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INFLUENCE OF THE STRAIN OF RATS ON THE INDUCTION OF HEXACHLOROBENZENE INDUCED PORPHYRIA

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Abstract—1. The present work undertakes a comparative study on the hexachlorobenzene (HCB) porphyria induction in female rats of Wistar and CHBBTHOM strains. The purpose was to characterize the CHBBTHOM strain with respect to the haem metabolic pathway, its regulatory mechanisms and its response to foreign drugs.

2. After 7 weeks of treatment it was observed that the hepatic porphyrins increased 140 times, ALA-synthase 4 times and PCL was 73% inhibited in the Wistar strain.

3. On the other hand the animals of CHBBTHOM strain showed lesser alteration on these parameters; hepatic porphyrins increased only 3-fold, ALA-synthase 1.7-fold and PCL was only 22% inhibited.

4. Total iron liver content was nearly equal in both strains of rats.

5. The results obtained would indicate that the lower susceptibility of the CHBBTHOM strain to acquire porphyria does not seem to be due to either: (1) congenital alterations of any parameters of the haem metabolic pathway, since the behaviour of normal animals from both strains was similar; or (2) a lower hepatic iron content in such animals.

6. These findings would suggest that the differential response to HCB to this strain would be looked for in another metabolic pathway, such as that involved in the metabolization process of the toxic.

INTRODUCTION

Hexachlorobenzene (HCB) is a polychlorinated hydrocarbon which has been widely used as pesticide. Such compound produced 30 years ago an epidemic of hepatic porphyria in the south-east of Turkey (Schmid, 1960; Cetingil and Ozen 1960; Cam and Nigogosyan 1963). Several studies performed in animals demonstrated the ability of HCB to induce a porphyria which resembles the human cutanea tarda type (Cam and Nigogosyan, 1963; San Martín de Viale *et al.*, 1970). The HCB provokes, in rats, a remarkable accumulation and excretion of uroporphyrin and heptacarboxylporphyrin (San Martín de Viale *et al.*, 1970, 1977; Taljaard *et al.*, 1972). This is a consequence of a remarkable decrease in the activity of the hepatic porphyrinogen carboxy-lyase (PCL); (Elder *et al.*, 1976; San Martín de Viale *et al.*, 1976) accompanied by an increase in the activities of δ-aminolaevulinate(ALA)-synthase, ALA-dehydratase, porphobilinogenase and ferrochelatase (Wainstok de Calmanovici *et al.*, 1984).

The experimental porphyria induced by chlorinated hydrocarbons could be associated with the hepatic siderosis (Smith and de Matteis, 1980). It has been reported that iron hepatic content increases in rats after HCB treatment (Saunders *et al.*, 1963; Hanstein *et al.*, 1981; Wainstok de Calmanovici *et al.*, 1985). It has been observed that the overload of iron accelerates and exacerbates the porphyria induced by HCB (Taljaard *et al.*, 1971; Smith and Francis, 1983). Moreover, Smith *et al.* (1979) reported that a strain of rats markedly susceptible to the porphyrinogenic effect of HCB, has a non-haem iron content higher than other strains less susceptible. On the other hand, it was reported that desferrioxamine, an iron chelating agent, was able to diminish and delay the porphyria produced by HCB in rats (Wainstok de Calmanovici *et al.*, 1986a).

Previous studies on the HCB porphyria induction using CHBBTHOM rats have suggested that this strain of rats is more refractory to acquire porphyria with this drug than the Wistar strain. Thus, the purpose of the present work is to compare the susceptibility of both the CHBBTHOM and the Wistar strains to HCB and to investigate if iron plays any role in the differential response to the porphyrinogenic agent in both strains of rats. With this purpose the urinary excretions of ALA, porphobilinogen (PBG) and porphyrins as a function of the time of treatment; the iron and porphyrins liver content and the hepatic activity of ALA-synthase and PCL were measured.

MATERIALS AND METHODS

Chemicals

HCB [commercial grade; composition: HCB 95%; tetra and pentachlorobenzene, 5% (w/w)] was generously provided by Compania Quimica S.A. (Buenos Aires, Argentina).

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Abbreviations: ALA—δ-aminolaevulinate; PBG—porphobilinogen; HCB—hexachlorobenzene; PCL—porphyrinogen carboxy-lyase.

