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2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) INDUCED ETHOXYRESORUFIN-*O*-DEETHYLASE (EROD) AND ALDEHYDE DEHYDROGENASE ($ALDH_3$) ACTIVITIES IN THE BRAIN AND LIVER

A COMPARISON BETWEEN THE MOST TCDD-SUSCEPTIBLE AND THE MOST TCDD-RESISTANT RAT STRAIN

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Abstract—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent inducer of ethoxyresorufin *O*-deethylase (EROD) and aldehyde dehydrogenase (EC 1.2.1.3., $ALDH_3$) enzyme activities in the liver. Little is known about their inducibility by TCDD in the brain, although it may be a target organ for TCDD toxicity. Two strains of rat, Long–Evans (L–E) and Han/Wistar (H/W) exhibit an over 1000-fold difference in their LD_{50}-values for TCDD. The induction of EROD and $ALDH_3$ in discrete brain regions and in the liver of L–E and H/W rats were now compared at 10 days after TCDD exposure to assess the role of these responses in the strain difference. Liver EROD and $ALDH_3$ were maximally induced at 5 μg/kg and 50 μg/kg, respectively, in both strains. In the brain 50 μg/kg TCDD was mostly needed to enhance EROD activity in both strains. The induction occurred especially in olfactory bulbs, but was also seen in the midbrain plus thalamus of both rat strains. The induced EROD activity in the olfactory bulb was almost totally abolished by a monoclonal antibody (Mab) 1-7-1 raised against CYP1A1. $ALDH_3$ activities were increased more dose dependently in olfactory bulbs of H/W than L–E rats. In other brain areas measured, $ALDH_3$ activities were induced more in L–E rats. Kinetic factors did not explain the differential induction of EROD and $ALDH_3$ among discrete brain regions. We conclude that both EROD and $ALDH_3$ are induced in the brain by TCDD although the activities are much lower than in the liver. The induction in the brain is region specific with olfactory bulbs being the most responsive area. As in the liver, the TCDD-induced activity of EROD in the brain is primarily associated with CYP1A1. According to the present findings, enzyme induction in the brain does not seem to have a crucial role in determining the strain susceptibility to the acute lethality of TCDD.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD‖) is a highly toxic environmental contaminant. Although the ultimate mechanism of TCDD toxicity remains unidentified, many biochemical responses have been associated with TCDD toxicity. One of the best understood actions of TCDD is the hepatic induction of a set of xenobiotic-metabolizing enzymes, particularly two members of the cytochrome P450 family of mono-oxygenases [1] as well as an isozyme of aldehyde dehydrogenase (ALDH) [2, 3]. The induction of P450 isozymes is mediated via cytosolic aromatic hydrocarbon (Ah) receptors [4, 5]. Numerous studies indicate that TCDD is a selective inducer of liver cytochrome P450 isozymes CYP1A1 and CYP1A2 [4–6]. The toxicity of TCDD has been postulated to be an Ah receptor-mediated response and therefore to be associated with the induction of mono-oxygenase activities.

ALDHs catalyse oxidation of aldehydes to corresponding carboxylic acids and may thus have a detoxifying function. The isozyme $ALDH_3$ is selectively induced by TCDD and other Ah receptor ligands in the rat liver [2, 3, 7]. A relationship between Ah receptors and $ALDH_3$ induction has been suggested [8]. A recent paper showed that TCDD may induce cytosolic but not microsomal $ALDH_3$ activity via an Ah receptor-dependent mechanism [9]. However, Dunn *et al.* [10] reported that TCDD induces the activities of $ALDH_3$ and CYP1A1 in a noncoordinate fashion in the rat liver. The relation of $ALDH_3$ induction to acute toxicity of TCDD has not been studied.

Studies in this laboratory have shown that two strains of rat, Long–Evans (L–E) and Han/Wistar (H/W) differ by a factor of about 1000 in their LD_{50}-values for TCDD [11, 12]. This interstrain disparity provides a physiologically appropriate animal model to study the biochemical alterations crucial for TCDD toxicity. We have previously reported that the rat strains did not differ in their levels of functional hepatic Ah receptors or in the induction

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‖ Abbreviations: Ah, aromatic hydrocarbon; AHH, aromatic hydrocarbon hydroxylase; ANOVA, analysis of variance; ALDH, aldehyde dehydrogenase; EROD, ethoxyresorufin *O*-deethylase; H/W, Han/Wistar; L–E, Long–Evans; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Mab, monoclonal antibody.

