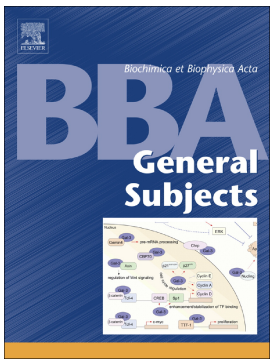


Effects of volatile anaesthetics on heme metabolism in a murine genetic model of acute intermittent porphyria. A comparative study with other porphyrinogenic drugs

Silvina Fernanda Ruspini, Johanna Romina Zuccoli, Jimena Verónica Lavandera, Marìa Del Carmen Martínez, Leda María Olivieri, Esther Noemí Gerez, Alcira María Del Carmen Batlle, Ana María Buzaleh

PII: S0304-4165(18)30055-2
DOI: doi:10.1016/j.bbagen.2018.02.013
Reference: BBAGEN 29050

To appear in:

Received date: 18 July 2017
Revised date: 13 February 2018
Accepted date: 19 February 2018

Please cite this article as: Silvina Fernanda Ruspini, Johanna Romina Zuccoli, Jimena Verónica Lavandera, Marìa Del Carmen Martínez, Leda María Olivieri, Esther Noemí Gerez, Alcira María Del Carmen Batlle, Ana María Buzaleh , Effects of volatile anaesthetics on heme metabolism in a murine genetic model of acute intermittent porphyria. A comparative study with other porphyrinogenic drugs. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbagen(2018), doi:10.1016/j.bbagen.2018.02.013

EFFECTS OF VOLATILE ANAESTHETICS ON HEME METABOLISM IN A MURINE GENETIC MODEL OF ACUTE INTERMITTENT PORPHYRIA. A COMPARATIVE STUDY WITH OTHER PORPHYRINOGENIC DRUGS

SILVINA FERNANDA RUSPINI[a #]: marvinsil@hotmail.com, JOHANNA ROMINA ZUCCOLI[a#]: johannazuccoli@hotmail.com, JIMENA VERÓNICA LAVANDERA[b]: chacabuco27@hotmail.com, MARÌA DEL CARMEN MARTÍNEZ[a,c]: mcmartin@qb.fcen.uba.ar, LEDA MARÍA OLIVIERI[a]: lemaoli@yahoo.com, ESTHER NOEMÍ GEREZ[a]: cuyro@yahoo.com, ALCIRA MARÍA DEL CARMEN BATLLE[a]: batllealcira@yahoo.com.ar, ANA MARÍA BUZALEH [a,c]: anamaria@qb.fcen.uba.ar

 # Both authors have had equal participation in the completion of this work

[a]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP), CONICET, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires, Argentina.

[b]Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

[c]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Argentina

Corresponding author: DR. ANA MARÍA BUZALEH

Av. Córdoba 2351

1120 - BUENOS AIRES

ARGENTINA

Phone 54 11 59508346

Fax 54 11 459508347

E-mail: anamaria@qb.fcen.uba.ar; anabuza@hotmail.com

ABSTRACT

Background: Acute intermittent porphyria (AIP) is an inherited disease produced by a deficiency of Porphobilinogen deaminase (PBGD). The aim of this work was to evaluate the effects of Isoflurane and Sevoflurane on heme metabolism in a mouse genetic model of AIP to further support our previous proposal for avoiding their use in porphyric patients. A comparative study was performed administering the porphyrinogenic drugs allylisopropylacetamide (AIA), barbital and ethanol, and also between sex and mutation using AIP (PBG-D activity 70% reduced) and T1 (PBG-D activity 50% diminished) mice.

Methods: The activities of 5-Aminolevulinic synthetase (ALA-S), PBG-D, Heme oxygenase (HO) and CYP2E1; the expression of ALA-S and the levels of 5-aminolevulinic acid (ALA) were measured in different tissues of mice treated with the drugs mentioned.

Results: Isoflurane increased liver, kidney and brain ALA-S activity of AIP females but only affected kidney AIP males. Sevoflurane induced ALA-S activity in kidney and brain of female AIP group. PBG-D activity was further reduced by Isoflurane in liver male T1; in AIP male mice activity remained in its low basal levels. Ethanol and barbital also caused biochemical alterations. Only AIA triggered neurological signs similar to those observed during human acute attacks in male AIP being the symptoms less pronounced in females although ALA-S induction was greater. Heme degradation was affected.

Discussion: Biochemical alterations caused by the porphyrinogenic drugs assayed were different in male and female mice and also between T1 and AIP being more affected the females of AIP group.

General significance: This is the first study using volatile anaesthetics in an AIP genetic model confirming Isoflurane and Sevoflurane porphyrinogenicity.

Keywords: 5-Aminolevulinic acid; porphobilinogen deaminase; heme metabolism; brain; volatile anaesthetics, porphyrinogenic drugs

1. Introduction

Acute intermittent porphyria (AIP) is a human disease because of having a dominantly inherited partial deficiency of the third heme enzyme, Porphobilinogen deaminase (PBG-D) [1-4]. It is characterized by a neurological syndrome consisting of acute abdominal pain with global or focal autonomic dysfunction of central nervous system and a predominantly motor polyneuropathy [5, 6].

The reduction of PBG-D activity is not enough for triggering such these symptoms and crisis may be precipitated by a number of factors, including physiological hormonal functions, fasting, stress, infection and also therapeutical drugs such as anaesthetics [2, 7, 8].

Several mechanisms have been postulated to explain the neuropsychiatric manifestations of acute Porphyrias, although the pathogenesis of acute attacks is yet unknown. 5-Aminolevulinic acid (ALA), the product of the first and regulatory enzyme ALA synthetase (ALA-S), generates reactive species leading to oxidative lesions in brain synaptic membranes, affecting GABAergic and glutamaergic systems. ALA accumulation in central nervous system could be one of the reasons for the neurological syndrome [9-12].

Heme Oxygenase (HO) is the key enzyme in heme catabolism because it catalyzes a rate-limiting step of this pathway [13-15].

Isoflurane and Sevoflurane are volatile anaesthetics that reproduce the typical biochemical signs of AIP when they were administered to *CF1* mice [16, 17]. Similar results were obtained when Isoflurane was given to other genetic models of Porphyrias, such as Erythropoietic Protoporphyria [18] and Hepatoerythropoietic Porphyria [19], showing that the use of these anaesthetics in porphyric patients should be avoided.

Porphyrinogenic activity of drugs may be observed from retrospective clinical data or by investigating their effect in animal models. Attempts to predict whether a drug will risk precipitating the symptoms in a carrier of acute porphyria should therefore focus on the ability of the substance to activate two mechanisms: to induce ALA-S and to inhibit its

physiological feedback-control via reduction of the regulatory heme pool by induction or destruction of the heme-based drug-metabolizing cytochrome P-450 (CYP) enzymes (8).

On the other hand, it has been reported by some of the authors of the present paper that porphyrinogenic agents altered heme metabolism, cholinergic and glutamatergic system, Phase I drug metabolizing system and also developed oxidative and nitrosative stress [20-25] in brain of *CF1* mice.

Dr. Meyer and his team developed in 1996 [26] a genetic mouse model of AIP that exhibit the typical biochemical and neurological characteristics of human AIP. This model is very useful to study the pathogenesis of the neurologic symptoms of AIP and to evaluate the porphyrinogenicity of drugs [27-29].

In the present work, using this AIP mouse model [26], we studied the effects on heme metabolism of volatile anaesthetics such as Isoflurane and Sevoflurane to further support our previous suggestion for avoiding its use in porphyric patients. A comparative study was performed with the porphyrinogenic drugs allylisopropylacetamide (AIA), barbital and ethanol. It was of interest to investigate if the effects were depending on sex due that human AIP is more frequently observed in women [3]. Moreover, taking into account that AIP could be symptomatic or latent, and it is not clear why some people develop the disease and others do not [3, 30], we used T1 mice and AIP mice which have PBG-D reduced 50% and 70% and are homozygote and composed heterozygote. Another difference between AIP mice and T1 is that only the latter strain accumulates porphyrin precursors after phenobarbital challenge [26]. The activities of ALA-S, PBG-D and HO, protein ALA-S expression and ALA levels were measured in different tissues. The activity of CYP2E1, isoform involved in the metabolism of Isoflurane and Sevoflurane [31] was also evaluated.

2. Materials and Methods

2.1 Chemicals

Isoflurane and Sevoflurane were from Abbott Laboratories S.A. USA. All other chemicals used were reagent grade obtained from Sigma Chem. Co., St. Louis, USA.

