA-S activity was observed 20 minutes after anaesthesia (Figure 1, inset). Hepatic PBG-ase and PBG-D were 30% (p<0.05) diminished 20 minutes after Sevoflurane administration (Figure 2 A, inset); while blood enzymes showed a similar profile but after 30 minutes treatment (Figure 2 B, inset).

The effects on HO, limited enzyme of heme catabolism, are shown in Figure 3. HO activity increased 150% (p<0.01) 10 minutes after anaesthetic administration, remaining induced during the times assayed.

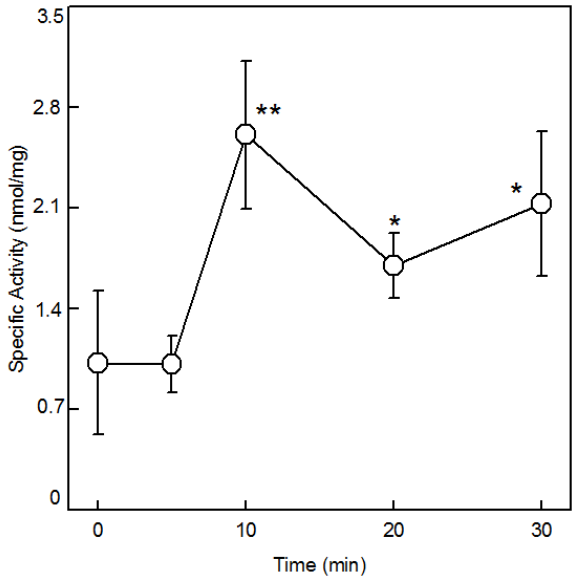

FIGURE 3. Effect of Sevoflurane on liver HO activity: Time response.
Animals received one dose of the anaesthetic (1.5 ml/kg, i.p.) and were sacrificed at different times after injection (5-30 minutes). Data represents mean value ± s.d. of at least 4-6 animals. (✱) p<0.05, (✱✱) p<0.01, significance of differences between treated and control groups. Other experimental details are described in Materials and Methods.

To further characterise this experimental model, the effects of Sevoflurane on Phase I drug metabolizing system was investigated measuring CYP levels, the activity and expression of CYP2E1 and the expression of CPR in animals receiving a dose of 1.5 ml/kg and sacrificed at different times after (5-60 minutes). Results are shown in Figures 4 and 5.

CYP levels were more than 200% (p<0.01) augmented after 5 minutes and remain in this high levels during the entire period assayed (Figure 4 A).

No alterations were observed in CYP2E1 activity even 60 minutes after Sevoflurane administration (Figure 4 B). So, it was of interest to evaluate if there were any modifications in protein expression levels. Western Blot analysis revealed the expected induction but only 30 minutes after anaesthetic administration; the expression was even greater 60 minutes latter (85%, p<0.05) (Figure 5 A).

CPR protein expression was 80% (p<0.05) augmented after 20 minutes of anaesthetic administration, remaining induced also after 30 minutes (Figure 5 B).

