TCDD Reduces Selectively Plasma β-Endorphin Levels In TCDD-Susceptible Long-Evans Rats But Not In TCDD-Resistant Han/Wistar Rats

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The predominant sign of the acute toxicity of TCDD and related compounds is a conspicuous suppression of feed intake accompanied by a prominent loss of body weight. The pathogenesis of this wasting syndrome is still unknown although it may involve a serious disorder of the regulatory systems for body weight and appetite[1].
Endogenous opioid peptides, foremost β-endorphin, appear to be important modulators of ingestive behavior[2]. Central administration of β-endorphin stimulates feeding[3] and, conversely, the nonselective opioid antagonist naloxone effectively inhibits various forms of eating (e.g. night time feeding and feed deprivation-induced feed consumption) in rats[4,5]. Hypothalamic, pituitary and/or plasma β-endorphin concentrations are also elevated in a number of genetic or experimental obesities in rats as well as in clinical obesity in humans[6,7]. A recent report implicated β-endorphin in the TCDD-induced wasting syndrome[8]. 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-endorphin-like immunoreactivity (β-END-LI) in Sprague-Dawley rats, while restricted feeding was without effect. The present study was designed to test the generality and reproducibility of the finding in the most TCDD-susceptible (Long-Evans [L-E; LD50 9.8 μg/kg]) and the most TCDD-resistant (Han/Wistar [H/W; LD50 >7200 μg/kg) rat strains[9]. Furthermore, the selectivity of the possible effect was addressed by analysing β-END-LI concurrently in several tissues and plasma.

Adult (10- to 12-week-old) female L-E and H/W rats 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%.
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. 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. Hypothalamus, pituitary, a piece of pancreas and duodenum were rapidly removed from the carcasses, frozen in liquid nitrogen and stored at the same temperature as the plasmas.
One ml of plasma was acidified with 0.2 ml 1 N HCl containing 1.6% glycine, applied to Sep-pak C-18 Cartridge (Millipore Co., Bedford, MA), 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[10]. 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 Ultraturrax. The homogenate was sentrifuged 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-lipotrophin which is devoid of opiate activity. In compliance with common practice, the results are yet given as β-endorphin equivalents.

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 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 in either strain (data not shown). 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.

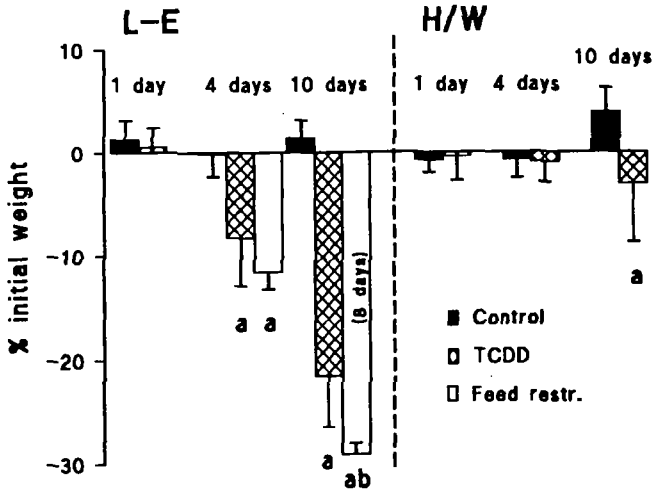

FIG. 1.
Body weight change as a function of time. The letters denote statistically significant differences ($p<0.05$) vs. control (a) or vs. TCDD (b). Mean ± SD of 6 rats.

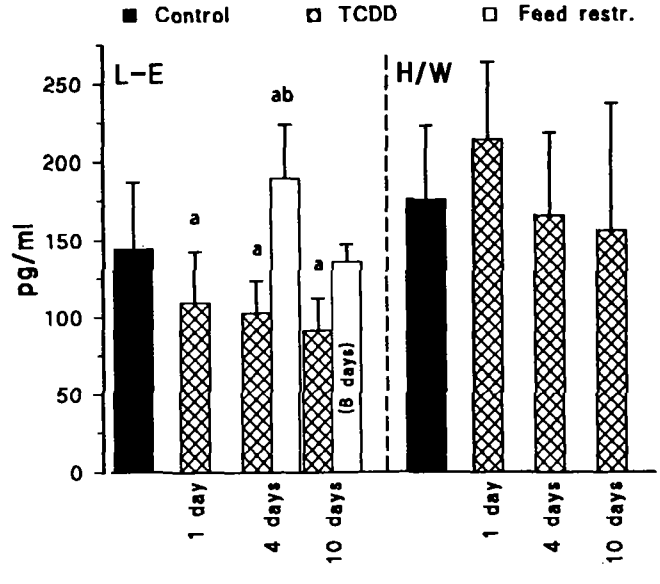

FIG. 2.
Plasma β-END-LI. As there were no statistically significant differences among the control values at the 3 time points, they were pooled strainwise (n=18). Other conditions are as in Fig. 1 (feed restriction group, day 8: data from 4 survivors).

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. Concomitantly, tissue β-END-LI levels remained unaffected even in L-E rats. 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. The 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.
In the present study, we could not replicate the recently reported effects of TCDD on hypothalamic β-END-LI found in male Sprague-Dawley rats[8]. 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. The elucidation of their detailed role in TCDD toxicity requires further studies.

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