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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes increases in expression of c-*erb-A* and levels of protein-tyrosine kinases in selected tissues of responsive mouse strains

DAVID W. BOMBICK, JERZY JANKUN, KATHRYN TULLIS, AND FUMIO MATSUMURA

Pesticide Research Center, Michigan State University, East Lansing, MI 48824

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ABSTRACT **2,3,7,8,-Tetrachlorodibenzo-*p*-dioxin (TCDD) administered *in vivo* causes drastic reduction in the weight of the mouse thymus at low doses (e.g., 30 μg/kg single i.p. injection), the reduction becoming statistically significant after 2 days. To understand the cause for such thymic involution TCDD-evoked changes in various biochemical parameters in this tissue were examined. The most noticeable change was observed in the increased activity of specific protein-tyrosine kinases and protein kinase C and an increased level of $p21^{ras}$-associated binding of [^{3}H]GTP. Since no significant change was observed with cAMP-stimulated protein kinases and cAMP levels, the above changes appear to be a selective effect on these special classes of proteins. As a result of a time sequence study it has become apparent that the rise in protein-tyrosine kinase activities becomes significant within 24 hr, whereas the rise in protein kinase C does not become significant until 48 hr. Among protein-tyrosine kinases, $pp60^{c\text{-}src}$ and probably $pp56^{lskT}$ were found to be significantly elevated by TCDD treatment. In view of similarities between TCDD and thyroid hormones in causing thymic involution, the levels of c-*erb-A* expression were assessed in the liver by using avian ^{32}P-labeled v-*erb-A* probe and RNA transfer blot hybridization technique. The results clearly indicate that TCDD has the property to elevate levels of mRNA bearing homology to v-*erb-A*. Such changes in c-*erb-A* expression and protein-tyrosine kinase occurred only in TCDD-susceptible (responsive) strains but not in tolerant (nonresponsive) strains of mice at the dose tested. Based on such observations a hypothesis has been proposed that TCDD owes its potency to its ability to stimulate the expression of one of a family of DNAs bearing homology to v-*erb-A* and that one of the major consequences of such an action is stimulation of various tyrosine kinases.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a very toxic, teratogenic, and carcinogenic chemical (1–4). In addition to these deleterious effects, TCDD is known to cause atrophy of thymus (5, 6) in most experimental animals. Since this lesion occurs at sublethal doses of TCDD at an early stage of poisoning, it has been used by several scientists (e.g., refs. 2, 7, 8) as a sensitive indicator of TCDD poisoning.

As for the possible cause for such a thymic lesion, Neal *et al.* (9) have shown that an increase in serum glucocorticoid levels is unlikely. Poland and Glover (7) have found that involution of thymus occurs at low doses of TCDD (30 μg/kg) in sensitive (responsive) strains of mice but only at high doses in tolerant (nonresponsive) strains of mice. These workers used such differential responses among mouse strains as a biochemical indicator for the involvement of specific cytosolic TCDD binding proteins (i.e., TCDD receptor) in the final expression of thymic involution.

Our research group has been studying the effects of TCDD on the hepatic plasma membrane receptors. To date, the plasma membrane-bound epidermal growth factor (EGF) receptor appears to be the most sensitive indicator of the effect of TCDD (10, 11). The cause for such a change in EGF receptor in the hepatic tissue has been proposed to be an increase in intracellular protein kinases (12). In view of the susceptibility of the thymus to TCDD, we have studied the basic cause for changes in thymic properties, particularly from the viewpoint of TCDD-evoked changes in protein kinase profiles.

MATERIALS AND METHODS

Animals. C57BL/6J and DBA/2J mouse strains were purchased from The Jackson Laboratory and were males of ≈25 g. Purina Rodent Chow and water were given ad libitum.

Chemicals. TCDD of >99.99% purity was a gift of Dow. TCDD was dissolved in corn oil/acetone, 9:1 (vol/vol), as a vehicle for i.p. injection of animals (11). Quercetin was administered i.p. at a dose of 50 mg/kg using the same corn oil/acetone. Other chemicals administered to mice were also dissolved in the corn oil/acetone vehicle. Radioisotopes ($Na^{125}I$, [γ-^{32}P]ATP, and [^{3}H]GTP) and a radioimmunoassay (RIA) kit for determination of cAMP levels were purchased from Amersham. A monoclonal antibody to $p60^{src}$ was obtained from Oncor (Gaithersburg, MD). All other biochemicals and chemicals were purchased from Sigma and were the highest purity obtainable.