2.2 Animals

A knockout mouse with targeted disruption of PBG-D, was used in the present study as an *in vivo* model of heme deficiency. AIP mice were gently provided by Dr. A. Fontanellas (University of Navarra, Spain) under the authorization of Dr. U. Meyer (University of Basel, Switzerland). This murine model of AIP is compound heterozygote of two different disruptions of the PBGD gene: T1 (C57BL/6-pbgd$^{tm1(neo)Uam}$) and T2 strain (C57BL/6-pbgd$^{tm2(neo)Uam}$) mutations. The study was performed using two groups: T1 (PBG-D activity 55% reduced), and AIP (PBG-D activity 70% reduced). A comparison was also done between male and female mice. Animals were maintained in controlled conditions with free access to food (Purina 3, Asociación de Cooperativas Argentinas, San Nicolás, Buenos Aires, Argentine) and water 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). All experiments were performed at the same time of the day.

2.3 Treatments

The design of drug administration was performed as previously described [22, 24]. In particular, the bases of doses and times used for anaesthetic administration were supported by our previously research [16, 17]. The experimental design for the other porphyinogenic drugs were the commonly used by other authors [32-34]. In all the cases, alterations on heme synthesis that mimic biochemical alterations observed in human AIP were observed in control mice. Treatment conditions were as follows:

- *Anaesthetics*: animals received a single dose of Isoflurane (2 ml/kg, i.p.) or Sevoflurane (1.5 ml/kg, i.p.) and were sacrificed 20 minutes after the injection.
- *AIA*: Animals received a single dose of 350 mg/kg (i.p.) 16 hours prior to sacrifice.
- *Barbital:* Animals were given one daily dose of 167 mg/kg (s.c.) during 3 days, and they were sacrificed 24 hours after the last dose.
- *Ethanol*: Animals received ethanol (30%, v/v) in the drinking water during 1 week.

Control animals received the vehicle (oil for the anaesthetics, ethanol:NaCl 0.9%; 1:3 v/v for AIA, and NaCl 0.9% for barbital) and were sacrificed at the same times as indicated for each particular treatment.

2.4 Tissue preparation and assays

Blood was obtained by cardiac puncture in heparinized tubes. Tissues were scissored and immediately processed. For ALA-S determination, whole liver, brain and kidney were used without perfusion. For PBG-D, HO, CYP2E1 and ALA determinations, tissues were perfused *in situ* with cold isotonic saline.

The activity of ALA-S was measured according to Marver et al. [35] in liver, kidney and brain. Tissues not perfused were homogenized (1:3, w/v) in Tris-HCl 10 mM pH 7.4; EDTA 0.5 mM and NaCl 0.9% and it was used as enzyme source.

The activity of PBG-D was measured using the method described by Batlle et al. [36] in liver, kidney, brain and blood. Liver, kidney and brain previously perfused with saline solution were homogenized (1:3, w/v) in sucrose 0.25 M and centrifuged at 15,000x*g* during 20 minutes. The resulting supernatant was used as enzyme cytosolic source. PBG-D was also measured in erythrocytes obtained from whole blood, and then hemolyzed with Triton X-100 (1:5 v/v) and diluted with buffer Tris-HCl 0.05 M pH 7.4 (1:4 v/v).

The activity of HO was determined according to Tehnunen et al. [37] in liver, kidney and brain. Tissues were processed in a same manner to that described for PBG-D activity using the supernatant of 15,000xg as enzyme source.

CYP2E1 activity was measured spectrophotometricaly in liver and brain using p-nitrophenol as substrate according to Reinke and Moyer [38]. Tissues previously perfused with saline solution were homogenized (1:3, w/v) in sucrose 0.25 M and centrifuged at 15,000xg during 20 minutes; the resulting supernatant was then centrifuged for 90 min at 105,000xg; the pellet obtained was resuspended in Tris-HCl buffer 0.05 M pH 7.4 and used as enzyme source.

The analysis of ALA-S protein expression was described in detail by Olivieri *et al.* [39]; polyclonal anti-ALA-S1 developed in our laboratory (1:800 v/v) was used as primary antibody that detected a protein of the predicted size for mitochondrial ALAS1 (65 kDa). Liver 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 phenylmethylsulphonylfluoride, 10 µg/ml leupeptin and 1 µg/ml pepstatin A. Gel loadings were normalized according to actine protein content.

Protein concentration was determined by the method of Lowry et al. [40] or Bradford [41].

2.5 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.

3. RESULTS

3.1 Effect of anaesthetics

3.1.1 ALA-S

The effects of Isoflurane and Sevoflurane on ALA-S activity are shown in Figure 1.

After Isoflurane administration, the activity of ALA-S was induced 160% ($p<0.01$) in the liver of T1 males without any modification in kidney and brain. In females, no alterations were observed (Figure 1 A). In the AIP group, ALA-S activity was only increased in kidney (120%, $p<0.01$) of males. On the contrary, in females AIP the induction was observed in liver (88%, $p<0.05$), kidney (60%, $p<0.05$) and brain (60%, $p<0.05$) (Figure 1 B).

When the anaesthetic tested was Sevoflurane, the variations were detected in liver (248%, $p<0.01$) and brain (550%, $p<0.01$) of T1 female group while in males the induction was observed only in liver (80%, $p<0.05$) (Figure 1 C). In the AIP group, the response was only found in females in which ALA-S was induced in kidney (100%, $p<0.05$) and brain (163%, $p<0.01$) but not in liver (Figure 1 D).

To determine if the observed induction of ALA-S activity was correlated with an increase of protein expression, western blot analysis was performed in those groups were the activity was augmented. No variations of ALA-S protein expression were observed in none of them (Figure 2).

Moreover, ALA levels in liver and brain were quantified with the aim to analyze if the induction of ALA-S activity provoked tissue ALA accumulation. In all the cases, ALA levels were unchanged (Figure 3).

3.1.2 PBG-D activity

The effects of Isoflurane and Sevoflurane on PBG-D activity are shown in Figure 4.

After Isoflurane treatment, PBG-D activity decreased 30% ($p<0.05$) in liver males T1 mice without any modification in kidney, brain and blood; no alterations were produced in female T1 group (Figure 4 A). The enzyme activity was unaltered in the AIP group, males or females (Figure 4 B).

In animals receiving Sevoflurane, PBG-D activity was reduced in liver (35%, $p<0.05$) and kidney (80%, $p<0.05$) of T1 females, but the activity remain unchanged in brain and blood, and also in all the assayed tissues of male group (Figure 4 C). In AIP males or females, no changes were detected either(Figure 4 D).

3.1.3 HO activity

The effects of Isoflurane and Sevoflurane on HO activity in T1 mice are shown in Figure 5.

When Isoflurane (Figure 5 A) was administered to T1 mice, no variations were detected in liver and kidney activity, while brain enzyme was 114% ($p<0.01$) induced in males and 150% ($p<0.01$) in female group.

Sevoflurane (Figure 5 B) caused no variation when it was administered to male or female T1 mice.

3.1.4 CYP2E1 activity

The effects of Isoflurane and Sevoflurane on CYP2E1 activity in T1 mice are shown in Figure 6

No induction of CYP2E1 activity was observed after anaesthetics administration to T1 mice independently on the sex.

3.2 Effect of porphyrinogenic drugs

3.2.1 ALA-S

The action of AIA, barbital and ethanol on ALA-S activity is shown in Figure 7.

When AIA was administered to male T1 mice, ALA-S activity was increased 130% ($p<0.01$) in liver and 140% ($p<0.01$) in kidney. In females T1 mice, the induction of activity was 60% ($p<0.05$) in liver and 110% ($p<0.05$) in kidney. Brain enzyme was unaltered in T1 males and females mice (Figure 7 A). In AIP group, ALA-S activity was strikingly increased in the liver of male (280%, $p<0.01$) and female (13 fold) mice; and remained in basal levels in kidney and brain of both males and females (Figure 7 B).

When the porphyrinogenic drug studied was barbital, ALA-S activity was 51% ($p<0.05$) increased in the liver of female T1 mice without any alteration in kidney or brain or in male group (Figure 7 C). In AIP animals, a 62% ($p<0.05$) induction of ALA-S was detected in male liver but the activity was unchanged in kidney and brain. A similar response was observed in female AIP group being ALA-S activity augmented only in liver (131%, $p<0.01$) (Figure 7 D).

The administration of ethanol to female T1 mice caused a significant increase of ALA-S in liver (100%; $p<0.05$) and kidney (220%; $p<0.01$), but not in brain enzyme. ALA-S activity of male T1 mice suffers no modifications in any of the studied tissues (Figure 7 E). In the AIP group receiving ethanol, ALA-S activity was only increased (80%, $p<0.05$) in female mice liver, without any alteration in male tissues (Figure 7 F).

