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Hepatic alteration of tryptophan metabolism in an acute porphyria model

Its relation with gluconeogenic blockage

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ABSTRACT

This study focuses on the alterations suffered by the serotonergic and kinurenergic routes of tryptophan (TRP) metabolism in liver, and their relation with gluconeogenic phosphoenolpyruvate-carboxykinase (PEPCK) blockage in experimental acute porphyria. This porphyria was induced in rats by a combined treatment of 2-allyl-2-isopropylacetamide (100, 250, 500 mg/kg bw) and 3,5-diethoxycarbonyl 1,4-dihydrocollidine (constant 50 mg/kg bw dose).

Results showed a marked dose-dependent increase of all TRP pyrrolase (TRPp) forms, active (holo, total) and inactive (apo), and a decrease in the degree of enzyme saturation by heme. Increases for holo, total, and apo-TRPp were 90, 150, and 230%, respectively, at the highest dose assayed (H). The treatment also impaired the serotonergic route of TRP metabolism in liver, causing a decrease in serotonin level (H, 38%), and a concomitant enhancement in TRP content (H, 23%).

The porphyrinogenic treatment promoted a blockage in PEPCK activity (H, 30%). This occurred in correlation to the development of porphyria, to TRPp alterations and to the production of hepatic microsomal thiobarbituric acid reactive substances. Porphyria was estimated through increases in 5-aminolevulinic acid-synthase (ALA-S) activity, ALA and porphobilinogen contents, and a decrease in ferrochelatase activity.

Thus, the TRP kynurenine route was augmented whereas the serotonergic route was reduced. PEPCK blockage could be partly attributed to quinoline generated from TRP by the increase of TRPp activity, which would be due to the effect of porphyrinogenic drugs on TRP. The contribution of ROS to PEPCK blockage is analyzed. Likewise, the implication of these results in the control of porphyrias by glucose is discussed.

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Abbreviations: AIA, 2-allyl-2-isopropylacetamide; ALA, 5-aminolevulinic acid; ALA-S, ALA-synthase; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; PCT, porphyria cutanea tarda; PEPCK, phosphoenolpyruvate-carboxykinase; PBG, porphobilinogen; Proto, protoporphyrin; ROS, reactive oxygen species; 5-HT, serotonin; TBARS, thiobarbituric acid reactive substances; TRP, tryptophan; TRPp, tryptophan pyrrolase.

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Fig. 1 – Effect of AIA and DDC on the heme pathway. Interrelation between heme and tryptophan pathways through tryptophan pyrrolase species. Apo, apoenzyme; Holo, holoenzyme; Cyt P-450, cytochrome P-450.

the liver can be increased by TRP administration or blood level elevation of adrenal cortical steroids. This hemeprotein, which has a very rapid turnover [19–21], is the rate-limiting enzyme in TRP degradation [22,23] and is closely regulated by the hepatic free heme pool [24–26]. In rat liver, TRPp exists in two forms. Generally, half of the hepatic TRPp protein is saturated with heme and functions as the already active holoenzyme. The other half appears as heme-free apoprotein (Fig. 1).

TRPp was found to be altered by certain porphyrinogenic drugs such as chlorinated dioxins [14,27], griseofulvin, collidines, and acetamides alkyl substituted, or by porphyria exacerbators such as phenobarbitone and carbamazepine [25,28].

Since AIA/DDC treatment induces a decrease in the gluconeogenic hepatic enzyme PEPCK in acute porphyria, and tryptophan was found to be a gluconeogenesis modulator, it seemed interesting to study some aspects of TRP metabolism in relation to PEPCK blockage in the already mentioned acute porphyria model. The purpose was to find possible alterations in this indole pathway leading to tryptophan accumulation, which would be one of the potential causes of PEPCK decrease, leading to depressed glucose levels. This might be the underlying mechanism of the “glucose effect” observed in hepatic porphyrias. Thus, TRP and 5-HT levels, as well as TRPp, ALA-S and ferrochelatase (as porphyria markers together with heme precursors levels), and PEPCK (as gluconeogenic key enzyme) enzymatic activities were measured in hepatic tissue of rats treated with different doses of AIA/DDC.

