Effect of TCDD on tryptophan and glucose homeostasis in the most TCDD-susceptible and the most TCDD-resistant species, guinea pigs and hamsters

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1.Introduction

In rats intoxicated lethally with TCDD, *de novo* biosynthesis and turnover of serotonin (5-HT) in the brain is elevated[1,2,3]. These effects appeared to be mediated via increased free tryptophan in the plasma of TCDD-treated Long-Evans (Turku AB; inbred) rats. Tryptophan is reversibly bound to albumin in the circulation[4]. The changes in free tryptophan occurred at doses known to elicit lethality in Long-Evans rats and correlated inversely with body weight change in this strain of rats. In contrast, TCDD-resistant Han/Wistar (Kuopio; outbred) rats showed no such effects in tryptophan metabolism at any dose tested[2] (up to 9600 μg/kg).

The guinea pig is the most TCDD-susceptible mammal with an LD_{50} in the range of 1-2 μg/kg for TCDD[5], and the hamster a highly TCDD-resistant with an ip LD_{50} greater than 3000 μg/kg[6]. Although interstrain comparisons may be a more meaningful model to study mechanisms of toxicity due to higher physiological similarity between the experimental subjects, the large difference in LD_{50} values between guinea pigs and hamsters offers an alternative approach to examine the mechanism of action of TCDD and to test for the validity of the results obtained with other models.

The present studies were undertaken to investigate whether or not the association between TCDD lethality and changes in tryptophan metabolism also holds true for guinea pigs and hamsters. Furthermore, decreased gluconeogenesis due to suppression of liver phosphoenoylpyruvate carboxykinase (PEPCK) has been implicated in the acute lethality of TCDD in some rat strains and the dose-responses for decreased PEPCK activity and increased serum tryptophan concentration were close to parallel in the Long-Evans rat[7]. Therefore, we also examined the effect of TCDD on PEPCK activity in the livers of these animal species.

2.Materials and Methods

Guinea pigs (Dunkin-Hartley) and Golden Syrian hamsters were kept in stainless steel wire mesh cages and provided with powdered K1 feed (Lactamin, Stockholm, Sweden; for guinea pigs) or pelleted R3 feed (Ewos, Södertälje, Sweden; for hamsters). At the onset of the experiments the guinea pigs were 8 and the hamsters 11 weeks old.

Experimental design. Guinea pigs were given a single ip dose of 0.3, 0.9, 1.6 or 2.7 μg/kg TCDD dissolved in corn oil and hamsters 900, 2700 or 4000 μg/kg. Controls received the same volume (5 ml/kg for guinea pigs and 7.5 ml/kg for hamsters) of corn oil alone. Doses for guinea pigs were chosen such as to range from nonlethal to lethal. For hamsters, all doses proved to be nonlethal (data not shown). Pair-fed control guinea pigs were given feed according to the amounts consumed by the 2.7 μg/kg TCDD dosage group. Trunk blood was collected in heparinized dishes. The brain and the liver were quickly removed and frozen in liquid nitrogen. Plasma and ultrafiltrate were separated from blood by centrifugation. All samples were then stored at −80 °C until analyses (1–2 months).

Biochemical analysis. Plasma and brain tryptophan, brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured by an HPLC method as described previously[1]. Plasma free fatty acids (FFA) and albumin were measured with a Wako test kit (Wako Chemicals GmbH, Neuss, Germany) and an Albuminie kit (BioMerieux, Charbonnieres-les-Bains, France), respectively. Plasma glucose was measured with Peridochrom Glucose®, (Boehringer Mannheim GmbH, Mannheim, Germany) test kits. Activity of liver PEPCK (EC 4.1.1.32) was measured with a bioluminescent method according to Wimmer[8]. Liver glycogen was measured by the method of Hultman[9] with minor modifications.

Mean ± SD were calculated for every variable. Data were stratified by time and assessed statistically by one-way analysis of variance (ANOVA) coupled with predetermined orthogonal contrasts. In the case of nonhomogeneous variances, the Kruskal-Wallis nonparametric one-way ANOVA followed by the Mann-Whitney *U* test were used.

3.Results

Body weight gain was dose-dependently inhibited in TCDD-treated guinea pigs (Table 1). There was virtually no growth in control hamsters and there was a slight tendency towards body weight loss (not significant) in TCDD-treated hamsters.