3.2.2 PBG-D activity

The action of AIA, barbital and ethanol on PBG-D activity is shown in Figure 8.

When AIA was administered to T1 mice, no alterations on PBG-D activity were observed in any of the tissues measured, independently on the sex (Figure 8 A). In the AIP group, PBG-D activity was 50% ($p<0.05$) reduced in liver of male group; while in females, this

activity was diminished in all the tissues assayed: 50% ($p<0.05$) in liver and blood, and 75% ($p<0.05$) and 50% ($p<0.05$) in kidney and brain respectively (Figure 8 B).

In barbital treated groups, no effects were produced on T1 enzyme (Figure 8 C). In AIP group, a diminution (46%, $p<0.05$) was only observed in blood enzyme of female mice without any effect on liver, kidney or brain and also on males mice (Figure 8 D).

After ethanol treatment, PBG-D activity of T1 male liver mice was 45% ($p<0.05$) diminished, without any alteration in kidney and brain. Ethanol produced no effect on female enzyme group in any of the studied

tissues (Figure 8 E). Although, in AIP group ethanol reduced PBG-D activity in liver and kidney (40%, $p<0.05$) of essayed female group; it was unchanged in brain and blood as well as in other AIP males tissues (Figure 8 F).

3.2.3 HO activity

The results obtained after AIA, barbital and ethanol treatments on HO activity in T1 mice are shown in Figure 9.

AIA (Figure 9 A) induced HO activity in liver (107%, $p<0.05$), kidney (133%, $p<0.01$) and brain (95%, $p<0.05$) of male T1 mice. However, in female group, the enzyme remained in basal levels in all the tissues measured.

When barbital (Figure 9 B) was administered to T1 males, the activity was 29% ($P<0.05$) increased in all the tissues studied; while no variations were detected in female tissues.

Ethanol administration (Figure 9 C) induced liver HO activity (67%, $p<0.05$) without any effect on kidney and brain enzyme. No significant alterations of HO activity were observed in female mice.

4. DISCUSSION

Heme controls its own intracellular level by modulating the production of the rate-limiting enzymes involved in its synthesis (ALA-S) and degradation (HO) [42, 43]. Mutations in human PBG-D, the third enzyme of this biosynthetic pathway cause AIP disease. The PBG-D deficient mouse has also been demonstrated to be a good model for the study of the pathogenesis of AIP [26-29].

In this work, the effect of Isoflurane and Sevoflurane on heme metabolism in a murine genetic model of AIP was investigated. A comparison was performed studying the effects of AIA, barbital and ethanol.

To study the response against xenobiotics, two lines T1 and AIP were used. Both lines differ in the PBG-D activity and when they received a porphyrinogenic drug [26]. There are many patients that, in spite of having the mutation of the disease, interestingly, remain in a latent form [3, 30].

Isoflurane caused biochemical alterations that affect further the diminished PBG-D activity as a consequence of the mutation. These findings were observed only in T1 male mice. This is a difference wih respect to human AIP which is mainly observed in females.

The action of Sevoflurane on PBG-D activity was only found in females T1, in this group, ALA-S was strikingly induced, something else occurring in brain. These results were different when the anaesthetic studied was Isoflurane.

When we compared these results with those obtained when Isoflurane or Sevoflurane were administered to control *CF1* mice [16, 17, 21], we realized that the effects on the PBG-D deficient model are more pronounced, indicating a higher sensibility to these xenobiotics.

ALA-S induction was not accompanied by an increase in protein synthesis. This result could be attributed to the short time of treatment but we observed alterations in mRNA ALA-S [21] and CYP2E1 protein expression [17] in CF1 mice using the same experimental protocol for anaesthetic administration.

In spite of the reduction of PBG-D activity and the induction of ALA-S, no accumulation of ALA in liver and brain was observed. This fact would indicate that heme pathway is able to metabolize ALA, thus avoiding ALA transport to the brain and the onset of neurological symptoms in this mice model by effect of anaesthetics.

Sex and strain different response were previously observed for Isoflurane [44]; alterations on ALA-S and PBG-D activities were detected in male CF1 mice without any modification in females and also in *C57* strain. Once again, we also observed a different behavior between Isoflurane and Sevoflurane in spite that they belong to the same family; and it could be related to its metabolism [16, 17].

CYP2E1 is one of the most studied cytochrome P-450 (CYP) enzymes in animals and humans, due to its role in several drugs metabolism and its participation in metabolic activation of some procarcinogens [45]. Moreover, CYP2E1 has particular relevance in several pathologies because high amounts of reactive oxygen species are producing during the detoxification process [45]. Hepatic and renal toxicity of volatile anaesthetics is due to their biotransformation to toxic metabolites mediated by CYP, being the isoform CYP2E1, responsible for the metabolism of these compounds in humans [46] (Miksys and Tyndale, 2013).

To improve the knowledge of the action of Isoflurane and Sevoflurane, and to justify the differential tissue response observed in heme enzymes measured, the activity of CYP2E1, isoform involved in anaesthetic metabolization, was studied.

In this work, no variations were observed in liver and brain CYP2E1 activity indicating that heme enzyme alterations in the AIP model due to anaesthetics, would not be related to an exacerbated metabolization of these xenobiotics. We have previously observed that this isoform was induced in liver after administration of Isoflurane to CF1 mice [46, 47]; although, when the anaesthetic was Sevoflurane, the activity was unchanged even 60 minutes after administration while protein expression was induced at 30 min [17]. Brain activity was also unchanged in brain of CF1 mice [25].

AIA produced the expected induction of ALA-S activity. AIA triggered in male AIP$^-$ mice neurological signs similar to those observed during human acute attacks; the symptoms were less pronounced in females in spite that ALA-S activity was several folds more induced. There was an earlier onset of the neurological symptoms that appeared 5 hours after AIA administration (data not shown). In T1 mice, ALA-S induction by AIA was less than in AIP mice without clinical alterations. This was an important difference with the action of the anaesthetics and the other drugs here assayed. AIA also affected PBG-D activity being a remarkable difference between the genetic model and control mice where a striking induction of ALA-S was observed but without any reduction of PBG-D activity [32].

Ethanol and barbital produced biochemical alterations depending on the group studied but without clinical manifestations.

Lindberg et al. [26] and Johansson et al. [29] characterized the regulation of ALA-S in the AIP mouse and measured the time-course expression of ALA-S in the liver during Phenobarbital treatment, a barbiturate like barbital. ALA-S activity and mRNA were not significantly enhanced by repeated Phenobarbital administration in control mice and were substantially induced in PBG-D deficient mice. Unzu et al. [49] also found hepatic ALA-S mRNA expression induced and a reduction of PBG-D activity when 7 doses of Phenobarbital were administered to AIP mice. Here, we report similar results after the administration of three doses of barbital to genetic models T1 and AIP that are different from our studies about the effect of barbital on ALA-S activity in brain [21].

The differences on tissue response observed when heme disturbance was challenged with the different drugs, is a fact previously observed in control animals [16, 17, 44] however, no explanation could we suggest for this phenomena. The activity of PBG-D in AIP mice was similarly diminished in all the tissues as was reported by Johansson et al [29], the same was observed when we compared basal activities of PBG-D of *C57BL/6* mice with that of T1 and AIP mice (data not shown).

HO is a known oxidative stress-inducible protein which plays a key role in heme catabolism, where heme, a potential prooxidant, is converted to bilirubin, an antioxidant [49]. HO increases markedly after different stimulus such as heat shock, ischemia, glutathione depletion [14, 15]. As expected HO was induced after drugs administration and this effect was mainly produced in male mice by action of AIA and barbital. Anaesthetics only affected the brain enzyme in male and female mice; while the action of ethanol was observed only in the liver of male group. These results are indicative of the induction of oxidative stress and are in concordance to those we have previously reported [16, 17, 21]. This tissue specific manner response was also reported by Gerjevic et al [50] due to ethanol.

Conclusions

In conclusion, when biochemical alterations were evaluated, we observed differences between males and females and also between T1 and AIP, with females being more affected in the AIP mice. Clinical results were different because neurological symptoms were developed in males AIP mice after AIA treatment, fact that is different to that observed in humans were the onset of AIP is mainly produced in females. Even the genetic mouse is a predictive model for AIP, extrapolation to human disease might be considered.

