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TCDD-Induced Hypophagia Is Not Explained by Nausea

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POHJANVIRTA, R., M. UNKILA AND J. TUOMISTO. *TCDD-induced hypophagia is not explained by nausea.* PHARMACOL BIOCHEM BEHAV **47**(2) 273-282, 1994.—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most potent known anorexigens with an unestablished mechanism of action. In the present study, the role of nausea in TCDD-induced hypophagia was assessed by a battery of behavioral (conditioned taste aversion [CTA], kaolin consumption, protein selection), biochemical (plasma oxytocin), and antiemetic drug intervention (trimethobenzamine, metoclopramide) approaches. Moreover, both the most TCDD-susceptible (Long-Evans [L-E]; IP LD50 ~10 μg/kg) and the most TCDD-resistant (Han/Wistar [H/W]; IP LD50 >3000 μg/kg) rat strains were employed in the experiments. L-E rats were exposed to a lethal dose of TCDD (50 μg/kg), whereas H/W rats were treated with high but nonlethal doses (50 or 1000 μg/kg). TCDD produced a positive CTA response in H/W rats alone. These animals also increased their kaolin consumption more than L-E rats of either gender after TCDD exposure. TCDD decreased the proportional intake of energy from high-protein diet in female L-E rats, but tended to increase it in male L-E and H/W rats. TCDD did not affect plasma oxytocin concentration by itself, but potentiated the elevation caused by the positive control compound, LiCl, in L-E rats on day 8. Neither antiemetic tested had any detectable influence on TCDD-induced wasting. These findings imply that the degree of nausea elicited by TCDD in the rat depends on strain and gender. However, nausea has only a minor, if at all, causal role in the lethal wasting syndrome characteristic of this compound.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin TCDD Acute toxicity Anorexia Hypophagia Body weight Nausea Pica Oxytocin Macronutrient selection Conditioned taste aversion Antiemetics Trimethobenzamine Metoclopramide

THE common environmental trace contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most potent anorexigens known. A single dose in the μg/kg range may lead to a rapid and irreversible decline of feed intake in rats. The accompanying body weight loss, which can amount to >50% of initial body weight over one to six weeks before death ensues, has been termed "wasting syndrome" (16). A single sublethal dose in turn may bring about permanent inhibition of body weight gain. These growth-stunted rats also actively defend their abnormally low body weights against external manipulations (changes in the caloric density or palatability of diet, feed restriction, etc.) (26), suggesting a specific effect of TCDD on body weight regulatory systems. Despite extensive studies, no histopathological or biochemical lesion has so far been detected that could account for the TCDD-induced wasting syndrome. The general behavior of TCDD-treated rats also remains intact (27). However, especially in view of the possible future use of TCDD as a pharmacological tool for studies on ingestive behavior, it would still be of paramount importance to make sure that the anorexia does not merely arise from general malaise or nausea.

The contribution of nausea to TCDD anorexia has attracted surprisingly little attention. This may result from both the general difficulty in demonstrating nausea in a species unable to vomit (rat) and the special problems related to TCDD. The temporal pattern and duration of TCDD-induced hypophagia differ notably from those of most other anorexigens. The suppression of feed consumption is progressive over the first week after a single treatment (16). This characteristic feature tends to render inapplicable some of the behavioral methods for detecting nausea [e.g., the approach suggested by Morley and coworkers (2), which relies on the differential influence of food deprivation on the potency of physiological satiety factors vs. nausea-producing agents].

However, there are still several ways to assess the degree of visceral distress in TCDD-exposed rats. A widely applied method is conditioned taste aversion (CTA), in which a distinct flavor (such as saccharin) added to drinking water is

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paired with exposure to the test substance. The extent of visceral illness produced correlates with aversion to the solution when offered subsequently (7).

Another method originally described (13) and later thoroughly characterized by Mitchell and his associates is based on the teleologically sound assumption that rats must have developed an alternative strategy to make up for their inability to vomit. In cases of visceral malaise, they resort to a specific form of pica, geophagia (eating clay or, under laboratory circumstances, kaolin). By way of the resultant adsorption of toxins, geophagia can relieve gastrointestinal discomfort under natural conditions. Numerous studies by Mitchell and others have confirmed that rats do reliably increase their kaolin intake in response to not only diverse chemical but also physical (such as rotation) sources of nausea (10–13). Furthermore, rats with conditioned aversion to saccharin engage in kaolin consumption when forced to drink saccharin solution (14).

A biochemical index of nausea in rats was recently introduced by Verbalis et al. (33). They discovered that a treatment with emetic model compounds, such as apomorphine, $CuSO_4$, and LiCl, led to a dose-dependent increase in the plasma concentration of oxytocin. Although other forms of stress (such as immobilization, foot shock, and swimming) also enhanced oxytocin secretion, the increase was less pronounced, amounting maximally to 25–30% of that observed after $CuSO_4$.

The proportional intake of separately offered macronutrients after exposure to a chemical may also give insight into the possible emetic properties of the compound. It has been reported that compounds causing gastrointestinal distress reduce energy consumption primarily by suppressing protein intake, whereas putative candidates for physiological mediators of satiety affect carbohydrate and fat more than protein intake (30,31).

A direct way to assess the importance of nausea in hypophagia induced by either chemical or physical agents is to eliminate it by means of antiemetics. Trimethobenzamide is a highly effective antiemetic drug acting at the chemoreceptor trigger zone in the area postrema (4). Previous studies have found that it prevents the hypophagic response of rats to acetyl salicylate (9) and attenuates LiCl-induced CTA response to saccharin (3). Metoclopramide in turn is one of the most widely used antiemetics in clinical medicine today. Although having central effects as well, it is thought to exert its antiemetic action mainly by synchronizing the motility of the upper gastrointestinal tract (8).

