2,3,7,8-Tetrachlorodibenzo-p-dioxin Causes Reduction of Glucose Transporting Activities in the Plasma Membranes of Adipose Tissue and Pancreas from the Guinea Pig*

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Toxicity from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure results in severe metabolic imbalances leading to a loss of fat stores in many animal species, a phenomenon known as the wasting syndrome. In this paper, we report that TCDD treatment at very low doses (0.03–1 μg/kg, single intraperitoneal injection) causes a profound reduction of glucose uptake by guinea pig adipose tissue, pancreas and brain. This effect of TCDD is dose-dependent, with a dose as small as 0.03 μg/kg resulting in a significant decrease. In adipose tissue, the decrease begins within 6 h of treatment and persists at least 28 days. The \( V_{\text{max}} \) of glucose transport was decreased by TCDD treatment, whereas the \( K_m \) was unchanged. Liver behaves oppositely to adipose tissue. At early stages of treatment (6–12 h) glucose uptake was depressed, while at later stages (24–96 h) it was increased. In situ (explant tissue culture) treatment with TCDD yields similar trends as in vivo studies for glucose uptake in all three tissues. In adipose tissue culture TCDD starts reducing glucose uptake after 30 minutes. The inhibitory potencies of three dioxin congeners on adipose glucose transport activities follows the same order of their toxicities in vivo. TCDD's effect on glucose transport is sensitive to cytochalasin B, a specific inhibitor of glucose transporter proteins. Based on these observations and the importance of glucose transporters to cellular energy maintenance, we conclude that at least in guinea pigs the reduction of glucose transporters in various tissues is one of the major causes for TCDD-induced wasting syndrome, which is so prominent in this species.

The biochemical mechanisms by which dioxin-type chemicals cause a variety of toxic symptoms are not well understood, although a sequence of events occurring after dioxin enters the cell has been established. These chemicals specifically bind first with a cytosolic receptor, the Ah-locus protein product, engage in subsequent processing in the cytosol, penetrate into the nucleus, and eventually interact with various DNA to cause changes in gene expression (1, 2). The toxic expressions of dioxins are numerous, varying from tissue to tissue and species to species (3). In vivo sensitivity to 2,3,7,8-

TCDD, the most potent congener of the dioxin family, also varies widely between species, ranging from an oral LD50 value of 0.6 μg/kg in male guinea pigs (4) to greater than 5,000 μg/kg in the golden Syrian hamster (5). Therefore, it has not been easy to pinpoint a major site of action or a common biochemical mechanism that would explain many diverse toxic endpoints (6).

One of the most consistently observed toxic manifestations of dioxins in many species is bodyweight loss (7, 8) accompanied with hypophagia (9), hyperlipidemia (10), and hyperinsulinemia (11, 12). Such observations indicate that the affected animals are unable to utilize the nutritional elements (e.g. glucose, triglycerides, cholesterol, etc.) available in their blood. Curiously, there appear to be no gross impairments of any of the major enzymes involved in catabolic utilization of lipids or carbohydrates (1, 2, 10). Also curious in this respect is the fact that the affected animals do not consistently exhibit hyperglycemic symptoms. Furthermore, the original hypothesis that the main cause of TCDD-induced wasting syndrome is malabsorption of nutrients through intestinal walls is no longer considered to be valid (9).

The current investigation was undertaken to answer the key question of why dioxin-treated animals appear to have difficulties in utilizing available glucose in their blood. During the course of our study, we discovered that TCDD causes a profound reduction in glucose uptake by guinea pig adipocytes and pancreatic cells. This decrease in transport activity is attributed to a reduction in the number of glucose transporter proteins (i.e. \( V_{\text{max}} \)) on the plasma membrane of these organs. These effects occur at very low doses of TCDD and persist for long periods. Thus we conclude that TCDD-induced reduction of glucose uptake in several tissues of the guinea pig represents an important factor in the development of wasting.

**EXPERIMENTAL PROCEDURES**

Chemicals—3-O-Methyl-D-[1-\( ^3 \text{H}\)]glucose ([\( ^3 \text{H}\)Me-Glc] (1 mCi/ml, 2–5 Ci/mmol) was purchased from Amersham Corp. Dulbecco's modified Eagle's medium (DMEM) powder (430–3800 EB) was purchased from GIBCO. d-Glucose, insulin, epidermal growth factor (EGF), dexamethasone, cycloheximide, epinephrine, bovine serum albumin (BSA), glucotic acid, pyruvic acid, and all the other biochemicals were purchased from Sigma.