2. Materials and methods

2.1. Materials

AIA was a gift from Roche Co. (Germany). DDC was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI). Dowex 1 and Dowex 50 W, ALA, bovine serum albumin (BSA), deoxyguanosine 5'-diphosphate, NADH, pyruvate, tryptophan, and serotonin were from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade.

2.2. Animals and treatment

Female Wistar rats (180–200 g) were purchased from the National Committee of Atomic Energy (CONEA, Argentina). Rats were maintained on food and water *ad libitum* and housed under conditions of controlled temperature (25 °C) and light (12 h light:12 h dark cycle; light from 06:00 to 18:00 h). Animals were fasted 24 h (8 h before treatment and 16 h after treatment) before being killed. This fasting period enhance the sensibility of the assays and at the same time allows standardizing the results obtained respect to the control. Since the effect of AIA depends on the previous diet [29], the 8 h of starvation previous to intoxication does not condition on the received diet, the effect of the drug.

Animals were treated according to the guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC),

and the Guide to the Care and Use of Experimental Animals issued by the Argentine Council on Animal Care.

AIA was dissolved in saline solution (0.15% w/v) [30] and DDC was dissolved in 2.5 ml corn oil [31]. Both solutions were freshly prepared before administration. Rats were fasted 8 h before being injected subcutaneously (sc) with three different AIA doses and intraperitoneally (ip) with a single DDC dose. Then, they were divided into the following groups: L (100 mg AIA + 50 mg DDC/kg body wt), M (250 mg AIA + 50 mg DDC/kg body wt), and H (500 mg AIA + 50 mg DDC/kg body wt). Control group (C) only received vehicles: saline, sc and corn oil, ip.

2.3. Liver preparation and enzymatic assays

Animals were decapitated 16 h after AIA/DDC administration. All assays were performed in rats with a total fasted period of 24 h. A liver fraction (1 g) was excised and immediately homogenized (1:3, w/v) in a solution containing 0.9% NaCl, 0.1 mM Tris-HCl, pH 7.4, and 0.5 mM EDTA to determine ALA-S activity.

To measure TRPp activity, liver was immediately homogenized in 6.5 volumes of 140 mM KCl 2.5 mM NaOH. The homogenate was incubated within 4–8 min of preparation. For PEPCK activity determination, liver was homogenized (1:3 w/v) in 0.25 M sucrose at 0–4 °C. The homogenate was then centrifuged for 120 min at $100,000 \times g$ to obtain the supernatant used in the assay. All procedures were carried out at 4 °C.

The rest of the organ was removed and part of it was homogenized in a Potter-Elvehjem in 4 volumes of 0.16 M perchloric acid plus 0.1% EDTA and 0.1% ascorbic acid to determine TRP metabolites by HPLC. Another portion of tissue was homogenized in 10 volumes of 0.9% NaCl and centrifuged at $100,000 \times g$ for 1 h to obtain total homogenate and microsomal fractions. Both fractions were used to determine lipid peroxidation. The whole liver homogenate was also used for ALA and PBG estimations.

2.3.1. 5-Aminolevulinic acid synthase (ALA-S) activity

ALA-S hepatic activity was assayed in the whole homogenate by the method of Marver et al. [32]. Mixtures containing 0.1 M glycine, 0.01 M EDTA, 0.08 M Tris/HCl buffer, pH 7.2, and 0.5 ml homogenate to a final volume of 2 ml were incubated at 37 °C for 60 min. Enzymatic activity was spectrophotometrically determined at 555 nm.

2.3.2. Phosphoenolpyruvate-carboxykinase activity

PEPCK was measured using deoxyguanosine 5'-diphosphate as nucleotide substrate. A 50 μ l aliquot of $100,000 \times g$ supernatant was used in the assays. Oxaloacetate formed during the reverse enzyme reaction was determined by reduction with malic-dehydrogenase (MDH) in the presence of NADH. Changes in absorbance were measured spectrophotometrically at 340 nm. The reaction was allowed to proceed for 2 min at room temperature [33].