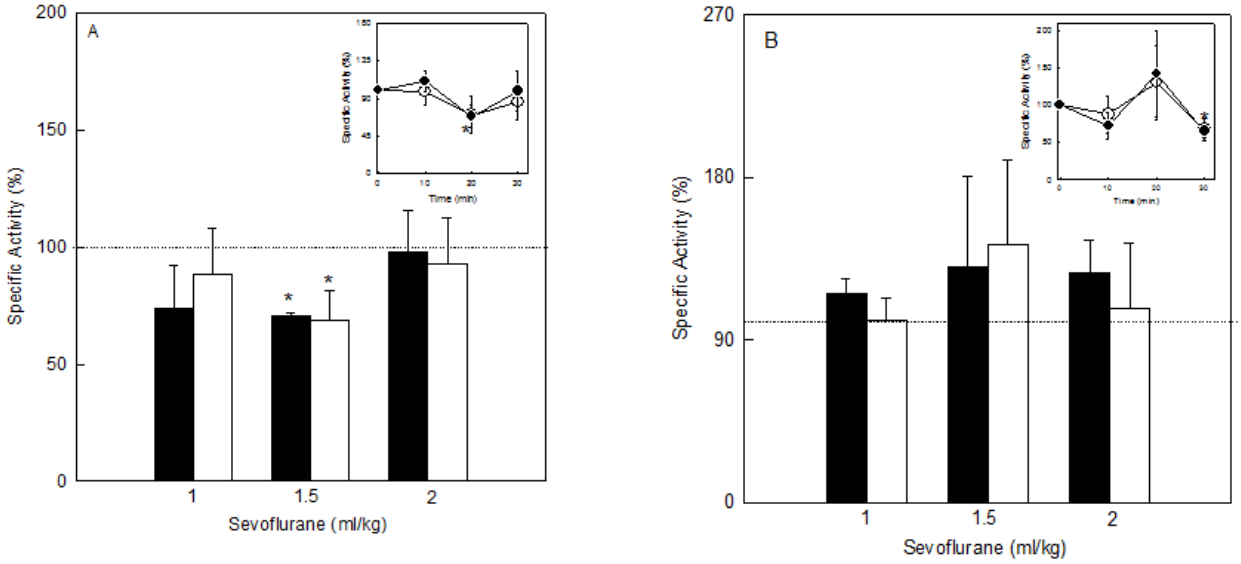

FIGURE 2. Effect of Sevoflurane on PBG-ase and PBG-D activities in liver and blood: Dose and time-response.
A: liver, B: blood. Animals received different doses of the anaesthetic (1 ml/kg, 1.5 ml/kg and 2 ml/kg, i.p.) and were sacrificed 20 minutes later. Inset: Animals received one dose of the anaesthetic (1.5 ml/kg, i.p.) and were sacrificed at different times after injection (10, 20 and 30 minutes), PBGase: black columns and circles, PBG-D: white columns and circles. Data represents mean value ± s.d. of at least 4-6 animals and are expressed relative to specific activity of control (‘’’’’’) value. Mean control value (nmol/mg): PBG-ase: Liver=0.405±0.060 (n=21), blood=0.101±0.024 (n=22); PBG-D: Liver=0.774±0.138 (n=20), blood=0.230±0.067 (n=20). (✱) $p<0.05$, significance of differences between treated and control groups. Other experimental details are described in Materials and Methods.