Our laboratory has established a remarkable disparity in susceptibility to the acute lethality of TCDD between two rat strains. The Long–Evans (Turku AB; L-E) strain is the most TCDD-sensitive and Han/Wistar (Kuopio; H/W) the most TCDD-resistant rat strain with IP LD50 values of ~10 and >3000 μg/kg, respectively (18) [for background information on the substrains, see (20)]. Gender does not play a major part in the susceptibility (17,23). Numerous studies with these strains have led us to conclude that the parameters correlating best with TCDD sensitivity appear to be feed intake and body weight gain. Although both strains initially decrease feed consumption after TCDD exposure, the suppression is reversible (in one to two weeks) in H/W but not in L-E rats [see (28) for review]. In the present study, these strains were exploited as a probe in assessing the contribution of nausea to the acute toxicity of TCDD by the methods described above. Female and male L-E rats were exposed to 50 μg/kg TCDD, which is lethal to all animals of this strain. For H/W rats, a high but nonlethal dose of TCDD (1000 μg/kg) was employed for comparative purposes.

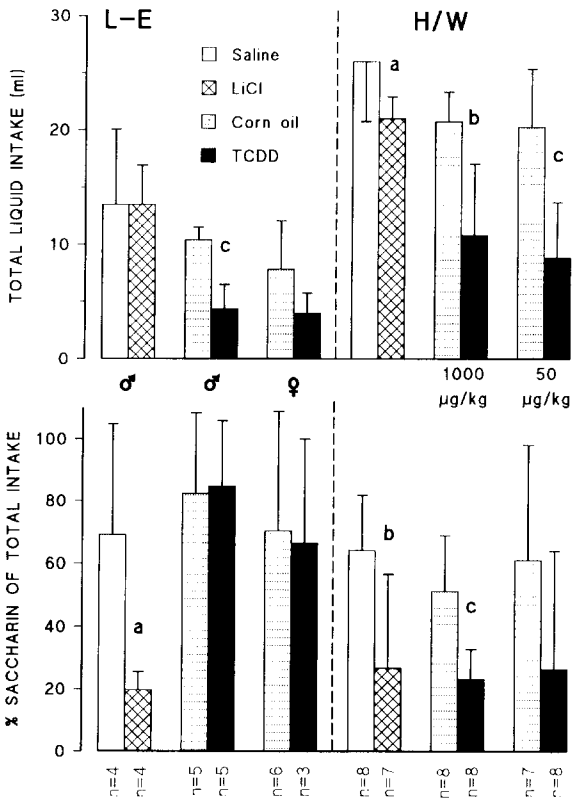

FIG. 1. The CTA response of male and female L-E rats as well as male H/W rats to IP saline (2 ml/kg), LiCl (84.8 mg/kg), corn oil (5 ml/kg), or TCDD (L-E, 50 μg/kg; H/W, 50 or 1000 μg/kg) three days after single conditioning. The upper panel shows the 3-h liquid intake (water plus saccharin), and the lower depicts saccharin intake relative to the total consumption. Mean ± SD. The letters denote statistically significant differences vs. control at the level of <0.05 (a), ≤ 0.01 (b), or ≤ 0.001 (c). The low group sizes for TCDD-treated L-E rats are due to the frequency of subjects with no liquid intake at the test.

METHOD

Animal Care

L-E and H/W rats of both genders were purchased from the National Laboratory Animal Centre, Kuopio, Finland. The rats were usually about 10 weeks old at the onset of the study; however, in the oxytocin experiment and the trial with pelleted kaolin they were somewhat older (15–19 weeks). The rats used for oxytocin analysis were housed in standard stainless steel wire-mesh cages in groups of five to six rats. In the kaolin and CTA experiments, the rats were kept singly in large stainless steel wire-mesh cages with a specially designed movable front wall. This wall was made of Plexiglas and contained three feeding tunnels leading to feed cups, and holders for two drinking bottles [for further details of the cages, see (19)]. The rats exploited for the intervention study with trimethobenzamide or metoclopramide were housed singly in plastic metabolic cages (Tecniplast® 7700). In these metabolic cages the rats had free access to powdered R3 rat feed (Ewos, Södertälje, Sweden) and tap water. In the oxytocin trial, the feed provided was pelleted R3. In the kaolin experiments the rats had a free choice of three diets all the time: R3, high-protein diet (R409, Ewos; protein content 77.5% [for thorough com-