Thymic Membrane Preparation. Each mouse thymus was homogenized in 200 μl of buffer (10 mM Tris·HCl, pH 7.4/250 mM sucrose/100 kallikrein units of aprotinin per ml) with 25 strokes in a glass/glass homogenizer. The homogenate was centrifuged for 60 sec on a table-top Eppendorf centrifuge (15,000 × *g*) to remove connective tissue and nuclei. The supernatant was assayed for protein content and used in subsequent analyses.

$p60^{src}$ Phosphorylation Assay. The method used was that of Collett and Erikson (13) with a minor modification (14).

Protein Kinase Activity Assay. The activity of general protein kinases was determined as before (12) and used histone as an exogenous substrate. The assay for protein-tyrosine kinase activity was basically that of Wong and Goldberg (15) as modified by Bombick and Matsumura (16). Individual protein-tyrosine kinases were examined with a Sephadex G-150 column (14).

Thymic membranes in homogenizing buffer were phosphorylated by using the protein-tyrosine kinase buffer system and allowing reaction with [γ-^{32}P]ATP (≈10^7 cpm; 0.01 μM) for 10 min at 30°C. The reaction was terminated by addition of an equal volume of electrophoresis treatment buffer and heating. The solubilized phosphorylated membranes were

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; T_3, 3,3′,5-triiodo-DL-thyronine; T_4, 3,3′,5,5′-tetraiodothyronine.

Table 1. Effects of *in vivo* treatment of TCDD-responsive (susceptible) C57BL/6J and -nonresponsive (tolerant) DBA/2J mice with various chemical agents on thymic weights (assayed 2 days after treatment)

Chemical	Dose, μg/kg	Thymic weight, mg	
		C57BL/6J	DBA/2J
Control		32.0 ± 1.8	37.9 ± 4.4
TCDD	30	23.3 ± 2.0*	36.1 ± 4.1
	60	20.8 ± 1.2*	33.9 ± 3.9
	115	15.0 ± 1.0*	22.7 ± 2.6*
3-Methylcholanthrene	40,000	16.0 ± 1.2*	30.4 ± 2.3
Dexamethasone	250	23.7 ± 1.5*	28.7 ± 4.0
Testosterone	400	38.4 ± 3.0	37.3 ± 3.7
Progesterone	400	33.3 ± 1.7	37.9 ± 5.4
17β-Estradiol	50	25.7 ± 2.0*	36.4 ± 4.1
T_3	105	19.2 ± 1.1*	30.0 ± 3.5
T_4	105	27.0 ± 1.6*	33.8 ± 3.9
3,5-Diiodothyronine	105	24.7 ± 2.5*	36.8 ± 1.7
Triac	105	24.4 ± 3.8	34.0 ± 5.4
Reverse T_3	105	24.3 ± 3.3*	35.7 ± 4.0
PMA	100	10.5 ± 2.4*	13.0 ± 0.9*

Data are expressed as mean ± SD. Results represent four determinations using four animals per test in all cases except for phorbol 12-myristate 13-acetate (PMA), where three animals were examined per test. T_3, 3,3',5-triiodo-DL-thyronine; reverse T_3, 3,3',5'-triiodo-L-thyronine; T_4, 3,3',5,5'-tetraiodothyronine.
*Statistically significant compared to control at $P \leq 0.01$ utilizing a two-tailed Student *t* test.

electrophoresed on a 10% $NaDodSO_4$/polyacrylamide gel following the procedure of Laemmli (17). The gels were dried and autoradiographed by using x-ray films.

Protein kinase C activity in thymic membranes was determined according to the method originated in Y. Nishizuka's laboratory using histone as an exogenous substrate (18, 19). A slight modification entailed solubilization of protein kinase C from thymic membranes with an EGTA/EDTA solution (20). After centrifugation at 100,000 × *g* for 1 hr at 4°C the supernatant was used as the enzyme source.