of hepatic cytochrome P450 isozymes by TCDD [11]. A striking difference between the rat strains emerges in their energy intake after TCDD. L–E rats respond to TCDD administration by drastically reducing their daily feed intake and body weight which are characteristics of TCDD-induced wasting syndrome. In contrast, H/W rats respond only marginally to TCDD in that respect [13]. Furthermore, our results have suggested that the central nervous system may be a target organ for TCDD toxicity [14]. On the other hand, information has been gathered indicating that the brain contains measurable levels of cytochrome P450-related enzyme activities [15, 16]. They may be distributed unevenly in discrete brain regions [17, 18] and are induced by xenobiotic compounds such as benzo-(*a*)-pyrene [15, 16]. Although the physiological role of cytochrome P450 systems in the brain is not well understood, they may be involved in the metabolism of endogenous catecholestrogens [19, 20] as well as xenobiotics. In addition to metabolism, there is evidence that cytochrome P450 is involved in the generation of active oxygen radicals and lipid peroxides [21, 22]. Thus, it may have a potential role in the generation of neurodegenerative changes by xenobiotics. The induction of $ALDH_3$ in the liver is well characterized but little is known about its induction in the brain [10].

The fact that TCDD is a strong inducer of cytochrome P450 and $ALDH_3$ in the liver and the recent reports indicating the existence and inducibility of these enzymes in the brain prompted us to examine whether the wide difference in susceptibility to the acute toxicity of TCDD between L–E and H/W rats could be associated with differential induction of ethoxyresorufin-*O*-deethylase (EROD) (a specific marker enzyme activity for CYP1A1) and $ALDH_3$ by TCDD in the central nervous system. Furthermore, it was of interest to compare the induction of EROD and $ALDH_3$ by TCDD in the brain with that in the liver between L–E and H/W rats.

MATERIALS AND METHODS

Chemicals. The purity of TCDD was >98% as assessed by gas chromatography–mass spectrometry. A calculated amount of TCDD was dissolved in ether and then mixed into corn oil, after which the ether was evaporated. The concentrations of TCDD in corn oil were 1 and 10 μg/mL. [^{3}H]TCDD (sp. act. 40 Ci/mmol; purity >97%) was purchased from Cambridge Isotope Laboratories (Woburn, MA, U.S.A.). Monoclonal antibody (Mab) 1-7-1 raised against rat liver 3-methylcholanthrene-inducible cytochrome P450 [23] was a kind gift from Dr H. V. Gelboin (NCI, NIH, Bethesda, MD, U.S.A.). All other chemicals were of at least analytical grade.

Treatment of animals and preparation of samples. Adult male H/W (Kuopio) and L–E (Turku AB) rats (for the current nomenclature of the strains, see [24]) were purchased from the National Laboratory Animal Centre (Kuopio, Finland). They were allowed to acclimatize to the new environment for more than 2 weeks prior to the experiment. Groups of rats of both strains were administered TCDD as a single i.p. injection at 5 or 50 μg/kg. Controls were given the same volume of the vehicle (5 mL/kg). A dose of 5 μg/kg is always non-lethal to both rat strains while 50 μg/kg is 100% lethal to L–E rats and non-lethal to H/W rats. Mortality is typically preceded by a dramatic body weight loss known as the wasting syndrome and it progressively develops within 2–3 weeks after exposure [25]. For this reason, the rats were weighed every 2 days. During the experiment the rats were housed individually and they were provided feed (R3, Ewos, Södertälje, Sweden) and tap water *ad libitum*. The ambient temperature in the animal room was 21.5 ± 1°, humidity 55 ± 10% and lighting cycle 12/12 hr light/dark.

The experiment was terminated 10 days after TCDD exposure, since around this time point both EROD [26] and $ALDH_3$ [10] attain their maximal activities in the liver, and the differential effect of TCDD on body weight between the strains is readily observable [27]. The rats were decapitated and the livers were excised and divided into two parts which were frozen in liquid nitrogen. The brains were rapidly removed and dissected on an ice-cold glass plate into eight regions as described previously [28]. To obtain a sufficient amount of material for enzyme activity measurements, the dissected brain regions were pooled from two to three animals. The brain samples were immediately placed into ice-cold 100 mM phosphate buffer containing 5 mM EDTA, 10% (v/v) glycerol and 0.1 mM phenylmethylsulfonyl fluoride. Once the desired pool size was attained (within 30–45 min), the pieces were blotted dry, weighed and homogenized on ice by a glass–teflon homogenizer in 3 vol. of the phosphate buffer. The homogenate was centrifuged at 10,000 *g* for 20 min at 4°. The resulting supernatant was then frozen in a freezer at −80° until further analyses.