**Animals**—Four-to-six-week-old (200–225 g) male English short-haired guinea pigs (Cavia porcellus) were used throughout the study. Animals were housed in suspended stainless steel cages and provided with food and water ad libitum. All animals were maintained on a 12-h light/12-h dark cycle at constant temperature and humidity.

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1 The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; [\( ^3 \text{H}\)Me-Glc, 3-O-methyl-D-[1-\( ^3 \text{H}\)]glucose; GLUT, glucose transporter; EGF, epidermal growth factor; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; \( T_h \), triiodothyronine; \( T_3 \), triiodothyronine; PeCDD, pentachlorodibenzo-p-dioxin.
vivo treatments were made by a single intraperitoneal injection of TCDD in a corn oil/acetone vehicle (9:1), control animals receiving an equal volume of the vehicle only. Animals were sacrificed between 8:00 and 9:00 a.m., and their adipose tissue, liver, and pancreas were removed rapidly, rinsed in cold distilled water, and chilled on ice. Three animals were used for each treatment point.

**Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) on Glucose Uptake—In Vivo Study**—To study dose-response relationships, glucose uptake was assayed 24 h after a single intraperitoneal injection of different TCDD doses (0.01, 0.03, 0.1, 0.3, and 1.0 μg/kg dose) and time-course effects of TCDD was studied by using animals held for measurements 2, 3, 5, 0.5, 1, 5, 10, and 28 days after a single 1 μg/kg (intraperitoneal) dosing. The effect of unlabeled D-glucose on [3H]Me-Glc uptake by adipose tissue was determined by adding five different concentrations of cold D-glucose (0.167, 3.33, 6.65, and 13.3 mM) to the assay medium. "Glucose uptake" was measured using its nonmetabolizable 3H-labeled analog, [3H]Me-Glc, according to Clancy and Czech (15) with minor modifications. Briefly, a 0.25-g portion of adipose tissue or liver and 0.1 g of pancreas from TCDD-treated or control guinea pigs were incubated in 1 ml of DMEM medium (gassed for 5 min with 95% O2, 5% CO2) supplemented with 584 mg/liter t-glutamate, 4026 mg/liter pyruvic acid, 3700 mg/liter NaHCO3, and 1% BSA. To study the effect of TCDD concentrations, the tissue was incubated for 4 h with different concentrations of TCDD (10^{-10} to 10^{-7} M), or the same volume of vehicle (acetone). The tissue was washed rapidly twice with 1 ml of fresh medium, transferred to a vial containing 4 ml of scintillation solution (Aquasol, Du Pont-New England Nuclear), incubated for over 3 h, and vortexed vigorously, and their radioactive contents were determined. Nonspecific glucose uptake and binding were measured by treating the tissue with cytochalasin B (1 μM) or 20 min prior to glucose uptake assay (14). The net [3H]Me-Glc uptake through cytochalasin B sensitive glucose transporters was calculated by subtracting the mean dpm of samples treated with cytochalasin B from mean total dpm of the corresponding experimental samples in each test series using the same source of tissues. All results are expressed as the mean ± standard deviation.

**In Situ TCDD Treatment Study**—A piece of adipose tissue (0.25 g) from a freshly killed, untreated guinea pig was incubated for 5 min in 10 ml of DMEM medium (gassed for 5 min with 95% O2, 5% CO2 prior to the adjustment of the pH to 7.5) supplemented with 584 mg/liter t-glutamate, 4026 mg/liter pyruvic acid, 3700 mg/liter NaHCO3, and 1% BSA. To study the effect of TCDD concentrations, the tissue was incubated for 4 h with different concentrations of TCDD (10^{-10} to 10^{-7} M), or the same volume of vehicle (acetone). The tissue was washed rapidly twice with 1 ml of fresh medium, transferred to a glass vial containing 1 ml of DMEM, and incubated for 5 min at 37°C. The glucose uptake reaction was initiated by the addition of 4 μCi of [3H]Me-Glc/g of tissue with and without 13.3 mM of nonlabeled D-glucose. After a 30-min incubation at 37°C, the medium was rapidly withdrawn using a pipette, and the tissue was washed three times with 1 ml of fresh DMEM. The tissues were transferred to a vial containing 4 ml of scintillation solution (Aquasol, Du Pont-New England Nuclear), incubated for over 3 h, and vortexed vigorously, and their radioactive contents were determined. Nonspecific glucose uptake and binding were measured by treating the tissue with cytochalasin B (1 μM) or 20 min prior to glucose uptake assay (14). The net [3H]Me-Glc uptake through cytochalasin B sensitive glucose transporters was calculated by subtracting the mean dpm of samples treated with cytochalasin B from mean total dpm of the corresponding experimental samples in each test series using the same source of tissues. All results are expressed as the mean ± standard deviation.