GTP Binding Assay and cAMP Analysis. Membrane protein (100 μg) in 75 μl of homogenizing buffer was added to GTP buffer (20 mM Tris, pH 7.4/1 mM dithiothreitol/5 mM $MgCl_2$/100 mM NaCl) containing 4 g of bovine serum albumin per 100 ml. Samples were incubated either with 100 pg of [^{3}H]GTP (2 μCi; 1 Ci = 37 GBq) alone or with 100 μg of unlabeled GTP for nonspecific binding. After incubation for 30 min at 30°C the reaction was stopped by adding 5 ml of ice-cold GTP buffer and filtered through a 0.45-μm HAWP Millipore filter. An additional 5-ml aliquot of ice-cold GTP buffer was passed through the filter. The filter was dried and assayed for radioactivity on a scintillation counter. Specific GTP binding was calculated by subtracting the nonspecific binding from total binding.

Determination of cAMP levels was made in thymic homogenates prior to centrifugation. The assay was described by Amersham, the producer of the particular RIA kit utilized in this study.

Determination of p21^{ras}-GTP Binding. Membrane protein (500 μg) in 50 μl of homogenizing buffer was added to 50 μl of GTP buffer containing 2 μCi of [^{3}H]GTP (100 pg). After 30 min at 30°C the membranes were solubilized by adding 50 μl of a 1:1 solution of 7 M guanidine·HCl/GTP buffer. The mixture was diluted with 10 vol of GTP buffer, and 10 μl of the p21^{ras} antibody (polyclonal sheep antibody to both Ha-*ras* and Ki-*ras*; Triton Bioscience, Houston, TX) was added, dispersed in a Vortex, and incubated for 2 hr at 4°C. A rabbit anti-sheep IgG antibody (10 μl; U.S. Biochemical, Cleveland, OH) was added and incubated for another hour at 4°C. A suspension of protein A-Sepharose CL-4B (12.5 mg in 100 μl of GTP buffer) was added; this was followed by frequent mixing for 30 min at 4°C and centrifugation for 2 min on an Eppendorf centrifuge as before. The p21^{ras} immunocomplex was washed twice with 400 μl of GTP buffer. The p21^{ras} immunocomplex was removed from the Sepharose by addition of 200 μl of electrophoresis treatment buffer and heated as before. Aliquots of the supernatant were then assayed for radioactivity in a scintillation counter. The background values were obtained by carrying out the identical experiment without the p21^{ras} antibody for each run and were subtracted from the experimental values. To determine the molecular mass of p21^{ras}, ^{3}H-labeled proteins were isolated (as above) and were analyzed on Sephadex G-50 calibrated with standard proteins. The radioactivity found in the 21-kDa fraction was 57.2% (control) to 60.8% (treated) of the total protein-bound radioactivities.

Preparation of DNA Probe and RNA Transfer Blot Hybridization. The avian AEV-10 2.5-kilobase (kb) *Pvu* II fragment was kindly provided by H. J. Kung. It is cloned into the *Pvu* II site of pBR322 plasmid in *Escherichia coli* (21). The *Pst* I fragment (0.5 kb) was obtained from isolated plasmids through restriction analysis, agarose gel electrophoresis, and electroeluting. ^{32}P nick-translation labeling was carried out (22) by using [α-^{32}P]dCTP from New England Nuclear, and the resulting labeled DNA was used for dot hybridization according to the method of Winberry *et al.* (23). The total RNA was isolated from individual livers by the method of Chirgwin *et al.* (24). Two hundred micrograms of RNA was used per assay. The above experiment was repeated once more with v-*erb-A* probe purchased from Oncor. After

Table 2. Various biochemical parameters assessed in thymus from mice treated with TCDD

Biochemical parameter	Strain tested	Time after treatment, days	TCDD dose, μg	Control	Treated
Total protein kinase, pmol/100 μg per 10 min	C57BL/6J	1	115	21.8 ± 4.4 (4)	16.6 ± 6.2 (4)
Protein-tyrosine kinase, pmol of P_i per 100 μg of protein per 10 min	C57BL/6J	1	115	4.0 ± 2.0 (4)	9.1 ± 3.0* (4)
pp60^{src}, pmol of P_i per 200 μg/5 min	C57BL/6J	1	60	2.50 ± 0.61 (4)	3.62 ± 0.90 (4)
	C57BL/6J	2	60	1.79 ± 0.26 (4)	4.72 ± 1.39* (4)
	DBA/2J	2	60	0.92 ± 0.10 (4)	0.90 ± 0.07 (4)
cAMP, pmol per thymus	C57BL/6J	1	115	0.57 ± 0.12 (3)	0.68 ± 0.22 (3)
GTP specific binding, pg/150 μg of protein	C57BL/6J	1	115	5.6 ± 1.1 (4)	11.4 ± 2.2* (4)
p21^{ras}-associated GTP binding, pmol of GTP per 500 μg of protein	C57BL/6J	0.5	115	1.2 ± 0.3 (4)	2.0 ± 0.4* (4)
^{125}I-labeled bovine serum albumin binding, ng/200 μg of protein	C57BL/6J	2	30	5.6 ± 0.9 (3)	5.1 ± 1.1 (3)