EROD and $ALDH_3$ assays. Due to the low recovery of microsomes from brain regions of this size, EROD activity from brain samples was measured from the 10,000 *g* supernatant fraction. A 100 μL aliquot of this fraction was incubated for 10 min in an end-point reaction mixture system for EROD activity as described earlier [29]. Each individual sample was measured against its own reagent blank. EROD activity was then calculated per original amount of wet tissue for every brain region.

For immunoinhibition of EROD, we determined in a preliminary test the amount of a Mab 1-7-1 raised against CYP1A1 which maximally inhibits TCDD-induced (*ca.* 75% or more) and control (*ca.* 20%) EROD activity in the liver and considered this amount of Mab 1-7-1 to be high enough to produce maximal inhibition of brain EROD, since the activities in the brain are substantially lower than those in the liver [18]. The saturating amount of 11 μg of Mab 1-7-1 or nonspecific IgG was incubated with 100- and 200-μL aliquots of 10,000 *g* supernatant from olfactory bulbs and cortex, respectively, for 15 min prior to the start of the reaction with NADPH.

The frozen livers were thawed and homogenized with a glass–teflon homogenizer in 4 vol. of ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 10,000 *g* for 15 min at −4° and the supernatant again at 105,000 *g* for 60 min in order

to separate the soluble and microsomal fractions. The microsomal fraction (the 105,000 *g* pellet) was resuspended in 0.25 M sucrose solution. The samples were stored at −80°. The liver microsomal EROD activity was measured according to Honkakoski and Lang [29].

Brain $ALDH_3$ was measured from the 10,000 *g* supernatant fraction and liver $ALDH_3$ from the 105,000 *g* supernatant fraction. Brain $ALDH_3$ activity was measured directly from the 10,000 *g* supernatant due to the scarcity of homogenate from most brain areas. Furthermore, in a pilot test we found no major differences in $ALDH_3$ activities when measured from 10,000 *g* (control: 1.73 ± 0.16, TCDD 50 μg/kg: 3.25 ± 0.33 nmol/mg protein/min) or 105,000 *g* supernatant fraction (control: 1.94 ± 0.25, TCDD 50 μg/kg: 3.65 ± 0.60 nmol/mg protein/min). The assays were carried out at 37° by monitoring the reduction of NADP at 340 nm. The assay mixture (final volume 1 mL) contained 70 mM sodium pyrophosphate buffer (pH 8.0), 5 mM NADP as cofactor and 5 mM benzaldehyde (dissolved in methanol, final concentration 1%) as substrate. The blanks containing no aldehyde were run for every sample. The activities were calculated as nmoles of NADPH formed/min/mg protein. Protein concentration was measured with a bicinchonic acid method [30] using bovine serum albumin as a standard.

[^{3}H]*TCDD distribution in brain, liver, fat and plasma.* Ten male rats of both L–E and H/W strains were given [^{3}H]TCDD as a single i.p. dose at 5 (^{3}H-activity 0.466 mCi/kg) or 50 μg/kg (^{3}H-activity 0.898 mCi/kg) TCDD (N = 5/strain/dose). Ten days later the rats were decapitated and the brain, liver, a sample of perirenal white adipose tissue and trunk blood were collected. Brain was dissected into eight regions in the same manner as performed for the enzyme activity determinations. Plasma was separated from the trunk blood. A representative sample of about 100 mg was taken from the liver and fat for radioactivity determinations. Since body weight decreased dramatically in L–E rats treated with 50 μg/kg TCDD, these rats had almost totally lost their perirenal white fat stores. Therefore, the fat sample for 50 μg/kg TCDD treated L–E rats was taken from the epididymal fat pad. For liquid scintillation counting, the tissue specimens were processed using Lumasolve® as solubilizer and Lipoluma® as scintillator (Lumac/3 M B.V., Schaesberg, the Netherlands). A 50 μL portion of plasma was used for plasma radioactivity measurement. The radioactivity was determined with a liquid scintillation counter (LKB Wallac, Turku, Finland).

Statistical analysis. The analysis of variance (ANOVA) was used to compare brain enzyme activities between the rat strains and among treatments. One-way ANOVA followed by the Duncan procedure as a post-hoc test was used for intrastrain comparisons of brain enzyme activities at each region among the three treatment groups. Due to non-homogenous variances for liver enzyme activities, a non-parametric test (Kruskall–Wallis one-way ANOVA followed by Mann–Whitney U-test) was employed for comparison among the different treatment groups. Repeated measures ANOVA was used to compare the effect of TCDD on body weight gain between the rat strains. For brain [^{3}H]TCDD distribution data, ANOVA with the randomized block design was used for comparison between the rat strains across all brain areas at the higher dose. For comparisons of the radioactivity data between the doses of strains in each brain region, two tailed Student's *t*-test for independent samples or Mann–Whitney U-test were employed. All calculations were performed with an SPSS-X programmed VAX computer.

