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Hexachlorobenzene as hormonal disruptor—studies about glucocorticoids: Their hepatic receptors, adrenal synthesis and plasma levels in relation to impaired gluconeogenesis

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ABSTRACT

In Wistar rats, hexachlorobenzene (HCB) depresses the gluconeogenic enzyme phosphoenolpyruvate-carboxykinase (PEPCK). In the liver, glucocorticoids (GC) normally regulate the glucose synthesis by acting on PEPCK. Thus, the aim of this work was to investigate, in a time-course study, the effects of HCB on plasma GC, its adrenal synthesis and stimulation, and the kinetic parameters of its hepatic receptors (GR) in relation to the gluconeogenic blockage produced by HCB.

Plasma corticosterone (CORT) concentration, urinary porphyrins and hepatic PEPCK were determined after 2, 4, 6 and 8 weeks of HCB-treatment. The effect of HCB on kinetic parameters of GR was studied in adrenalectomized porphyric rats after 2, 4 and 8 weeks of treatment. Additionally, adrenal CORT synthesis in the same weeks was measured with or without ACTH. Results show that plasma CORT in intoxicated animals dropped significantly after 2 and 4 weeks of treatment (23% and 58%, respectively), and then remained constant until the 8th week. HCB also promoted a reduction in the number of hepatic GR (50–55%) without modifying affinity. After 8 weeks, when porphyria was well established (40–50-fold increase in urinary porphyrins), a reduction (52%) in hepatic GR number, as well as a decrease in PEPCK activity (56%) were observed. Moreover, CORT biosynthesis in adrenals from intoxicated animals significantly decreased (60%) without changes in ACTH effect.

Briefly, this paper shows that HCB causes a disruption in GC and GR. This disturbance could contribute to the negative effect on glucose synthesis through PEPCK regulation, thus modulating porphyria. These results enhance the knowledge about the hormonal disruption produced by chlorinated xenobiotics.

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Abbreviations: AA, arachidonic acid; ACTH, adrenocorticotropin; ALA-S, ALA-synthase; CORT, corticosterone; DEX, dexamethasone; GC, glucocorticoid; GR, glucocorticoid receptor; HCB, hexachlorobenzene; PCT, porphyria cutanea tarda; PEPCK, phosphoenolpyruvate carboxykinase; PL, phospholipid; PMSF, phenyl methyl sulfonylfluoride; SM, sphingomyelin; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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1. Introduction

Hexachlorobenzene (HCB) is a persistent global pollutant widely distributed in ecosystems and used as fungicide to control pests in seed grains [1]. HCB is produced in large quantities as a by-product in the manufacture of chlorinated solvents [2].

Several studies performed in animals have demonstrated the ability of HCB to induce a type of porphyria resembling human porphyria cutanea tarda (PCT) [3–5]. Both in human and experimental PCT the key enzyme blocked in the heme pathway is uroporphyrinogen decarboxylase (URO-D) [6,7].

HCB impacts on many metabolic pathways by disrupting several biochemical processes [8]. In fact, the pollutant alters not only the heme pathway but also carbohydrate and lipid metabolisms, among others.

The organochloride compound causes a blockage in the gluconeogenic pathway through the decrease of pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase activities [9]. HCB also enhances the hepatic glycogen content due to the increase of glycogen synthase and the decrease of glycogen phosphorylase activities [10]. In addition, this fungicide elicits a lower glucose uptake by the liver [10]. As a consequence of all these disturbances, a lower glucose plasma level is observed.

PEPCK, the rate-limiting enzyme in gluconeogenesis, plays a central role in the synthesis of glucose in liver and kidney, its activity being blocked by HCB. PEPCK gene expression can be increased by several factors, among them cyclic AMP, thyroid hormones and glucocorticoids (GC). Insulin, on the other hand, inhibits this gene expression [11].