Data are expressed as mean ± SD, with the number of animals tested given in parentheses.
*Statistically significant compared to control at $P \leq 0.05$ utilizing a two-tailed Student *t* test.

Table 3. Protein-tyrosine kinase activities in thymus and serum from control and TCDD-treated mice

Strain	Tissue	Tyrosine kinase activity, pmol of P_i per 100 μg of protein per 10 min	
		Control	Treated*
Responsive			
C57BL/6J	Thymus	4.8 ± 2.8 (7)	8.2 ± 2.5† (7)
	Serum	0.88 ± 0.46 (4)	2.12 ± 1.22† (4)
A/J	Thymus	1.9 ± 1.1 (4)	3.2 ± 2.0 (4)
Nonresponsive			
DBA/2J	Thymus	4.9 ± 2.9 (4)	4.8 ± 2.7 (4)

Data are expressed as mean ± SD, with the number of animals tested given in parentheses.
*TCDD: 115 μg/kg (single dose i.p.) assayed 2 days after treatment.
†Statistically significant from respective control at $P \leq 0.05$ utilizing a two-tailed Student *t* test.

hybridization, washed and dried discs were radioassayed for radioactivity. The purity of both probes was ascertained by using agarose gel electrophoresis with subsequent blotting to nitrocellulose sheets (21) and autoradiography.

RESULTS

In view of the clear-cut difference in sensitivity toward TCDD among mouse strains (7, 8) we first surveyed the *in vivo* effects of various chemical agents on thymic weights in a susceptible strain, C57BL/6J, and a tolerant strain, DBA/2J. TCDD caused a dose-dependent thymic involution in the C57BL/6J strain by day 2, whereas such an effect was only evident at the highest dose tested in the tolerant strain (Table 1). Such a differential effect was observed not only with a high dose of 3-methylcholanthrene as previously shown (7, 8) but also with thyroxine analogs and 17β-estradiol. On the other hand, although PMA caused a drastic decline in thymic weight, such an effect was found equally in both strains. The effect of dexamethasone was intermediate in this regard, the susceptible strain showing a more profound effect than the tolerant strain.

In the second series of tests we examined various biochemical changes occurring in the thymus mainly in the susceptible strain. There are several biochemical parameters showing significant effects of TCDD: relative levels of $pp60^{src}$, specific GTP binding proteins, particularly $p21^{ras}$, and total protein-tyrosine kinases (Table 2). The increase in $p21^{ras}$ indicates that TCDD may have caused directly or indirectly an activation of c-*ras* DNA. When the effects of TCDD on total protein-tyrosine kinases were tested in susceptible and tolerant mouse strains, it became evident that the changes were observable only in the susceptible strains (Tables 2 and 3).

An examination of the time sequence of TCDD-caused changes in these protein kinase activities revealed that the change in protein-tyrosine kinase precedes that of protein kinase C, the former event occurring as early as 12 hr after TCDD administration (Fig. 1). To confirm that the TCDD-caused change in thymic protein kinase activities involves activation of some protein-tyrosine kinase, the total membranes phosphorylated with [γ-^{32}P]ATP without angiotensin were digested with strong acid, and the resulting phosphoamino acids were analyzed by TLC (25). The extent of labeling at the tyrosine residue was (31% ± 17%, mean ± SD, four animals each) higher in the preparation from the treated animals (C57BL/6J, 115 μg/kg, analyzed after 48 hr) than that from the control animals.