Table 1. Body weight at 10 days after dosing relative to the initial body weight.

Guinea pigs		Hamsters	
Dose μg/kg	Body weight Change (%)	Dose μg/kg	Body Weight Change (%)
0	14.8±3.7	0	−0.3±2.7
0.3	12.6±3.4	900	−4.1±2.9
0.9	4.9±9.6*	2700	−2.4±2.5
1.6	4.3±1.9*	4600	−3.0±2.4
2.7	−9.9±11*		
pair-fed	−8.6±2.7*		

*Significantly different from ad libitum fed controls ($p<0.05$).
Mean±SD, N=3–8.

Brain 5-HT metabolism was not affected by any dose of TCDD in guinea pigs as evidenced by unaltered levels of brain tryptophan, 5-HT and its main metabolite, 5-HIAA (data not shown). In addition, plasma levels of neither free nor total tryptophan were affected in TCDD-treated guinea pigs.

Similarly, brain 5-HT and 5-HIAA were unaffected in hamsters at any dose or time point after TCDD administration (data not shown). Increases were seen in brain tryptophan, plasma free and total tryptophan concentrations but without dose-dependence. The bound fraction of tryptophan in plasma remained, however, the same (around 25 %), over the whole dose range.

Table 2. Effects of TCDD in hamsters.

Day postexp	Dose µg/kg	Brain trp* nmol/ g tissue	Plasma free trp mg/ l	Plasma total trp mg/ l	Plasma FFA mmol/ l	Plasma Glucose mmol/ l	Liver glycogen mg/g
Day 4	0 (5)	24.1±11.7	5.56±0.64	25.3±4.82	0.70±0.31	3.78±0.43	32.4±14.8
	900 (6)	36.0±3.79	9.87±2.62*	39.6±7.57*	0.67±0.22	4.34±1.16	36.2±3.7
	2700 (5)	31.0±5.10	10.3±0.84*	35.2±5.01*	0.66±0.18	4.05±0.61	35.8±15.0
	4600 (5)	33.2±8.74	8.22±2.02*	33.2±7.12	0.62±0.14	4.75±0.56	37.9±12.2
Day 10	0 (6)	19.3±2.47	5.91±1.15	22.4±3.23	1.10±0.39	4.07±0.62	47.4±21.8
	900 (6)	24.7±3.04*	8.04±1.24*	31.5±3.78*	0.74±0.22	3.87±0.51	24.1±14.4
	2700 (6)	29.2±2.60*	8.29±1.00*	34.0±1.49*	0.69±0.25	3.58±0.60	29.6±4.9
	4600 (6)	27.9±3.34*	7.69±0.70*	31.1±4.39*	0.52±0.14*	4.22±0.58	26.0±7.0

For details, see table1. *tryptophan

Plasma albumin was unaffected by TCDD in both guinea pigs or hamsters (data not shown). FFAs were dose-dependently elevated in TCDD-treated guinea pigs at 4 and 10 days after exposure (Table 3). There was at least an equal increase in pair-fed guinea pigs. In contrast, FFA concentrations were dose-dependently decreased in hamsters at the last time point (Table 2).

The activity of PEPCK was not affected by TCDD in guinea pigs (Fig. 1, top) whereas in hamsters it was profoundly suppressed (Fig. 1, bottom). Nevertheless, both guinea pigs and hamsters treated with TCDD maintained normal glucose levels during the observation period (Tables 2 and 3). Liver glycogen levels were not much affected in hamsters (Table 2) but they tended to be dose-relatedly decreased in guinea pigs although not as much as in pair-fed controls (Table 3).

Table 3. Effects of TCDD in guinea pigs.

Day postexp	Dose µg/kg	Plasma FFA mmol/ l	Plasma Glucose mmol/ l	Liver glycogen mg/g
Day 4	0 (6)	0.18±0.10	7.48±1.00	54.7±26.8
	0.3 (6)	0.22±0.10	7.43±0.83	32.0±3.78
	0.9 (6)	0.30±0.15	7.77±0.96	26.0±11.9
	1.6 (6)	0.40±0.13	7.50±0.66	25.0±9.58
	2.7 (6)	0.55±0.19*	7.78±0.37	24.0±4.87
Day 10	0 (7)	0.15±0.29	7.44±0.98	72.8±16.3
	0.3 (4)	0.18±0.09	7.68±1.28	69.8±10.7
	0.9 (7)	0.34±0.34*	7.13±0.37	40.0±10.5*
	1.6 (3)	0.27±0.04*	7.63±0.75	39.0±9.61*
	2.7 (8)	0.54±0.24*	7.55±1.28	28.8±10.9*
	P-f (6)	0.78±0.09*	6.22+1.06	15.7±5.91*

For details, see table 1.