This report has been the first study of volatile anaesthetics administration to a genetic mouse model of AIP, confirming Isoflurane and Sevoflurane porphyrinogenicity. Although Isoflurane has been reported to have neuroprotective properties and it had been proposed as an alternative medicine in children with neonatal hypoxia ischemia [51], we again suggest taking care of its administration to porphyric patients.

The relevance of the results presented in this paper is supported by the fact that at present little information is known about the mechanisms acting on brain and involved in the triggering of acute attacks in AIP. The understanding of the widespread action of the anaesthetics and other porphyrinogenic drugs affecting other metabolisms, besides the

heme pathway, is very important in translational medicine and would allow us to extrapolate results from the animal model to human Porphyria.

Acknowledgements

J. Lavandera, A.M. Buzaleh, E. Gerez and A. Batlle hold the post of Assistant, Independent and Superior Scientific Researchers at the Argentine National Research Council (CONICET). M. del C. Martinez is a Researcher from the University of Buenos Aires. S. Ruspini and J. Zuccoli are fellows from CONICET. We want to especially mention Dr. Urs Meyer for his authorization to use his AIP model and to Dr. A. Fontanellas who kindly provide us the mice. Moreover we want to acknowledge the Animal facility of our School of Sciences for animal care and the aid for the establishment of the lines.

Fundings: This work has been supported by grants from the CONICET (PIP 11220130100557CO, the Scientific and Technology Agency (PICT 2007-0142) and the University of Buenos Aires (UBACYT 2011-2014, 01/W772; 2014-2017, 01/Q839), Argentina.

Competing interests

The authors declare that they have no competing interests

Abbreviations

AIA: allylisopropylacetamide; AIP: Acute Intermittent Porphyria; ALA: δ-Aminolevulinic acid; ALA-S: δ-Aminolevulinic acid Synthetase; CYP: cytochrome P-450; PBG-D: Porphobilinogen deaminase; HO: Heme oxygenase; s.d.: Standard deviation

References

[1] A. Batlle, Porfirias Humanas. Signos y Tratamientos. In Porfirias y Porfirinas. Aspectos clínicos, bioquímicos y biología molecular, Acta Bioquim. Clin. Latinoamer. 3(2) (1997) 37-69.

[2] A. Schüler and R. Salamanca, "Metabolismo de las Porfirinas y Porfirias", Ed. Complutense, Madrid. 2000.

[3] G. Cerbino, E. Gerez, L. Varela, V. Melito, V. Parera, A. Batlle and M. Rossetti, Acute Intermittent Porphyria in Argentina. An update, J. Biomed. Biotech. 4-2015 (2015) 1-7.

[4] M. Hrdinka, H. Puy, P. Martasek, May 2006 update in porphobilinogen deaminase gene polymorphisms and mutations causing acute intermittent porphyria: comparison with the situation in Slavic population, Physiol. Res. 55, Suppl 2 (2006) S119-136.

[5] E. Pischik, R. Kauppinen, Neurological manifestations of acute intermittent porphyria**,** Cell. Mol. Biol. (Noisy-le-grand) 55(1) (2009) 72-83.

[6] S. Besur, W. Hou, P. Schmeltzer, H. Bonkovsky, Clinically Important Features of Porphyrin and Heme Metabolism and the Porphyrias, Metabolites 4(4) (2014) 977-1006.

[7] E. Park, K. Yi Seul, L. Kyung-Jee, L. Hye Kyoung, L. Soo Kyung, C. Hyun, K. Mae-Hwa, Severe neurologic manifestations in acute intermittent porphyria developed after spine surgery under general anesthesia -a case report, Korean J. Anesthesiol. 67(3) (2014) 217-220.

[8] S. Thunell, E. Pomp, A. Brun, Guide to drug porphyrogenicity prediction and drug prescription in the acute porphyrias, Br. J. Clin. Pharmacol. 64(5) (2007) 668–679.

[9] M. Demasi, C. Penalti, R. DeLucia, E. Bechara, The prooxidant effect of 5-aminolevulinic acid in the brain tissue of rats: implications in neuropsychiatric manifestations in porphyrias, Free Rad. Biol. Med. 20(3) (1996) 291-299.

[10] T. Emanuelli, F. Pagel, L. Porciuncula, D. Souza. Effects of 5-aminolevulinic acid on

the glutamatergic neurotransmission. Neurochem. Int. 42 (2003) 115-121.

[11] A. Adhikari, C. Penatti, R. Resende, H. Ulrico, L. Britto, E. Bechara, 5-aminolevulinate and 4, 5-dioxovalerate ions decrease GABA(A) receptor density in neuronal cells, synaptosomes and rat brain, Brain Res. 1093(1) (2006) 95-104.

[12] N. Felitsyn, C. McLeod, A. Shroads, P. Stacpoole, L. Notterpek, The heme precursor delta-aminolevulinate blocks peripheral myelin formation, J. Neurochem. 106(5) (2008) 2068-2079.

[13] A. Takeda, Y. Itoyama, T. Kimpara, R. Kutty, N. Abraham, B. Dwyer, R. Petersen, G. Perry, M. Smith, Role of heme catabolism in neurodegenerative diseases, In: Abraham, N.G., ed. Heme Oxygenase in Biology and Medicine, Kluwer Acad. Publ., N.Y. (2002) 135-143.

[14] M. Maines, P. Gibbs, 30 some years of heme oxygenase: from a “molecular wrecking ball” to a “mesmerizing” trigger of cellular events, Biochem. Biophys. Res. Comm. 338(1) (2005) 568-577.

[15] N. Abraham, A. Kappas, Pharmacological and Clinical Aspects of Heme Oxygenase, Pharmacol. Rev. 60(1) (2008) 79–127.

[16] A. Buzaleh, R. Enriquez de Salamanca, A. Batlle, Administration of the anesthetic Isoflurane to mice: A model for Acute Intermittent Porphyria?, J. Pharmacol Toxicol. Methods 28(4) (1992) 191-197.

[17] R. Sampayo, J. Lavandera, A. Batlle, A. Buzaleh, Sevoflurane: its action on heme metabolism and Phase I drug metabolizing system, Cell. Mol. Biol. (Noisy-le-grand) 55(2) (2009) 140-146.

[18] A. Buzaleh, M. Morán-Jiménez, M. García-Bravo, A. Sampedro, A.Batlle, R.Enríquez de Salamanca, A. Fontanellas, Induction of Hepatic Aminolevulinate Acid Synthetase Activity by Isoflurane in a Genetic Model for Erythropoietic Protoporphyria, Cell. Mol. Biol. 55(1) (2009) 38-44.

[19] A. Buzaleh, M. García Bravo, S. Navarro, M. Morán Jimenez, M. Méndez, A. Batlle, A. Fontanellas, R. Enríquez de Salamanca, 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 Bio. 36(2) (2004) 216-222.

[20] J. Rodríguez, A. Buzaleh, M. Fossati, J. Azcurra, A. Batlle, The effects of some porphyrinogenic drugs on the brain cholinergic system, Cell. Mol. Biol. 48(1) (2002) 103-110.

[21] J. Rodriguez, M. Martinez, E. Gerez, A. Batlle, A. Buzaleh, Heme oxygenase, aminolevulinate acid synthetase and the antioxidant system in the brain of mice treated with porphyrinogenic drugs, Cell. Mol. Biol. (Noisy-le-grand) 51(5) (2005) 487-494.

[22] J. Lavandera, A. Batlle, A. Buzaleh, Metabolization of porphyrinogenic agents in brain: involvement of the Phase I drug metabolizing system. A comparative study in liver and kidney, Cell. Mol. Neurobiol. 27(6) (2007) 717-729.

[23] J. Lavandera, M. Fossatti, J. Azcurra, A. Batlle, A. Buzaleh, Glutamatergic System: another target for the action of porphyrinogenic agents, Cell. Mol. Biol. 55(1) (2009) 23-28.

[24] J. Lavandera, A. Batlle, A. Buzaleh, Mice brain nitric oxide synthase response induced by anaesthetics and other porphyrinogenic drugs, Drug Metab. Lett. 5(1) (2011) 25-29.

[25] J. Lavandera, S. Ruspini, A. Batlle, A. Buzaleh, Cytochrome P450 expression in mouse brain: specific isoenzymes involved in phase I metabolizing system of porphyrinogenic agents in both microsomes and mitochondria, Biochem. Cell Biol. 92(1) (2014) 1-6, http://dx.doi.org/10.1139/bcb-2014-0088.

[26] R. Lindberg, C. Porcher, B. Grandchamp, B. Ledermann, K. Bürki, S. Brandner, A. Aguzzi, U. Meyer, Porphobilinogen deaminase deficiency in mice causes a

neuropathy resembling that of human hepatic porphyria, Nat. Genetics 12(2) (1996) 195-199.