To study the nature of protein-tyrosine kinase changes, the total membranes from the thymus of control and treated animals (obtained 12 hr after treatments) were dissolved with a detergent and subjected to Sephadex G-150 column separation, and protein-tyrosine kinase activity of each fraction was measured. The effect of TCDD treatment in this regard was most significant in the protein fractions with molecular mass ranges of about 50–60 and 70–80 kDa (Fig. 2). An electrophoresis examination of the [^{32}P]phosphoproteins resulting from incubation of total thymic membranes with [γ-^{32}P]ATP (Fig. 3) revealed that TCDD treatment *in vivo* caused an increase in protein phosphorylation in several protein bands in the 50- to 60-kDa range. Densitometric scanning of the band intensity of the most prominent protein, pp56, indicated that the extent of increases in the TCDD-treated preparation was 151.7% ± 1.7% (expressed as % of control, three independent experiments each with three replicate runs) at day 2 among animals treated with 115 μg of TCDD per kg (single i.p.). It is known that certain protein-tyrosine kinases are inhibited by quercetin, a bioflavonoid

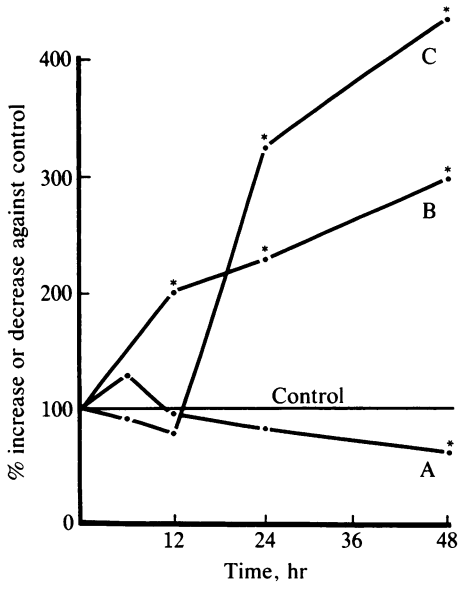

FIG. 1. Time course of TCDD-caused changes in thymic weights (A), protein-tyrosine kinase activity (B), and protein kinase C activity (C) in thymus of C57BL/6J mice. The mice were initially treated with a single dose of 115 μg/kg (i.p.) and were sacrificed after given time intervals, and their thymuses were analyzed. Values below 100% indicate a reduction as compared to those of the control, which are expressed as 100%. Asterisks denote a significant difference at $P \leq 0.05$.

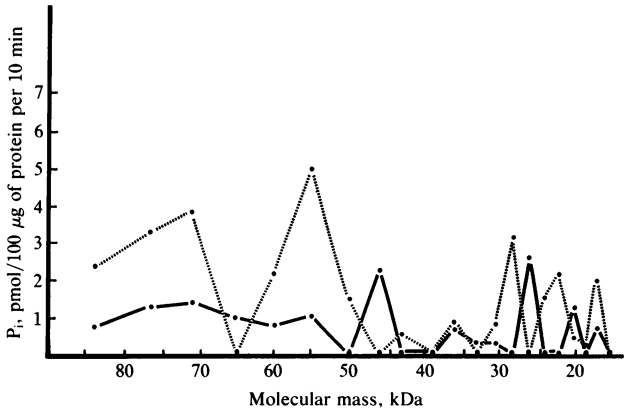

FIG. 2. Sephadex G-150 column elution pattern of solubilized thymic membrane preparations from control (solid line) and TCDD-treated (dashed line) C57BL/6J mice. Mice were treated with TCDD at 115 μg/kg and 12 hr later were sacrificed to obtain the thymus. Control animals were similarly treated with corn oil/acetone only.

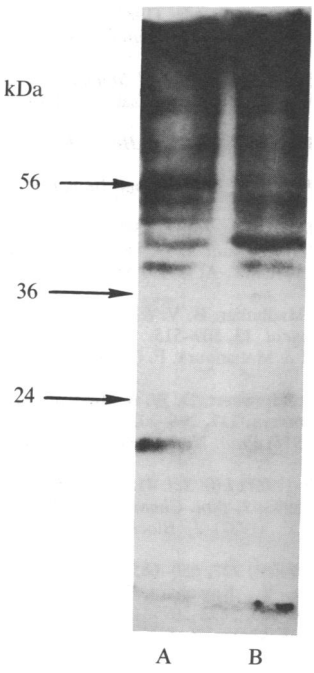

FIG. 3. $NaDodSO_4$/polyacrylamide slab gel electrophoretogram/autoradiogram of solubilized thymic membrane phosphoproteins from male C57BL/6J mice labeled with [γ-^{32}P]ATP under conditions favoring protein-tyrosine kinases. Lane A, TCDD-treated (115 μg/kg, day 2); lane B, control (treated with corn oil/acetone only, day 2).

