Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic retinyl ester hydrolase activities in the rat.

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) disrupts normal homeostasis of vitamin A (retinoids), most clearly evident as changed tissuel levels of retinoids in several organs of several species (reviewed in reference 1). In the liver, a dose-dependent decrease of retinoids is observed after TCDD treatment of rats (2). TCDD affects the storage form of vitamin A, the retinyl esters (Brouwer et al. 1989 (3)). The decreased hepatic stores of retinoids in TCDD-treated rats involve both decreased accumulation of newly administered retinoids (4) and increased mobilization of endogenous retinoid stores (5, 6). The decreased accumulation of retinoids may be due to the decreased activity of the retinyl ester-forming enzyme lecithin:retinol acyltransferase (LRAT) in hepatic stellate cells of TCDD-treated rats (7). The majority of the hepatic retinoids is found in the hepatic stellate cells, which is where the main effect of TCDD on hepatic retinoids is observed (8). Mobilization of hepatic retinoid stores must involve hydrolysis of the stored retinyl esters to retinol. Several enzymes capable of hydrolysing retinyl esters to retinol exist, although their roles in physiological retinoid metabolism is still unclear (reviewed in reference 9). To investigate if TCDD increased hepatic retinoid mobilization by inducing retinyl ester hydrolyzing enzyme activities, we measured four retinyl ester hydrolase (REH) activities in livers of rats treated with TCDD in three different experiments, as described below.

Materials and Methods

Chemicals TCDD (CIL, Andover, MA, USA, lot no. MLB-682-49; Dow Chemical, Midland, Michigan, USA) lot no. 851:144-II) was prepared as previously described (4). All other chemicals were of appropriate analytical grade and were commercially obtained. Light conditions were adjusted to avoid degradation of retinoids by UV light.

Study designs Male Sprague-Dawley rats were obtained from B&K Universal AB (Sollentuna, Sweden). *Time course experiment*: During a one week acclimatization period and throughout the experiment, the rats had free access to R34 diet (Lactamin, Stockholm, Sweden), stated to contain 12 000 IU of vitamin A per kg. Rats weighing 239 ± 12 g were given a single oral dose of 10 µg TCDD/kg body weight (bw) in a vehicle of corn oil (4 ml/kg bw), or the vehicle only. At different time points (1, 3, 7, 28, 56 and 112 days after administration), six control and six TCDD-treated rats were sacrificed. Livers were excised, weighed, frozen in liquid nitrogen, and stored at -70 C. *Dose effect experiment:* During a two month acclimatization period and throughout the experiment, the rats had free access to R34 diet (Lactamin, Stockholm, Sweden), formulated to contain 4 000 IU per kg. Rats weighing 418 ± 23 g, divided in groups of six rats, were given a single oral dose of 0, 0.1, 1.0, 10, or 100 µg/kg bw, in a vehicle of corn oil (1 ml/kg bw). Three days after administration, all rats were sacrificed and livers were collected as above. *Liver perfusion experiment:* Rats fed R34 diet stated to contain 12 000 IU vitamin A per kg (Lactamin,

Stockholm, Sweden), weighed 228 ± 16 g at the time of administration. Four rats were given a single oral dose of 10 µg TCDD/kg bw, in a vehicle of corn oil (4 ml/kg bw). Four other rats were given the vehicle only. After seven days, the livers of anaesthetized rats were perfused using a collagenase/pronase method (7). The hepatic caudate lobe was removed prior to perfusion with the collagenase buffer. Liver cells were separated by differential centrifugation into one parenchymal cell fraction consisting of hepatocytes, and one non-parenchymal cell fraction containing stellate cells together with other non-parenchymal cells.

Retinoid analyses Retinoids in liver homogenates (20% w/v in water) and sonicated liver cell suspensions were extracted using diisopropyl ether (10), and separated on a Nucleosil C_{18} 5µ HPLC column (Macherey-Nagel, GmbH, Germany) using an ethanol:water gradient elution. Retinol, retinyl acetate (internal standard used for quantification), retinyl palmitate and retinyl stearate were detected with a JASCO 821-FP fluorescence detector (λ_{ex}= 325 nm, λ_{em}= 475 nm).