**Hormonal Effects on Glucose Uptake**—Adipose tissue (0.25 g) from untreated animals was incubated with or without TCDD (10^{-7} M) plus or minus one of the following agents: insulin (1 μg/ml), epinephrine (10 μM), EGF (1 μg/ml), cycloheximide (500 μM), deoxymethasone (400 μM), and T3 and T4 (1 ng/ml) in 10 ml of DMEM at 37°C (all concentrations expressed as final). After 4 h, the medium was withdrawn, the tissue was washed twice with 1 ml of DMEM, and [3H]Me-Glc uptake was assayed as above. Each assay was run in triplicate, and the data are the mean ± S.D. of three independent experiments.

**In Situ Effects of TCDD Congeners on Glucose Uptake**—One concentration (10^{-10} M) of 2,3,7,8-TCDD and two concentrations (10^{-7} and 10^{-6} M) of 1,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin were incubated with 0.25 g of adipose tissue from untreated guinea pigs in 10 ml of DMEM. After 4 h, the medium was withdrawn and the glucose uptake was assayed as described.

**Glucokinase Assay**—Adipose tissue from TCDD (1 μg/kg) in vivo treated animals (6, 12, 24 h) and in situ TCDD-treated system (4 and 8 h) was used as source for this enzyme. The enzyme activity was assayed according to the method of Porter and Chassy (15).

**RESULTS**

**In Vivo Studies: Dose and Time Course of TCDD's Effect on Glucose Uptake**—The dose response of in vivo administered TCDD on [3H]Me-Glc uptake is shown in Fig. 1 (A–C). As expected the uptake of [3H]Me-Glc was less in the presence of a high concentration of nonlabeled D-glucose than without it in all cases. The response of these three tissues to the TCDD treatment, however, was markedly different. Glucose transport in adipose tissue was less with increasing doses of TCDD (i.e. TCDD dose-dependent decrease). Maximum observed decrease (50% of control) occurred at 1 μg/kg (Fig. 1A) in the presence of D-glucose. In the absence of D-glucose, the same TCDD dose (1 μg/kg) reduced the [3H]Me-Glc uptake by 35% of control (Fig. 1A). In the pancreas, glucose uptake was maximally decreased at 0.03 μg/kg and 1 μg/kg TCDD with and without D-glucose, respectively (Fig. 1B). Interestingly, the liver was affected in an opposite way: 1 μg/kg TCDD stimulated glucose uptake to 155% and 134% of control with and without D-glucose, respectively (Fig. 1C).

As illustrated in Fig. 2, A–C, TCDD showed a sustained time-course effect on the uptake of [3H]Me-Glc in adipose tissue and pancreas. A decrease in transport was observed as early as 6 h after treatment in both tissues; however, the pattern of response varied. In adipose tissue, glucose transport was continuously decreased throughout the time course to a maximum reduction of 80% of the control at day 28. In contrast, pancreatic glucose transport decreased only by 30% of the control, and the maximal effect occurred after only 24 h with no further changes at longer exposures to TCDD. In both tissues, reduction is greater with 13.3 mM D-glucose in
the incubation medium during the glucose uptake assay than without D-glucose. Again, liver responded in a different manner. The glucose transporting activity was initially modestly depressed, and then gradually increased to reach a maximum stimulation level after 24–48 h before recovering to a near-normal level. There were no significant changes at the longest time of exposure.