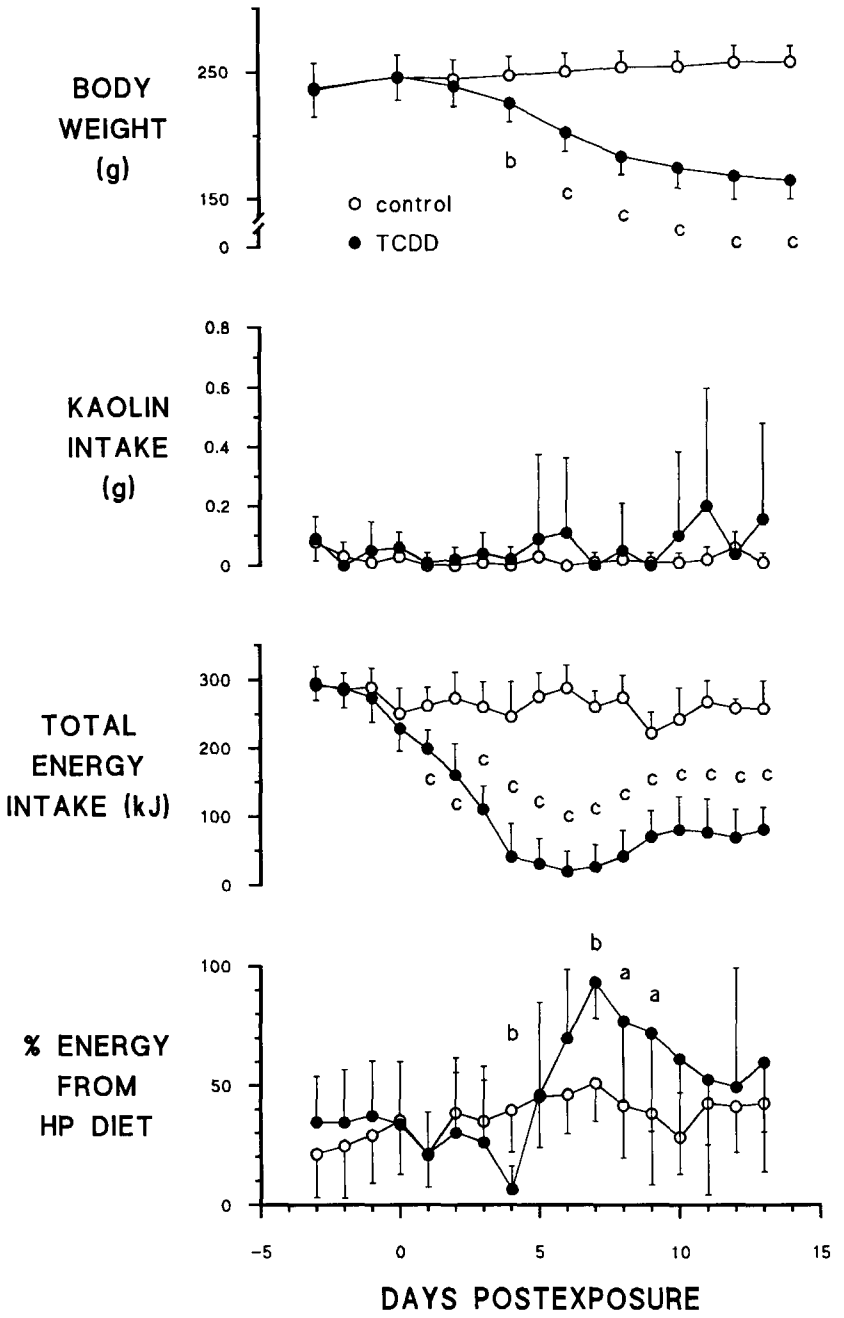

FIG. 2. Effect of TCDD (50 μg/kg) on body weight, kaolin intake, total energy consumption (chow plus high-protein diet), and proportional intake of the high-protein diet in male L-E rats (mean ± SD; n = 10). The rats had simultaneous access to powdered varieties of kaolin, chow, and R409 high-protein diet. TCDD was administered IP on day 0. The letters denote statistically significant differences vs. control at the level of <0.05 (a), ≤ 0.01 (b), or ≤ 0.001 (c).

position, see (19)]), and kaolin. These diets were in most cases powdered, but female L-E rats were also tested with pelleted forms of the diets. To this end, 99 parts of pharmaceutical grade kaolin or the high-protein diet was mixed with 1 part of acacia (w/w) in water to form a thick paste which was extruded through a syringe on a clean surface and dried at 120°C. After drying, the strands were broken into aggregates of similar size to R3 pellets. Finally, the rats employed for the CTA test were provided powdered R3 feed (in one of the three feeding units only). The animal room was artificially illuminated with lights on from 0700 to 1900. The ambient temperature in the animal room was 21.5 ± 1.0°C (during the CTA tests the temperature accidentally decreased to 18°C for two days) and relative humidity 55 ± 10%.