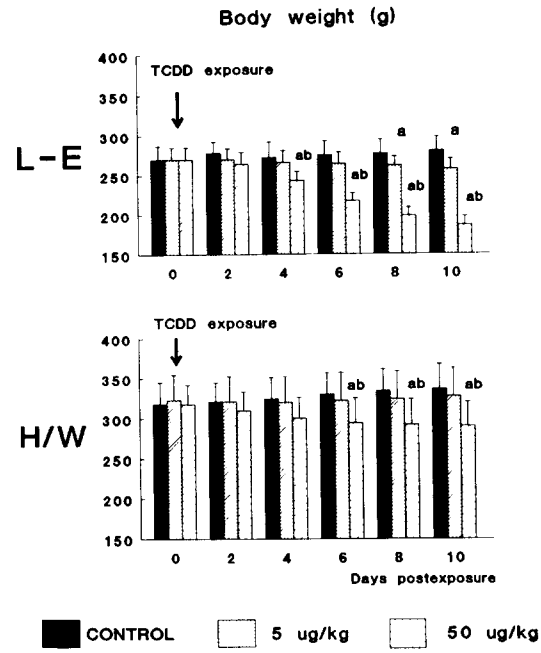

Fig. 1. Body weights (g) of L–E and H/W rats. Statistically significant differences are denoted by "a" ($P < 0.05$ vs control) and "b" ($P < 0.05$ vs 5 μg/kg). Mean ± SD.

RESULTS

None of the animals succumbed during the 10-day period after exposure. As expected, TCDD decreased body weight strikingly in L–E rats whereas H/W rats responded only marginally ($P < 0.001$ for the strain difference) to TCDD administration (Fig. 1).

Liver EROD activities were maximally increased already at the lower 5 μg/kg dose level (Fig. 2) in accordance with previous findings [11]. Also in line with the previous report, the degree and pattern of EROD induction was comparable between L–E and H/W rats. In contrast to EROD, the liver $ALDH_3$ activities were higher at 50 than 5 μg/kg TCDD (Fig. 2). Furthermore, ANOVA revealed a significant strain-by-treatment interaction term in liver $ALDH_3$ induction ($P < 0.001$). This resulted from a notably greater induction at 5 μg/kg TCDD in L–E than H/W rats. However, at 50 μg/kg TCDD the induction of $ALDH_3$ was comparable in magnitude between both strains.

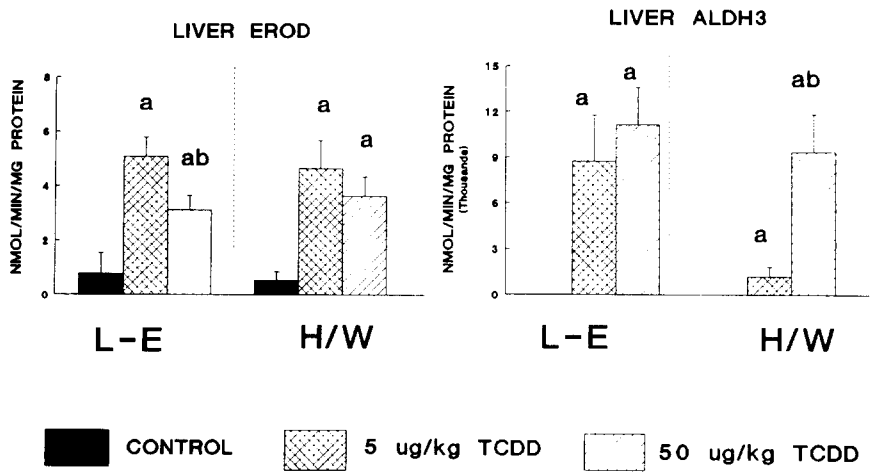

Fig. 2. Liver EROD and $ALDH_3$ activities 10 days after TCDD treatment. The symbols are as in Fig. 1. N = 5–12. Control $ALDH_3$ activities are too low to show: L–E, 20.0 ± 5.84; H/W, 4.0 ± 1.71 nmol/min/mg protein, mean ± SD.

