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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

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Abstract

Hepatoerythropoietic porphyria (HEP) is the homozygous form of Porphyria Cutanea Tarda (PCT), characterized by an accumulation of porphyrins due to uroporphyrinogen decarboxylase deficiency.

Fluorinated volatile anaesthetics are often used to produce general anaesthesia. Anaesthesia has certainly been implicated in the triggering of acute porphyria crisis.

The effects of volatile anaesthetics in a B-lymphocyte cell line established from HEP patients (LBHEP) on heme metabolism have been investigated.

LBHEP cells were exposed to sodium phosphate buffer containing dissolved Enflurane, Isoflurane or Sevoflurane (10 mM) during 20 min.

Aminolevulinate synthase (ALA-S) activity, the regulatory enzyme of heme synthesis, was 300% induced by the anaesthetics. A 25–30% diminution of porphobilinogenase (PBG-ase) activity was found when Isoflurane or Sevoflurane were added to the cells, while no significant changes were detected after Enflurane treatment.

Although some oxidative stress has been induced by the anaesthetics, reflected by the 35% diminution of glutathione (GSH), no alteration in heme oxygenase (HO) activity, the enzyme involved in heme breakdown and frequently induced as a response to stress stimuli, was observed.

Studies using animals inoculated with LBHEP cells were also performed. Findings here described mimic biochemical alterations in the heme pathway, which are characteristic of another hepatic porphyria, similar to those previously reported

Abbreviations: AIP, Acute Intermittent Porphyria; ALA-S, aminolevulinate synthase; GSH, glutathione; HEP, hepatoerythropoietic porphyria; HO, heme oxygenase; LBHEP, B-lymphocyte cell line established from HEP patients; PBG-ase, porphobilinogenase; PBS, phosphate buffer saline; PCT, Porphyria Cutanea Tarda

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when these anaesthetics were administered to animals, and they also advertise about the possible unsafe use of these drugs in the case of hepatic non-acute porphyrias.

Keywords: Volatile anaesthetics; Porphyrias; Heme pathway; LBHEP cell lines

1. Introduction

Porphyrias are inherited metabolic diseases characterized by specific enzyme defects along the heme pathway (Batlle, 1997). Hepatoerythropoietic porphyria (HEP) is the homozygous form of familial Porphyria Cutanea Tarda (PCT), where high deficiency of uroporphyrinogen decarboxylase is leading to accumulation of uro and heptacarboxylic porphyrins in bone marrow and liver (Castaño Suarez, Zamarro Sanz, Guerra Tapia, & Enríquez de Salamanca, 1996).

Erythropoietic porphyrias are characterized by extreme skin sensitivity, there are not neurological alterations and they are not associated either with drug-precipitated crisis. However, there is one report on the neurological symptoms in a child with HEP (Parsons, Sahn, Holden, & Pai, 1994).

We have shown that administration of volatile anaesthetics such as Enflurane and Isoflurane (Buzaleh, Enriquez de Salamanca, & Batlle, 1992a,b; Buzaleh & Batlle, 1996) or Sevoflurane (unpublished results) to mice produced heme alterations mimicking the pattern of acute porphyrias. We have observed a striking induction in aminolevulinate synthase (ALA-S) activity, the regulatory enzyme of heme pathway, secondarily induced in all porphyrias and a reduced of porphobilinogenase (PBG-ase) activity, the deficient enzyme in Acute Intermittent Porphyria (AIP).

Although acute porphyrias are of major anaesthetic relevance (Jensen, Fiddler, & Striepe, 1995), it was also of interest to examine the effects of volatile anaesthetics on non-acute porphyrias and to evaluate whether these drugs produce any alterations in parameters related with the acute crisis such as an increase of ALA-S activity or a diminution in heme concentration.

The aims of this work have been to investigate the actions of volatile anaesthetics on heme metabolism in a B-lymphocyte cell line established from HEP patients (LBHEP) and in mice inoculated with this same cell line, which develop a cutaneous tumor. To this end, the activities of ALA-S, PBG-ase and heme oxygenase (HO), the first enzyme involved in heme catabolism (Maines, 1988), were measured. Because it has been found that GSH levels were dimished after administration of these anaesthetics to mice (unpublished results), GSH concentration was also determined.

2. Materials and methods

The anaesthetics used were kindly provided by Abbott Laboratories (Buenos Aires, Argentina). All other chemicals used were reagent grade.

2.1. Experimental design

2.1.1. In vitro studies

Peripheral blood lymphocytes were isolated from a patient with HEP carrying the G281E mutation in the uroporphyrinogen decarboxylase gene. B-lymphocyte cell line was established from these lymphocytes after Epstein-Barr virus infection (LBHEP) (Fontanellas et al., 1999). This cell line was maintained in RPMI 1640 (20 mM HEPES) medium supplemented with 20% fetal calf serum, 100 U of penicillin/ml and 1 mg/ml of streptomycin, at 37 °C in 5% CO_2 atmosphere.