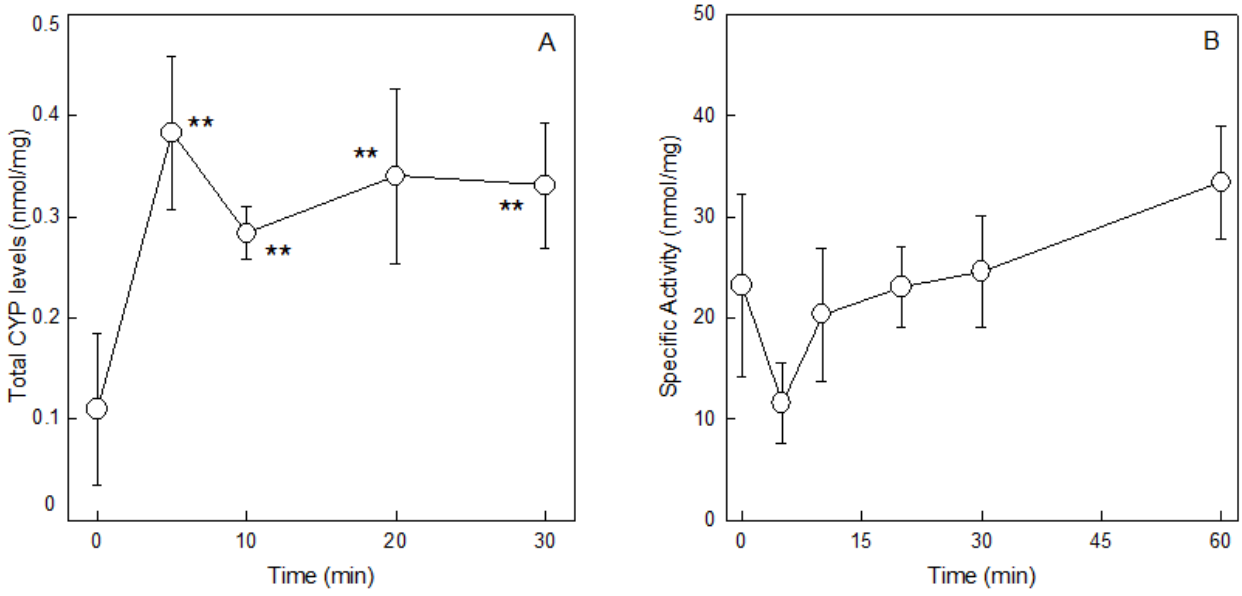

FIGURE 4. Effect of Sevoflurane on CYP levels and CYP2E1 activity: Time-response.
A: CYP, B: CYP2E1. Animals received one dose of the anaesthetic (1.5 ml/kg, i.p.) and were sacrificed at different times after injection (5-60 minutes). Data represents mean value ± s.d. of at least 4-6 animals. (✱✱) $p<0.01$, significance of differences between treated and control groups. Other experimental details are described in Materials and Methods.