EROD was induced in the midbrain plus thalamus of L–E rats at the dose of 5 μg/kg, and in several brain areas of both strains at the dose of 50 μg/kg (Fig. 3). As a whole, EROD activity was very low in the brain as compared with the liver and this resulted in a relatively wide variation of the data. However, in the olfactory bulbs the induction of EROD was more consistent and greater (3- and 10-fold for L–E and H/W rats, respectively; note high control activity in L–E rats) than in other brain regions (2–3-fold). There was no difference in TCDD-induced EROD activities in olfactory bulbs between the rat strains. The actual values of EROD activity were considerably higher (*ca.* 70 pmol/min/g tissue) at 50 μg/kg TCDD in olfactory bulbs than in any other brain region for both strains. The Mab 1-7-1 did not affect the constitutive activity of EROD in either olfactory bulbs or in the cortex (Table 1). The activity of EROD induced by 50 μg/kg TCDD in the olfactory bulb and cortex was inhibited by 85 and 45%, respectively, by Mab 1-7-1 reverting the activity of EROD to control levels. This indicates that the EROD induction in olfactory bulbs and cortex is for the greater part due to increased expression of CYP1A1.

The baseline activity of $ALDH_3$ was highest in olfactory bulbs, and the activity was also significantly induced in this brain area in H/W rats. In other brain areas measured, $ALDH_3$ activities were more induced in L–E rats (Fig. 4). ANOVA confirmed a statistically significant (or almost significant) TCDD-by-strain interaction term for the olfactory bulbs, medulla and cortex ($P = 0.01$, 0.015 and 0.059, respectively).

To assess whether the larger inductions of EROD and $ALDH_3$ in olfactory bulbs were due to higher accumulation of TCDD in that area, the rats were given [^{3}H]TCDD at 5 and 50 μg/kg. The retention of ^{3}H-activity in the brain regions at 10 days after [^{3}H]TCDD administration is shown in Table 2. In the present study, the chemical identity of ^{3}H-activity was not analysed. In a previous study with ^{14}C-labelled TCDD in the two rat strains, however, all radioactivity in the liver turned out to stem from the parent compound and the proportional distribution of the dose between the brain and the liver was similar to that in the present study [32]. At 5 μg/kg, there was no significant difference in the accumulation of ^{3}H-activity among the discrete brain regions. The pattern of accumulation between the two rat strains was also similar at this dose. At the higher 50 μg/kg dose there was a somewhat greater (*ca.* 2-fold) retention of ^{3}H-activity than at 5 μg/kg (as percent of dose), especially in L–E rats. Furthermore, the values were approximately twice as high in L–E as in H/W rats, although the hippocampus was the only brain region where the difference attained statistical significance. When all the brain regions from the rats treated with the higher dose of [^{3}H]TCDD were assessed together with a randomized block design, there was a significant strain effect ($P < 0.001$) indicating greater accumulation of [^{3}H]-TCDD in the brains of L–E than of H/W rats. As a rule, ^{3}H-activities in the liver, fat, plasma and pituitary tended to be higher in L–E than in H/W rats at 50 μg/kg TCDD (Table 3). This difference was (as well as that seen in the brain) probably attributable to the serious wasting syndrome in L–E rats resulting in loss of body fat stores and consequently leading to enhanced mobilization of TCDD.

DISCUSSION

In the present study, we studied the ability of TCDD to induce EROD and $ALDH_3$ in two possible target tissues of TCDD toxicity, the brain and the liver. The main objective was to compare the enzyme induction in the CNS between two rat strains, which differ widely (about 1000-fold) in their LD_{50} values for TCDD [11, 12], i.e., whether the difference in sensitivity would be reflected in the degree and

