EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON PLASMA AND TISSUE BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY IN THE MOST TCDD-SUSCEPTIBLE AND THE MOST TCDD-RESISTANT RAT STRAIN

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Summary

The salient sign of acutely lethal 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication in rats is hypophagia along with a prominent body weight loss. Endogenous opioid peptides have been implicated as modulators of food intake. In the present study, female rats of both the most TCDD-susceptible (Long-Evans [L-E]; LD50 9.8 μg/kg) and the most TCDD-resistant strain (Han/Wistar [H/W]; LD50 >7200 μg/kg) were exposed to a single dose of 50 μg/kg TCDD ip. This treatment is usually lethal within 1–6 weeks to all L-E rats and nonlethal to all H/W rats. The animals were killed at 1, 4 or 10 days after the treatment. β-Endorphin-like immunoreactivity (β-END-LI) was determined by a validated RIA method in the hypothalamus, pituitary, pancreas, duodenum and plasma. TCDD decreased plasma β-END-LI concentration by 24–37% at every time point of measurement in L-E rats alone. By contrast, feed-restricted controls exhibited an increase of similar magnitude on day 4. Pancreatic β-END-LI was also elevated in feed-restricted controls at that time point as compared with either the ad libitum control or TCDD group. TCDD appeared to shrink the pituitary gland in both strains by day 4. Pituitary weight was similarly lowered in TCDD-treated rats and feed-restricted controls at the last time point and this reduction was reflected in pituitary β-END-LI content. Thus, TCDD affects selectively plasma β-END-LI levels and this impact correlates with its lethality in these strains.

The major sign of the acute toxicity of the extremely potent environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), in laboratory animals is suppression of feed intake along with a striking body weight loss. Animals exposed to a lethal dose of TCDD do not die until they have lost a prominent portion (up to >50%) of their initial body weight, which takes 1–6 weeks postexposure (1). Though suggested to result from a serious disorder of body weight regulation (2), the biochemical and morphological lesions so far detected do not readily account for this wasting syndrome. Another characteristic feature of TCDD is a wide inter- as well as intraspecies variation in susceptibility. For example, in the rat the LD50 values span from 9.8–17.6 to >7200 μg/kg with...
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β-Endorphin is an endogenous opioid peptide which seems to play an as yet poorly defined role in the control systems for food intake and body weight (for reviews, see refs. 5–8). Central administration of β-endorphin stimulates feeding (9) and genetically obese mice (ob/ob) display elevated β-endorphin–like immunoreactivity (β-END-LI) levels in the ventromedial and dorsomedial hypothalamic nuclei (10) as well as in the pituitary (11,12). Pituitary β-END-LI may also be increased in rats with either genetically (fa/fa) (11) or experimentally (ventromedial hypothalamic lesion) induced obesity (13). In some studies, pituitary and/or hypothalamic β-END-LI has been found to increase in response to food deprivation (14,15) and, conversely, decrease in response to muscimol–stimulated feeding (15). However, there are also reports contradictory of the abovementioned ones, and the physiological relationship between this peptide and feeding is overall still obscure.

In addition to the hypothalamus and pituitary, β-END-LI has been detected in plasma and in certain peripheral organs. Plasma β-END-LI levels usually covary with those of ACTH and are elevated by stress states or adrenalectomy, and depressed by dexamethasone and hypophysectomy (16). Circulating β-endorphin may also be associated with ingestive processes as suggested by the findings that it increases together with increased appetite in several experimental situations (such as a short–term fast, 2-deoxyglucose–induced feeding, night time feeding, e.g., cases responsive to naloxone antagonism) in rats (17) and that both obese rats with hypothalamic lesions (13) and obese women (18) display elevated levels of it. In the pancreas, β-endorphin might stimulate insulin and glucagon secretion (19). Duodenal β-END-LI has been reported to be higher in 48 h fasted than control rats (20). That β-endorphin has discrete control systems in distinct organs is suggested by its differential alterations e.g. in obesity resulting from ventromedial hypothalamic lesion: in plasma and pancreas the levels deviate upwards but in duodenum downwards as compared with those in control rats (13).