[27] R.L.P. Lindberg, R. Martini, M. Baumgartner, B. Erne, J. Borg, J. Zielasek, K. Ricker, A. Steck, V. Toyka, U.A. Meyer. Motor neuropathy in porphobilinogen deaminase-deficient mice imitates the peripheral neuropathy of human acute porphyria. J. Clin. Invest. 103 (1999) 1127-1134.

[28] R. Jover, F. Hoffmann, V. Scheffler-Koch, R. L. P. Lindberg. Limited heme synthesis in porphobilinogen deaminase-deficient mice impairs transcriptional activation of specific cytochrome P450 genes by Phenobarbital. Eur. J. Biochem. 267 (2000) 7128-7137.

[29] A. Johansson, C. Möller, J. Fogh, P. Harper, Biochemical chacterization of Porphobilinogen Deaminase-deficient mice during phenobarbital induction of heme synthesis and the effect of enzyme replacement, Mol. Med. 9 (2003) 193-199.

[30] M. Balwani, R. Desnick, The porphyrias: advances in diagnosis and treatment, Blood 120(23) (2012) 4496-4504.

[31] S. Miksys, E. Hoffman, R.F. Tyndale, R.F. Regional and cellular induction of nicotine-metabolizing CYP2B1 in rat brain by chronic nicotine treatment, Biochem. Pharmacol. 59 (2000) 1501-1511.

[32] P. Ortiz de Montellano, B. Mico, G. Yost, Suicidal inactivation of cytochrome P450. Formation of heme-substrate covalent adduct, Biochem. Biophys. Res. Commun. 83(1) (1987) 132-137.

[33] C. Handschin, J. Lin, J. Rhee, A.K. Peyer, S. Chin, P.H. Wu, U.A. Meyer, B.M. Spiegelman (2005) Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha, Cell. 122(4) (2005) 505-515.

[34] S.R. Paredes, P.A. Kozicki, H. Fukuda, M.V. Rossetti, A. Batlle. S-adenosyl-L-methionine: its effect on aminolevulinate dehydratase and glutathione in acute ethanol intoxication. Alcohol 4(2) (1987) 81-85.

[35] H. Marver, D. Tschudy, M. Perlroth, A. Collins, Delta-aminolevulinic acid synthetase. I. Studies in liver homogenates, J. Biol. Chem. 241(12) (1996) 2803-2809.

[36] A. Batlle, E. Wider de Xifra, A. Stella, A simple method for measuring erythrocyte porphobilinogenase and its use in the diagnosis of acute intermittent porphyria, Int. J. Biochem. 9(12) (1978) 871-875.

[37] R. Tenhunen, H. Marver, R. Schmid, The enzymatic catabolism of hemoglobin: stimulation of microsomal heme oxygenase by hemin, J. Lab. Clin. Med. 75(3) (1970) 410-421.

[38] L.A Reinke, M.J. Moyer. p-Nitrophenol hydroxylation. A microsomal oxidation which is highly inducible by ethano, Drug Metab. Dispos. 13(5) (1985) 548-552.

[39] L.M. Oliveri, C. Davio, A. M. Del C. Batlle, E.N. Gerez. ALAS1 gene expression is down-regulated by Akt-mediated phosphorylation and nuclear exclusion of FOXO1 by vanadate in diabetic mice, Biochem. J. 442 (212) 303–310.

[40] O. Lowry, N. Rosebrough, A. Farr, R. Randall, Protein measurement with the folin-phenol reagent, J. Biol. Chem. 193(1) (1951) 265-275.

[41] M.M. 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. 72 (1976) 248-254.

[42] K. Furuyama, K. Kaneko, P. Vargas, Heme as a magnificent molecule with multiple missions: heme determines its own fate and governs cellular homeostasis, Tohoku J Exp. Med. 213(1) (2007) 1-16.

[43] D. Chiabrando, F. Vinchi, V. Fiorito, S. Mercurio, E. Tolosano, Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes, Frontiers Pharmacol. (2014) doi: 10.3389/fphar.2014.00061. eCollection.

[44] A. Buzaleh, A. Batlle, Strain and sex differences in the effect of Enflurane and Isoflurane on heme metabolism in mice. Gen. Pharmac. 27(6) (1996) 1009-1012.

[45] L. Knockaert, B. Fromenty, M.A. Robin. Mechanisms of mitochondrial targeting of cytochrome P450 2E1: physiopathological role in liver injury and obesity, FEBS J. 278(22) (2011) 4252-4260.

[46] A.M. Buzaleh, E. Vázquez, R. Enríquez de Salamanca, A. Batlle. An overview on Anesthesia and Porphyrias (Review), Endocrinol. Metab. 4 (1997) 267-279.

[47] M. del C. Martínez, A.M. Buzaleh, A. Batlle. Efecto de la administración de los anestésicos Enflurano e Isoflurano a ratones con niveles inducidos y deprimidos de Citocromo P450. Estudios sobre el sistema enzimático metabolizante de drogas. Acta Bioquím. Clin. Latinoam. 39 (2000) 37-42.

[48] C. Unzu, A. Sampedro, E. Sardh, I. Mauleon, R. Enrıquez de Salamanca, J. Prieto, E. Salido, P. Harper, A. Fontanellas, Renal failure affects the enzymatic activities of the three first steps in hepatic heme biosynthesis in the Acute Intermittent Porphyria mouse, PLoS ONE 7(3) (2000) e32978 1-9.

[49] M. Tomaro, A. Batlle, Bilirubin: its role in cytoprotection against oxidative stress, Int. J. Biochem. Cell Biology 34(3) (2002) 216-220.

[50] L. Gerjevic, S. Lu, J. Chaky, D. Harrison-Findik, Regulation of heme oxygenase expression by alcohol, hypoxia and oxidative stress, World J. Biol. Chem. 2(12) (2011) 252-260.

[51] B. Burchell, J. Dixon, Tang, J. Zhang, Isoflurane provides neuroprotection in neonatal hypoxic ischemic brain injury, J. Inv. Med. 61(7) (2013) 1078-1083.

LEGEND TO FIGURES

Figure 1: Activity of ALA-S after Isoflurane (A, B) and Sevoflurane (C, D) administration to T1 (A, C) and AIP (B, D) mice.

T1 Male, T1 Female, AIP Male, AIP Female. Li: Liver, Ki: Kidney, Br: Brain, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. (*) $p<0.05$, (**) $p<0.01$: significance of differences between treated and control groups. A unique control value is given, because no significant differences were obtained in any of the controls after the administration of the vehicle. Other experimental details are described in Materials and Methods section.

Figure 2: Effect of Isoflurane and Sevoflurane on liver ALA-S proteinexpression.

a) Western blot of ALA-S 1. An equal amount of total protein was loaded in each lane.

b) Columns represent normalized signals of control and treated animals that were quantified using an Image Analyzer. Gel loadings were normalized according to total protein content and actin. ISO: Isoflurane; SEVO: Sevoflurane; 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% (Dotted line). Experimental details are described in the text.

Figure 3: ALA levels after Isoflurane (A) and Sevoflurane (C) administration to T1 mice.

T1 Male, T1 Female, AIP Male, AIP Female. Li: Liver, Br: Brain, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. A unique control value is given, because no significant differences were obtained in any of the controls after the administration of the vehicle. Other experimental details are described in Materials and Methods section.

Figure 4: Activity of PBG-D after Isoflurane (A, B) and Sevoflurane (C, D) administration to T1 (A, C) and AIP (B, D) mice.

Control, T1 Male, T1 Female, AIP Male, AIP Female.

Li: Liver, Ki: Kidney, Br: Brain, Bl: Blood, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. (∗) $p<0.05$: significance of differences between treated and control groups. Other experimental details are described in Materials and Methods section.

Figure 5: Activity of HO after Isoflurane (A) and Sevoflurane (B) administration to T1 mice.

Control, T1 Male, T1 Female.

Li: Liver, Ki: Kidney, Br: Brain, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. (∗) $p<0.05$: significance of differences between treated and control groups. Other experimental details are described in Materials and Methods section.

Figure 6: Activity of CYP2E1 after Isoflurane (A) and Sevoflurane (B) administration to T1 mice.

Control, T1 Male, T1 Female.

Li: Liver, Br: Brain, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. Other experimental details are described in Materials and Methods section.

Figure 7: Activity of ALA-S after AIA (A, B), Barbital (C, D) and ethanol (E, F) administration to T1 (A, C, E) and AIP (B, D, F) mice.