Uroporphyrin III was purchased from Porphyrin Products (Logan, Utah, U.S.A.) Porphyrinogens were prepared with sodium amalgam as described by Mauzerall and Granick (1958). The ion exchange resins Dowex 1 (X8) and Dowex 50 W (X8) (both 200–400 mesh) A.G. grade and the Sephadex G-25 (coarse) were purchased from Sigma Chemical Co. (St Louis, Mo, U.S.A.).

Animals and treatment

Female Wistar and CHBBTHOM rats weighing 160–180 g at the start of the experiment were housed individually in metabolic cages for collection of 24 hr urine and were fed Purina 3 diet (Cabeca S.C.A., Buenos Aires, Argentina) and water, *ad libitum*.

Animals of each strain were divided into two groups: (1) normal, untreated control; (2) treated with HCB administered daily by stomach tube (1 g/kg body wt); the drug was suspended in water (40 mg/ml) containing Tween 20 (0.5 ml/100 ml). Rats were killed by decapitation after 7 weeks of HCB treatment. Livers were immediately removed, perfused, weighed and divided into two portions. (1) Homogenates were made with 0.154 M KCl (1:5 w/v) for estimation of porphyrins, iron content and PCL activity. Homogenates were centrifuged at 11,000 *g* at 0–2°C for 20 min and the supernatant thus obtained was used as enzyme source. Homogenate supernatants from porphyric livers were then filtered through a Sephadex G-25 column (2.4 × 30 cm), equilibrated and eluted with 0.134 M potassium phosphate, pH 7.0. The eluates with little or no fluorescence, arising from porphyric livers were pooled and used for enzymic determination. (2) Livers were homogenized in 0.9% (w/v) NaCl/0.5 mM EDTA/10 mM Tris–HCl buffer, pH 7.4 (1:3 w/v) for determination of ALA-synthase activity.

Hepatic and urinary content of porphyrins and precursors

Porphyrin content in liver was determined in 0.1–1 ml portions of whole homogenates as total free porphyrins in 5% (w/v) HCl as described by San Martín de Viale *et al.* (1977). Analyses of ALA, PBG and porphyrins were carried out weekly on 24 hr urinary specimens. They were determined in aliquots of 0.3–1 ml of urine. Two separate ion-exchange columns were used according to the method described by Wainstok de Calmanovici *et al.* (1984).

Enzyme activities

ALA-Synthase was assayed in whole homogenates by the method of Marver *et al.* (1966). PCL activities in 11,000 *g* supernatant fraction from normal or in eluates of Sephadex G-25 from porphyric livers were determined according to Wainstok de Calmanovici *et al.* (1984) except that porphyrins formed were separated and quantified by high performance liquid chromatography (Wainstok de Calmanovici *et al.*, 1986).

Total iron estimation

Aliquots of 0.5 ml of homogenates were dried in a stove and calcinated at 550°C in a muffle furnace with successive dissolutions in water after disappearance of the organic matter, iron was dissolved with HCl 20% and the colorimetric reaction with *o*-phenanthroline and hydroquinone was carried out reading the absorbance at 508 nm (Sandell, 1959). $FeCl_3.6H_2O$ was used as standard, the solution being estimated by gravimetry after calcination. Thus an extinction coefficient of $E_M = 6.7 \times 10^3$ for iron was determined.

Protein determination

Proteins were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Statistical treatment of results

Results are expressed as the arithmetic means ± SEM, and the means were compared by Student's test. To obtain the value of P a degree of freedom $n_1 + n_2 - 2$ was used throughout.

RESULTS

Urinary excretion of precursors and porphyrins

The contents of ALA, PBG and porphyrins were determined weekly in 24 hr urine specimens. After 1 month of HCB administration, the urinary levels