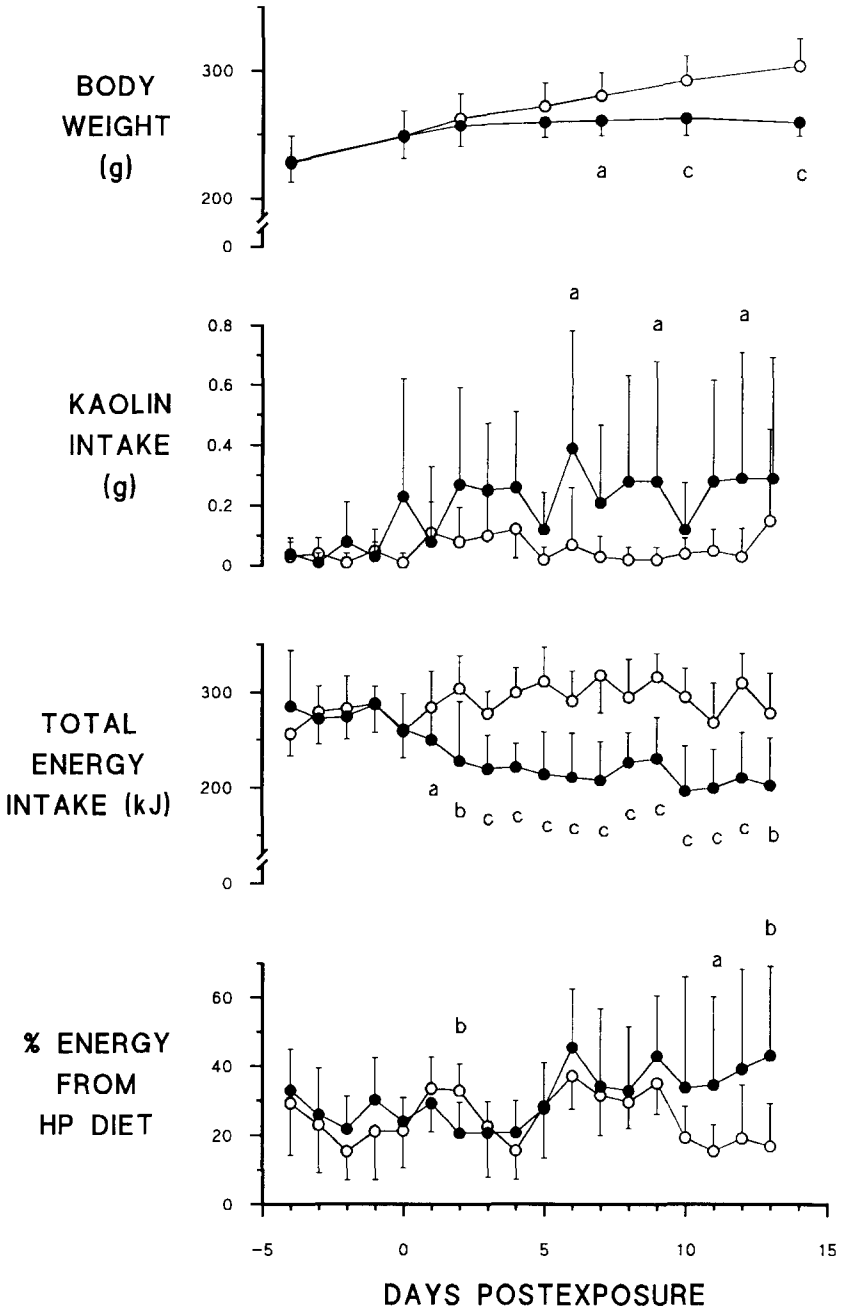

FIG. 3. Effect of TCDD (1000 μg/kg) on body weight, kaolin intake, total energy consumption (chow plus high-protein diet), and proportional intake of the high-protein diet in male H/W rats (mean ± SD; $n = 10$). The rats had simultaneous access to powdered varieties of kaolin, chow, and R409 high-protein diet. TCDD was administered IP on day 0. ○ Control, ● TCDD. The letters denote statistically significant differences vs. control at the level of <0.05 (a), ≤ 0.01 (b), or ≤ 0.001 (c).

Chemicals

TCDD (purity >99%) was dissolved in corn oil as described previously (21). The concentrations were adjusted in such a manner that the volume administered was 5 ml/kg IP for both L-E and H/W rats, although the doses differed substantially. TCDD concentrations were verified by adding small quantities of ^{3}H-labelled TCDD (Cambridge Isotope Laboratories, Woburn, MA) to the unlabelled substance, determining the radioactivity relative to TCDD concentration, and using this ratio for TCDD analysis in the final solutions. Trimethobenzamide (Tigan) was obtained from Sigma (St.

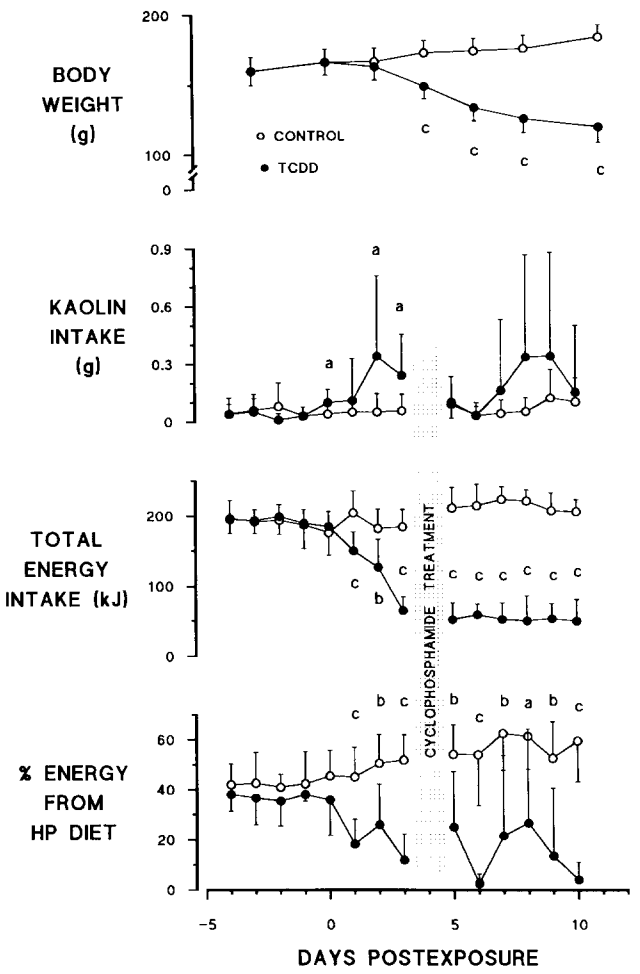

FIG. 4. Effect of TCDD (50 μg/kg) on body weight, kaolin intake, total energy consumption (chow plus high-protein diet), and proportional intake of the high-protein diet in female L-E rats (mean ± SD; $n = 10$). The rats had simultaneous access to powdered varieties of kaolin, chow, and R409 high-protein diet. TCDD was administered IP on day 0. The letters denote statistically significant differences vs. control at the level of <0.05 (a), ≤ 0.01 (b), or ≤ 0.001 (c). On day 4, the rats were additionally treated with cyclophosphamide or saline (see Table 1).

Louis), metoclopramide (Primperan®) from H. Lundbeck A/S (Valby, Denmark), and cyclophosphamide (Syklofosfamidi®) from Orion-Lääkefarmos (Turku, Finland).