(26–28). When this agent was coinjected at 50 mg/kg with TCDD, the effects of the latter compound on the thymic weight could be reversed (Table 4). At the same time the portion of the protein-tyrosine kinase activity that was elevated by TCDD was reduced by the coinjected quercetin.

We also examined the effects of various agents on thymic weights and protein-tyrosine kinase activities (Table 5). At certain doses 17β-estradiol and dexamethasone were found to reduce thymic weights and elevate protein-tyrosine kinase activities. Phenobarbital did not cause stimulation of protein-tyrosine kinase even at 50 mg/kg.

The above observations raise a natural question whether there is a common denominator among the agents that cause thymic weight reduction and alteration of the levels of protein-tyrosine kinases. One important clue is the difference in responsiveness to TCDD among various mouse strains (Table 1). To examine the extent of parallelism between their responsiveness to TCDD vs. that to other agents, T_3 was injected into various strains, and its effects on the thymic weight were monitored. The results (Table 6) clearly indicate that the parallelism is a perfect one among all strains tested. It has been recently shown that DNAs encoding receptors for estradiol (28–30), steroid hormones (30, 31), and thyroid hormones (32, 33) bear extensive homology to v-*erb-A*, an oncogene of avian erythroblastosis virus (33, 34). To test the hypothesis that the common denominator of these agents including TCDD is to increase the expression of c-*erb-A*, the level of specific TCDD-stimulated mRNAs was assessed by RNA transfer blot hybridization with ^{32}P-labeled v-*erb-A* DNA probe and total RNA extracts from treated and control mice. TCDD has the property of increasing c-*erb-A* expression in the responsive mice but not in the nonresponsive ones at the time–dose regimen tested (Table 7).

Table 4. Effect of quercetin, an inhibitor of protein-tyrosine kinase, on TCDD-evoked changes in thymic weight and tyrosine kinase activities in C57BL/6J mice 1 day after treatment

Treatment	Thymic weight, mg	Protein-tyrosine kinase activity, pmol of P_i per 100 μg of protein per 10 min
Control	42.8 ± 3.0	4.2 ± 0.9
Quercetin	43.4 ± 5.3	4.5 ± 1.4
TCDD	33.6 ± 3.8	6.4 ± 1.1*
+ quercetin	46.5 ± 4.8	5.0 ± 1.3

Data are expressed as mean ± SD. Results represent four independent determinations involving one animal per determination. Quercetin and TCDD were administered at 50 mg/kg and 115 μg/kg, respectively.
*Statistically different from others at $P \leq 0.01$ according to a Student *t* test.

Table 5. Effect of *in vivo* treatment by different compounds on the body and thymic weights and total thymic tyrosine kinase activities of C57BL/6J mice

Compound	Dose, mg/kg	Thymic weight,* % of control	Total protein-tyrosine kinase activity,† % of control
3-Methylcholanthrene	4	91.8 ± 29.8	96.9 ± 7.7
	20	80.5 ± 17.8	81.5 ± 15.4
Dexamethasone	0.4	84.0 ± 18.8	168.3 ± 14.6‡
	1.6	49.3 ± 7.0	72.5 ± 17.6
17β-Estradiol	0.1	104.8 ± 21.5	88.7 ± 28.3
	0.4	97.0 ± 18.5	152.9 ± 25.4‡
Sodium phenobarbital	50	104.3 ± 9.8	93.9 ± 15.6
TCDD	0.115	88.0 ± 5.8	109.5 ± 11.7

Data are expressed as mean ± SD.
*Results of three or four determinations.
†Average protein-tyrosine kinase activity of untreated mice is 5.8 ± 3.4 pmol of P_i incorporated per 100 μg of thymus protein per 10 min.
‡Statistically significant compared to control at $P < 0.05$ utilizing a two-tailed Student *t* test.