LBHEP cell lines were grown at 0.2×10^6 to 1×10^6 cells/ml for 30–40 days. 500×10^6 cells were harvested by centrifugation at 1500 rpm ($420 \times g$) during 10 min, washed twice with phosphate buffer saline (PBS) and counted. The cells were subjected to 20 min exposure to a solution of 10.4 mM of the anaesthetics (20 μl in 15.5 ml of PBS). Then, cells were centrifuged and pellets were resuspended in the corresponding solution depending on the parameter to be measured and sonicated (three times for 10 s each).

2.1.2. In vivo studies

BALBc immunodeficient mice were inoculated with LBHEP cells (2×10^6, s.c.). Animals (six per group) weighing 25–30 g were maintained in controlled sterile conditions and allowed free access to food and water. Animals received human care and were treated in

accordance to guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals. When tumor was macroscopically visualized (45 days after) animals received a single dose of Isoflurane (2 ml/kg, i.p., 0.06/0.03 ml oil (v/v)) and were sacrificed 20 min after injection. Controls received the vehicle (with oil) and were sacrificed at the same time as treated animals.

2.2. Parameters measured

- *ALA-S activity*: The cell pellet was resuspended in a solution of NaCl 0.9% containing EDTA 0.5 mM and buffer Tris–ClH 10 mM pH 7.4 and sonicated. The resulting homogenate was used as enzyme source and the activity was measured according to Marver, Tschudy, Perlroth, and Collins (1966).
- *PBG-ase and HO activity*: The cell pellet was resuspended in KCl 0.1% and sonicated. The resulting homogenate was centrifuged at 10,000 × *g* during 20 min and the supernatant was used as enzyme source. PBG-ase activity was measured as described by Anderson and Desnick (1982) and HO activity according to Tenhunen, Marver, and Schmid (1970).
- *GHS levels*: The cells were treated as described for PBG-ase and HO activity. GSH levels were determined in the 10,000 × *g* supernatant following the method of Rossi, Cardaioli, Scaloni, Amiconi, and Di Simplicio (1995).
- Protein concentration was determined by Bradford's method (Bradford, 1976) using a Bio-Rad Protein Assay (Bio-Rad Laboratories, München, Germany).

2.3. Statistical analysis

The mean and S.D. of every parameter for each group were calculated. The significance of differences between groups was analyzed using the analysis of variance (ANOVA) and $P < 0.05$ was regarded as significant.

3. Results

3.1. In vitro studies

When the anaesthetics were added to LBHEP cell lines, ALA-S activity was 300% ($P < 0.01$) increased

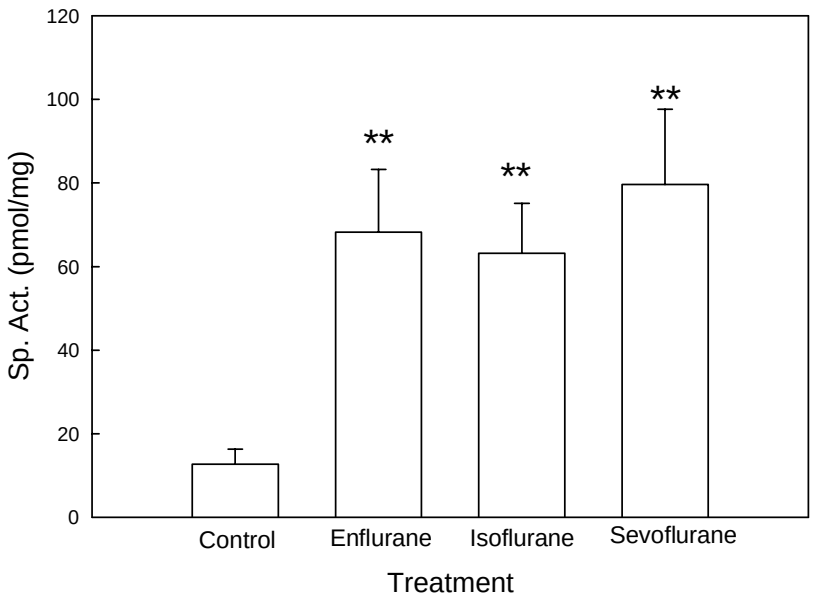

Fig. 1. In vitro effect of Enflurane, Isoflurane and Sevoflurane on ALA-S activity in LBHEP cells. Cells were exposed for 20 min to a solution of 10.4 mM of the anaesthetics (20 μl in 15.5 ml of PBS). Data represent mean value ± S.D. of two experiments carried out in triplicate. (**) $P < 0.01$, significance of differences between treated and control cells. Other experimental details are described in the text.