Regarding lipid metabolism, a disruption in phospholipid (PL) metabolism has been reported in liver of female rats after HCB administration [12–14]. Billi de Catabbi et al. [12] observed that PL metabolism shows a biphasic behavior not only in PL levels but also in their *de novo* syntheses, processes associated with membrane proliferation and destruction and with the fluidizing effects of HCB which is incorporated between the fatty acid chains of membrane lipids [15]. As an exception, sphingomyelin (SM) content showed a continuous decrease [13]. This decrease, related to a rise in neutral and acidic sphingomyelinases, could be a protective mechanism acting as an early cellular response to the liver injury elicited by HCB [13]. In addition, HCB affects fatty acid metabolism. Rats treated for two weeks with HCB showed a reduction in fatty acid content as well as an increase in free arachidonic acid (AA) levels in their hepatic microsomal membranes [14]. Regarding AA, an increase in liver CYP-dependent AA metabolism in hepatic prostaglandin E production, as well as a biphasic behavior in cytosolic phospholipase A2 activity has been recently reported by Billi de Catabbi et al. [16] in HCB treated rats. These changes were related to different aspects of HCB-induced liver toxicity such as alterations of membrane fluidity and membrane-bound protein function, particularly Na^+/K^+ ATPase involved in ion and water transport [16].

On the other hand, fat and endocrine tissues concentrate HCB [17], suggesting that HCB could have a toxic effect on endocrine systems. In fact, it has been reported that this chlorinated pesticide induces a hypothyroid-like state by reducing serum thyroxin (T4) levels [17,18] through an increase in hepatic T4 deiodination without altering thyroid hormone synthesis [18]. T4 was described as a sensitive parameter in HCB intoxication [18]. Furthermore, Foster et al. [19] found that ovariectomized rats treated with HCB show alterations in plasma concentration of CORT and progesterone, but no changes in aldosterone levels. Other chlorinated and porphyrinogenic drugs such as chlordane and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were reported to produce persistent and significant increases in plasma CORT concentration. The effect of chlordane on CORT was observed over the lifespan of offspring mice prenatal-exposed to non teratogenic doses of this pesticide [20]. Moreover, an increase in plasma CORT was also reported in TCDD-treated male Sprague–Dawley rats [21].

This study was undertaken based on the following facts: (1) HCB blocks gluconeogenesis; (2) PEPCK is a key regulatory enzyme of gluconeogenesis positively regulated by GC; (3) GC are lipid compounds synthesized from cholesterol; (4) HCB promotes hypercholesterolemia accompanied by an increase in hepatic synthesis of cholesterol in mice [22]; (5) HCB is able to alter different kinds of lipid metabolisms; (6) TCDD, a porphyrinogenic drug mechanistically related to HCB, is able to alter glucocorticoid receptor (GR) [23,24]; (7) there are no reports about the effect of HCB neither on the number nor on the kinetic parameters of GR.

The aim of the present work was to investigate the effect of HCB treatment on the level of plasma GC, the number and affinity of hepatic GR, and on GC biosynthesis in adrenal tissue. These determinations were undertaken in relation to the gluconeogenic blockage caused by HCB in liver of rats since this blockage is involved in the modulation of porphyria.

2. Materials and methods

2.1. Materials

HCB (commercial grade), composed of 95% HCB and 5% tetrachlorobenzene and pentachlorobenzene, was a gift from Compañía Química S.A., Argentina. Heparin, pyruvate, 2′-deoxyguanosine 5′-diphosphate, malic dehydrogenase, NADH, ACTH and DEX were supplied by Sigma Chemical Co. (St. Louis, MO, USA). [^{3}H]CORT (76.5 Ci/mmol) and [^{3}H]DEX (35 Ci/mmol) were from NEN (Boston, MA, USA). Scintillation cocktail for all samples was OptiPhase-Hi safe 3 (Wallac Co., Turku, Finland). The antiserum against CORT was kindly provided by Dr. Celso Gomez Sanchez from the University of Mississippi Medical Center (MS, USA). All other chemicals were of the highest available grade.