2.3.3. Tryptophan pyrrolase (TRPp) activity

Enzymatic activity was determined in liver homogenates measuring the formation of kynurenine from L-TRP either in the absence (holoenzyme activity) or the presence (total enzyme activity) of hematin. Apoenzyme activity, calculated

by difference, was then used to measure holoenzyme/apoenzyme activity ratio, which indicates the degree of heme saturation of the apoenzyme. Activity was measured in the presence 7.5 mM L-tryptophan as described by Badawy and Evans [20]. When necessary, hematin was dissolved in 0.1 M NaOH and was included in overall the mixture to obtain a final 2 μ M concentration as described Badawy and Evans [34].

2.4. 5-Aminolevulinic acid (ALA) and porphobilinogen (PBG) hepatic contents

To determine ALA and PBG hepatic content, portions of whole liver homogenate were deproteinized with 0.6 M TCA [32]. Supernatants (0.3–1 ml) were adjusted to pH 4.5–6.0. Contents were determined by the method of Piper et al. [35]. Two separated columns were used; PBG was eluted from a Dowex 1 column with 1 M acetic acid, whereas ALA was eluted from a Dowex 50 W column with 1 M sodium acetate after removing urea. ALA pyrrol and PBG were measured colorimetrically as described by Mauzerall and Granick [36].

2.5. Lipid peroxidation in homogenate and microsomes

Lipid peroxidation in liver was determined measuring the production of thiobarbituric acid reactive substances (TBARS) in total homogenate and microsomes resuspended in 0.9% NaCl. The red MDA–thiobarbituric acid complex produced was extracted with butanol to avoid interference by endogenous porphyrins. TBARS contents were determined from the absorbance at 532 nm using a molar absorption coefficient of 156,000 $M^{-1} cm^{-1}$ [37].

2.6. Hepatic content of tryptophan (TRP) and serotonin (5-HT)

Liver samples from normal and treated rats were prepared according to Wolf and Kuhn [38]. An aliquot of 12,000 $\times g$ homogenate supernatant was kept at 4 °C in the dark until use. All samples were filtered using 0.45 μ m pore-size Millipore filters before being injected. TRP and 5-HT contents were determined by the isocratic chromatographic HPLC method described by Moran and Fitzpatrick [39].

2.7. Apparatus

A Spectra SERIES (Thermo Separation products) Model P200 liquid chromatograph and a FL2000 fluorescence detector set at excitation and emission wavelengths of 290 and 330 nm, respectively, were used in the assays. Separations were carried out on a C18 reverse-phase column [39].

2.8. Protein determination

Protein concentrations were measured following Lowry et al. [40] using bovine serum albumin as standard.

2.9. Statistical analysis

Values expressed in figures and tables are the means of three different experiments performed in duplicate and involving

two animals each. They indicate mean values \pm S.E.M. Data were submitted to one-way ANOVA. When the overall F-statistic was significant, multiple comparisons among treatment groups were performed using Tukey–Kramer's test. The level of significance used was 0.05.

3. Results

In order to determine the onset of porphyria, hepatic ALA-S and ferrochelatase activities, and heme precursor levels were measured. AIA/DDC combined treatment resulted in the development of an acute type of porphyria. Fig. 2 shows that ALA-S, the rate-limiting enzyme of the heme pathway, increased according to the AIA dose used. Increases were statistically significant at the two higher AIA doses assayed; 215% for the medium and 233% for the highest dose with respect to the control. Differences in ALA-S activity in groups H and M were statistically significant when compared to group L.

AIA/DDC administration half reduced ferrochelatase activity in all the groups treated with 50 mg/kg bw of DDC, independently of the AIA dose assayed. Values were 0.6 ± 0.2 , 0.7 ± 0.2 , and 0.8 ± 0.2 nmol heme/mg h for groups L, M, and H, respectively. Differences were statistically significant among groups and with respect to the control (1.4 ± 0.2 nmol heme/mg h).