T1 Male, T1 Female, AIP Male, AIP Female.

Li: Liver, Ki: Kidney, Br: Brain, M: Male, F: Female

Data represent mean value ± S.D. of 4-6 animals (∗) $p<0.05$, (∗∗) $p<0.01$: significance of

differences between treated and control groups. Other experimental details are given in legend to Figure 1.

Figure 8: Activity of PBG-D after AIA (A, B), barbital (C, D) and ethanol (E, F) administration to T1 (A, C, E) and AIP (B, D, F) mice.

Control, T1 Male, T1 Female, AIP Male, Female.

Li: Liver, Ki: Kidney, Br: Brain, Bl: Blood, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. (∗) $p<0.05$: significance of differences between treated and control groups. Other experimental details are described in Materials and Methods section.

Figure 9: Activity of HO after AIA (A), barbital (B) and ethanol (C) administration to T1 mice.

Control, T1 Male, T1 Female.

Li: Liver, Ki: Kidney, Br: Brain, M: Male, F: Female.

Data represent mean value ± S.D. of 4-6 animals. (∗) $p<0.05$: significance of differences between treated and control groups. Other experimental details are described in Materials and Methods section.

HIGHLIGHTS

EFFECTS OF VOLATILE ANAESTHETICS ON HEME METABOLISM IN A MURINE GENETIC MODEL OF ACUTE INTERMITTENT PORPHYRIA. A COMPARATIVE STUDY WITH OTHER PORPHYRINOGENIC DRUGS

Questions related to neuropathology of Acute Intermittent Porphyria were addressed.

Porphyrinogenic drugs altered Heme pathway in AIP mice depending on sex and mutation

Isoflurane/Sevoflurane use in AIP patients should be avoided or used with caution.

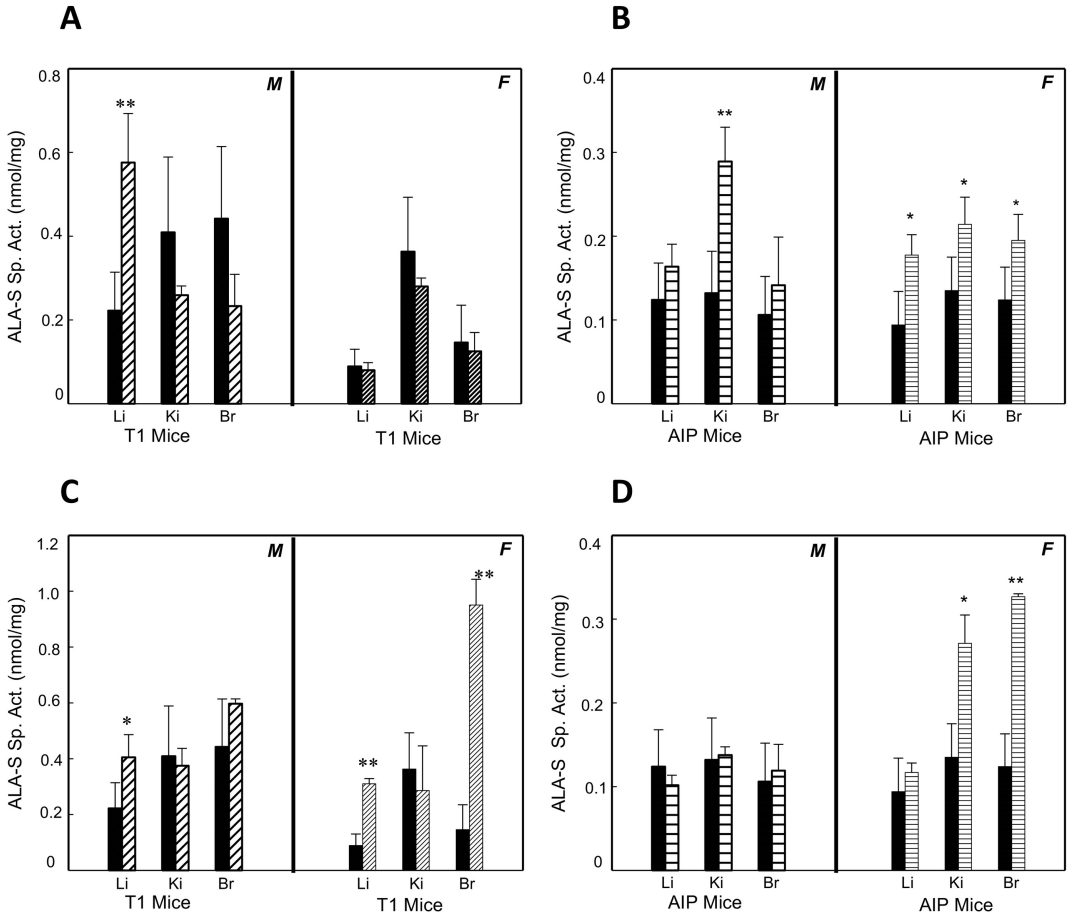
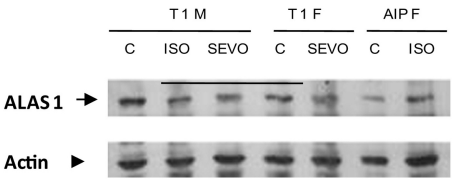