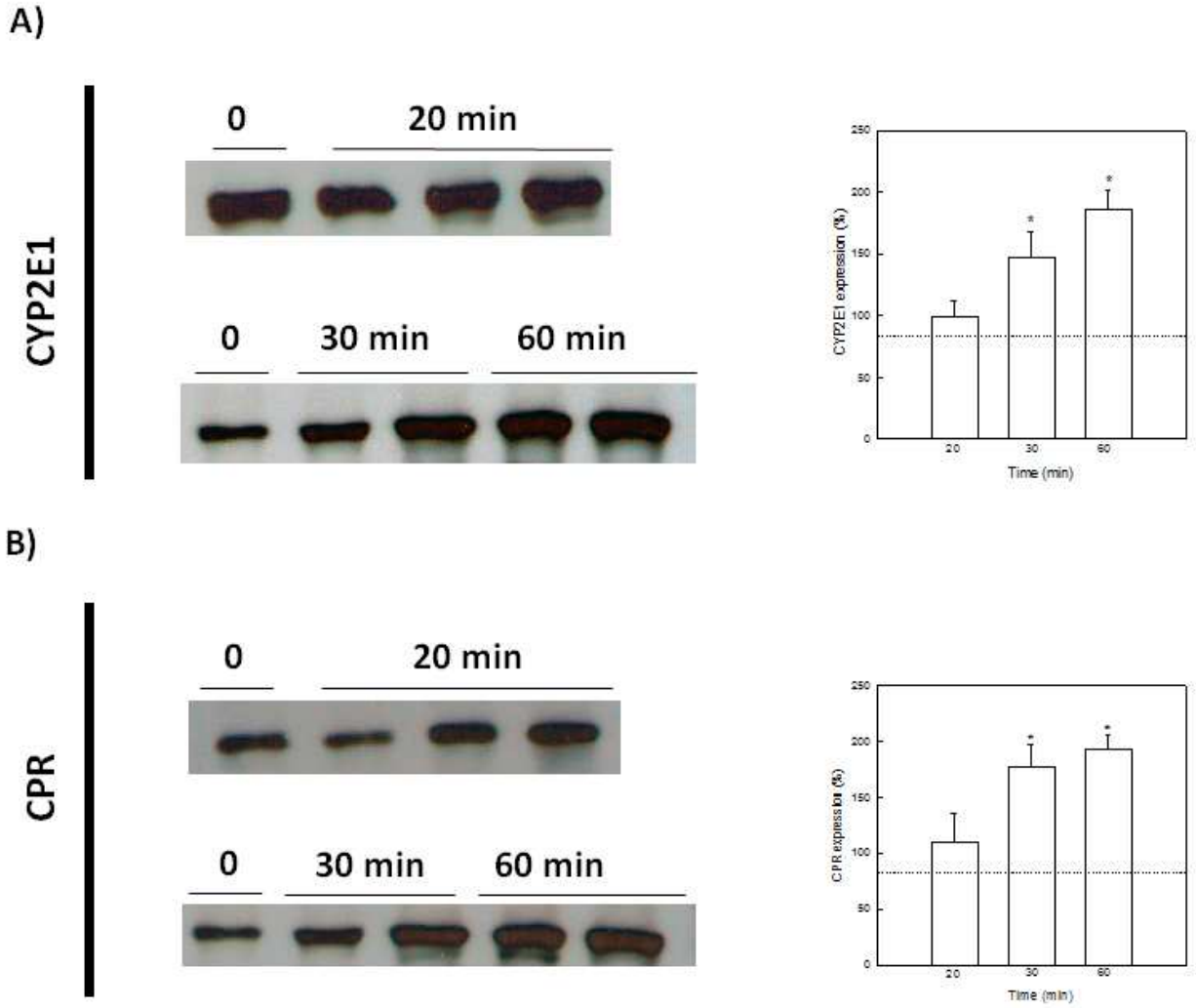

FIGURE 5. Western blot analysis of CYP2E1 and CPR: Time response.
A: CYP2E1, B: CPR. Animals received one dose of the anaesthetic (1.5 ml/kg, i.p.) and were sacrificed at different times after injection (20-60 minutes). Band indicated as "0" correspond to control group. On the right-hand side, columns represent normalized signals in the control and treated animals that were quantified using an Image Analyzer. Values are expressed as mean of at least three determinations run in duplicate and are expressed as a percentage taking the control group (·····) as 100%. (✱) $p<0.05$, significance of differences between treated and control groups. Other experimental details are described in the text.

DISCUSSION

The effect of Sevoflurane, an inhalational volatile anaesthetic, was investigated on heme synthesis and drug metabolizing system in mice.

Data revealed important alterations in the enzymes involved in AIP, such as an induction in hepatic ALA-S activity and a diminished PBG-ase and PBG-D activities in liver and blood 20 minutes after Sevoflurane administration to mice in a dose of 1.5 ml/kg.

When we compared these findings with our previous studies (5, 6), we observed that the dose of Sevoflurane leading to heme enzyme alterations was lower than that for Enflurane and Isoflurane (1.5 mg/kg vs. 2 ml/kg), although the time-response was the same in the case of ALA-S. However, here, Sevoflurane reduced both liver and blood PBG-ase and PBG-D activities, effects that had been only detected in blood for Enflurane and Isoflurane anaesthesia.

The HO pathway represents a major cell and organ protective system in the liver that is induced by a variety of stressors, including cytokines, hypoxia, and reactive oxygen species, generated under certain disease conditions, including ischemia-reperfusion (15, 20, 32, 35). Hoetzel et al. (15) showed that Isoflurane and Sevoflurane up-regulate the inducible isoform of HO (HO-1). The early induction of HO here observed would indicate the onset of oxidative stress.

CYP is one of the most important families of proteins involved in the metabolic response of

many living organisms to foreign chemicals. CPR acts as an electron bridge, transferring electrons between NADPH and CYP enzymes (30), and also as electron donor in the degradation of heme catalyzed by HO (20).

Results also indicate the involvement of CYP in the metabolization of Sevoflurane, but in this case total CYP levels were enhanced and only protein expression varied for CYP2E1 without any effect on its activity. This is another difference between Sevoflurane and other anaesthetics of the same family such as Enflurane and Isoflurane (7). Moreover, the fact that CYP was increased is an index of a great requirement for heme, producing a greater induction of ALA-S, enzyme which is de-represed as a consequence of the diminished heme synthesis.

So, it seems that the effects of Sevoflurane in mice would be more severe than those of Enflurane and Isoflurane and also very likely in porphyric patients when we intend to extrapolate these findings to humans. At this respect we have chosen a different way of administration than that used for humans (endotracheal way) on the basis of other authors (12, 24) so to compare with our own investigations performed with Enflurane and Isoflurane. Fry et al. (12) studying the metabolism of methoxyflurane in mice, found that the i.p. administration of this anesthetic lead to more consistent data than administration by inhalation.

In conclusion, although Sheppard and Dorman (29) reported a case history of the anesthetic management in a child with a severe form of AIP, describing the safe use of Sevoflurane in the maintenance of anesthesia, our results in mice would suggest that Sevoflurane should be avoided for its use in porphyric patients or at least it should be used with caution and very careful control.