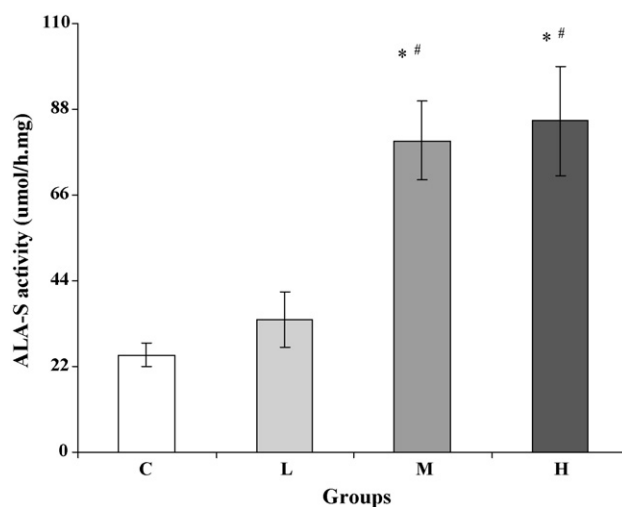


Fig. 2 – Effect of AIA/DDC treatment on ALA-S activity according to AIA doses. The development of porphyria was estimated measuring ALA-S activity. Each value represents the means \pm S.E.M. of six animals. Animals were injected subcutaneously (sc) with three different AIA doses and intraperitoneally (ip) with a single DDC dose, and were divided into the following groups: L (100 mg AIA + 50 mg DDC/kg body wt), M (250 mg AIA + 50 mg DDC/kg body wt), and H (500 mg AIA + 50 mg DDC/kg body wt). Control group (C) only received vehicles: saline solution, sc and corn oil, ip. ALA-S activity is expressed as μ mol/h mg protein. * $P < 0.05$ is significantly different from C. # $P < 0.05$ is significantly different from L. Multiple comparisons between groups were performed using Tukey–Kramer's test.

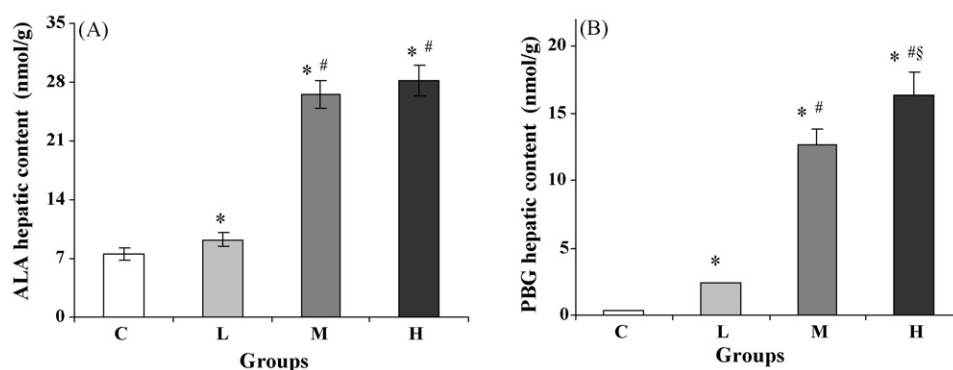


Fig. 3 – Dose-related effect of AIA/DDC treatment on ALA (A) and PBG (B) contents. ALA and PBG hepatic contents are expressed as nmol/g wet liver. Each value represents the means \pm S.E.M. of six animals. Animals were injected with the drugs and divided in four groups as detailed in Fig. 1. * $P < 0.05$ is significantly different from C. # $P < 0.05$ is significantly different from L. § $P < 0.05$ is significantly different from M. Multiple comparisons between groups were performed using Tukey–Kramer’s test.

The effect of the drug treatment on heme pathway precursors is described in Fig. 3. Both precursors, ALA (Fig. 3A) and PBG (Fig. 3B), were increased in all the groups assayed (L, M, and H) when compared to the control (C). Increase percentages for ALA were 25, 250, and 280%, and for PBG were 650, 3900, and 5000% for L, M, and H, respectively. For both precursors, differences in groups H and M were statistically significant with respect to group L. In the case of PBG, increases in group H also differ statistically from those observed in group M.