Experimental Design

CTA. Twenty-eight male and 20 female L-E as well as 48 male H/W rats were adapted to the cages for three days. Over that period, water was available from two bottles. The rats were then kept without water for 24 h to ensure drinking of a 0.15% saccharin solution (a few rats refused to drink and were excluded from the experiment), which was offered for exactly 1 h before IP exposure to TCDD (L-E, 50 μg/kg; H/W, 1000 μg/kg), 1 M LiCl (2 ml/kg; 84.8 mg/kg), corn oil (5 ml/kg), or NaCl (2 ml/kg). After the injections, water was substituted for saccharin. The next day the position of the water bottle in the cage was changed. Starting on day 2 morning, the rats were water-deprived again for 24 h. They were then concomitantly presented with water and the 0.15% saccharin solution (the rats were allowed to lap four to five times from both bottles before these were placed available). The 3-h intake of both liquids was measured. Only rats with total consumption of ≥2 ml were included in the final results.

Pica and protein selection. Twenty rats were used in each trial. The rats were allowed to adapt to the cages for at least seven days prior to TCDD exposure. On day 0, the rats were assigned to two groups with matched body weights. One group was administered TCDD at 50 (L-E) or 1000 (H/W) μg/kg IP, and the other received corn oil. The order of the three diets available in the feeding units was regularly rotated. Daily intake recordings were corrected for spillage. The rats were killed 11 or 14 days after exposure.

Since the kaolin response turned out to be less pronounced in male L-E than male H/W rats, the experiment was repeated in female L-E rats. These animals were additionally subdivided to groups of five on day 4. Half the rats of both TCDD and control groups were then treated with 50 mg/kg cyclophosphamide (20 mg/ml in sterile water) IP, and the rest with saline. This dose of cyclophosphamide has previously been shown to augment kaolin consumption in rats (13).

When it appeared that female but not male L-E rats markedly decreased their consumption of the high-protein diet after TCDD exposure, another set of female L-E rats was tested with pelleted diets. In this case, however, no extra treatment was incorporated in the scheme.

Oxytocin. A total of 48 and 30 female L-E and H/W rats, respectively, were treated IP with 50 (L-E) or 1000 μg/kg TCDD (H/W) or corn oil on day 0. On days 2 (for L-E rats alone) and 8, half the animals in the TCDD and control groups were administered IP LiCl (the same dose as in the CTA test), while the rest were dosed with saline. Exactly 30 min afterwards, the rats were killed by decapitation and trunk blood was collected in EDTA-containing (20 mg) plastic dishes. Plasma was separated by centrifugation and stored at −80°C until analysis.

Drug intervention.

Trimethobenzamine. Twenty female L-E rats were placed in metabolic cages two days before the onset of the experiment. On day 0, the rats were sedated with the highly selective α_2-agonist medetomidine (Domitor®, Orion-Lääkefarmos; 750 μg/kg SC). An Alzet® osmotic minipump, filled with either saline or trimethobenzamide (300 mg/ml), was placed SC in the nuchal area. The pump had a pumping rate of 0.462 μl/h (about 3.3 mg/rat/day; adjusted to the initial body weights, 19 mg/kg/day) and a working time of 14 days. After the pumps were installed, the rats of the trimethobenzamine groups were given a loading dose of 5 mg/kg SC; controls received the same volume (1 ml/kg) of saline. Half the trimethobenzamine- and saline-dosed animals were then injected IP with TCDD (50 μg/kg), and the rest with corn oil (5 ml/kg). The rats were eventually awakened by administering the α_2-antagonist atipamezole (Antisedan®, Orion-Lääkefarmos) IM (0.5 mg/rat; 2.9 mg/kg). The feed intake of the rats was recorded daily, and body weight every two days.

Metoclopramide. The experimental design was mainly the same as with trimethobenzamine. However, to compensate for the rapid elimination of metoclopramide in the rat (1), one-week varieties of Alzet® osmotic minipumps with a higher delivery rate (0.97 μl/h) were used. Additionally, two doses of metoclopramide were tested. The lower concentration was