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REFERENCES

1. Battle, C., In: Porfirias y Porfirinas. Aspectos clínicos, bioquímicos y biología molecular. *Acta Bioquím. Clin. Latinoam.*. 1997, Suppl. No. 3, Chapter II, pp. 37-69.
2. Batlle, A., Wider de Xifra, E. and Stella, A.M., A simple method for measuring erythrocyte porphobilinogenase and its use in the diagnosis of acute intermittent porphyria. *Int. J. Biochem.* 1978, **9**: 1-12.
3. Behne, M., Wilke, H.J. and Harder, S. Clinical pharmacokinetics of sevoflurane. *Clin. Pharmacokinet.* 1999, **36(1)**: 13-26.
4. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976, **72**: 248-254.
5. Buzaleh, A.M., Enriquez de Salamanca, R. and Batlle, A.M. del C., Porphyrinogenic properties of the anesthetic enflurane. *Gen. Pharmac.* 1992, **23:** 665-669.
6. Buzaleh, A.M., Enriquez de Salamanca, R. and Batlle, A.M. del C., Administration of the anesthetic Isoflurane to mice: A model for Acute Intermittent Porphyria? *J. Pharmac. Methods.* 1992, **28**: 191-197.
7. Buzaleh, A. M., Martínez, M. del C. and Batlle, A., The relevance of P-450 levels on enflurane and isoflurane action action in mice. Studies on heme pathway. *Clin. Exp. Pharmacol. Physiol.* 2000, **27:** 796-800.
8. Buzaleh, A.M., García Bravo, M., Navarro, S., Morán Jimenez, M.J., Méndez, M., Batlle, A., Fontanellas, A. and Enríquez de Salamanca, R., Volatile anaesthetics induce biochemical alterations in the heme pathway in a B-lymphocyte cell line established from hepatoerythropoietic porphyria patients (LBHEP) and in mice inoculated with LBHEP cells. *Int. J. Biochem. Cell. Biol*. 2004, **36**: 216-222.
9. Buzaleh, A.M., Morán-Jiménez, M.J., García-Bravo, M., Sampedro, A., Batlle A., Enríquez de Salamanca R. and Fontanellas, A., Induction of Hepatic Aminolevulinate Acid Synthetase Activity by Isoflurane in a Genetic Model for Erythropoietic Protoporphyria. *Cell. Mol. Biol.* 2009, **55:** 38-44.
10. Eger EI 2nd. New inhaled anesthetics. Review. *Anesthesiology* 1994, **80(4)**: 906-922.
11. Ellencweig, N., Schoenfeld, N and Zemishlany, Z., Acute Intermittent Porphyria: Psychosis as the only clinical manifestation. *Int. J. Psychiatry Relat. Sci.* 2006, **43**: 52-56.
12. Fry, B.W., Ciardone, A.E. and Fairclot, R.E., Early appearance of methoxyflurane fluor metabolites in mice. *Pharmacol. Ther. Dent.* 1980, **5**: 79-86.
13. Goa, K.L., Noble, S. and Spencer, C.M., Sevoflurane in Paediatric Anaesthesia. A Review. *Paediatric Drugs* 1999, **1**: 127-153.
14. Herrick, A.L. and McColl, K.E.L., Acute intermittent porphyria. *Best Practice Res. Clin. Gastroenterol.* 2005, **19**: 135-249.
15. Hoetzel, A., Geiger, S., Loop, T., Welle, A., Schmidt, R., Humar, M., Pahl, H.L., Geiger, K.K. and Pannen, B.H., Differential effects of volatile anesthetics on hepatic heme oxygenase-1 expression in the rat. *Anesthesiology* 2002; **97**: 1318–1321.
16. Jensen, N.F., Fiddler, D.S. and Striepe, V., Anesthetic considerations in porphyrias. *Anesth. Analg.* 1995, **80**: 591-599.
17. Kharasch, E.D. and Thummel, K.E., Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. *Anesthesiology* 1993, **79(4)**: 795-807.
18. Kharasch, E.D., Armstrong, A.S., Gunn, K., Artru, A., Cox, K. and Karol, M.D., Clinical sevoflurane metabolism and disposition. II. The role of cytochrome P450 2E1 in