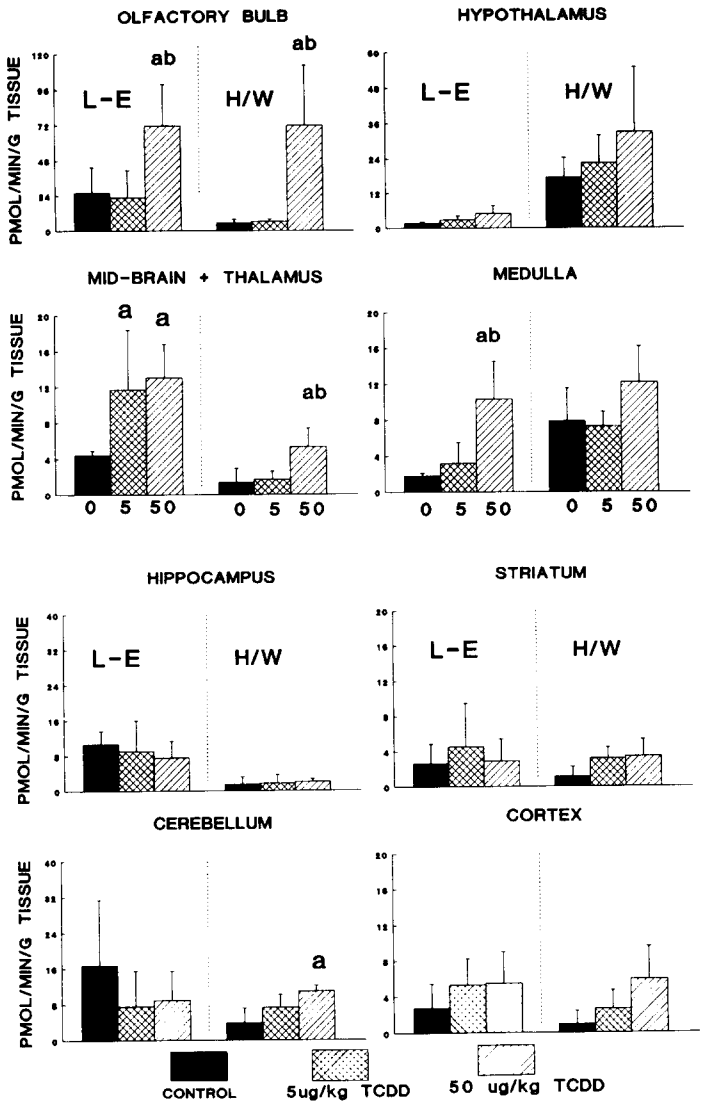

Fig. 3. EROD activity in various brain areas 10 days after TCDD treatment. The symbols are as in Fig. 1, N = 4.

Table 1. EROD activity (pmol/min/g tissue) in olfactory bulbs and cortex from control and 50 μg/kg TCDD-treated H/W rats with and without preincubation with Mab 1-7-1

	Olfactory bulb		Cortex	
	Control	TCDD	Control	TCDD
IgG	8.2, 9.6	78.0, 98.6	1.0, 1.6	2.6, 2.0
Mab 1-7-1	5.4, 10.3	11.1, 18.0	0.9, 1.8	1.1, 1.0

N = 2, both values are shown.

location of EROD and $ALDH_3$ induction in the CNS.

TCDD enhanced the activities of both EROD and $ALDH_3$ in the brain. An earlier study had shown that TCDD may induce aromatic hydrocarbon hydroxylase (AHH) activity in whole brain although no increase in CYP1A1 amount could be detected [31]. Immunoblotting studies revealed that in addition to the liver, 3-methylcholanthrene may also induce CYP1A1 in rat brain [16]. Dunn *et al.* [10], who studied the gene expression of CYP1A1 and $ALDH_3$, found non-coordinate expression of these enzymes in the liver and could not demonstrate any induction at all of either CYP1A1 or $ALDH_3$ in the brain by TCDD. To our knowledge, the present study is the first to show a clear induction response to TCDD of $ALDH_3$ and CYP1A1 (with EROD as a marker) within the CNS.

The induction of EROD in the brain was region specific without any noticeable difference between L-E and H/W rat strains. Thus it is not likely that the induction of CYP1A1 in the brain would be a