Retinyl ester hydrolase assays At neutral pH, REH activities in liver homogenates from the time course and dose effect experiments were measured in the presence (bile salt-stimulated) or absence (bile salt-independent) of 20 mM sodium cholate (11). Retinyl-^{14}C-palmitate (2 nmol) in 10 µl ethanol was added as substrate to samples containing 20 µg homogenate protein and, when measuring the bile salt-stimulated REH, 20 mM sodium cholate. The final volume was 200 µl. Following a 60 min incubation at 37°C, the reaction product ^{14}C-palmitatic acid was extracted and counted in a liquid scintillation counter. Results were corrected for non-enzymatic product formation. Microsomes from whole liver, parenchymal and non-parenchymal cells from the liver perfusion experiment were analyzed for neutral, bile salt-independent REH activity as described above, with the exception that 8 nmol of retinyl-^{14}C-palmitate in 10 µl ethanol were added as substrate to samples containing 150 µg microsomal protein. Incubation time was 30 min.

An acidic bile salt-independent REH activity was measured in liver homogenates from the time course study as descibed above, but at an acid pH (12). Also at an acid pH, using as substrate 300 µM retinyl palmitate incorporated in liposomes, REH activity was measured in liver homogenates from the time course and dose effect experiments (13). Liver homogenates containing 500-800 µg protein were incubated for 90 minutes, in a final volume of 0.5 ml. Retinoids were extracted from the incubation medium, and retinol was measured by reversed-phase HPLC (14). Results were corrected for endogenous retinol and retinol originating from non-enzymatic formation.

Results and discussion

Rats exposed to TCDD showed the expected increased liver weights and decreased thymus weights, as well as induction of hepatic ethoxyresufin-*O*-deethylase activity (data not shown). Liver retinyl ester levels in TCDD-treated rats were lower than corresponding control levels in all studies (see Figures 1 and 2, and Table 1).

Neutral bile salt-independent REH activity [shown to be highly related or identical to rat liver carboxylesterase ES-2 (15)], was unaffected by TCDD treatment in liver homogenates from rats in the time course (Figure 3) and dose effect (Figure 4) experiments, and in liver and hepatic parenchymal and nonparenchymal liver cell fractions in the liver perfusion experiment (Table 1). Neutral bile salt-independent REH activity most likely hydrolyzes dietary chylomicron-derived retinyl esters (16), and may also be involved in the mobilization of hepatic retinoid stores in stellate cells (17).

No neutral bile salt-stimulated REH activity [which is is identical to the pancreatic carboxylester lipase (18)] was detected in any livers (data not shown). A similar absence of hepatic bile salt-stimulated REH activity has been observed earlier (19). Bile salt-stimulated REH activity may play a role in the hydrolysis of dietary-derived retinyl esters as well as in the mobilization of endogenous stores of retinyl esters (20).

Of the two hepatic REH activites measured at an acidic pH, the activity assayed using retinyl palmitate in liposomes as substrate was decreased during the first weeks following exposure to TCDD in the time course study (Figure 5). No treatment-related effects of TCDD were seen in the dose effect study (Figure 6). This acidic REH activity may be involved in retinyl ester mobilization in the hepatic stellate cells (13).
An acidic REH activity assayed using retinyl-^{14}C-palmitate in ethanol as substrate, was increased seven days after exposure to TCDD, but unaffected at all other time points (Figure 7). This acidic REH activity is most likely involved in hydrolysing dietary retinyl esters (12).

Thus, no clear increase in any of the investigated retinyl ester hydrolase activites was found following TCDD treatment. The lack of increased REH activity in livers from TCDD-treated rats is intriguing, since previous studies (5, 6) indicate an increased mobilization of vitamin A stores; a mobilization that by its size should involve hydrolysis of hepatic retinyl ester stores. The decreased levels of hepatic retinyl ester levels in TCDD-treated rats may be due to an imbalance between the retinoid esterification and hydrolysis pathways. This is supported by the decreased retinol esterification activity in hepatic stellate cells from TCDD-treated rats (7). Alternatively, if there is a continuous removal of product (i.e., retinol) presumably by oxidation (21) or glucuronidation (22), no measurable increased retinyl ester hydrolysis activity may be needed to explain the decreased retinyl ester levels in livers following TCDD treatment.