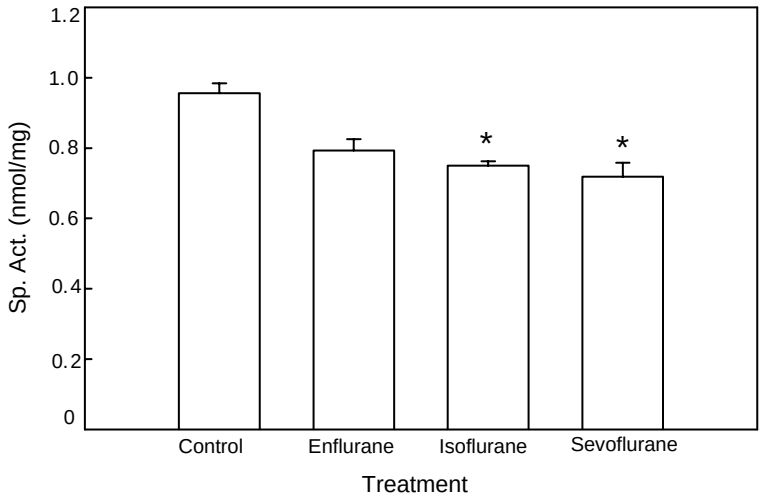

Fig. 2. In vitro effect of Enflurane, Isoflurane and Sevoflurane on PBG-ase activity in LBHEP cells. Experimental details are indicated in legend to Fig. 1. (*) $P < 0.05$.

(Fig. 1). PBG-ase was 25–30% ($P < 0.05$) diminished when Isoflurane or Sevoflurane were added to the cells, while no significant variation was found after Enflurane treatment (Fig. 2). No alterations were observed in HO activity (Fig. 3). GSH levels were 35% ($P < 0.05$) diminished in all cases (Fig. 4).

3.2. In vivo studies

The effect of Isoflurane in liver and tumor of animals inoculated with LBHEP cells on heme metabolism and GSH levels was examined.

It is interesting to point out that significant alterations on heme metabolism due to only LBHEP cells

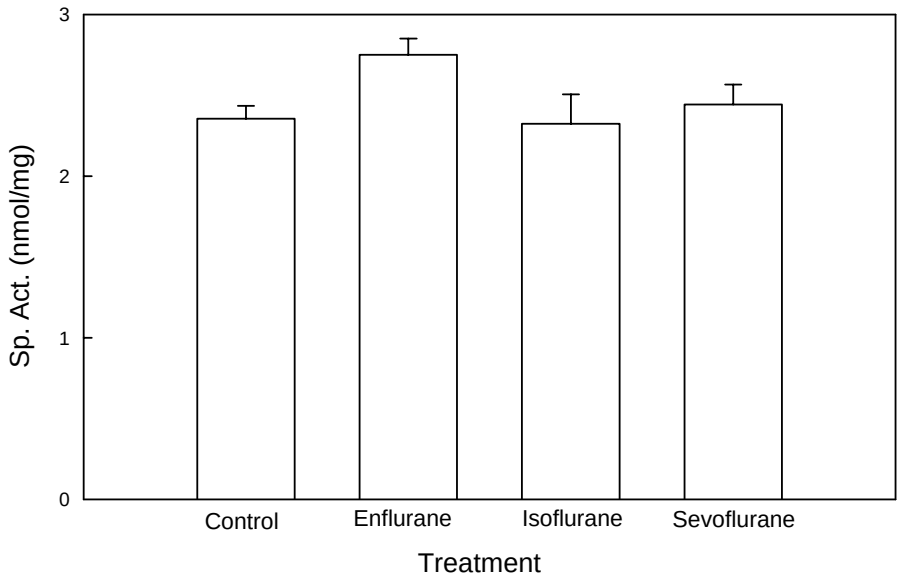

Fig. 3. In vitro effect of Enflurane, Isoflurane and Sevoflurane on HO activity in LBHEP cells. Experimental details are indicated in legend to Fig. 1.