Effect of D-Glucose on L3H\textsuperscript{3}Me-Glc Transport—The concentration effect of exogenously added nonlabeled D-glucose in the tissue incubation medium was studied (Fig. 3). TCDD treatment resulted in a significantly lower transport of L3H\textsuperscript{3}Me-Glc in adipose tissue at all concentrations of D-glucose tested compared to the control. However, in adipose and pancreas, the percentages of TCDD-caused reduction were greater at high glucose concentration (13.3 mM) than at low glucose concentration (0 mM). A Lineweaver-Burke plot of the adipose tissue data indicated that the primary effect of TCDD on glucose uptake is on transporter numbers rather than on the transporters' affinities. The V\textsubscript{max} of the glucose transporting activity was reduced to 4.2 pg/g of tissue/min compared to the control value of 8.3 pg/g of tissue/min. The K\textsubscript{s} remained constant (10 mM) for both groups (Fig. 4). Pancreas showed the same tendency as adipose tissue (Fig. 3B), whereas liver responded in an opposite manner (Fig. 3C).

In Situ TCDD Effect Studies: Effect of TCDD Concentration on L3H\textsuperscript{3}Me-Glc Transport—Incubation of adipose tissue in situ with TCDD for 4 h significantly reduced the glucose transporting activity at concentrations as low as 10\textsuperscript{-10} M. Such an effect was TCDD concentration-dependent. Glucose uptake was reduced to approximately 50% of the control value in the presence of 13.3 mM D-glucose with 10\textsuperscript{-7} M of TCDD and about 10% in the absence of D-glucose. This difference in glucose uptake plus and minus D-glucose indicates that TCDD's effect is more pronounced on low affinity glucose transporters (e.g. GLUT4) than on high affinity glucose transporters (e.g. GLUT3) in adipose tissue treated in situ (Fig. 5).

The results of a time-course study showed that glucose uptake is decreased when the adipose tissue is incubated in situ with TCDD for 0.5, 1, 2, or 8 h either plus or minus D-glucose (Table I). The TCDD-induced reduction in glucose transport in the plasma membrane became significant as early as 0.5 h and continued throughout the test period. Pancreas reacted differently according to the incubation time with TCDD. After 2 h, an increase in glucose uptake was noticed both with and without D-glucose, but after 8 h, uptake was decreased, like adipose tissue. Liver's glucose transport activity was again opposite to adipose tissue and pancreas. After an 8-h incubation with TCDD, glucose uptake increased up to 115 and 146% of control with and without D-glucose, respectively (Table I). In two separate experiments, 10\textsuperscript{-8} M
TCDD Effect on Glucose Transport

**In situ effects of TCDD (10^-8 M) on 3-O-methyl[1-3H]glucose uptake through glucose transporters in the plasma membrane of three different guinea pig organs.**

Four guinea pigs were used separately. Each assay was run in triplicate. Significantly different from control (*, p ≤ 0.05) or (**, p ≤ 0.01) (Student's t test).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Incubation time (h)</th>
<th>[3H]Me-GLC Uptake (pg/g tissue/30 min) (X ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control TCDD</td>
<td>Without D-glucose With 13.3 mM D-glucose</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.5</td>
<td>282 ± 7 206 ± 13* 265 ± 14 212 ± 7*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>289 ± 10 211 ± 17* 263 ± 9 225 ± 14*</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>287 ± 9 201 ± 13* 264 ± 10 150 ± 8**</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>269 ± 9 180 ± 11* 250 ± 11 149 ± 9**</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.0</td>
<td>116 ± 11 196 ± 10 82 ± 7 124 ± 9*</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>170 ± 9 105 ± 11 92 ± 9 87 ± 7</td>
</tr>
<tr>
<td>Liver</td>
<td>2.0</td>
<td>63 ± 7 27 ± 3* 59 ± 6 59 ± 8</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>28 ± 3 41 ± 4* 39 ± 4 45 ± 6</td>
</tr>
</tbody>
</table>

* Each organ was incubated in DMEM with and without 10^-8 M TCDD for different times. The control received same volume of the solvent (acetone).