In order to study the gluconeogenic pathway, its key enzyme (PEPCK) was measured in the four groups. Fig. 4 shows

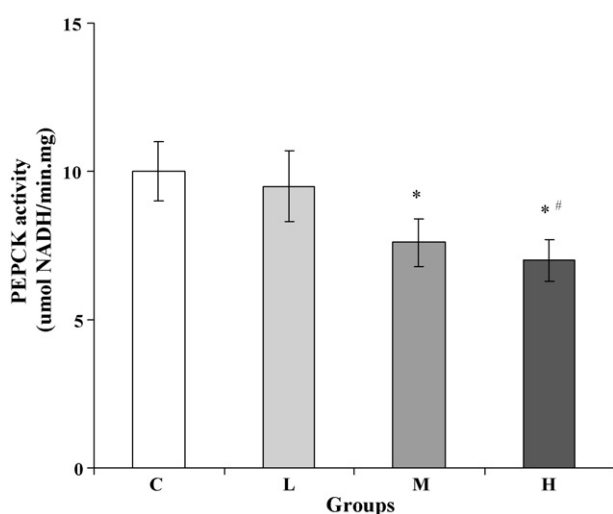


Fig. 4 – Dose-related effect of AIA/DDC treatment on hepatic PEPCK activity. PEPCK activity is expressed as μ mol NADH oxidized/min mg protein. Each value represents the means \pm S.E.M. of six animals. Animals were injected with the drugs and divided in four groups as detailed in Fig. 1. * $P < 0.05$ is significantly different from C. # $P < 0.05$ is significantly different from L. Multiple comparisons between groups were performed using Tukey–Kramer’s test.

there was a partial blockage in the activity of this cytosolic enzyme that was slight at a low dose (L), higher and statistically significant (24%) at a medium dose (M), and marked (30%) at the highest dose (H) of AIA assayed. The reduced activity detected in group H was significantly different from that observed in group L.

To determine the effect of ROS and the damage they may cause on primary biomolecules such as lipids, TBARS production was measured in two fractions: total homogenate and hepatic microsomes, a subcellular fraction rich in membranes. Results described in Table 1 showed that TBARS increased in both fractions, and that this dose-dependent increase was more significant in the microsomal fraction, about four times greater. Thus, in the total homogenate fraction, malondialdehyde increases with respect to control were 22, 33, and 72% for groups L, M, and H, respectively, whereas in the microsomal fraction values were 75, 150, and 330%, respectively, for the same groups.

To evaluate the impact of this porphyrinogenic treatment on tryptophan metabolism, serotonergic and kynurenergic routes were explored in liver. Thus, 5-HT and TRP levels and TRPp activity were measured. As shown in Table 2, AIA/DDC combined treatment promoted a decrease in serotonin level, which was more marked at higher AIA/DDC doses (20% in group M, and 38% in group H) and was significantly different from the control group. The decrease in 5-HT level in group H was statistically different from that in group M. On the other hand, TRP hepatic concentration was enhanced 23% at the highest AIA/DDC dose assayed with respect to the control.

The TRP degradation pathway was tested measuring hepatic TRPp activity. Results demonstrated that there was a marked and statistically significant increase in the activity of all TRPp forms (holo, apo, and total enzyme), which was dose-dependent with respect to the control group. Activity was measured in the presence or absence of exogenous heme. Table 3 shows 30, 70, and 120% increases for holo, total, and apo-TRPp, respectively, at the medium dose assayed (group M), and 90, 150, and 230% increases, respectively, at the higher dose assayed (group H). Activity values of all the enzyme species both at the highest and medium doses assayed (groups

Table 1 – Lipid peroxidation estimated as MDA formation in whole liver homogenate and microsomes of AIA/DDC treated and control rats

Group	MDA in homogenate (nmol MDA/g wet liver)	MDA in microsomes (nmol MDA/mg protein)
C	128 ± 10	20 ± 5
L	156 ± 12 [*]	35 ± 3 [*]
M	170 ± 13 [*]	50 ± 8 ^{*,#}
H	220 ± 12 ^{*,#,\$}	86 ± 9 ^{*,#,\$}

Malondialdehyde (MDA) is evaluated as TBARS. Each value represents the means ± S.E.M. of six animals. Animals were injected subcutaneously (sc) with three different AIA doses and intraperitoneally (ip) with a single DDC dose, and were divided into the following groups: L (100 mg AIA + 50 mg DDC/kg body wt), M (250 mg AIA + 50 mg DDC/kg body wt), and H (500 mg AIA + 50 mg DDC/kg body wt). Control group (C) only received vehicles: saline solution, sc and corn oil, ip. Multiple comparisons between groups were performed using Tukey–Kramer's test.

