TCDD-Induced Increase In Brain Serotonin Turnover: Dose-Response Relationship To Plasma Free Tryptophan But Not To Inhibition Of Liver Tryptophan Pyrrolase

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Introduction The most prominent sign of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity at lethal doses is suppression of food intake, the mechanism of which is not understood. This laboratory has established a new rat model for mechanistic studies of TCDD toxicity. It is based on a susceptibility divergence between two rat strains, Long-Evans (L-E; LD_{50} ca. 10 μg/kg) and Han/Wistar (H/W; LD_{50} >7200 μg/kg)[1]. The strain difference is reflected in the severity and pattern of feed refusal after TCDD administration. We have previously reported that TCDD increases brain serotonin metabolism in the TCDD-susceptible L-E but not in the TCDD-resistant H/W strain[2]. Serotonin is a neurotransmitter able to modulate food consumption. Here we have further explored the mechanism by which the acceleration of brain serotonin metabolism occurs. The possible role in these responses of the main tryptophan metabolizing enzyme, liver tryptophan pyrrolase (2,3-dioxygenase), was also studied, since this enzyme has been reported to be inhibited by TCDD[3].

Materials and methods Adult male L-E rats were given 5, 10, 15, 20 or 50 μg/kg TCDD in corn oil (4 ml/kg ig b.i.d. for two days) or vehicle alone. Five μg/kg is nonlethal while 50 μg/kg is 100% lethal to L-E rats. Pair-fed L-E rats were given corn oil and then fed an equal amount of food their 50 μg/kg TCDD-treated counterparts had eaten on the previous day (corrected for spillage). H/W rats were treated with doses of 50, 500, 1000, 5000 or 9600 μg/kg TCDD (4 ml/kg ig b.i.d. x 2) or corn oil. Even a dose as high as 7200 μg/kg is nonlethal to H/W rats[1]. 9600 μg/kg has never been tested before for this strain and therefore we ran parallelly a small- scale experiment where H/W rats were given 9600 μg/kg TCDD (4 ml/kg ig b.i.d. x 2) or vehicle and the rats were then followed for lethality for 42 days. On day 6 postexposure, the rats assigned to the dose-response experiment were decapitated and trunk blood was collected on a heparinized dish containing 25 IU heparin per ml blood. The brain and liver were also rapidly removed and frozen in liquid nitrogen. Blood was centrifuged at 1000 x g for 10 min and the resultant plasma was further centrifuged in Ultrafree® MC 30 000 NMWL ultrafiltration tubes (Millipore, Kogyo, Japan) at 4700 x g for 20 min. All centrifugations were carried out at room temperature. Brain indoleamines (serotonin, its metabolite 5-hydroxyindoleacetic acid [5-HIAA] and precursor tryptophan) as well as plasma free tryptophan were determined by an HPLC-EC method[4]. Liver tryptophan pyrrolase was determined according to Metzler[5].

Results and Discussion L-E rats decreased their daily food intake in a dose-dependent manner during the six-day period after the exposure while in H/W rats the reduction was marginal and did not exhibit a clear dose-response pattern despite the three orders of magnitude higher doses of TCDD used for them (fig 1). The 9600 μg/kg dose proved to be nonlethal to H/W rats since none of the animals succumbed during the 42-day follow-up time after the exposure. The average body weight loss from initial levels was 8% for 9600 μg/kg TCDD-treated H/W rats while control rats gained 16% extra weight over the 42-day follow-up period.

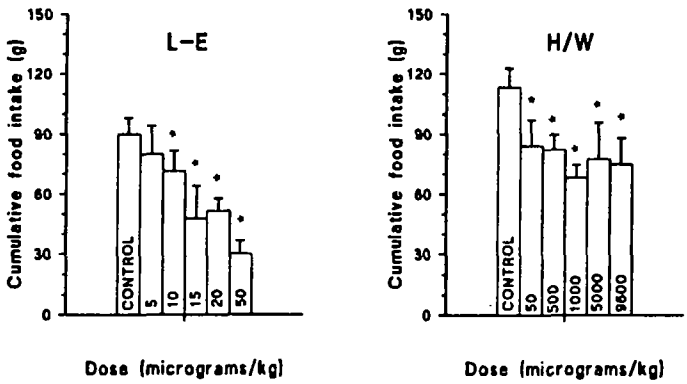

Figure 1. The effect or varying doses of TCDD on cumulative food consumption in L-E and H/W rats during the 6 days postexposure. Asterisk denotes a statistically significant difference ($p<0.05$) vs control level. N=5-6.