<table>
<thead>
<tr>
<th>Treatment agents (concentration)</th>
<th>[3H]Me-GLC Uptake (pg/g tissue/30 min) (X ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>Control TCDD</td>
</tr>
<tr>
<td>Insulin (1 μg/ml)</td>
<td>445 ± 7 316 ± 15**</td>
</tr>
<tr>
<td>Epinephrine (10 μM)</td>
<td>283 ± 17 216 ± 12*</td>
</tr>
<tr>
<td>EGF (1 μg/ml)</td>
<td>207 ± 8 180 ± 6</td>
</tr>
<tr>
<td>Cyclohexamide (500 μM)</td>
<td>229 ± 13 199 ± 3*</td>
</tr>
<tr>
<td>Dexamethasone (400 mM)</td>
<td>276 ± 10 239 ± 11</td>
</tr>
<tr>
<td>8-Br-cAMP (1 μM)</td>
<td>210 ± 12 133 ± 5**</td>
</tr>
<tr>
<td>T₄ (1 ng/ml)</td>
<td>222 ± 15 230 ± 11</td>
</tr>
<tr>
<td>T₃ (1 ng/ml)</td>
<td>207 ± 10 178 ± 11</td>
</tr>
</tbody>
</table>

* Control tissue was incubated under same conditions except that the same volume of acetone (without TCDD) was added to the medium at the beginning.

When cyclohexamide was added at 0 min followed by TCDD at 60 min, and glucose transporting activity was measured at 150 min, the corresponding values were: cyclohexamide alone (total 150 min incubation) 210 ± 11 pg/g tissue, and cyclohexamide (150 min) plus TCDD (90 min) 275 ± 5 pg/g tissue.

* Significantly different from the control (319 ± 9) value (p ≤ 0.01) by Student's t test.

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**Fig. 4. Inhibition by D-glucose of [3H]Me-Glc uptake in control and TCDD-treated adipose tissue.** Experimental conditions are identical to those described under Fig. 3. The data indicate that the Kₑₐ is the same for both control and TCDD-treated tissue (10 mM), but the Vₘₐ₅ value of the treated samples is lower (42.2 pg/g of tissue/min) than the control (82.3 pg/g of tissue/min). Each point is the mean of three separate experiments. Data are expressed as pg of [3H]Me-Glc uptake/g of tissue/30 min.

**Fig. 5. Dose-response curve of TCDD concentration versus glucose uptake in adipose tissue treated in situ.** Portions of adipose tissue (0.5 g) from untreated guinea pigs were incubated with the indicated concentrations of TCDD in DMEM media for 4 h. Glucose uptake was assayed using [3H]Me-Glc as described under “Experimental Procedures.” *, without or ■, with 13.3 mM D-glucose. Each point represents the mean (± S.D.) of three animals. Data are expressed as pg of [3H]Me-Glc uptake/g of tissue/30 min.

TCDD was found to cause a 12 ± 1.5 to 15 ± 2.1% reduction in glucose uptake as compared to controls in 0.25 g of brain slices after 4 h.

**Combined Effects of TCDD and Certain Hormones on Glucose Uptake**—The results shown in Table II indicate that the glucose uptake in control tissue was consistently increased by insulin and reduced by T₄, T₃, epinephrine, EGF, dexamethasone, cyclohexamide, and 8-Br-cAMP as expected. As a whole, treatment with TCDD in the presence of each agent caused further reduction, or an increase in the case of insulin, over the level already achieved by each agent alone or by TCDD alone.

Under the same conditions, adipose tissue was incubated with TCDD or vehicle alone (control) for 4 h, washed twice, and incubated with 1 μM of cytochalasin B for 20 min at 37 °C prior to glucose uptake assay. The results showed...
that in the absence of cytochalasin B, \([^{3}H]\)Me-Glc uptake was 352 ± 13 and 243 ± 11 pg/g of tissue/30 min for control and TCDD-treated tissue, respectively. In the presence of cytochalasin B, however, the corresponding values were 35 ± 2.1 and 29 ± 1.9 pg/g of tissue/30 min, indicating that TCDD's effect could be completely blocked by cytochalasin. The action of cytochalasin B in this regard was essentially identical in all other tissues tested (data not shown). Therefore, we have subtracted the cytochalasin B-insensitive part of glucose uptake from all data (except the above case) to arrive at the specific glucose transporter-dependent glucose uptake values in this paper.

**In Situ Effects of Active and Less Active TCDD Congeners on Glucose Uptake**—Three dioxin congeners of varying toxicity (high = 2,3,7,8-TCDD; moderate = 1,2,3,7,8-PostDD; low = 1,3,7,8-TCDD) were tested for activity against glucose transport of guinea pig adipose tissue plasma membranes in situ (Table III). 2,3,7,8-TCDD reduced the glucose uptake by 50% compared to control, while 1,2,3,7,8-PostDD reduced glucose uptake by 30 and 49% at 10⁻² and 10⁻³ M, respectively. 1,3,7,8-TCDD showed only 25% reduction of glucose uptake even at 10⁻³ M.