2.2. Animals and treatments

Female Wistar rats (180–200 g) were purchased from the National Committee of Atomic Energy (CONEA Argentina). Animals were maintained on food (Purina 3 diet) and water ''ad libitum'', and housed in stainless steel cages under conditions of controlled temperature (25 °C) and light (12 h light–dark cycle, light from 06:00 to 18:00 h). Total urine was collected using metabolic cages, over 24 h periods, weekly. Urine was kept at 4 °C and protected from light until

evaluation. Animals were treated according to International Guidelines (Guide for Care and Use of Laboratory Animals, National Research Council, USA, 1996, the Council of the European Communities Directive, 86/609/ECC) and also to the guidelines from the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC). They were randomly divided into two groups—group I: controls ($n = 28$); group II: HCB-treated ($n = 28$). Rats in the HCB group received a total 500 mg HCB/kg body weight in the form of five consecutive daily doses of 100 mg/kg body weight dissolved in corn oil (10 ml/kg body weight) by gastric intubation [25,26]. Control rats received corn oil alone (10 ml/kg body weight). Sixteen control and 16 HCB-treated rats were used for PEPCK, urinary porphyrins and plasma CORT determinations. These animals were killed by decapitation 2, 4, 6 and 8 weeks after dosing (controls, $n = 4$; HCB-treated, $n = 4$, each week). Animals were sham-adrenalectomized 48 h before PEPCK determination. For porphyrin estimation, urine was collected during 24 h before decapitation and animals were food-deprived during this period. Just before death, blood samples were collected with a heparinized capillary tube from the ophthalmic venous plexus. Samples were centrifuged at $1000 \times g$ for 15 min at 4 °C. Plasma samples were kept at −20 °C until CORT determination. In parallel, 12 controls and 12 HCB-treated adrenalectomized rats (controls, $n = 4$; HCB-treated, $n = 4$, each week) were killed by decapitation 2, 4 and 8 weeks after drug suspension in order to determine the number of GR and their kinetic parameters. CORT biosynthesis in adrenals was measured using fresh glands obtained during adrenalectomy. All these animals were adrenalectomized 48 h before death, and deprived of food for 24 h.

2.3. Determinations

2.3.1. Corticosterone (CORT) production by adrenal glands

Adrenals were obtained from control and HCB-treated animals. Adrenals were quickly isolated and placed on ice-cold saline. Each adrenal was cut into four pieces. Tissue was incubated in 2 ml Krebs–Ringer-glucose medium containing 10 mM Hepes (pH 7.4) in the presence and absence of 1 nM ACTH. Incubations were carried out at 37 °C with continuous shaking in an atmosphere of air and stopped after 1 h by chilling the samples. Media were quickly separated and stored at −20 °C until CORT determination by RIA.

2.3.2. Plasma corticosterone (CORT)

Plasma CORT levels were measured by using RIA as previously described [27]. Briefly, plasma was extracted twice with ethyl ether, evaporated and re-dissolved in 100 mM borate buffer, 0.1% gelatin, pH 8.0. Losses during the entire procedure were calculated in parallel samples by adding [^{3}H] CORT. The antiserum against CORT was used in a final dilution of 1:22,500. The cross-reactivity with aldosterone was less than 0.1% [28]. The sensitivity of the assay was 50 pg/ml. Intra and inter-assay coefficients of variation were below 8% and 12%, respectively. CORT was assayed in triplicate.

2.3.3. Phosphoenolpyruvate carboxykinase (PEPCK) activity

Assay of PEPCK was carried out in fresh livers of sham-adrenalectomized animals. PEPCK activity was determined according to Petrescu et al. [29]. For its determination, liver was homogenized in three volumes of 0.25 M sucrose at 0–4 °C. Cytosolic fraction was prepared from the homogenate by differential centrifugation. After sedimentation of the mitochondrial fraction at $11{,}000 \times g$ for 20 min, the cytosolic fraction was separated from the microsomal fraction by centrifugation at $105{,}000 \times g$ for 60 min. An aliquot of cytosolic fraction (20 μl) was used in each assay. The oxaloacetate formed during the reverse enzyme reaction was determined by reduction with malic dehydrogenase in the presence of NADH. Changes in absorbance were measured spectrophotometrically at 340 nm in triplicate. The reactions were allowed to proceed for 2 min at room temperature.

2.3.4. Rat urinary porphyrins

Total urine was collected employing metabolic cages, and porphyrins were determined according to Mauzerall and Granick [30] with modifications [31]. Urine was kept at 4 °C and protected from light until evaluation performed by the use of Dowex 1 anion exchange resin column. 0.3–1 ml samples of urine were used in each determination and porphyrins were eluted from the column with 10% (w/v) HCl and determined spectrophotometrically by using Rimington and Sveinsson [32] correction formula.