fluoride and hexafluoroisopropanol formation. *Anesthesiology* 1995, **82(6)**: 1379-1388.
19. Lowry, O., Rosebrough, N., Farr, A. and Randall, R., Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* 1951, **193**: 265-275.
20. Maines, M.D. and Gibbs, P.E., 30 some years of heme oxygenase: from a "molecular wrecking ball" to a "mesmerizing" trigger of cellular events. *Biochem. Biophys. Res. Commun*. 2005, **338**: 568-577.
21. Marver, H., Tschudy, D., Perlroth, M. and Collins, A., δ-Aminolevulinic acid synthetase I. Studies in liver homogenates. *J. Biol. Chem.* 1966, **241**, 2803-2809.
22. Omura, T. and Sato, R., The carbon monoxide-binding pigment of liver microsomes. Evidence for its hemoprotein nature. *J. Biol. Chem*. 1964, **239**: 2370-2378.
23. Parera, V.E., De Siervi, A., Varela, L., Rossetti, M.V. and Batlle, A.M. del C., Acute porphyrias in the Argentinean population: A Review. *Cell. Mol. Biol.* 2003, **49**: 493-500.
24. Pantuck, E.J., Pantuck, C.B., Ryan, D.E. and Conney, A.H., Inhibition and stimulation of Enflurane metabolism in the rat following a single dose of chronic administration of ethanol. *Anesthesiology* 1985, **62**: 255-262.
25. Patel, S. S. and Goa, K.L. Sevoflurane: a review of its pharmacodynamic and pharmacokinetic properties and its clinical use in general anaesthesia. *Drugs* 1996, **51**:658-700
26. Poblete-Gutiérrez, P., Wiederholt, T., Merk, H.F. and Frank, J., The Porphyrias: clinical presentation, diagnosis and treatment. *Eur. J. Dermatol*. 2006, **16**: 230-240.
27. Reinke, L.A. and Moyer, M.J., A microsomal oxidation which is highly inducible by ethanol. *Drug Met. Disp*. 1985, **13**: 548-552.
28. Sakai, E.M., Connolly, L.A. and Klauck, J.A., Inhalation anesthesiology and volatile liquid anesthetics: focus on isoflurane, desflurane, and sevoflurane. *Pharmacotherapy* 2005, **25(12)**: 1773-1788.
29. Sheppard, L. and Dorman, T., Anesthesia in a child with homozygous porphobilinogen deaminase deficiency: a severe form of acute intermittent porphyria. *Paediatr. Anaesth.* 2005, **15(5)**: 426-428.
30. Shimada, T., Mernaugh, R.L. and Guengerich, F.P., Interactions of mammalian cytochrome P450, NADPH-cytochrome P450 reductase, and cytochrome b_5 enzymes. *Arch. Biochem. Biophys.* 2005, **435**: 207-216.
31. *Sigston, P.E., Jenkins, A.M., Jackson, E.A., Sury, M.R., Mackersie, A.M. and Hatch, D.J., Rapid inhalation induction in children: 8% sevoflurane compared with 5% halothane.* Br. J. Anaesth. *1997, **78(4)**: 362-365.*
32. Suematsu, M., COHb: a stress-induced marker reflecting surgical insults. *J. Gastroenterol. Hepatol.* 2002, **17**: 519–520.
33. Tenhunen, R., Marver, H.S. and Schmid, R., The enzymatic catabolism of hemoglobin: Stimulation of microsomal heme oxygenase by hemin. *J. Lab. Clin. Med.* 1970, **75**: 410-421.
34. Thadani, H., Deacon, A. and Peters, T., Diagnosis & management of porphyria. *B.M.J.* 2000, **320**: 1647-1651.
35. Tomaro, M.L. and Batlle, A.M. del C., Bilirubin: its role in cytoprotection against oxidative stress. *Int. J. Biochem. Cell Biol.* 2002, **34**: 216-220.
36. Wallin, R.F., Regan, B.M., Napoli, M.D. and Stern, I.J., Sevoflurane: a new inhalational anesthetic agent. *Anesth. Analg.* 1975, **54**: 758-766.
37. Wandel, C., Neff, S., Kepppler, G., Bohrer, H., Stockinger, K., Wilkinson, G.R., Wood, M. and Martin, E.**,** The relationship between cytochrome P4502E1 activity and plasma fluoride levels after Sevoflurane anesthesia in humans. *Anesth. Analg.* 1997, **85**: 924-930.
38. Young, C.J. and Apfelbaum, J.L., Inhalational anesthetics desflurane and Sevoflurane. *J. Clin. Anesth*. 1995, **7**: 564-577.