**Glucokinase Activity**—TCDD did not inhibit glucokinase activity either after 12 h (in vivo) or 4 h (in situ), but enzyme activity was reduced to 53 and 60% after 24 h (in vivo) and 8 h (in situ) after TCDD treatment. Therefore, early reduction of glucose uptake could not be attributed to the inhibition of this enzyme.

**DISCUSSION**

In the current study we have demonstrated that TCDD at a dose as low as 0.03 \(\mu\)g/kg (single intraperitoneal) causes a significant reduction in the glucose transporting activity of guinea pig adipose tissue and pancreas. This level of sensitivity is extraordinary, occurring at 1/10 to 1/100 of the doses known to cause other toxicological lesions in this and other species (3). To our knowledge, this is the lowest in vivo dose of TCDD reported to cause any biochemical changes in male guinea pigs. TCDD's effect is that TCDD specifically affects one form of glucose transport in adipose tissue (see Table III) supports such a view. At the same time, it is not likely that the phenomenon is caused by some blockage in glucose metabolism per se, since \([^{3}H]\)Me-Glu is not metabolized. Furthermore, glucokinase activity was not affected by TCDD treatment at early time points (12 h in vivo and 4 h in situ) when glucose transporting activities had already been reduced. In addition, epinephrine, which is well known to enhance glucose metabolism, did not influence the action of TCDD on glucose transporting activities in adipose tissue.

The main question raised by the current work is how TCDD causes a reduction in glucose transporter activities in adipose tissue, pancreas, and, to a lesser extent, in the brain. It is generally accepted that TCDD's initial action is mediated through binding to a cytosolic receptor, Ah-receptor (1-3), followed by processing, translocation into the nucleus, and eventual activation of a variety of genes for transcriptional processing. Therefore, it is unlikely that TCDD directly binds with the glucose transporter protein to cause inhibition of its transporting capability. The fact that the in vivo action of TCDD results in an elevation of total glucose uptake in the liver, rather than reductions in all other organs studied so far, supports such a view. At the same time, it is not likely that the phenomenon is counteracted by some blockage in glucose metabolism, since glucokinase activity was not inhibited by TCDD treatment.

**TABLE III**

<table>
<thead>
<tr>
<th>Glucose Uptake in Guinea Pig Adipose Tissue</th>
<th>(X ± S.D.)*</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>193 ± 8</td>
<td>100</td>
</tr>
<tr>
<td>2,3,7,8-TCDD (10⁻² M)</td>
<td>97 ± 3*</td>
<td>50</td>
</tr>
<tr>
<td>1,3,7,8-TCDD</td>
<td>174 ± 11</td>
<td>90</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>145 ± 9</td>
<td>75</td>
</tr>
<tr>
<td>1,2,3,7,8-PostDD</td>
<td>135 ± 12</td>
<td>70</td>
</tr>
<tr>
<td>10⁻³ M</td>
<td>99 ± 10*</td>
<td>51</td>
</tr>
</tbody>
</table>

* Means of three experiments. Each assay was run in triplicate. TCDD and its congeners were incubated for 4 h with the adipose tissue as described under "Materials and Methods." Significantly different from control (\(p \leq 0.01\)* (Student's \(t\) test).
may be that the liver is a unique organ, by having its GLUT system tightly coupled to gluconeogenesis (20). Since TCDD is known to lower the activity of phosphoenol pyruvate carboxykinase, a key enzyme in gluconeogenesis (23, 24), it is possible that the unexpected response of liver might be related to this unique feature. The fact that at very early stage of poisons TCDD causes a slight but significant reduction in glucose uptake in vivo supports such a view. More work would be needed to clarify this point.

The five members of the GLUT family exhibit tissue-specific gene expression (16). Therefore, differences in glucose transport by various organs following TCDD treatment may indicate selective susceptibilities of GLUT to dioxin. Based on our results, GLUT4 appears to be the most affected form. In contrast to pancreas and liver, adipose tissue primarily expresses GLUT4, an insulin-recruitable transporter, and GLUT3 (16). The observation that insulin increased glucose uptake while both epinephrine and 8-Br-CAMP reduced glucose transport in experiments with isolated adipose (Table II) indicates that GLUT4 is the most prevalent form of GLUT in this tissue (19). Two lines of evidence suggest that TCDD can affect GLUT4. First, TCDD’s effect on glucose transport is greater in the presence of insulin than in its absence.