2.3.5. Determination of glucocorticoid receptor (GR)

For binding assays, livers from adrenalectomized animals were quickly removed, cut into small pieces and rinsed repeatedly with ice-cold GR buffer (10 mM Hepes with 5 mM EDTA, 10% (v/v) glycerol, 20 mM Na_2MoO_4, 2 mM DTT and 0.1 mM PMSF, pH 7.4) and homogenized (650 mg of tissue/ml) in the same buffer. The cytosolic fraction was prepared from the homogenate by differential centrifugation [33]. After the sedimentation of the mitochondrial fraction at $11{,}000 \times g$ for 20 min, the cytosolic fraction was separated from the microsomal fraction by centrifugation at $105{,}000 \times g$ for 60 min. All steps were carried out at 4 °C. Binding was assayed in triplicate using 400–600 mg cytosolic proteins and 1.25 nM [^{3}H]DEX in GR buffer. Binding parameters were obtained by the displacement of [^{3}H]DEX specific binding with seven concentrations of unlabelled DEX (0.5–100 nM). All the incubations were carried out in a final volume of 0.5 ml at 4 °C. After equilibrium was reached, unbound [^{3}H]DEX was removed by the addition of an equal volume of charcoal–dextran [2% (w/v):0.2% (w/v)] in phosphate buffer saline (PBS), pH 7.4. Specific binding was calculated by subtracting non-specific binding obtained in parallel samples after the addition of an excess of unlabelled DEX. Apparent dissociation constants (Kd_{app}) and apparent number of binding sites (B_{app}) were obtained employing the Ligand Programme (Ligand Software David Rodbard, NIH). All the determinations were carried out between 10 and 12 h to avoid the influence of daily variations.

2.3.6. Proteins

Proteins were determined according to the method of Lowry et al. [34] using bovine serum albumin as standard.

2.3.7. Statistical analysis

Results were expressed as means ± S.E.M. Binding parameters Kd_{app} and B_{app} were obtained employing the Ligand Programme

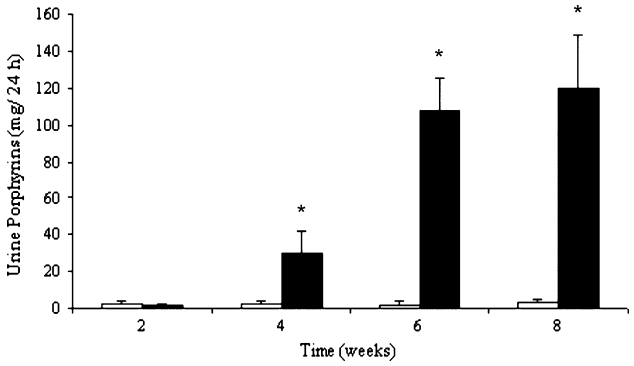

Fig. 1 – Time-course effect of hexachlorobenzene on urinary porphyrin content. Each bar represents mean ± S.E.M. of duplicate determinations performed from four animals. Open bars represent controls; solid bars, HCB treated rats. Porphyrins were estimated spectrophotometrically in 10% (w/v) HCl after passing the urine through Dowex 1 anion exchange resin column as described in Section 2. Results are expressed as mg porphyrins /total urine from 24 h. *Significant differences with control rats ($p < 0.05$).

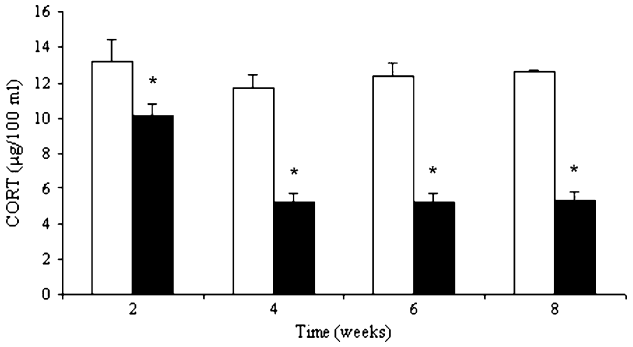

Fig. 2 – Time-course effect of hexachlorobenzene on plasma corticosterone levels (CORT). Each point represents mean ± S.E.M. of duplicate determinations performed from four animals. Open bars represent controls; solid bars, HCB treated rats. Plasma CORT levels were measured by RIA as described in Section 2. CORT concentration is expressed as μg of CORT/100 ml plasma. *Significant differences with control rats ($p < 0.05$).