Figure 1


T1 M
T1 F
AIP F
C
ISO
SEVO
C
SEVO
C
ISO
ALAS 1
Actin

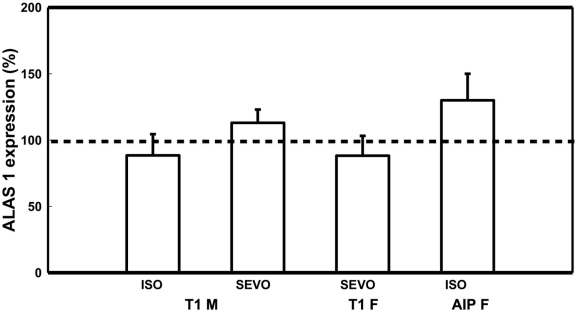

200
150
100
50
0
ALAS 1 expression (%)
ISO
SEVO
SEVO
ISO
T1 M
T1 F
AIP F

Figure 2

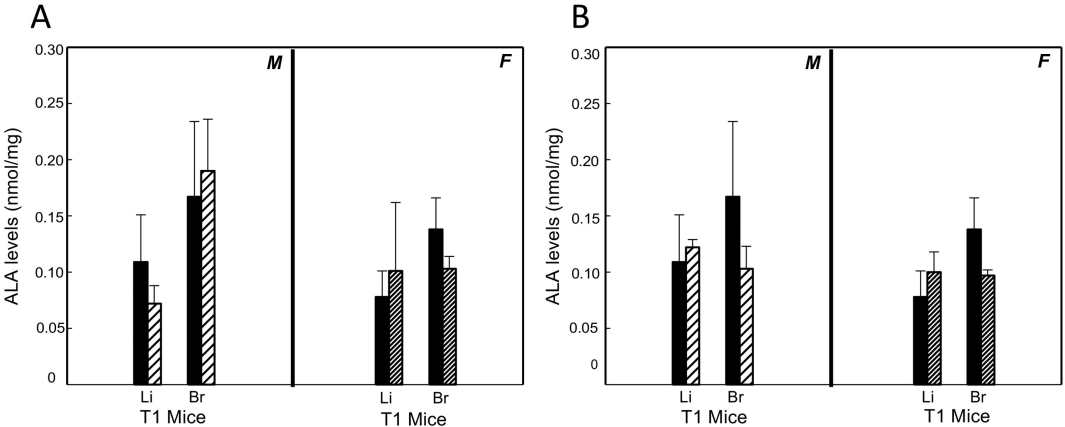


Figure 3

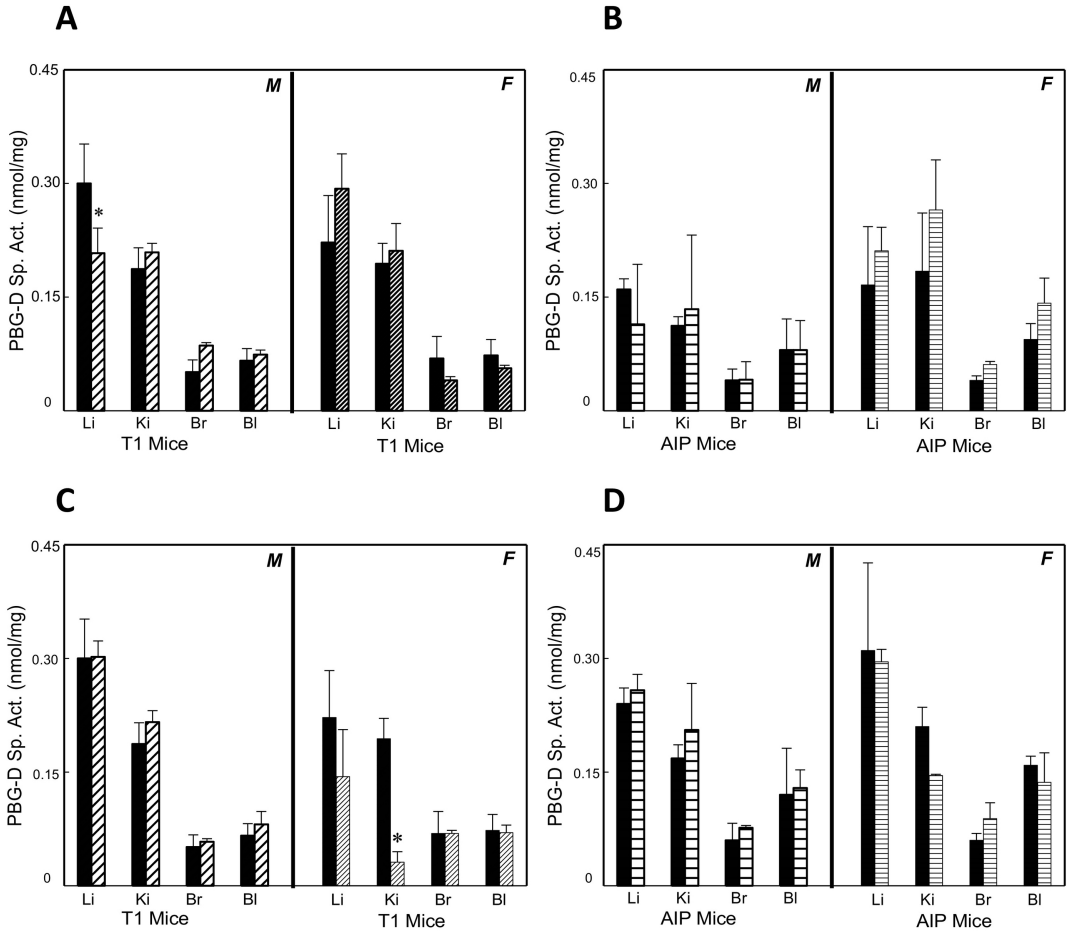

A
B
C
D
M
F
PBG-D Sp. Act. (nmol/mg)
0.45
0.30
0.15
0
Li
Ki
Br
Bl
T1 Mice
AIP Mice
*