A recent report implicated β-endorphin in the TCDD–induced wasting syndrome (21). TCDD was shown to initially increase (by 66% on day 1 postexposure) and then depress (to 39–49% of control values on days 2 and 3) hypothalamic β-END-LI concentration in Sprague–Dawley rats, while restricted feeding was without effect. This was potentially an extremely important finding, since it was one of the very first tenable explanations for the wasting. To confirm the finding, assess its relation to TCDD lethality and extend it to peripheral organs and plasma, the effects of TCDD on β-END-LI were compared in the present study in L–E and H/W rats.

**Materials and Methods**

**Animal husbandry**: Adult (10– to 12-week-old) female inbred L–E and outbred H/W rats weighing 196.8 ± 9.3 g and 208.7 ± 12.2 g (mean ± SD at the onset), respectively, were used. The rats were purchased from the National Laboratory Animal Centre, Kuopio, Finland, at the age of 4 weeks. They were housed singly in stainless steel wire–mesh cages and had free access (except for the feed restriction groups) to pelleted R3 rat feed (Ewos, Södertälje, Sweden) and tap water. The animal room was artificially illuminated with lights on from 07.00 hr to 19.00 hr. The ambient temperature in the animal room was 21.5±1.0°C and relative humidity 55±10%

**TCDD**: TCDD was over 99% pure as confirmed by gas chromatography–mass spectrometry. It was dissolved in corn oil (10 μg/ml) as described previously (22). The correctness of the concentration was verified by adding a small quantity of 3H–labelled TCDD (Cambridge Isotope Laboratories, Woburn, MA, USA) to the unlabelled substance, determining the radioactivity relative
Study design: On day 0, the rats were injected ip with 0 (corn oil 5 ml/kg) or 50 μg/kg TCDD. This dose of TCDD is usually lethal to all L-E rats, but nonlethal to all H/W rats. The rats were killed by decapitation on day 1, 4 or 10. Additionally, two groups of 6 female L-E rats served as feed restriction controls. They were administered corn oil on day 0, and were maintained thereafter on a predetermined feeding schedule based on feed intake records of female L-E rats treated with 50 μg/kg TCDD in our previous studies. One of these groups was intended to be killed on day 4 and the other one on day 10. However, 2 of the 6 rats in the latter group died on day 8 and since the remaining 4 were also extremely debilitated, they were euthanized at that time point. L-E rats pair-fed to their counterparts, treated with a lethal dose of TCDD, often develop fatal gastrointestinal ulcers in response to the feed deprivation (23). This phenomenon has been described in pair-fed Sprague-Dawley rats as well (24). TCDD treatment may confer protection against gastrointestinal erosion and ulceration by substantially promoting the plasma levels of gastrin (25-27), which acts as a mucoprotectant (28). The amounts of feed given daily to the restricted animals were as follows (day 0 through 7): 9.8, 8.4, 7.0, 5.6, 4.2, 0.7, 0.7 and 1.4 g.

Trunk blood was collected from the decapitated animals in plastic cups containing 1 ml of an EDTA solution (20 mg/ml). Plasma was separated and stored at -80 °C until analysis. Duodenum was excised and cleared of its contents by washing in saline. In addition, hypothalamus, pituitary and a piece of pancreas were rapidly removed from the carcasses. The tissues were frozen in liquid nitrogen and stored at the same temperature as the plasmas.

Analysis of β-END-LI: One ml of plasma was acidified with 0.2 ml 1 N HCl containing 1.6% glycine, applied to a Sep-pak C-18 Cartridge (Millipore Co., Bedford, MA, USA), washed with 0.1 N acetic acid and eluted with 60% acetonitrile in 0.1 N acetic acid. The eluates were evaporated, reconstituted with radioimmunoassay (RIA)-buffer and assayed by a validated β-endorphin RIA (29). The tissue samples were weighed and boiled in 4.5 parts (40 parts in the case of the pituitary gland) of destilled water (w/v) for 10 min. Then 4.5 parts (resp. 40 parts) of 4 M acetic acid was added and the sample was homogenized for 1 min with an Ultra-Turrax (Janke & Kunkel Co., Staufen, Germany). The homogenate was centrifuged for 20 min at 13,000 rpm, and the supernatant was lyophilized and reconstituted with RIA-buffer for the β-endorphin RIA. Recovery of the extraction was over 90% and the values were not corrected for that. The antiserum used in the β-endorphin RIA recognizes both β-endorphin and β-lipotropin which is devoid of opiate activity. In compliance with common practice, the results are yet given as β-endorphin equivalents.