(Ligand Software David Rodbard, NIH). Non-transformed data were tested for one-site or two-site models. Data in the time course studies were submitted to one-way ANOVA and differences between means by the *F*-test. In all cases, 0.05 was used as the level of significance. Before statistical analysis data were tested for normality and homoscedasticity using Lilliefors and Bartlett's tests, respectively.

3. Results

In order to determine if HCB is able to produce alterations in GC content in coincidence with the onset of the porphyria, CORT concentration was measured in plasma of HCB-treated and control rats at different times (2, 4, 6 and 8 weeks) in parallel with the determination of urinary porphyrin content. Analysis of total porphyrins in urine from HCB-treated rats showed 15-fold and 40–50-fold increase at the 4th and 8th week, respectively (Fig. 1). This important increase of excreted porphyrins indicates that a significant porphyria develops in HCB-treated animals. Fig. 1 also shows that HCB intoxication was effective and that once porphyrin accumulation starts it continues increasing during several weeks after the treatment was finished.

Fig. 2 shows that after a 2-week treatment with HCB a significant drop in plasma CORT level was observed (23% decrease) when compared with vehicle treated animals. This reduction in plasma CORT values reaches its maximum after 4 weeks (58%), remaining constant from the 4th week onwards.

PEPCK activity was assayed to determine the effect of HCB on glucose neosynthesis. As shown in Fig. 3, the activity of this enzyme remains unaltered after two weeks while a significant decrease can be observed from the 4th week onwards (34% compared to control). The activity continues decreasing on the 6th week (44% compared to control), the highest inhibition being obtained on the 8th week (56% compared to control).

In order to analyze the effect of HCB treatment on the hepatic response to GC, the number and affinity of GR was analyzed in each experimental condition. Table 1 describes how HCB treatment produces an early and significant loss in the number of cytosolic GR. It also shows that the affinity of GR remains constant throughout the treatment while the number of binding sites (B_{app}) significantly decreases from the 2nd (16%) to the 4th (40%) and 8th weeks (52%) when compared to control animals.

In order to test if the decrease on plasma CORT could be due to a disruption in CORT production by the adrenal gland, the biosynthesis of CORT was studied. Table 2 shows that from the 4th week onwards the basal biosynthetic ability of the

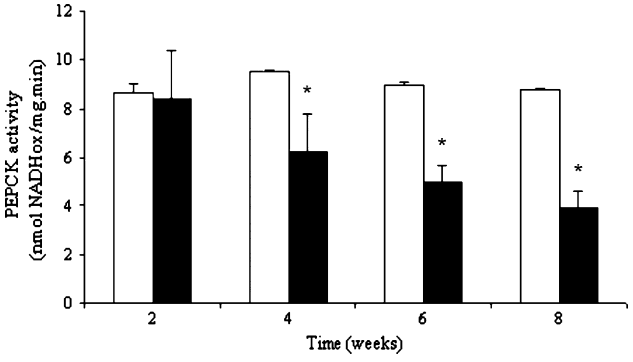

Fig. 3 – Time-course effect of hexachlorobenzene on hepatic phosphoenol-pyruvate carboxykinase activity. Each bar represents mean ± S.E.M. of triplicate determinations performed from four animals. Open bars represent controls; solid bars, HCB treated rats. PEPCK activity was measured spectrophotometrically at 340 nm using 105,000 × *g* liver cytosolic fraction as enzyme preparation as described in Section 2. The activity is expressed as nmol of NADH oxidized /min. mg protein (for the coupled reaction). *Significant differences with control rats ($p < 0.05$).