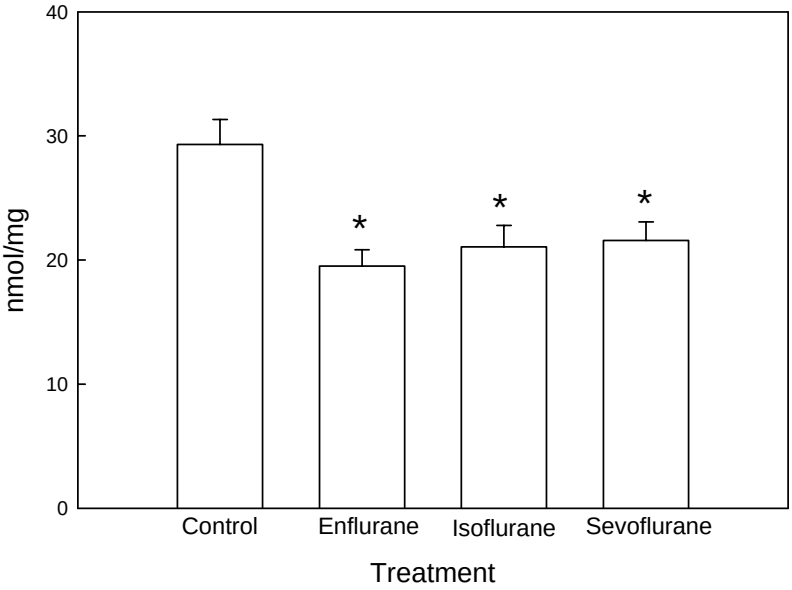

Fig. 4. In vitro effect of Enflurane, Isoflurane and Sevoflurane on GSH levels in LBHEP cells. Experimental details are indicated in legend to Fig. 1. (*) $P < 0.05$.

injection were observed (data not shown). It has been found that in tumor, the activities of ALA-S and HO were 90 and 50% induced, while PBG-ase activity was 25% reduced. In the liver of these animals, 60% reduction of HO activity was observed. GSH levels were strikingly diminished in liver (60%) and in tumor (90%).

Table 1
In vivo effect of Isoflurane in mice inoculated with LBHEP cells

	Relative activity (%)	
	Liver	Tumor
ALA-S	90.47 ± 37.46	70.84 ± 46.89
PBG-ase	83.52 ± 11.12	83.04 ± 29.76
HO	105.26 ± 46.35	40.43 ± 22.49*
GSH	122.95 ± 14.98	98.38 ± 14.63

Activities and GSH levels were expressed as a percentage of their values in LBHEP inoculated mice not receiving Isoflurane, taken as controls. BALBc immunodeficient mice were inoculated with LBHEP cells, when tumor was macroscopically visualized (45 days after) animals received a single dose of Isoflurane (2 ml/kg, i.p.) and were sacrificed 20 min after injection. All other experimental details are described in the text.

* $P < 0.05$.

As is it shown in Table 1, ALA-S and PBG-ase activities and GSH levels were unchanged in the liver and tumor of animals injected with LBHEP cells and treated with Isoflurane, while tumoral HO was 60% ($P < 0.05$) diminished, when they were all compared with the group of animals inoculated only with LBHEP cells.

4. Conclusions

The effects of the volatile anaesthetics Enflurane, Isoflurane and Sevoflurane, of common use in clinics, on the synthesis and degradation of heme in a B-lymphocyte cell line established from HEP patients were investigated.

ALA-S activity was 300% induced in cells by all of these anaesthetics, while PBG-ase was inhibited only after Isoflurane and Sevoflurane administration. These results are indicating that biochemical alterations alike those seen in patients with AIP can be induced in the LBHEP cell lines when grown in the presence of the volatile anaesthetics here assayed.

Although oxidative stress was shown to occur, reflected by GSH diminution, no alteration in HO

activity, the enzyme involved in heme breakdown and frequently induced as a response to stress stimuli (Buzaleh & Batlle, 2000; Takahashi, Akagi, Shimizu, Hirakawa, & Sassa, 2002), was observed.

The effect of Isoflurane on the synthesis and degradation of heme in liver and tumor of animals inoculated with LBHEP cells was also investigated.

HO activity was higher in tumor than in liver, in agreement with the results of Tozer et al. (1998), using subcutaneous transplants of the P22 rat carcinosarcoma. Alterations in heme metabolism were also earlier reported in liver nodules and tumors by Stout and Becker (1987).

When LBHEP inoculated animals received Isoflurane, only HO was diminished in tumor, but unchanged in liver, while all the other parameters measured, both in tumor and liver of these animals were not altered either. Actually this is a contradictory result if we recall that HO activity was induced when LBHEP cells were treated with this anaesthetic and also when it was administered to control mice (Buzaleh et al., 1992a; Buzaleh & Batlle, 1996), however we cannot offer yet any explanation for these findings.

So, our present results would be indicating that the alterations produced on heme metabolism due to the presence of the tumor are so severe that they could not be exacerbated further by administration of the anaesthetic.

Both in vitro and in vivo results described here mimic the biochemical alterations found in the heme pathway, which are characteristic of another hepatic porphyria and are similar to those previously reported when these anaesthetics were administered to animals (Buzaleh, Vazquez, Enríquez de Salamanca, & Batlle, 1997).

It is already known that most anaesthetics are among the so-called porphyrinogenic drugs considered unsafe for their use in the acute porphyrias. The findings here reported are indicating that much care should also be taken when these anaesthetics need to be used in cases of hepatic non-acute porphyrias, such as HEP and very likely PCT.

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