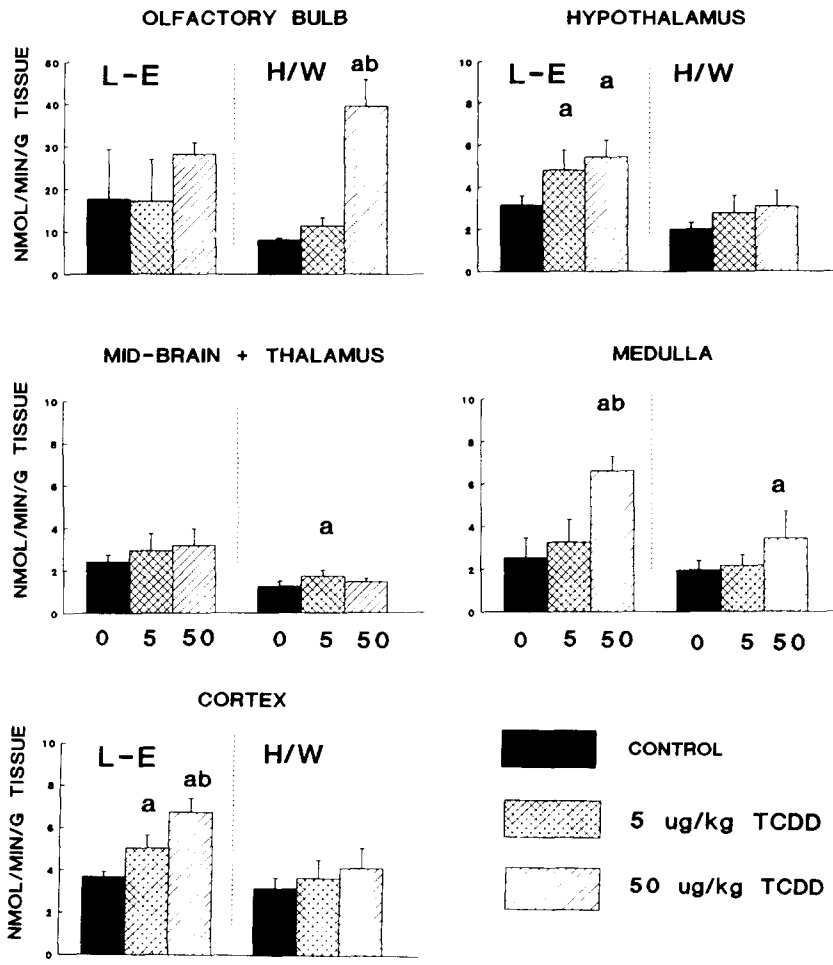

Fig. 4. $ALDH_3$ activity in various brain areas 10 days after TCDD treatment. The symbols are as in Fig. 1, N = 4.

Table 2. ^{3}H-Activity in various brain regions 10 days after [^{3}H]TCDD administration

Region	Dose (μg/kg)	Strain	
		L-E	H/W
Olfactory bulb	5	0.016 ± 0.005	0.017 ± 0.003
	50	0.042 ± 0.024	0.026 ± 0.006
Hypothalamus	5	0.024 ± 0.013	0.019 ± 0.003
	50	0.051 ± 0.031	0.034 ± 0.022
Medulla	5	0.015 ± 0.006	0.016 ± 0.003
	50	0.060 ± 0.036*	0.026 ± 0.008*
Midbrain + thalamus	5	0.013 ± 0.007	0.015 ± 0.004
	50	0.049 ± 0.026*	0.021 ± 0.006
Hippocampus	5	0.013 ± 0.005	0.020 ± 0.014
	50	0.048 ± 0.032†	0.016 ± 0.007
Striatum	5	0.015 ± 0.006	0.016 ± 0.003
	50	0.052 ± 0.036	0.023 ± 0.007
Cerebellum	5	0.012 ± 0.005	0.014 ± 0.003
	50	0.043 ± 0.024*	0.020 ± 0.006
Cortex	5	0.014 ± 0.006	0.014 ± 0.003
	50	0.047 ± 0.030	0.021 ± 0.006

* Denotes difference ($P < 0.05$) vs 5 μg/kg, two-tailed Student's t-test. † Denotes difference ($P < 0.05$) between the rat strains at the corresponding dose level, Mann–Whitney U-test. The values represent percentage of the dose administered per g wet tissue. Mean ± SD. N = 5.

Table 3. ^{3}H-Activity in the liver, white fat pituitary and plasma 10 days after [^{3}H]TCDD administration

Tissue	Dose (μg/kg)	Strain L–E	Strain H/W
Liver	5	0.90 ± 0.419	1.36 ± 0.504
	50	3.40 ± 1.991	1.77 ± 0.483
Fat	5	0.95 ± 0.874	0.83 ± 0.323
	50	5.41 ± 2.452†	1.27 ± 0.474
Plasma	5	0.11 ± 0.039	0.12 ± 0.045
	50	0.51 ± 0.328†	0.15 ± 0.046
Pituitary	5	0.08 ± 0.037	0.10 ± 0.023
	50	0.16 ± 0.056*	0.13 ± 0.069

The conditions are the same as for Table 2.

key biochemical alteration of TCDD intoxication and explain the strain difference in TCDD acute toxicity. In contrast to EROD, brain $ALDH_3$ showed more strain-dependent induction by TCDD. In H/W rats the olfactory bulbs were almost the only responsive area while in L–E rats there was fairly uniform induction across the brain areas measured. The importance of this slight difference in $ALDH_3$ induction pattern between the two rat strains with regard to TCDD susceptibility remains to be determined. Furthermore, similar to the liver, $ALDH_3$ activity correlated better with dose than that of EROD in the brain. In the liver, the lower 5 μg/kg dose resulted in greater induction of CYP1A1 than 50 μg/kg. This is in sharp contrast to the outcome in the brain where EROD induction was discernible only at the higher dose. This organ-derived divergence may be due to lower accumulation of TCDD in the brain as compared with the liver as was revealed by the distribution experiment.