Table 1 – Binding parameters of GR

Week	Group	Kd_{app}	B_{app}
2	Control	3.24 ± 0.02	523.1 ± 4.3
	HCB	3.35 ± 0.26	453.9 ± 23*
4	Control	3.31 ± 0.03	533.0 ± 19
	HCB	3.58 ± 0.33	332.2 ± 14*
8	Control	3.50 ± 0.39	567.1 ± 8.9
	HCB	3.42 ± 0.59	267.9 ± 12*

Binding parameters were obtained by the displacement of [^{3}H]DEX (1.25 nM) specific binding with different concentrations of unlabelled DEX (0.5–100 nM). Experiments were performed with 400–600 μg cytosolic proteins in GR buffer. Kd_{app} and B_{app} were calculated using the Ligand programme. Kd_{app} is expressed in 10^{-10} M and B_{app} in fmol per mg protein. Values represent means of triplicate determinations performed in four rats ± S.E.M.
* Significant differences with control animals, $p < 0.05$.

adrenals from intoxicated animals is significantly impaired (46% and 51% at 4th and 8th week, respectively). A similar degree of impairment was observed in ACTH-stimulated assays. However, adrenal weight were not significantly modified [control: 35.2 ± 1.3 mg; HCB-treated: 36.7 ± 1.6 mg (8th week)] suggesting that the differences in CORT production is not due to a decrease in adrenal weight.

4. Discussion

There are several determinants to set the level of the biological action of GC. Undoubtedly, the level of plasmatic GC is an important issue to take into account, but the number and affinity of GR are also significant aspects to bear in mind, in particular, when we study the effect of a pollutant, in this case HCB, on the glucose metabolism. This compound induced a significant decrease in plasmatic CORT in agreement with results reported for Sprague–Dawley ovariectomized rats [19]. In this respect, Foster et al. [19] reported that the higher decrease in plasma CORT occurred at the lower dose assayed.

Table 2 – Basal and ACTH-stimulated CORT production by adrenal glands

Treatment		CORT (pg/ml)	
Week	Group	(−ACTH)	(+ACTH)
2	Control	135 ± 24	945 ± 61
	HCB	141 ± 38	923 ± 39
4	Control	158 ± 44	998 ± 41
	HCB	86 ± 23*	679 ± 42*
8	Control	149 ± 42	1012 ± 82
	HCB	73 ± 15*	457 ± 56*

Adrenals were obtained from control and HCB-treated animals. Tissue was incubated in the absence (−ACTH) and presence (+ACTH) of 1 nM ACTH. Incubations were carried out at 37 °C and stopped after 1 h by chilling samples. Media were quickly separated and stored at −20 °C until CORT determination by RIA. Values represent means of duplicate experiments performed with four rats ± S.E.M.
* Significant differences with control animals with $p < 0.01$.

The disruption that HCB produces in hormonal status is an early process not only for CORT [19] (Fig. 2 from this paper), but also for T4 [18]. The effect on plasma levels is achieved after two weeks of intoxication, i.e. earlier than the disturbance elicited by HCB in the heme pathway. In fact, the disruption in heme biosynthesis is evident only from the 4th week onwards (Fig. 1). The effect was determined by the increase of urinary porphyrins, biomarkers of the onset and development of porphyria, as previously described [18,35]. In these previous reports, a significant decrease in URO-D was also observed in the 4th week. Consequently, the results herein presented, obtained working with the same strain and under the same experimental conditions, could indicate that CORT level is a more sensitive parameter for determining HCB intoxication than porphyrin excretion or URO-D activity. Similar results regarding an early alteration of plasma CORT levels were also reported for another chlorinated pesticide, TCDD, which promotes a rise in GC levels as early as 4 days after treatment [21].

However, circulating CORT decreases before a significant impairment of the adrenal biosynthetic ability is detected, suggests that other factors different from adrenal biosynthesis could be involved. In a previous paper, San Martin de Viale et al. [36] reported an increase in hepatic glucuronyl transferase activity (GT) in HCB-porphyric rats. Thus, it is possible to speculate that a higher rate of hepatic hormone degradation could be responsible for CORT diminution through the formation of conjugates with uridine diphosphate glucuronic acid (UDP-GlcUA) by the action of GT, or with sulfuric phosphoadenosine–phosphosulfate (PAPS). Another explanation involves corticosteroid binding globulin (CBG). This protein binds GC with high affinity and therefore could regulate the availability of CORT. The binding of CORT to CBG may serve as a tissue buffer against potentially deleterious effects of elevated CORT by regulating the availability of free hormone to target tissues, or altering CORT clearance rates [37,38]. Taking this in mind, a decrease in CBG could compensate the decrease in total CORT by keeping free hormone constant. However, this possibility needs to be demonstrated.