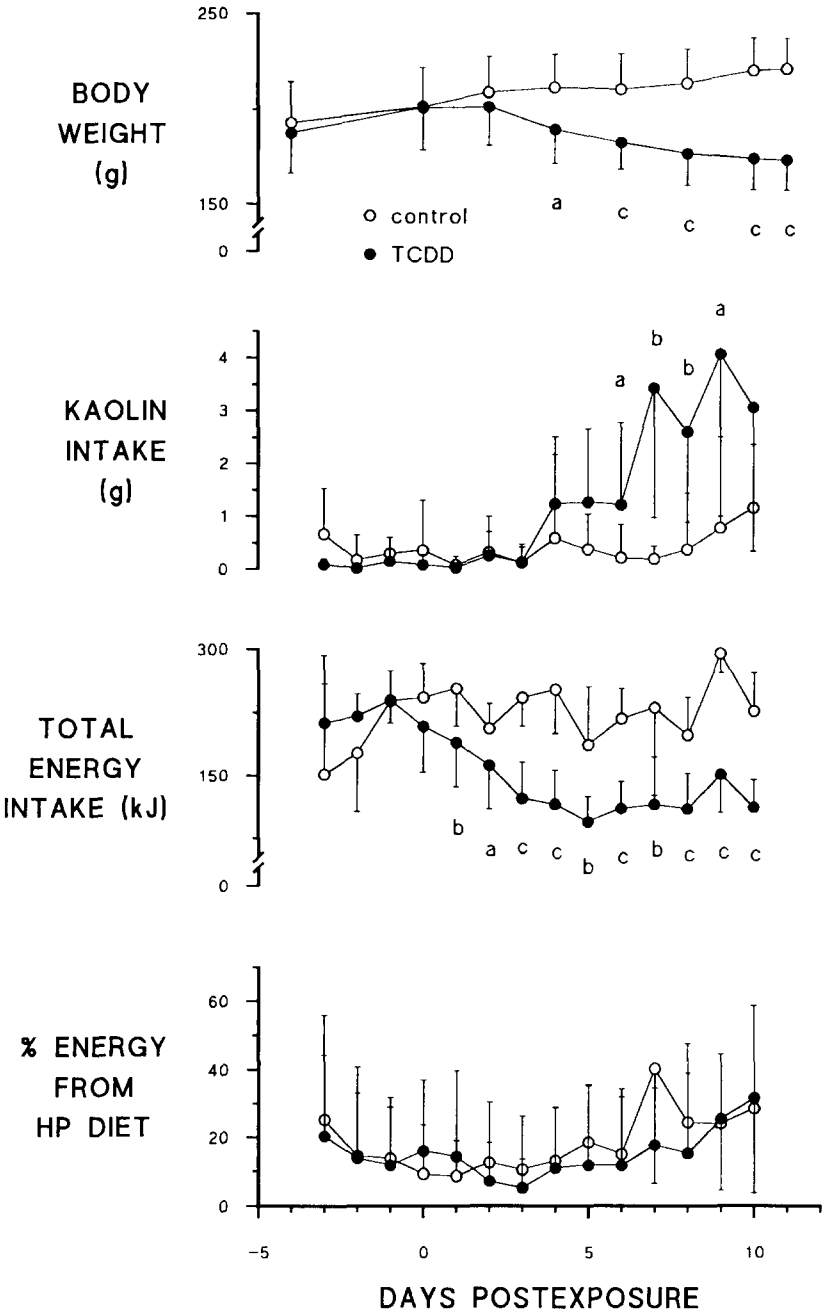

FIG. 5. Effect of TCDD (50 μg/kg) on body weight, kaolin intake, total energy consumption (chow plus high-protein diet), and proportional intake of the high-protein diet in female L-E rats (mean ± SD; n = 10). The three diet varieties were offered in a pelleted form (see Method). The rats had simultaneous access to powdered varieties of kaolin, chow, and R409 high-protein diet. TCDD was administered IP on day 0. The letters denote statistically significant differences vs. control at the level of <0.05 (a), ≤ 0.01 (b), or ≤ 0.001 (c).

5 mg/ml (resulting in a dose of about 0.12 mg/rat/day), and the higher one 100 mg/ml (2.3 mg/rat/day). As with trimethobenzamine, the rats of the metoclopramide groups received a loading dose of 10 mg/kg SC right after the insertion of the pumps.

Oxytocin Analysis

One-milliliter aliquots of the EDTA plasma samples were extracted with Sep-pak C-18 Cartridges (Millipore Co., Bedford, MA) according to the manufacturer's instructions. After

TABLE 1

INTAKE OF ENERGY, KAOLIN, AND PROPORTIONAL ENERGY FROM THE HIGH-PROTEIN DIET OVER 24 h IN FEMALE L-E RATS TREATED WITH TCDD AND/OR CYCLOPHOSPHAMIDE

Treatment 1	Treatment 2	Energy (kJ/day)	Kaolin (g/day)	% Protein
Corn oil	NaCl	195.5 ± 27.9	0.02 ± 0.05	46.2 ± 9.5
Corn oil	Cyclophosphamide	156.1 ± 18.5*	0.36 ± 0.26*	46.4 ± 3.9
TCDD	NaCl	45.8 ± 27.3	0.22 ± 0.29	27.3 ± 32.8
TCDD	Cyclophosphamide	22.2 ± 14.0	0.38 ± 0.63	22.9 ± 27.6

The rats were exposed to TCDD (50 μg/kg) or corn oil four days before cyclophosphamide (50 mg/kg) or saline dosing. Mean ± SD (n = 5). *Significantly different from the corresponding saline group ($p < 0.05$).

evaporation, the specimens were dissolved in assay buffer and measured for oxytocin by radioimmunoassay using a specific oxytocin antiserum (kindly provided by Dr. Lasslo Ferenc) and ^{125}I-labelled oxytocin. The recovery was 76%, and inter- and intra-assay variations were <15% and <10%, respectively. In reverse-phase high-performance chromatography (HPLC), the endogenous immunoreactivity moved like synthetic oxytocin.

Statistics

All data are given as mean ± SD, if not otherwise indicated. The CTA results were evaluated by Student's two-tailed t test for independent samples. In the kaolin experiments, body weight and energy intake data were also analysed by the t test. The Mann-Whitney U test was employed for kaolin and proportional protein intake data due to the frequency of nonhomogenous variances. The cyclophosphamide (pica experiment) and oxytocin data were subjected strainwise to two-way analysis of variance (ANOVA).