Statistics: The data are shown as mean ± SD. Two-tailed Student's t-test for independent samples was used in cases where only two groups were to be compared. If there were more than two groups (e.g. the 4- and 10-day results from L-E rats), the data were statistically assessed by means of one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (with level of significance set at 0.05). In case of nonhomogeneous variances (assessed by Bartlett's and Cochran's tests), Kruskal-Wallis nonparametric one-way ANOVA together with two-tailed Mann-Whitney U test were employed. Comparisons between the strains were performed by three-way ANOVAs with strain, treatment (feed-restricted rats excluded) and time as the main factors.
**Results**

TCDD caused a striking body weight loss in L-E rats. At 10 days, the mean decline amounted to >20% of initial body weight (Fig. 1). By contrast, there was only a negligible effect in H/W rats with a 3% body weight loss by 10 days. The differential outcome between the strains was confirmed by ANOVA (strain x treatment x time interaction: p<0.001). The feed-restricted L-E rats lost slightly more weight by 4 days than the corresponding TCDD group. On day 8, when the remaining survivors of the feed-restricted rats had to be euthanized (see above), the decrease was significantly greater (attaining to almost 30%) than that in TCDD-treated L-E rats at 10 days.

TCDD-treated L-E rats exhibited a rapid and persistent reduction of plasma β-END-LI (Fig. 2). This change was statistically significant at every time point of measurement and varied in magnitude from 24 to 37%. The feed-restricted L-E rats showed a similarly large shift, but in the opposite direction, on day 4. The increase vanished by day 8. There was no departure from the control level at any stage in H/W rats.

Tissue concentrations of β-END-LI were not appreciably affected by TCDD (Table I). However, TCDD turned out to diminish the weight of the pituitary gland. This alteration became apparent by 4 days in both strains, although in L-E rats statistical significance was reached on day 4 in comparison with feed-restricted controls only (p=0.051 vs. *ad libitum* controls). At 8 days the feed-restricted L-E rats displayed pituitary atrophy as well. The smaller size of the pituitary along with its unchanged β-END-LI concentration resulted in a decreased total pituitary content of the peptide on day 10 in TCDD-exposed rats of both strains, and on day 8 in the feed-restricted L-E rats. The feed restriction group deviated from *ad libitum* controls by two additional parameters: the pituitary and pancreatic β-END-LI concentrations were elevated at 4 and 8 days, respectively.
Plasma β-END-LI. As there were no statistically significant differences among the control values at the 3 time points, they were pooled strainwise (n=15). Other conditions are as in Fig. 1.

Discussion

The major finding of the present study was a selective effect of TCDD on plasma β-END-LI. The plasma concentration of the peptide was reduced in the TCDD-susceptible L-E strain alone, at a dose that is usually 100% lethal. The alteration emerged at an early phase of TCDD intoxication and persisted over the entire observation period. There was no such effect in the TCDD-resistant H/W strain to which the dose of TCDD employed is always nonlethal. Concurrently, tissue β-END-LI levels remained unaffected even in L-E rats. It is also pertinent to note that despite the drastic disparity in TCDD sensitivity, there are surprisingly few biochemical or morphological parameters that would be dissimilarly influenced by TCDD in L-E and H/W rats (3,30). These facts imply a correlation, direct or indirect, between plasma β-endorphin and the acutely lethal action of TCDD. This contention is also supported by the outcome in feed restricted rats. Body weight changes revealed that the restriction was adequate. Yet those rats responded by increasing their plasma β-END-LI levels at 4 days, probably as a result of metabolic stress (16). The increase subsided by 8 days. This pattern of shifts resembles findings in man: total fasting elevated plasma β-END-LI over the first 5 days, but thereafter the change tended to level off by day 10 (31). The entirely differential outcome in TCDD-treated and feed restricted L-E rats advocates specificity of TCDD's impact; it did not clearly arise as a secondary change to hypophagia. The depressive effect of TCDD on feed intake is paralleled by changes in body weight (1). Previous studies had further convincingly proved that the dose of 50 μg/kg TCDD employed here has only a marginal influence on feed consumption in H/W rats (32). Inferred from the body weight data, TCDD did
<table>
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<th>Strain</th>
<th>Time</th>
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<th>Duodenal concentration (ng/g)</th>
<th>Pancreatic concentration (ng/g)</th>
<th>Hypothalamus weight (mg)</th>
<th>Hypothalamus concentration (ng/g)</th>
<th>Hypothalamus content (ng)</th>
<th>Pituitary weight (mg)</th>
<th>Pituitary concentration (μg/g)</th>
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<td>75.3±22.5</td>
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<td>ND</td>
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<td>67.1±9.31</td>
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<td>Control</td>
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<td>89.1±14.3</td>
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* Significantly different (p<0.05) from ad libitum control