^{*} P < 0.05 is significantly different from C.

[#] P < 0.05 is significantly different from L.

^{\$} P < 0.05 is significantly different from M.

H and M) were significantly different from those measured with the lower dose (group L), with the exception of holoenzyme activity in group M. In addition, apo-TRPp activity in group H was also significantly different from that observed in group M. As it can also be seen in Table 3, AIA/DDC treatment promotes a dose-dependent decrease in the degree of enzyme saturation (holoenzyme/apoenzyme). This decrease was 8.8, 40.8, and 42.4% for groups L, M, and H, respectively, when mean values of these treated groups were compared with those of the control. The loss in the heme saturation degree observed in the two groups M and H were statistically significant when compared either with the control or with group L.

4. Discussion

Results show that the AIA/DDC intoxication schedule assayed promotes the development of an acute type of porphyria, evidenced by the alteration of the heme pathway through biomarkers such as the enhancement of its rate-limiting enzyme (ALA-S), the accumulation of its precursors (ALA and PBG), and the half depressed activity of ferrochelatase.

The study also shows that AIA/DDC combined treatment impairs the serotonergic route of TRP metabolism causing a decrease in 5-HT level and a concomitant enhancement in TRP content. This moderate TRP accumulation would trigger TRPp

enhancement. In fact, it has been reported that TRP produces a substrate-type TRPp enhancement involving the reduced degradation of the preexisting apoenzyme at a normal synthesis rate [20,21,23]. The effects caused by moderate increases of TRP hepatic concentration *in vivo* such as those observed in the porphyria model studied, together with the activation of the enzyme *in vitro*, have been suggested to be involved in the promotion of the apoenzyme conjugation with heme and the subsequent reduction of the oxidized holoenzyme [23]. After promoting this conjugation, TRP sequesters the active form and protects it against degrading enzymes, diminishing the dissociation of the enzyme to porphyrin and inactive apoenzyme (Fig. 5), thus favoring tryptophan pyrrolase over other proteins in the competition for the prosthetic group. This leads to an increase in holoenzyme activity, as the one observed here in porphyric rats treated with AIA/DDC.

It can be speculated that AIA/DDC treatment increases the apoenzyme synthesis and consequently its activity. This speculation was based on the following considerations: (a) the proposal that TRP increases TRPp at a normal enzyme synthesis rate as discussed, (b) the present results showing that AIA/DDC treatment promotes high activity increases in the apoenzyme greater than in the holoenzyme or in the total enzyme, and (c) previous reports showing that apo-TRPp has a short half-life (four-fold shorter than the holoenzyme) and thus it is a species highly susceptible to protein synthesis modulators [20,26].

Assays show a dose-dependent increase in all TRPp forms, active (holo, total) and inactive (apo), and a decrease in the degree of enzyme saturation by heme as consequence of AIA/DDC combined treatment. These results agree with those from Badawy and Evans [41], who reported that the administration of these porphyrinogenic drugs alone and in a fixed dose (AIA, 400 mg/kg body wt or DDC, 150 mg/kg body wt) to male rats (inbred Wistar) increased total pyrrolase activity and simultaneously decreased heme saturation in the newly formed apoenzyme. However, the administration of DDEP (a DDC analogue, suicide inhibitor of cytochrome P-450 and acute heme depletor) together with phenobarbital (a hepatic cytochrome P-450 inducer) was reported to reduce TRPp activity [42]. This difference could be attributed to the fact that, although the porphyrinogenic drugs DDEP and PB produce heme depletion, they do not promote hepatic TRP accumulation, as in the porphyria model herein studied. Instead of TRP,

Table 2 – Serotonin (5-HT) and tryptophan (TRP) levels in liver of AIA/DDC treated and control rats

Group	5-HT (μg/g wet liver)	TRP (μg/g wet liver)
C	0.195 ± 0.017	2.281 ± 0.121
L	0.180 ± 0.015	2.350 ± 0.103
M	0.156 ± 0.013 [*]	2.580 ± 0.191
H	0.121 ± 0.010 ^{*,§}	2.802 ± 0.224 [*]

Each value represents the means ± S.E.M. of six animals. Animals were injected with drugs and divided in four groups as detailed in Table 1. TRP and 5-HT contents are expressed as μg tryptophan or serotonin/g wet liver. Multiple comparisons between groups were performed using Tukey–Kramer's test.