Regarding the activities of EROD and $ALDH_3$ in the liver, the present findings are in agreement with previous reports suggesting a non-coordinate regulation of these enzyme systems in the liver [10]. The doses needed to produce maximal induction are considerably higher for $ALDH_3$ than for CYP1A1 [10] as also supported by the present study. In the dose–response respect, the induction of $ALDH_3$ more closely parallels the appearance of the wasting syndrome in L–E rats. Moreover, at a non-lethal dose level (5 μg/kg), L–E rats may be slightly more sensitive to the induction of $ALDH_3$ than H/W rats. However, the induction is comparable between the rat strains at a dose lethal to L–E rats alone. This fact tends to exclude a major determinant role for liver $ALDH_3$ induction in the mechanism of acute toxicity of TCDD.

In the [^{3}H]TCDD distribution experiment, there were no differences between the strains at 5 μg/kg, but at 50 μg/kg there was two to four times greater accumulation of [^{3}H]TCDD in L–E rats. This phenomenon is probably related to the wasting syndrome which emerged in L–E rats at the higher dose. Although the retention of ^{3}H-activity was clearly greater in 50 μg/kg-treated L–E than H/W rats, the induction in L–E rats did not further increase in the liver nor in the brain as compared with that seen in H/W rats. On the other hand, one could ask the role of this kinetic divergence as a mediator of the strain difference. The notion that is without importance is supported by the finding that H/W rats may tolerate doses up to >7200 μg/kg without any dose-related acute mortality [12]. Secondly, toxicokinetic studies argued against the view that TCDD metabolism or disposition would account for this strain difference in TCDD lethality [32].

A peculiar feature in EROD as well as $ALDH_3$ induction in the brain is the regional specificity. Especially EROD activity was induced strikingly more in the olfactory bulbs than in other brain areas. It must be noted, however, that the constitutive activities were also higher in olfactory bulbs than in other areas. This finding is in line with reports indicating that olfactory bulbs have the highest brain mitochondrial AHH activity in rats [15] and in monkeys [33]. However, in monkeys there was no difference in EROD activity between olfactory bulbs and other brain areas when measured in 10,000 *g* supernatant fractions [33] as was done in the present study. This suggests that there may be species differences in the subcellular distribution of this metabolic activity. One plausible explanation for the greater induction of the enzyme activities in the olfactory bulbs could have been exceptionally high accumulation of TCDD therein. According to the [^{3}H]TCDD distribution experiment, this does not seem to be the case. Another explanation might be related to the Ah receptors which mediate the induction of CYP1A1, at least in the liver [5]. Specific clustering of Ah receptors in the olfactory bulbs could conceivably account for the greater sensitivity to EROD induction of this site as compared with the rest of the brain. Carlstedt-Duke [34] demonstrated that rat brain contains measurable amounts of TCDD binding. According to that report, the Ah receptor concentration in the brain was 20% of that in the liver. However, Gasiewicz and Rucci [35] attempted to measure Ah receptor from different brain regions but failed to find any binding.

The inhibition experiment clearly indicated that the TCDD-inducible portion of EROD activity in olfactory bulbs (and probably in other brain areas) is of CYP1A1 origin. This finding is not surprising keeping in mind that CYP1A1 is the only established subtype of cytochrome P450 induced by TCDD and 3-methylcholanthrene in extrahepatic tissues such as the lung, intestine, kidney and spleen [31, 36]. It is also important to note that since CYP1A1 induction is an Ah receptor mediated response [5], the brain is now the second target tissue for TCDD in which no difference could be discovered in Ah receptor function between the most TCDD susceptible and the most TCDD resistant rat strain, the same result being reported previously for the liver [11]. As for the brain, it should be remembered that in certain regions (such as the cerebellum) xenobiotic metabolizing enzyme systems mainly reside in glial cells, not in the neurons themselves [18].

Taken together, both EROD and $ALDH_3$ are induced in the brain by TCDD although the activities are much lower than in the liver. The induction in the brain is region specific with olfactory bulbs being

the most responsive area. As in the liver, the TCDD-induced brain EROD activity is associated with CYP1A1 in the brain. The induction of brain CYP1A1 does not seem to have a crucial role in determining strain susceptibility to the acute lethality of TCDD. Likewise, the induction of EROD or $ALDH_3$ in the liver appear to lack causal relationship with TCDD lethality.

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