As to the effects of TCDD on brain neurochemistry, L-E rats exhibited increased serotonin turnover in the brain as evidenced by dose-dependently increased levels of the serotonin metabolite 5-HIAA and its precursor amino acid tryptophan at doses of 15 and 50 μg/kg (fig 2). In pair-fed L-E rats there was a slight tendency towards increased levels of brain 5-HIAA when compared with ad libitum-fed controls. The elevation in pair-fed rats was, however, lower than that seen in L-E rats treated with 15 or 50 μg/kg TCDD. In H/W rats, there was also evidence of increased levels of brain 5-HIAA and tryptophan by TCDD. A statistically significant difference was reached, however, only at the 9600 μg/kg dose which is 192 times as high as the highest dose used for L-E rats. In general, the departures in brain 5-HIAA and tryptophan from control level by TCDD were less prominent in H/W than in L-E rats. A striking difference was seen in the effect of TCDD on plasma free (ultrafiltrable) tryptophan levels. In L-E rats they proved to be greatly elevated at doses ≥ 15 μg/kg (fig 2, 3rd panels). The most important point to note is that the increases of brain 5-HIAA and tryptophan followed meticulously the patterns of plasma free tryptophan. In H/W rats there was a slight nonsignificant tendency towards elevated plasma free tryptophan at the highest 9600 μg/kg dose which was also parallelled by similar alterations in brain 5-HIAA and tryptophan at this dose. There were no changes in either rat strain in plasma total tryptophan (data not shown). It has been proposed that the mechanism of TCDD-induced appetite suppression might be mediated via inhibition of the main tryptophan metabolizing enzyme, tryptophan pyrrolase, in the liver and the consequent accumulation of tryptophan in the body[3]. In L-E rats, there was a slight and nonsignificant trend towards inhibited liver tryptophan pyrrolase