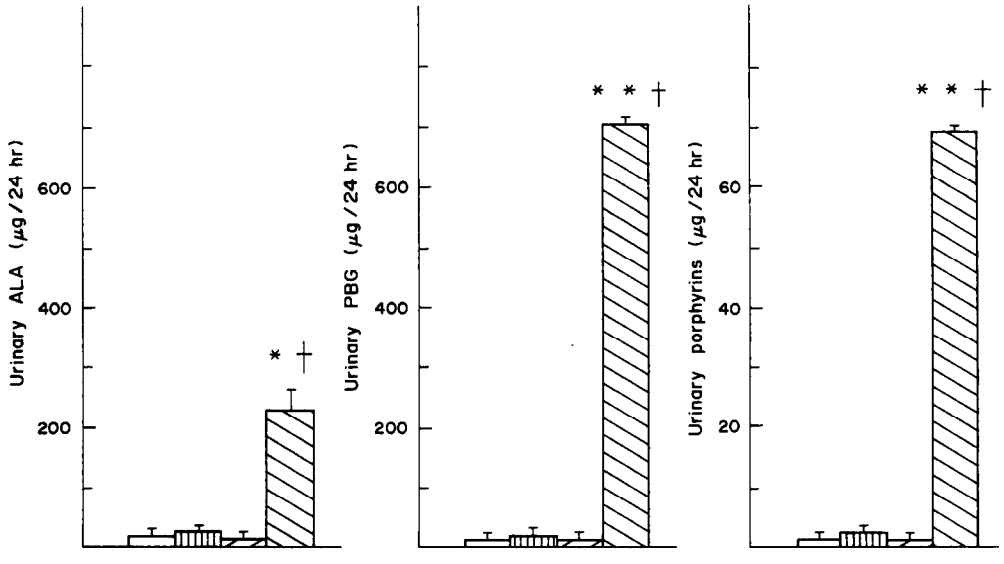

Fig. 1. Excretion of ALA, PBG and total porphyrins in normal CHBBTHOM □, normal Wistar ▥, HCB CHBBTHOM ▨ and HCB Wistar ▧. Experimental conditions and methodology were as described in the text. Data are expressed as means ± SEM of 6 rats, $P < 0.01$(*) and $P < 0.001$ (**) when compared with the normal or $P < 0.001$ (†) when compared with the HCB group.

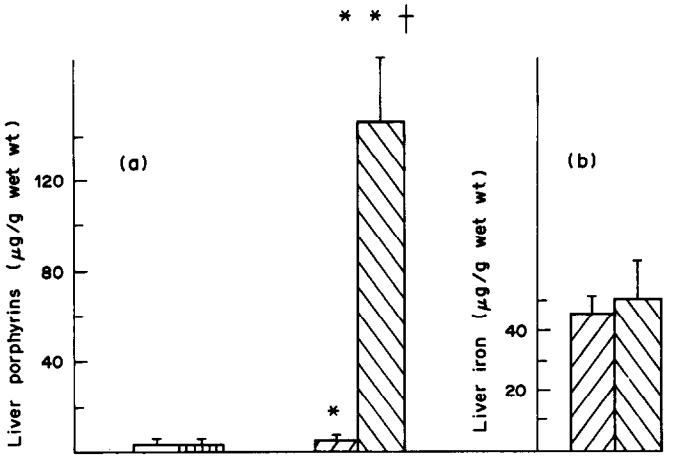

Fig. 2. Effect of HCB on hepatic porphyrin accumulation (a) and iron content (b) in normal CHBBTHOM □, normal Wistar ▥, HCB CHBBTHOM ▨ and HCB Wistar ▧. Experimental conditions and methodology were as described in the text. Data are expressed as means ±SEM for 6 rats, $P < 0.05$ (*) and $P < 0.001$ (**) when compared with the normal or $P < 0.001$ (†) when compared with the HCB treated group.

of ALA, PBG and porphyrins significantly exceeded the control values in the Wistar strain; instead the corresponding levels in the CHBBTHOM animals remained within normal range. Clinical manifestations were also different between strains. While no alterations were observed in the CHBBTHOM animals, the Wistar rats showed a typical intoxication picture. Two animals of the latter strain died prematurely, having an hepatic porphyrin content of 191.0 and 292.1 μg/g liver.

Figure 1 shows the pattern of urinary excretion of ALA, PBG and porphyrins of normal and HCB treated rats belonging to the CHBBTHOM and Wistar strains after 7 weeks of intoxication. It can be seen that the animals of CHBBTHOM strain excreted normal amounts of precursors and porphyrins while these parameters were greatly augmented in the HCB treated Wistar strain. Increases of 11 times for ALA, 122 times for PBG and 11.8 times for porphyrins, considering the means values of 4–6 animals, were observed. For Wistar animals maxima of 415.8 μg/24 hr for ALA, 1262.8 μg/24 hr for PBG and 91.6 μg/24 hr for porphyrins were reached, while in the CHBBTHOM strain values of only 26.2 μg/24 hr for ALA, 10.7 μg/24 hr for PBG and 2.1 μg/24 hr for porphyrins were attained.

