Tetrachlorodibenzo-p-dioxin Alters Rat Hypothalamic Endorphin and Mu Opioid Receptors

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BESTERVELT, L. L., C. J. NOLAN, Y. CAI, P. MAIMANSOMSUK, C. A. MOUSIGIAN AND W. N. PIPER. Tetrachlorodibenzo-p-dioxin alters rat hypothalamic endorphin and mu opioid receptors. NEUROTOXICOL TERATOL 13(5) 495--497, 1991.—The present study was undertaken to assess if hypothalamic β-endorphin (βE) and/or brain mu opioid receptors are associated with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) (50 μg/kg)-induced hypophagia and body weight decline in rats. Hypothalamic βE concentrations were initially increased to 166% of controls on day 1, and then were depressed to 39% and 49% of control values on days 2 and 3, respectively. Brain mu opioid receptor number was increased 60% in TCDD-treated rats at day 3 without a change in the binding affinity. Food-restricted rats did not exhibit changes in hypothalamic βE concentrations or brain mu opioid receptor number. These results indicate that TCDD causes early perturbations in hypothalamic βE concentrations and brain mu receptor number, which may contribute to the mechanisms by which TCDD leads to decreased food intake and progressive weight loss. TCDD is an undesired by-product produced during the synthesis of various chlorinated phenolic compounds, incineration of waste, and bleaching of paper pulp. There is considerable concern of potential health hazards associated with the release of this substance into the environment. Animals exposed to a single, oral dose of TCDD typically begin to exhibit reduced food intake after two to three days that is associated with progressive weight loss, and mobilization of adipose tissue stores (5, 7, 9). Furthermore, loss of appetite and decreased body weight have been reported in humans exposed to TCDD in the work place (16, 17, 19). The TCDD induced anorexia may be due to a specific effect on the regulatory systems of food intake. The regulation of food intake and appetite is an extremely complex process which involves numerous peptides and hormones (10,12). The hypothalamus has been considered to play a central role in this regulation (11). The hypothalamus maintains the nutritional homeostasis of the organism by activating or deactivating the food-seeking behaviors of the animal. It is known that endogenous opioid peptides (EOP) such as βE play a physiological role in appetite and regulation of food intake (2, 6, 13). Different areas of the hypothalamus have been associated with EOP mediated modulation of eating. The hypothalamus, like the pituitary, contains proopiomelanocortin (POMC), the precursor molecule for the opioid βE. βE, a by-product of the precursor POMC peptide, is present in high concentrations in the hypothalamus (10). It has been suggested that decreased hypothalamic βE concentration is a mechanism for the down regulation of feeding behavior to conserve energy during periodic food shortages (4). It has also been shown that opioid receptor receptor blockade (i.e., antagonist binding) reduces food intake and body weight (2, 6, 13). The possibility exists that TCDD intoxication may affect levels of EOP and their receptors, which could have a profound impact on the regulation of feeding behavior. Thus this study was performed to assess if hypothalamic βE and its brain receptor (μ) are associated with TCDD-induced anorexia and body weight decline.

METHOD

TCDD (50 μg/kg) was administered in a single, oral dose to adult, male Sprague-Dawley rats (200--220g). This is a dose less than the reported LD₅₀ for adult, male Sprague-Dawley rats (60

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RESULTS

Body weights did not differ at days 1 and 2 for control, food-restricted control or TCDD groups. At day 3, body weights of TCDD-treated animals (196 ± 5) were significantly lower than control (223 ± 4) or food-restricted control (220 ± 6) groups, which did not differ from each other. Rat hypothalamic βE concentrations were initially found to be significantly higher than controls at day 1, and then were significantly depressed at days 2 and 3 following the administration of TCDD (Fig. 1). Hypothalamic βE levels were increased to 166% of controls on day 1, and then were significantly depressed at days 2 and 3 following the administration of TCDD. The effects of treatment were due to TCDD and not to a decrease in food intake. All animals were maintained on a controlled light cycle (6:00 a.m. lights on; 6:00 p.m. lights off), with restricted access to minimize environmental disturbances to the rats for the duration of the experiment. Rats were killed (9:00 a.m.) by decapitation, their brains rapidly removed, and hypothalamic blocks dissected with the following limits: cuts were made posterior to the optic chiasm, anterior to the mammillary bodies and through the lateral hypothalamic sulci, with a depth of 2 mm. The hypothalami were frozen in liquid nitrogen, lyophilized and extracted in 0.1 N HCl (15). Hypothalamic extracts were analyzed for βE content using a radioimmunoassay kit obtained from INCSTAR (Stillwater, MN). The sensitivity of the assay was 10 pg/ml. The data were statistically evaluated by analysis of variance (p<0.05) with differences between means evaluated by Tukey’s Test (p<0.05).

Membranes from rat cerebrum were prepared and mu opioid receptor binding assays were performed (3). The binding-assay reaction consisted of 190 μl of membrane suspension (approximately 0.6 mg/ml protein), 20 μl of distilled H2O, 25 μl of either distilled H2O or excess (2 μM) (D-Ala2, NMe-Phe4, Glyol)-Enkephalin (DAGO), and 25 μl of the appropriate concentration of [3H]-DAGO (0.125–10 nM) in 8 ml polypropylene tubes. The final volume of the assay was 260 μl. Specific binding of the radioligand is defined as the difference between binding in the absence and presence of an appropriate excess of DAGO. After incubation for 80 minutes at 25°C (reflecting binding equilibrium), the samples were filtered through glass fiber filters (GF/C). The filtered samples were washed with ice cold 50 mM Tris-HCl, pH 7.4, and placed into polyethylene counting vials. After addition of 1 ml absolute ethanol followed by 10 ml of biodegradable scintillation fluid, vials were subjected to liquid scintillation counting. Receptor number (Bmax) and binding affinity (Kd) were determined by Scatchard analysis (18). Protein concentration was determined by the method of Lowry and coworkers (8). The data were statistically evaluated by analysis of variance (p<0.05) with differences between means evaluated by Tukey’s Test (p<0.05).

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opioid peptides at their binding site (2, 6, 13). A characteristic onists reduce food intake and body weight by displacement of tion of feeding behavior to conserve energy during periodic food shortages (4). It has also been shown that opioid receptor antag- in hypothalamic 13E levels is a mechanism for the down regula- tion 210:1271-1272; 1980.

REFERENCES