References

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Table 1. *Retinyl palmitate levels and bile salt-independent REH activity in liver and liver cell fractions from the liver perfusion experiment.*

	Liver	Hepatocytes/ PC fraction	Stellate cells /NPC fraction
Retinyl palmitate (*nmol / g liver or nmol / 10^6 cells)*			
control	280 ± 67	0.14 ± 0.12	23.2 ± 8.6
TCDD	198 ± 70	1.01 ± 0.98	17.5 ± 3.8
Bile salt-independent REH activity (*pmol / min * mg)*			
control	24.5 ± 2.6	20.8 ± 2.3	34.2 ± 7.9
TCDD	25.0 ± 1.4	21.5 ± 2.6	27.0 ± 6.0

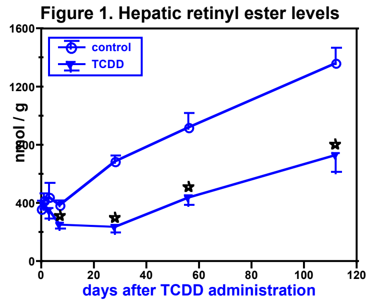
Figure 1. Hepatic retinyl ester levels
control
TCDD
nmol / g
days after TCDD administration

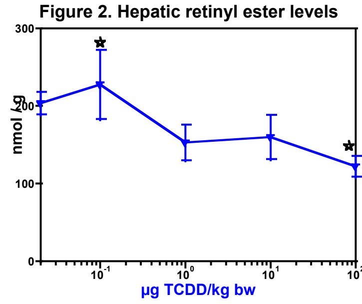
Figure 2. Hepatic retinyl ester levels
nmol / g
µg TCDD/kg bw

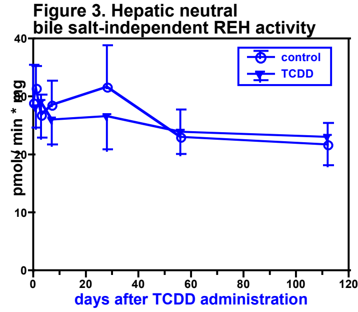
Figure 3. Hepatic neutral
bile salt-independent REH activity
control
TCDD
pmol / min * mg
days after TCDD administration

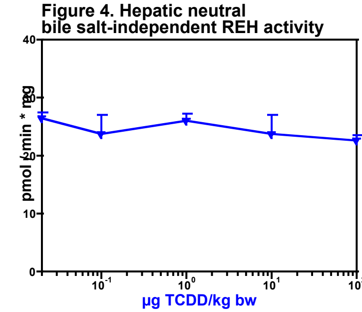
Figure 4. Hepatic neutral
bile salt-independent REH activity
pmol / min * mg
µg TCDD/kg bw

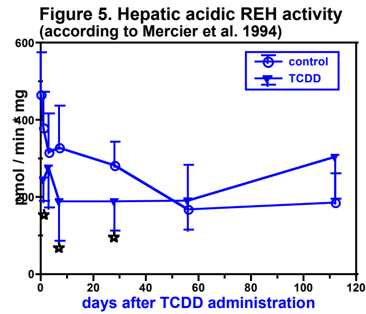
Figure 5. Hepatic acidic REH activity
(according to Mercier et al. 1994)
control
TCDD
pmol / min * mg
days after TCDD administration

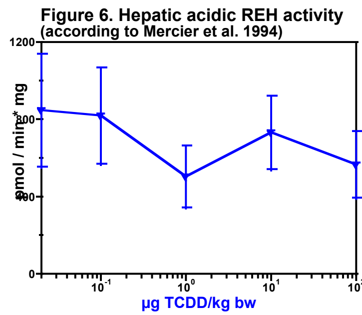
Figure 6. Hepatic acidic REH activity
(according to Mercier et al. 1994)
pmol / min * mg
µg TCDD/kg bw

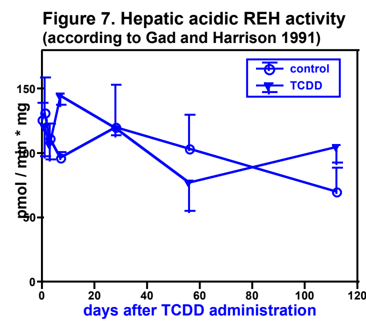
Figure 7. Hepatic acidic REH activity
(according to Gad and Harrison 1991)
control
TCDD
pmol / min * mg
days after TCDD administration