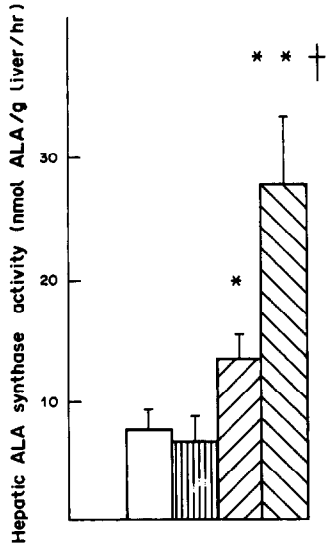

Fig. 3. Effect of HCB on the activity of ALA-synthase normal CHBBTHOM □, normal Wistar ▥, HCB CHBBTHOM ▨ and HCB Wistar ▧. Experimental conditions and methodology were as described in the text. Data are expressed as means ±SEM for 6 animals, $P < 0.05$ (*) and $P < 0.01$ (**) when compared with the normal or $P < 0.05$ (†) when compared with the HCB treated group.

Hepatic porphyrin and iron contents

The hepatic porphyrin content (Fig. 2a) was the same in the normal animals of both strains: CHBBTHOM (1.1 ± 0.1 μg/g liver) and Wistar (1.0 ± 0.1 μg/g liver). In the animals treated with HCB for a period of 7 weeks an important difference between strains was observed, thus liver porphyrin levels of Wistar rats was increased 140 times, while enhancement in the CHBBTHOM strain was only 3 times. However, no differences were found in the analysis of hepatic total iron content (Fig. 2b) of both strains.

Enzyme activity of ALA-synthase and PCL

The enzyme activity levels for ALA-synthase and PCL were found to be the same for normal animals from CHBBTHOM and Wistar strains (Figs 3 and 4).

However the response of the animals receiving HCB differed in both strains. Thus, ALA-synthase activity (Fig. 3) was, as mean, 1.7 times greater than the normal for the CHBBTHOM animals and 4 times greater for the Wistar.

As can be seen in Fig. 4 Wistar rats showed an inhibition of PCL of 73% for the first stage and 98% for the second stage of uroporphyrinogen III decarboxylation, while in the CHBBTHOM animals

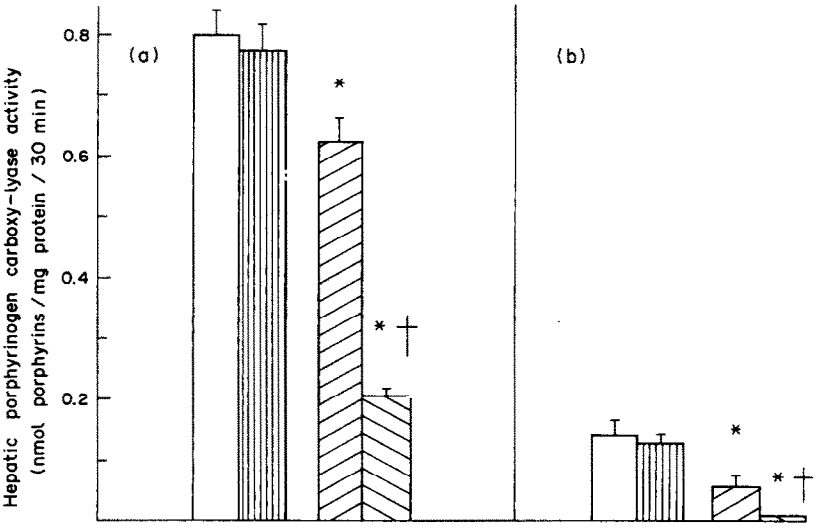

Fig. 4. Effect of drug administration on PCL, normal CHBBTHOM □, normal Wistar ▥, HCB CHBBTHOM ▨ and HCB Wistar ▧. Experimental conditions and methodology were as described in the text. The first stage (a) of PCL, i.e. uroporphyrinogen decarboxylation, was measured as the formation of hepta + hexa + penta + tetra carboxyporphyrinogens, with uroporphyrinogen III as substrate. The second stage (b) of PCL activity was measured as coproporphyrinogen formation. The amount of protein incubated ranged from 5 to 7 mg. Data are expressed as means ±SEM for 6 animals, $P < 0.001$ when compared with normal (*) or with the HCB treated group (†).

the degree of inhibition was only 22 and 55%, respectively.