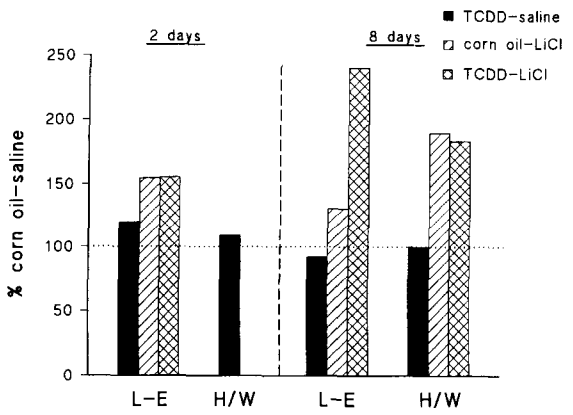

FIG. 6. Plasma oxytocin concentrations in female L-E and H/W rats treated with TCDD and/or LiCl relative to corn oil-saline controls. TCDD (50 or 1000 μg/kg for L-E and H/W rats, respectively) was administered IP on day 0. On days 2 or 8, the rats were dosed with 1 M LiCl (2 ml/kg) 30 min before decapitation. The control values (pg/ml) were day 2, 38.1 ± 8.4 (L-E), 40.3 ± 7.1 (H/W); day 8, 67.1 ± 21.0 (L-E), 40.3 ± 9.9 (H/W). N = 5-6 for L-E and 4-5 for H/W rats.

RESULTS

CTA

In agreement with previous findings (19), the total liquid consumption of L-E rats was only about half the volume drunk by H/W rats, and TCDD exposure diminished drinking by ~50% in both strains (Fig. 1, upper panel). Administration of LiCl resulted in a statistically significant decrease in the selection of saccharin versus water in both strains (Fig. 1, lower panel), thereby demonstrating the validity of the experimental setting. There was no CTA effect discernible in either gender of L-E rats treated with 50 μg/kg TCDD. By contrast, both doses of TCDD tested (50 and 1000 μg/kg) suppressed saccharin drinking in male H/W rats by approximately 55%, although the change did not attain statistical significance at the lower dose due to a large variability in the response.

Pica and Protein Selection

TCDD caused a rapid and sustained body weight loss in both genders of L-E rats (Figs. 2, 4, and 5), whereas it merely curbed further growth of male H/W rats (Fig. 3) despite the fact that the dose was 20 times as high for the latter. The same difference was seen in total energy consumption. Kaolin intake tended to increase in response to TCDD administration, especially in H/W and female L-E rats (Figs. 3 and 4). The effect was more distinct when kaolin was provided in a pelleted form (Fig. 5). A gender-related divergence was discernible in proportional energy consumption from the powdered high-protein diet. While it displayed a swift and persistent suppression in female L-E rats (Fig. 4), in male L-E as well as H/W rats there was an upward trend during the latter half of the observation period (Figs. 2 and 3). When female L-E rats were presented with pelleted high-protein diet, the effect vanished, primarily because of low intake values in the control animals (Fig. 5).

Cyclophosphamide increased kaolin intake over 24 h in a statistically significant manner in control rats alone (Table 1). However, the rats cotreated with TCDD and cyclophosphamide ate an amount of kaolin almost equal to that consumed by the corn oil-cyclophosphamide group. ANOVA revealed that both TCDD ($p < 0.001$) and cyclophosphamide (p = 0.007) significantly decreased energy intake and TCDD additionally suppressed the consumption of the high-protein diet (p = 0.047). No interaction term between TCDD and cyclophosphamide reached statistical significance.

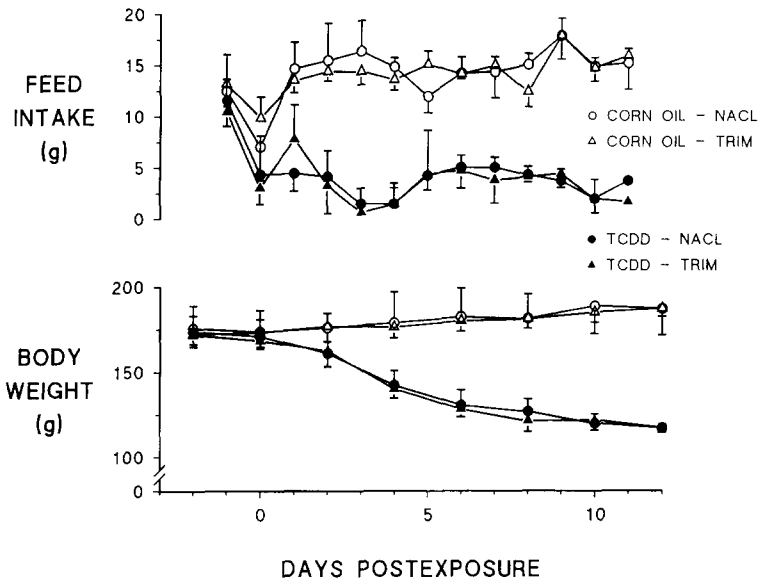

FIG. 7. Changes in body weight and feed intake in female L-E rats treated with 50 μg/kg TCDD or corn oil together with trimethobenzamide (5 mg/kg plus continous infusion [see Method]) or saline. $N = 5$.

Oxytocin

Two days after TCDD exposure, LiCl stimulated oxytocin secretion by about 50% ($p = 0.001$) in L-E rats without any interference of TCDD in the response (Fig. 6). TCDD itself did not have a statistically significant effect on plasma oxytocin concentration in either strain. On day 8, there was again no departure from control values in L-E and H/W rats dosed with TCDD alone. The oxytocin response to LiCl was also equal in TCDD-exposed and control H/W rats (drug effect, $p < 0.001$; interaction term, $p = 0.84$). However, in L-E rats TCDD markedly potentiated the impact of LiCl (interaction term, $p = 0.001$).

Drug Intervention

The antiemetic agents did not show any influence on the wasting syndrome elicited by TCDD (Fig. 7; only data from trimethobenzamine-dosed animals presented). Nor did they affect feed intake or body weight gain in control L-E rats.