Moreover, a similar behavior was described for the thyroid hormone in HCB treated rats, in which the level of serum T4 decreases as a consequence of an enhanced degradation elicited by the increase of hepatic T4 dehalogenation [18]. Nonetheless, at higher HCB intoxication times (4 weeks onwards) the decrease in CORT level could be ascribed both to hepatic degradation and impaired adrenal function. As regards adrenal biosynthesis, the availability of cholesterol, the biosynthetic precursor of steroid hormones, seems not to be responsible for it. In mice, it has been reported that plasma cholesterol concentration is increased by the treatment with HCB as well as by other porphyrinogenic drugs such as allylisopropyl-acetamide (AIA) and griseofulvin [22].

This study also shows that HCB provokes a decrease in the biosynthetic ability of the adrenal gland probably due to a failure in one or more steroidogenic enzymes without modifying the adrenal weight. In this respect, it is worth mentioning that polychlorinated biphenyls, which share with

HCB the common feature of being porphyrinogenic and chlorinated compounds, produce a decrease in adrenal cytochrome P-450 C21-hydroxylase when administered to guinea pigs [39]. This enzyme has a key role in GC biosynthesis. The effect of HCB on adrenal biosynthesis could be the response of this gland to the accumulation of the drug. In fact, Foster et al. [19] reported residual levels of HCB in the adrenal gland greater than those detected in liver and serum.

In addition to the effect on CORT, HCB also promotes an early and important decrease (50–60%) in the number of hepatic GR without modification of the dissociation constant. These results agree with those previously described in mice [24] and female Sprague–Dawley rats [23] treated with TCDD, a porphyrinogenic drug mechanistically related to HCB. After 1-day treatment, TCDD also reduced the binding ability of the hepatic GR (38–50%), without affecting Kd [23,24]. Moreover, in these papers, B_{max} values observed were 300 fmol/mg in male or female, haired or hairless HRS/J mice and about 400 fmol/mg in female Sprague–Dawley rats. The number of hepatic GR was in agreement with that herein reported for female Wistar rats (500 fmol/mg). As liver GR from mice (2 nM) and Sprague–Dawley rats (0.5 nM), Kd values for female Wistar rats were around 3 nM.

Taking into account the early response of plasma CORT to HCB intoxication preceding its effect on hepatic GC-regulated PEPCK activity, it is possible to speculate that the decrease in the gluconeogenic enzyme could be a consequence of the earlier hormonal disruption caused by HCB on CORT and its receptors. Therefore, in this experimental porphyria the negative effect exerted by HCB on PEPCK through GC and its receptor would be added to that previously reported for reactive oxygen species (ROS) [9]. With respect to insulin, which depresses PEPCK in the liver, the disturbances promoted by HCB on the status of this hormone [9] would suggest that they are not the primary cause of the reduced activity of PEPCK herein observed in HCB-treated animals. On the contrary, insulin levels would adapt to the variations in glucose levels; i.e. HCB-induced changes in insulin homeostasis would be a compensatory response of the organism to stimulate gluconeogenesis [9]. In this respect, Mazzetti et al. [9], working with rats treated with HCB in the same conditions as those of the present work, reported plasma insulin levels lower than in control animals and a pancreatic insulin secretion not affected by HCB treatment. As these authors suggest, disturbances were probably due to increased hormone degradation in the liver.

In summary, this paper shows that xenobiotic HCB, in addition to producing severe disturbances in the heme pathway giving rise to porphyria, promotes an early hormonal disruption of GC that results in a gluconeogenic blockage. In fact, the pollutant elicits a drastic drop in its adrenal synthesis and its circulating level, as well as in the number of its hepatic receptor. This hormonal disruption could contribute to the negative control of glucose synthesis through PEPCK regulation, modulating porphyria. In fact, glucose represses the heme pathway regulatory enzyme ALA-S [40], which is induced in porphyrias [41]. The disturbances promoted by HCB in the GC status herein reported also contribute to increasing the knowledge about hormonal disruption produced by these high persistence xenobiotics.

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