These results indicate that these two enzymatic activities were affected to a different extent in both strains, after 7 weeks of fungicide treatment, the Wistar strain being more susceptible than the CHBBTHOM to the action of HCB.

DISCUSSION

The results of the present work show that a great difference, in the intensity and in the time necessary to produce a disturbance in the haem metabolism, exist between the two strains of rats. While the Wistar showed important changes already after 1 month of drug treatment, the CHBBTHOM animals exhibited normal clinical behaviour during the same period. After 7 weeks of treatment a severe picture of porphyria was observed in the Wistar rats, i.e. a significant increase in the excretion of precursors and porphyrins, a noticeable enhancement of porphyrins and the hepatic ALA-synthase activity as consequence of a strong decrease observed in the PCL enzyme activity (73% for uroporphyrinogen III decarboxylation). Within the same period of intoxication, the CHBBTHOM rats did not show a clear cut porphyria picture, since the urinary excretion of precursors and porphyrins was normal, the levels of hepatic porphyrins and the activity of ALA-synthase exceeded only slightly the normal values. This lack of response can be due to the fact that the blockade of the key enzyme PCL, was only 20%.

It is important to point out that decreases near to or higher than 50% in the uroporphyrinogen III decarboxylation process must be reached to impair the haem pathway regulation and therefore leading to the clinical and biochemical manifestations of this enzymatic failure (Wainstok de Calmanovici *et al.*, 1984).

In order to dilucidate the reasons of the different response of both strains of rats to the porphyrinogenic action of HCB; hepatic iron contents were determined, because it was claimed that iron plays a synergistic role in the production of porphyria. So, it was reported that in the HCB porphyria the decrease of PCL is enhanced by iron overload (Taljaard *et al.*, 1971; Low *et al.*, 1977). It was also observed that iron would not be a direct inhibitor of PCL (Low *et al.*, 1977; Smith *et al.*, 1979; Smith and Francis 1983; Wainstok de Calmanovici *et al.*, 1986b) but that it would be required for HCB metabolization (Sinclair and Granick, 1974; Wainstok de Calmanovici *et al.*, 1986a) a necessary process for decreasing PCL activity. Moreover, a direct role of iron for induction of ALA-synthase has been proposed by several authors (Stein *et al.*, 1970; De Matteis and Sparks, 1973; White *et al.*, 1978).

With respect to the relation iron-porphyria is timely to mention the work of Smith *et al.* (1979) who observed that Agus rats with an hepatic iron content higher than the Porton exhibited a greater decrease in the PCL activity and a higher increase in the ALA-synthase activity and consequently they were more susceptible to the porphyrinogenic effect of HCB.

However, in the present work the lower susceptibility of the CHBBTHOM strain to acquire porphyria seems not to be directly related with the total iron content, since there were not differences between both strains when the hepatic content of this metal was measured. Such lower susceptibility can not be ascribed either to congenital alterations of some of the haem metabolic pathway parameters, because no differences in their haem metabolism were detected between the normal animals of the Wistar and CHBBTHOM strains.

Therefore, we can conclude that the different susceptibility to acquire porphyria of the CHBBTHOM strain could not be ascribed to differences in either the haem pathway or in the hepatic iron content. More likely the problem could be related to another metabolic pathway, such as that involved in HCB metabolization, which leads to the formation of certain active metabolites. In this respect, previous studies with phenobarbitone (Wainstok de Calmanovici *et al.*, 1984) have demonstrated that the porphyrinogenic action of HCB would be mediated through the action of some of its metabolites. Thus, possibly differences between the two strains might reside in the rate of HCB metabolization, which in turn would promote disturbances in the haem metabolic pathway, but at a different time and to a different extent.

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