† Significantly different (p<0.05) from TCDD-treated group

ND = not determined

ND = not determined

For the feed restriction group, 8 days

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not affect the feed intake of H/W rats more than to a modest extent in the present study either, thereby justifying the decision to omit feed-restricted controls from this strain.

β-Endorphin originates from the same large precursor molecule, pro-opiomelanocortin, as ACTH. The principal source of plasma β-endorphin is the anterior lobe of the pituitary, where the peptide is colocalated with ACTH (33). We have previously shown an equal dose of TCDD to that employed in this study to markedly elevate serum corticosterone values in L–E rats irrespective of time of day (30 and unpublished data). This, together with the reported 1.5-fold increase in circulating ACTH in Sprague–Dawley rats 2 days after the same dose of TCDD (34), implies an opposite effect by TCDD on plasma β-endorphin and ACTH. Although rare, this outcome is not without precedent. For example, acute restraint stress lowered plasma β-END-LI in sheep afflicted by moderate to severe cutaneous myiasis, but concurrently there was an increase in cortisol and ACTH (35). Part of the circulating β-END-LI stems from the neurointermediate lobe of the pituitary, with its secretion being controlled by systems distinct from those operating in the anterior lobe (36,37). Thus, TCDD could selectively inhibit the secretory activity of the neurointermediate lobe. Alternatively, TCDD might interfere with peptide processing in the anterior lobe (leading to altered ratios among ACTH, β-endorphin and β-lipotropin), or accelerate the elimination of β-endorphin/β-lipotropin from the body. These possibilities remain to be tested.

The lowered plasma β-END-LI levels were not reflected in the pituitary concentrations in L–E rats. An unexpected outcome was the atrophy of the pituitary gland. To our knowledge, this finding has not been reported before. Since it occurred in both strains and since it emerged earlier in TCDD-treated L–E rats than in their feed restricted counterparts, it appears to represent a primary effect of TCDD. A histomorphological approach would have been required to ascertain whether the discrete lobes of the pituitary were uniformly affected. The shrinkage resulted in a diminished total content of β-END-LI per pituitary which may have contributed to some extent to the low plasma β-END-LI values in L–E rats at the last time point. However, plasma β-END-LI was reduced already on day 1 in this strain but remained unaffected over the whole period in H/W rats despite their decreased pituitary weights thereby indicating that there must be other, more important mechanistic factors involved.

TCDD was recently reported to affect hypothalamic β-END-LI by first increasing (day 1) and then decreasing (day 2) the levels in male Sprague–Dawley rats (21). In the present study, no statistically significant departures from control levels were detected in the hypothalamus over the 10–day observation period. Since the dose of TCDD was the same in both studies, the discrepancy may be related to differences in the strain and/or gender of the rats. Assessed together, however, these two studies argue for endogenous opioid peptides as targets for TCDD. In support of this assertion, we have previously reported that H/W rats exposed to a 20 times higher dose than the one employed here, show an attenuated response to naloxone inhibition of fast-induced feeding (38) and do not respond by eating to 2-deoxyglucose (a phenomenon mediated by endogenous opioids) (39). It is also noteworthy that in the hamster, which is highly resistant to the acute lethality of TCDD, endogenous opioids do not appear to function as appetite inducers (5).

In conclusion, we found an association between reduced plasma β-END-LI following TCDD exposure and TCDD susceptibility in rats. The nature of the relationship as well as the mechanism(s) await further studies.
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References