DISCUSSION

The most noticeable difference between L-E and H/W rats in responses to TCDD appears in feed intake and body weight gain (28). The wasting syndrome is also the hallmark of acute TCDD toxicity in most, if not all, laboratory animal species. Although its relationship with TCDD lethality is still obscure, the exceptionally long duration of anorexia and the drastic magnitude of body weight loss after a single dose merit a detailed scrutiny of its pathogenesis in its own right. A crucial but overlooked question in this respect is whether the wasting results from a selective disorder of regulation for energy metabolism by TCDD or whether it just arises due to general malaise. The present study is one of the very first attempts to address this point. Since TCDD is distinguished from other anorexigens by both a long elimination half-life [about three weeks (24)] and gradually progressive nature of effects, which pose serious problems to the experimental design, a battery of appropriate methods was employed instead of any single approach. The methods chosen have proved their validity and concordance of outcomes [e.g., in the case of another anorexigen, cholecystokinin, high doses of which have been shown to induce a positive CTA response (5), enhance kaolin consumption (11), stimulate oxytocin secretion (32), and to be antagonized by trimethobenzamide (15)]. In the case of TCDD, the best applicable and the most useful of the methods tested turned out to be kaolin consumption. It allowed continuous monitoring of a proven indicator of nausea over a prolonged period of time in contrast to both the CTA response, which only measured the first 6 h after exposure (7), and the biochemical index, plasma oxytocin, which just provided information of the situation at specific time points. Kaolin consumption also gave consistently positive results for female L-E rats in the two experiments performed (with powdered and pelleted formulations), while the outcomes with the other parameter suited for continuous follow-up, proportional protein intake, were at variance with one another.

The low consumption of liquids characteristic of L-E rats (19), along with the suppressing influence of TCDD on drinking, hampered proper conductance of CTA trials. However, it was still clear that the response of L-E rats to TCDD markedly differed from that to a model toxic compound, LiCl. This was not the case in H/W rats. In this strain, both doses employed (50 and 1000 μg/kg) decreased saccharin selection much to the same extent as LiCl, although only the effect of the higher dose attained statistical significance due to wider interindividual variation at 50 μg/kg. The important point to note here, however, is that the CTA response displayed inverse correlation with TCDD susceptibility.

The effect of TCDD on feed intake and body weight gain was lesser in H/W rats despite the fact that the dose employed for H/W rats was 20 times as high as that for L-E rats. Yet the opposite was true for kaolin consumption. In H/W rats, this parameter tended to stay above the control level from

exposure to termination, whereas L-E rats displayed more fluctuation in it. Moreover, there appeared to be a gender-related difference, with female L-E rats showing greater responsiveness than males. The induction of kaolin consumption in female L-E rats by TCDD was confirmed by offering kaolin in a pelleted form. Compared with the powdered variety, the rats increased their intake of pelleted kaolin more gradually but to a notably (about 10 times) higher level, which was reached during the last four days of the observation period.

Another remarkable disparity between female and male rats occurred in proportional consumption of the powdered high-protein diet. While male rats of both L-E and H/W strains showed an upward trend, there was a rapid and persistent decline in female L-E rats. The decrease was not seen with pelleted protein diet, but the low intake rate in control rats makes this finding questionable, suggesting an untoward effect of the heating procedure on the gustatory properties of the feed. Prototypes of nausea-producing compounds decrease preferentially protein ingestion (30,31). Hence, the differential response of female L-E rats may reflect greater gastrointestinal discomfort, partially in support of the data for L-E rats on kaolin consumption. Another explanation might be a selective impact on protein as a nutrient. There appears to be unlearned specific appetite for protein (6). In a previous study with male L-E and H/W rats we failed to find any influence of TCDD on macronutrient self-selection, when the three macronutrients were provided simultaneously as complete diets, which led us to conclude that TCDD primarily reduces the drive for energy (19). The present findings suggest that in female L-E rats TCDD may affect the specific protein appetite as well. Furthermore, the slight increments in protein intake detected in male rats of both strains imply that the outcome may partly depend on experimental setting, and warrant selection studies with pure macronutrients.

TCDD did not alter plasma oxytocin concentration on any occasion. The oxytocin response to LiCl was also intact in female L-E rats two days after TCDD exposure, in accordance with the lack of interaction between TCDD and cyclophosphamide on kaolin consumption in these animals. However, at eight days TCDD markedly potentiated the effect of LiCl in L-E but not H/W rats. Since there is evidence that physiological satiety associated with feeding can under certain conditions result in nausea as assessed by enhanced oxytocin secretion (32), it is possible that the hyperresponsiveness of L-E rats to nausea-eliciting stimuli at later stages of TCDD intoxication contributes to their reduced feeding. This phenomenon may be operative also in H/W rats, as we have previously shown them to develop one to two months after exposure to 1000 μg/kg TCDD an exaggerated satiety response to feed energy, which manifests itself in, for example, preloading experiments (22). The pathogenetic mechanism of the augmented oxytocin response in L-E rats remains to be determined but may be related to accelerated serotonin turnover in the brain by TCDD (29), since enhanced serotonergic neurotransmission has been shown to stimulate oxytocin secretion in the rat (25).

Neither of the antiemetic compounds tested showed any ability to ameliorate the TCDD-induced wasting syndrome. It is of course possible that the doses examined were not optimal. For example, in a previous study with single IP injections a dose of 5 mg/kg trimethobenzamine proved to be more effective in attenuating LiCl-induced CTA response than either 2.5 or 10 mg/kg (3). As the body weights of the TCDD-treated rats declined, the doses (per unit weight) gradually increased. Nevertheless, considered in the context of the other results, the completely negative findings with trimethobenzamine and metoclopramide argue for the view that, at least during the early stages of TCDD intoxication, nausea has a minor role to play. This conclusion, in turn, advocates the notion that TCDD exerts a specific action on the regulatory systems for food intake and/or body weight.

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