^{*} P < 0.05 is significantly different from C.

[§] P < 0.05 is significantly different from M.

Table 3 – Kynurenine synthesis triggered by TRP pyrrolase in rat liver with and without exogenous heme				
Group	Holoenzyme (μmol kynurenine formed/g fresh liver h)	Total (μmol kynurenine formed/g fresh liver h)	Apoenzyme (μmol kynurenine formed/g fresh liver h)	Holoenzyme/apoenzyme
C	3.07 ± 0.14	5.51 ± 1.02	2.44 ± 0.89	1.25 ± 0.18
L	3.29 ± 0.19	6.17 ± 0.67	2.87 ± 0.47	1.14 ± 0.15
M	4.04 ± 0.69*	9.48 ± 1.41* [#]	5.43 ± 1.37* [#]	0.74 ± 0.14* [#]
H	5.81 ± 1.04* [#]	13.87 ± 1.54* [#]	8.06 ± 1.31* [#] [§]	0.72 ± 0.09* [#]

Each value represents the means ± S.E.M. of six animals. Animals were injected with drugs and divided in four groups as detailed in Table 1. Total enzyme: activity in the presence of exogenous heme. Holoenzyme: activity in the absence of exogenous heme. Apoenzyme: difference between total enzyme and holoenzyme. Degree of heme saturation: ratio between holoenzyme/apoenzyme. Activities are expressed as μmol kynurenine formed/g fresh liver h. Multiple comparisons between groups were performed using Tukey-Kramer's test.

* P < 0.05 is significantly different from C.
P < 0.05 is significantly different from L.
§ P < 0.05 is significantly different from M.

which as discussed enhance TRPp, they elicited hepatic 5-HT accumulation. In the serotonergic route of TRP, these drugs would have a different effect than that of AIA/DDC.

Data showing that TRPp was increased in all its forms, whereas enzyme saturation by heme was reduced (Table 3) seem to be a paradox considering the hemoproteic nature of the enzyme (TRPp) and the heme depletory effect of the porphyrinogenic drugs used in the hepatic acute porphyria studied. This heme decrease, in fact, would be expected to lower TRPp activity instead of increasing it. AIA enhanced liver heme destruction, and DDC promoted ferrochelatase inhibition, thus lowering heme synthesis and increasing heme degradation [2,6]. In spite of the decrease in the heme pool, the increase in apoenzyme synthesis elicited by the treatment

herein assayed accounts for lower enzyme saturation by heme, and enhanced holoenzyme formation, in order to compete with other proteins which require this kind of porphyrin-complex as prosthetic group. Higher holoenzyme formation is also a consequence of the multiple and above discussed positive effects of TRP.

In the porphyria model studied, TRP metabolism is enhanced in the kynurenine route, i.e. the NAD pathway of TRP metabolism, and reduced in the serotonergic route. Results showed greater kynurenine formation in porphyric animals, in agreement with previous works that reported that porphyric patients exhibit greater urinary excretion of hepatic TRP metabolites of the kynurenine pathway [43]. The pattern of metabolite excretion in urine reported by Price [43] is