Discussion

Results of these experiments demonstrate that hypotheses generated by studies in rats[3,10,11] are not capable of explaining species differences in the acute toxicity of TCDD between guinea pigs and hamsters. The implication is

that one should be very careful in differentiating between causality and coindicence among various manifestations of TCDD toxicity. Many different consequences may have a common origin, but they may not bear a causal relationship among themselves, and due to different physiology of different species, the spectrum of consequences varies.

No association could be found between changes in tryptophan metabolism and TCDD lethality, as evidenced by the lack of effect of a lethal dose of TCDD on brain 5-HIAA, tryptophan and free tryptophan concentrations in the plasma of TCDD-sensitive guinea pigs. In contrast, the hamster exhibited increased brain tryptophan levels although there was no lethality at the doses studied.

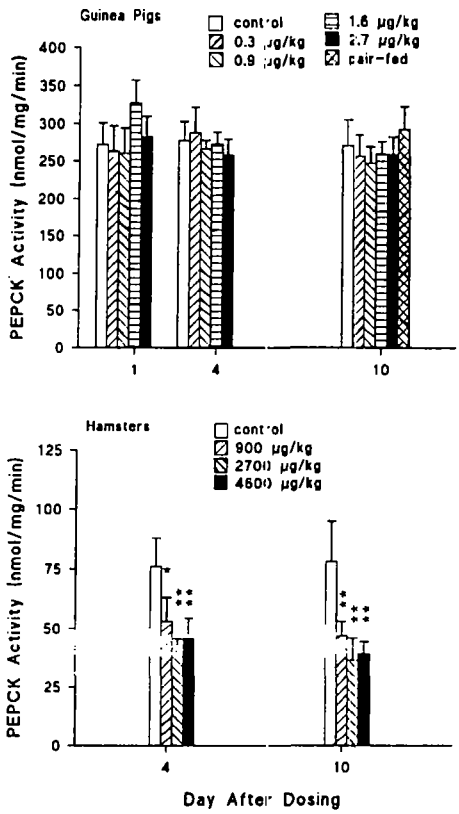

Fig. 1. The effect of TCDD on liver PEPCK activity. N=6-8. * and ** denote statistially significant differences ($p<0.05$ and $p<0.01$, respectively).

These findings stand in sharp contrast to our previous observations from two rat strains with a thousandfold difference in susceptibility to TCDD. Those studies showed that in TCDD-susceptible Long-Evans rats, brain 5-HT metabolism, free tryptophan in the plasma, body weigh loss and lethality are all dose-dependently increased by TCDD in a temporally concurrent way[1,2]. In contrast, TCDD-resistant Han/Wistar rats did not exhibit these changes at any dose tested (the highest 9600 µg/kg). Hence the wasting syndrome and lethality were associated with changes in tryptophan metabolism in rats. Assuming a causal role for the increased indoles in anorexia would dictate similar increases in brain 5-HIAA, brain tryptophan and plasma free tryptophan across TCDD sensitive and TCDD-resistant species. According to the present results this was not the case.

In rats the augmented levels of free tryptophan may be mediated via increased FFA in the plasma[13]. In the present study, FFA levels were elevated in TCDD-treated guinea pigs but still these animals had normal plasma free tryptophan levels. It is not known at this stage whether the discrepancy between rats and guinea-pigs follows from a difference in the plasma lipid profile or from different conformational effects of fatty acids on the albumin of these species.

In the present study, hepatic PEPCK activity, a proposed key target of TCDD acute toxicity in rats[9,10] was affected in hamsters but not in guinea pigs. Furthermore, plasma glucose remained unaltered in both species. Finally, liver glycogen was depressed in guinea pigs alone with the greatest decrease in pair-fed controls suggesting that the change was probably due to hypophagia. Therefore, dysregulation of glucose homeostasis and inhibition of gluconeogenesis cannot constitute a universal mechanism for the acute lethality of TCDD either, but rather these phenomena can be viewed as various consequences of toxicity.

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