Furthermore, TCDD’s effects are more pronounced when a high concentration of exogenously added d-glucose (13.3 mM) is present in the medium (Fig. 5); GLUT4 has a much lower glucose affinity (Km = 5 mM) than GLUT3 (Km < 1 mM) (16). On the other hand, GLUT4 cannot be the only target of TCDD, since the brain, which has only GLUT1 and GLUT3, also shows decreased glucose uptake. Without a doubt, other types of GLUT are also affected by TCDD, but future in-depth analyses of specific GLUT gene products would be needed to explain the precise nature of TCDD-susceptible types of GLUT.

Another important factor in body weight loss is hypophagia. It is generally acknowledged that the blood glucose level is one of the major determinants of appetite control in animals (25, 26). Since the blood glucose levels in TCDD treated (1 μg/kg single intraperitoneal) male guinea pigs are known to be either slightly elevated or equal to their matched, pair-fed controls during the 10-day post-treatment period (10, 26), it is reasonable to assume that the affected animals do not receive extra feeding signals in the central nervous system and that the lack of glucose transport in TCDD treated animals per se does not trigger hunger sensation. The situation is also similar in TCDD-treated rabbits (27), which clearly exhibit hypoinsulinemia during the early stage (1-48 h), and yet the blood level of glucose remain same or transiently elevated as compared to pair-fed control animals during the same time period. By drastically restricting foods given, Peterson and associates (9, 20) could produce similar bodyweight loss as TCDD-treated counterparts in untreated rats. Thus, hypophagia plays an important role in producing bodyweight loss. On the other hand, Gasiewicz et al. (28) subjected TCDD-treated and control rats to total parenteral nutrition procedure. By bypassing the intestinal absorption route, they counteracted TCDD-induced bodyweight loss. However, neither of these nutritional manipulations prevented the TCDD-treated animals from meeting their eventual death.

It should be clear from the results of our current study that cellular glucose starvation due to the blockage of glucose transporting activities on the plasma membrane of critical tissues of TCDD-treated animals cannot be totally overcome by excess nutrition alone. Even when plenty of blood glucose is available, insulin production and secretion by β-cells of pancreas is likely to be hampered because of the lack of intracellular glucose sensing. Indeed, Brewster and Matsunura (17) observed that intravenous injection of excess glucose resulted in sudden death in many of TCDD-treated guinea pigs but not in pair-fed control animals.

The current finding helps to explain at least two of the major mysteries associated with TCDD-induced wasting syndrome. One is its action to reduce lipoprotein lipase production, which is known to be tightly coupled to glucose transport in vivo (10, 17), and the second toxic endpoint is hypoinsulinemia. The current consensus on the insulin production mechanism is that it is largely controlled through intracellular glucose sensing by the beta cells of pancreas (16). Glucose transport, and not the level of blood glucose or insulin per se, is now acknowledged to be tightly coupled to glucose sensing and to insulin production. Therefore, the blockage of glucose transporter activities in pancreas by TCDD should logically lead to the lowered insulin production.

In conclusion, we have firmly established that TCDD causes a reduction in glucose transporting activities on the plasma membranes of adipose tissue, pancreas, and brain at very low doses and at early stages of TCDD poisoning. To our knowledge, this is the first time that such an effect of dioxin-type chemicals has been reported. The phenomenon is consistent with a number of observed tissue specific toxic symptoms that this group of chemicals is known to cause. While the biochemical mechanism by which TCDD causes such reductions in glucose transporting activities in selected tissues remains unsolved, the phenomenon itself warrants future attention in view of the toxicological significance of this compound, particularly with regard to causing massive loss of body fat accompanied with hypertriglyceridemia (8, 17), premature atherosclerosis (29) and peculiar diabetic effects (i.e. hypoinsulinemia without hypo- or hyperglycemia (12, 15)). Moreover, TCDD appears to be a very interesting molecular probe for understanding the mechanisms by which glucose transporting activities are controlled in various tissues.

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