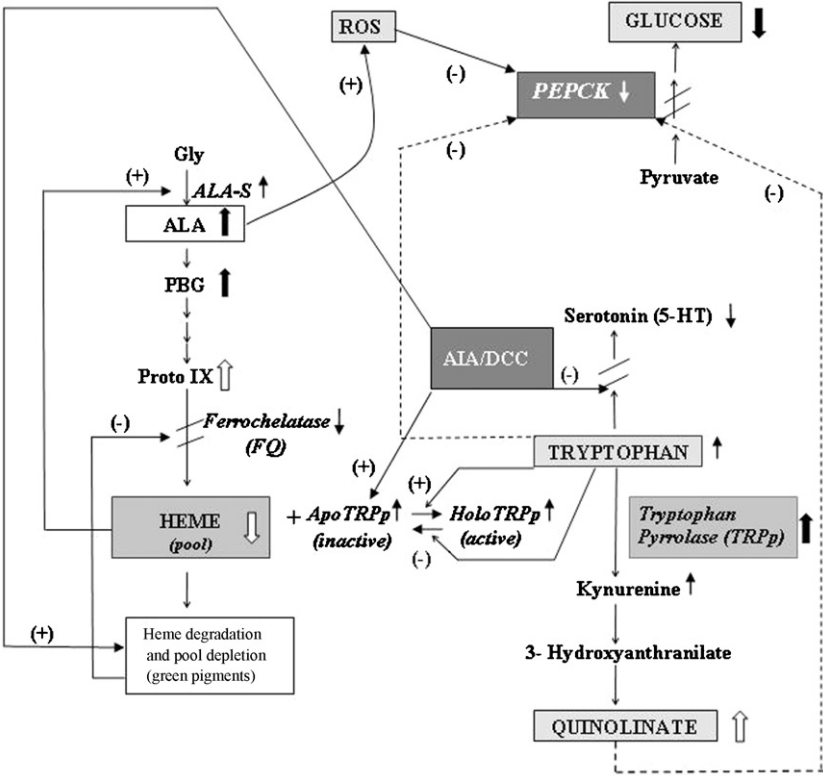


Fig. 5 – Scheme showing direct and indirect actions of AIA/DDC treatment on different metabolic routes (tryptophan and heme pathway) and the impact on the gluconeogenic blockage of PEPCK level, leading to glucose decrease.

consistent with enhanced TRPp activity leading to a functional vitamin B6 deficiency that manifests itself in the abnormal excretion of some metabolites involved in reactions catalyzed by pyridoxal phosphate-requiring enzymes along the pathway [41]. Moreover, it has been reported that pyridoxine-deficient rats excrete abnormally large amounts of kynurenine and xanthurenic acid [44].

Since the results herein obtained show that kynurenine formation is higher in porphyric animals (90% increase), that kynurenine via 3-hydroxyanthranilate forms quinolinate, and that TRP might inhibit gluconeogenesis by being converted to quinolinic acid, a compound whose ferrous coordination complex inhibits PEPCK [16], it could be speculated that PEPCK blockage induced by AIA/DDC treatment would be caused by quinolinic acid generated from TRP (Fig. 5). Results from our laboratory (unpublished) demonstrate that total NAD level is enhanced by AIA/DDC treatment in this porphyria model. This might reflect the increase of quinolinate level since NAD is formed from quinolinate through nicotinic acid.

On the other hand, TRP direct action on PEPCK could not be discarded, but in a lower degree since the amount of TRP accumulated is lower than its derived metabolites.

This work also demonstrates that ROS, generated from ALA accumulation, has a damaging effect on lipids, and especially membrane lipids of microsomes, in the same way as the loss of PEPCK activity does. Therefore, it is worth mentioning that, in addition to the effect of TRP and its kynurenine metabolites, these ROS would also affect PEPCK, damaging its protein structure as proposed Lelli et al. [9]. As a consequence of these combined actions, a defined gluconeogenic blockage would occur at the level of PEPCK activity. This blockage, together with the impairment of glycogen phosphorylase, promotes a state of low hepatic glucose generated by the porphyrinogenic drugs AIA/DDC [9].

Fig. 5 summarizes the direct and indirect effects of AIA/DDC treatment on different metabolic routes (TRP and heme pathway) and their impact on gluconeogenic PEPCK blockage leading to glucose decrease.

The mechanism through which the impairment of glucose neosynthesis occurs would provide the molecular basis to explain the beneficial role played by carbohydrate administration to patients suffering from acute hepatic porphyrias [1,4], which in some cases seems to be life-saving [3,5]. It would also be useful to explain the fact that high glucose intake in animals prevents the development of experimental porphyria by AIA [45], and to better understand the metabolic interrelations and inter-regulations among glucose, heme, and TRP metabolisms.

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