Figure 4

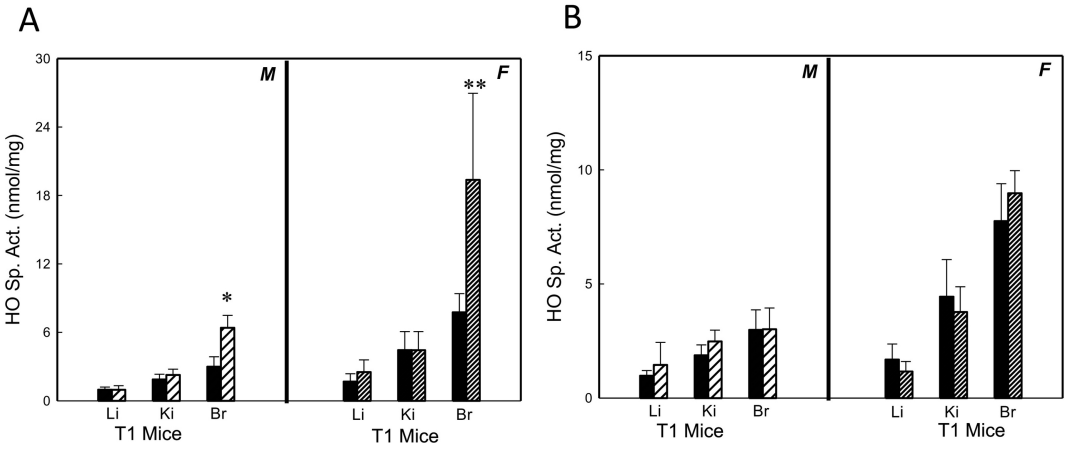


Figure 5

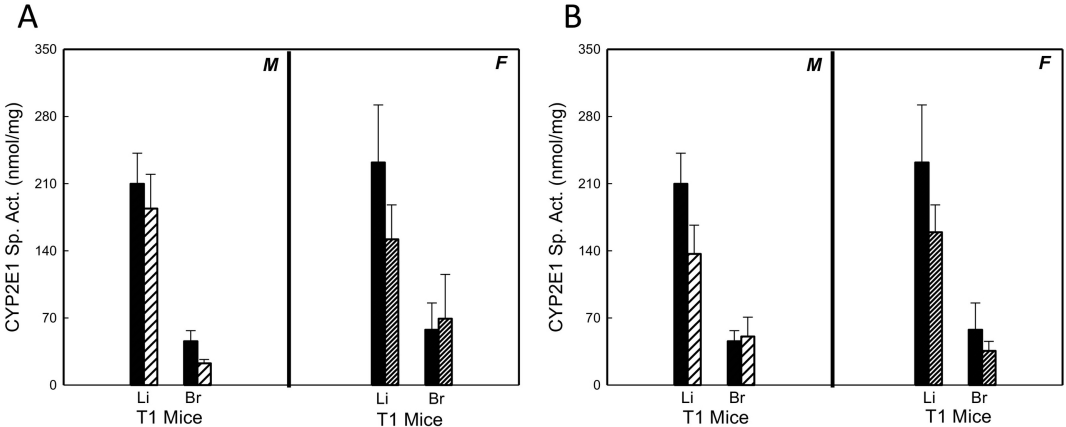


Figure 6

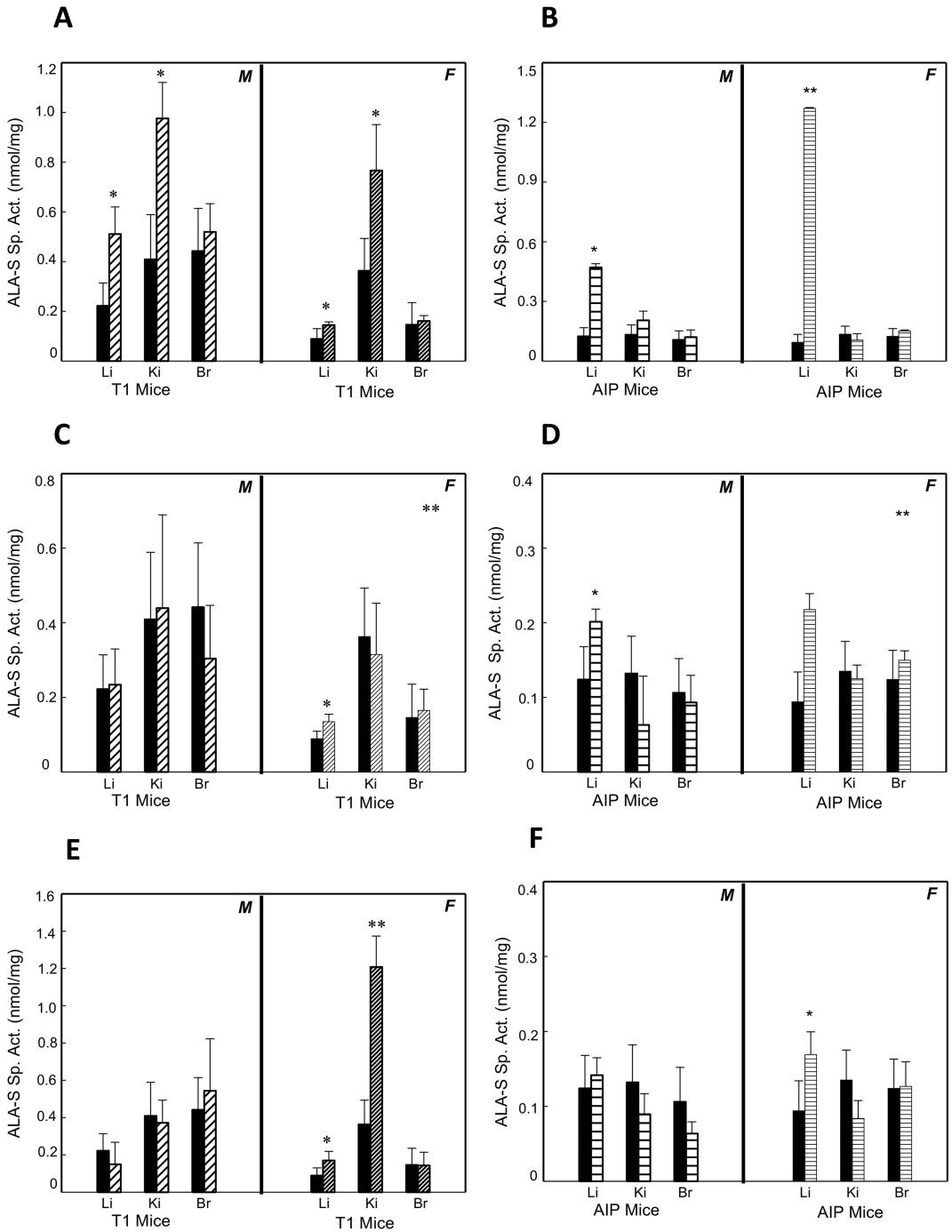


Figure 7

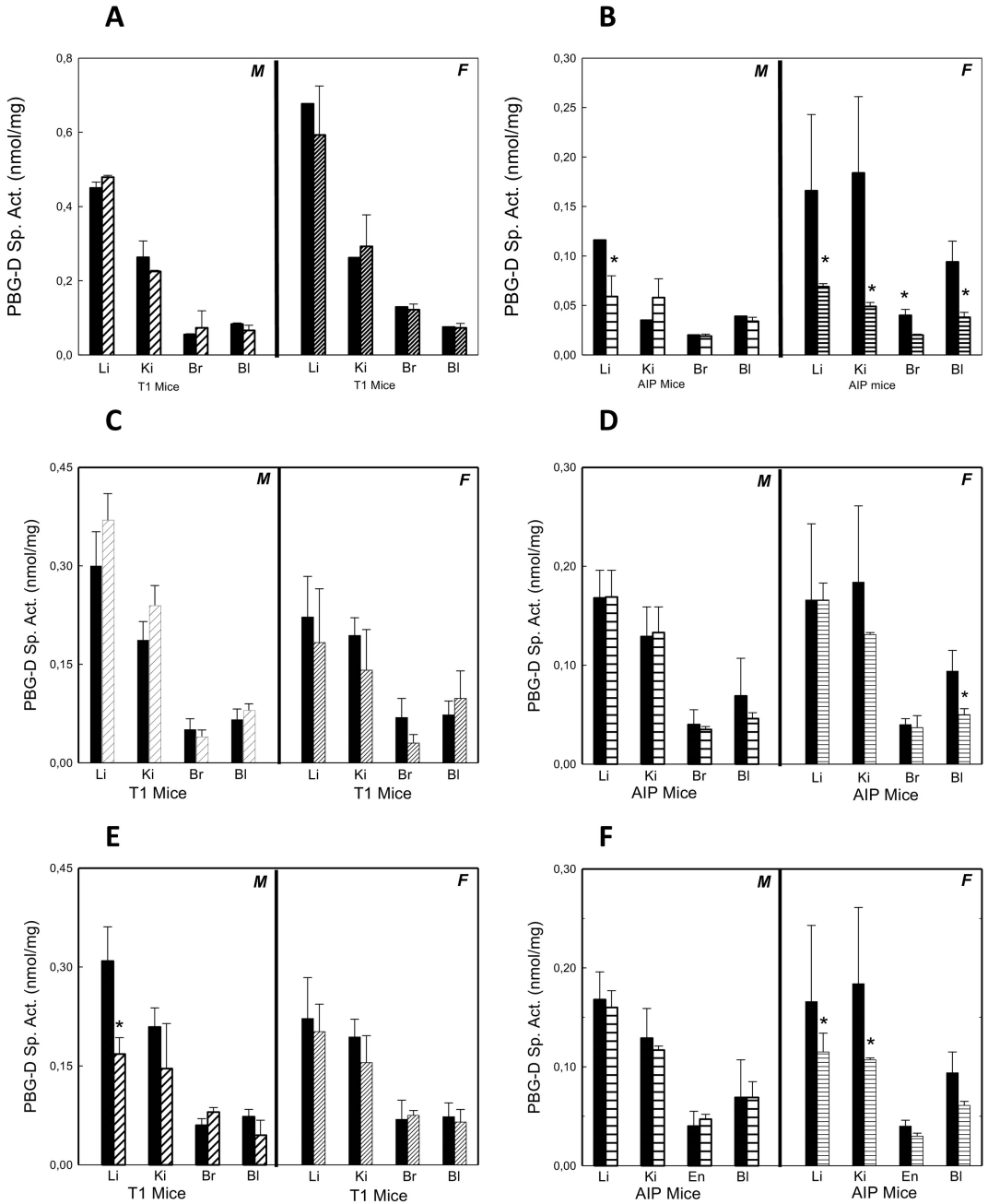

A
B
C
D
E
F
M
F
PBG-D Sp. Act. (nmol/mg)
Li
Ki
Br
Bl
En
T1 Mice
AIP Mice
AIP mice
*

Figure 8

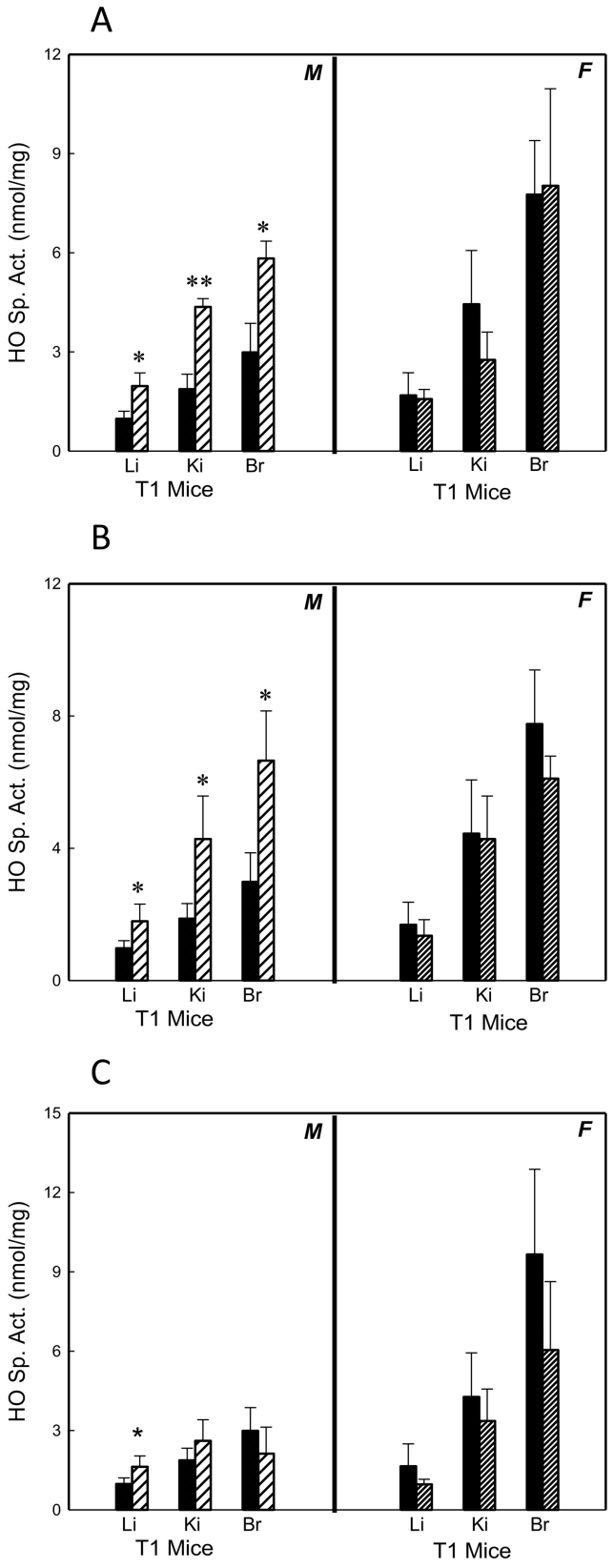

A
M
F
HO Sp. Act. (nmol/mg)
12
9
6
3
0
*
**
*
Li
Ki
Br
T1 Mice
B
M
F
HO Sp. Act. (nmol/mg)
12
8
4
0
*
*
*
Li
Ki
Br
T1 Mice
C
M
F
HO Sp. Act. (nmol/mg)
15
12
9
6
3
0
*
Li
Ki
Br
T1 Mice

Figure 9