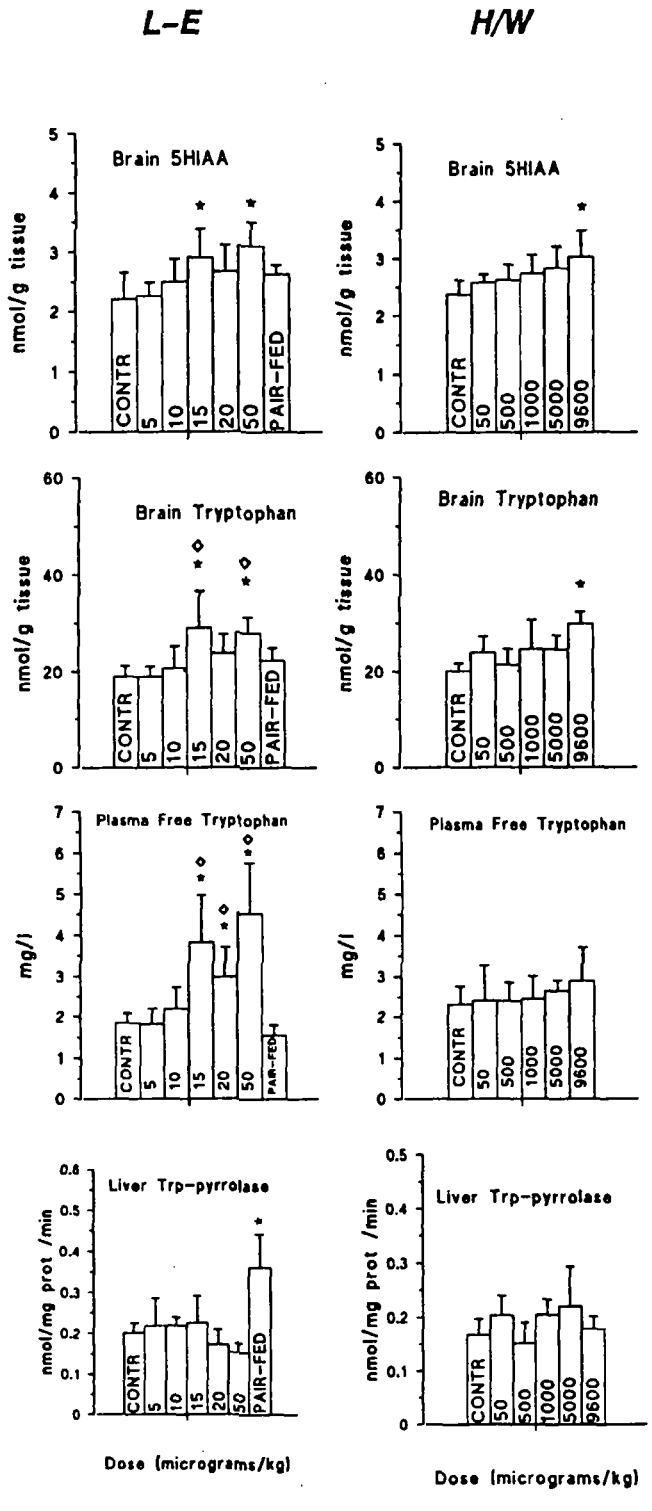

Figure 2. The dose-response effect of TCDD on various parameters of L-E and H/W rats. Asterisk denotes a statistically significant difference ($p<0.05$) vs. ad libitum (closed) or pair-fed (open) controls. N=5-6.

activity at 20 and 50 μg/kg when compared with ad libitum controls (fig 2, bottom panels). It is interesting to note that the pyrrolase activity was clearly induced in pair-fed control rats (ca. 2-fold). This suggests that there is abnormal regulation of this metabolic activity in TCDD-treated rats. Taken together, it seems clear from the dose-response pattern, that the inhibition of liver tryptophan pyrrolase activity in L-E rats cannot explain the increase in brain serotonin metabolism nor the elevation of plasma free tryptophan. For example, at 15 μg/kg there was an almost maximal increase in brain tryptophan and plasma free tryptophan but no evidence of inhibited liver tryptophan pyrrolase activity. In H/W rats, TCDD did not have any marked effect on liver tryptophan pyrrolase activity.

Keeping in mind that 15 μg/kg to L-E rats is very close to the LD_{50}-value for this strain, it is interesting to note that the increases in brain 5-HIAA, tryptophan and plasma free tryptophan emerged at this dose. This further strenghtens the view that there is an associaton between enhanced brain serotonin turnover and TCDD lethality. It does not, however, indicate any

causal relationship between these phenomena.
Of the parameters measured, perhaps the most sensitive and best correlated with TCDD lethality was plasma free tryptophan. In L-E rats there was a striking increase in this parameter at doses eliciting lethality (≥15 μg/kg). Tryptophan is the only amino acid in plasma that is to a large extent bound to plasma albumin while a small (ca. 10%) fraction circulates free in a form able to penetrate into the brain. It is known that the ratio of free and bound tryptophan in plasma affects brain tryptophan levels. Additional factors influencing brain tryptophan levels are other large neutral amino acids competing for the same transport carrier from plasma to brain. The balance between bound and free tryptophan is controlled by other ligands which compete with tryptophan for binding to albumin. At least free fatty acids are physiological competitors of tryptophan in that respect[6]. This mechanism may be important in the case of TCDD, since TCDD has been reported to increase plasma free fatty acids[7]. The possibility of TCDD itself as a direct displacer of tryptophan from albumin is ruled out by the finding that a 600 times higher dose of TCDD for H/W rats than the lowest effective dose in L-E rats rats did not increase appreciably plasma free tryptophan.
According to the present results, TCDD increases brain serortonin turnover in TCDD - sensitive L-E rats at the same dose range in which acute lethality occurs. It is likely that the changes seen in brain tryptophan may be secondary to elevated plasma free tryptophan levels. Future studies will explore whether the increase in plasma free tryptophan involves plasma free fatty acids or perhaps alterations in plasma albumin concentration. The inhibition of tryptophan metabolism in the liver can not explain the increase in brain and plasma free tryptophan levels. In H/W rats, all doses tested were nonlethal and reliable increases in brain serotonin turnover and related parameters were seen only at the highest dose. The present results further strenghten the association between TCDD lethality and brain serotonin metabolism. They also reveal a new indicator, plasma free tryptophan, which seems to be a sensitive correlate of TCDD acute lethality.

References

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