DISCUSSION

We have found that thymic involution in mice can be caused by a variety of agents other than TCDD, 3-methylcholanthrene, and dexamethasone as reported (see ref. 2). Of those agents, thyroxine derivatives and estradiol are of great interest, since there is a clear-cut interstrain difference between TCDD-responsive and TCDD-nonresponsive strains in their susceptibilities toward these agents.

Such an observation supports the view that TCDD receptor belongs to the family of c-*erb-A* products and that a mutation on the c-*erb-A* DNA in these nonresponsive mice makes them less responsive to all of these agents, whose actions are mediated through c-*erb-A* expressions.

Perhaps the most pertinent observation to the understanding of the above hypothesis is that v-*erb-A*, when introduced to cultured erythroid cells *in vitro* by way of DNA transformation, has been shown to cooperate with the expression of *src*, *seafps*, *erb-B*, and Ha-*ras* (35). The latter oncogenes are

Table 6. Differential effects of T_3 on thymus weights of TCDD-responsive and -nonresponsive strains of mice

Strain*	Thymic weight, mg	
	Control	Treated†
Responsive		
BALB/cJ	38.6 ± 3.9	27.6 ± 6.6‡
A/J	27.0 ± 3.5	21.6 ± 1.6‡
Nonresponsive		
RF/J	61.5 ± 5.6	65.0 ± 5.5
AKR/J	80.3 ± 6.4	75.8 ± 7.9
SWR/J	38.8 ± 2.7	37.5 ± 6.2

Data are expressed as mean ± SD (five mice per test).
*Also see Table 1 for C57BL/6J (responsive) and DBA/2J (nonresponsive) mice.
†T_3: 105 mg/kg (single dose i.p.) assayed after 48 hr.
‡Significant difference at $P \leq 0.05$ using a Student *t* test.

Table 7. Transfer blot analysis of mouse liver RNA with a ^{32}P-labeled v-*erb-A* DNA probe

Strain	Hybridization, pg of DNA bound per 100 μg of RNA	
	Control	TCDD-treated
C57BL/6J	2.95 ± 0.62 (7)	4.35 ± 0.51* (8)
DBA/2J	3.40 ± 0.63 (8)	2.65 ± 0.47 (8)

Data are expressed as mean ± SD, with the number of determinations given in parentheses. The specific activity of the DNA probe was 64.7×10^6 cpm/μg.
*Statistically significant difference at $P \leq 0.001$ by a Student t test.

those known to produce protein-tyrosine kinases, or, in the case of Ha-*ras*, known to cooperate with protein-tyrosine kinases to enhance their expressions (36). Thus, TCDD showed the property of increasing the levels of p21^{ras}, p60^{src}, and probably p56^{lskT} in the mouse thymus. Among c-*erb-A*-related agents tested in this work T_3 appears to be closest to TCDD in its ability to affect thymic weight. In fact, the dose–time range of the former to cause thymic involution is no different from the latter in this species. Thyroid hormones are also known to modulate protein-tyrosine kinases (37, 38). It is possible that in this species T_3 and TCDD act on the same receptor.

One may ask: What is the relationship between TCDD-induced thymic atrophy and changes in protein-tyrosine kinase activities? The general relevance of these two phenomena may be inferred, since quercetin, which is known to inhibit certain protein-tyrosine kinases *in vitro*, has been shown to prevent TCDD from causing thymic involution *in vivo*. In agreement with an earlier observation in the liver (39), the most conspicuous change is in protein-tyrosine kinase activities in the molecular mass range of 50–60 kDa. One of the protein kinases whose activity is stimulated is pp60^{src} protein. Unfortunately, the role of pp60^{src} in thymus is not well known. As to the nature of 56-kDa protein-tyrosine kinase there are a number of reports indicating that it is of lymphocyte origin (39–45). That activation of protein kinases associated with lymphocytes is in some way related to maturation of lymphocytes has been considered by several groups (40–46). The oncogenic property of the viral gene encoding p56 has been recognized and two designations, *lsk*^{T} (46) and *tck* (see ref. 47), have been assigned. Marth *et al.* (46) speculate that, if p56 tyrosine kinase activity is associated with T-cell blastogenesis, a functional role for pp56 might reside in transducing mitogenic signals from lymphocyte-specific receptors such as those for interleukin 2.

Another supporting fact is that such a change in protein-tyrosine kinases is accompanied by an increase in p21^{ras} protein, indicating an activation of c-*ras*, which appears to have the property of being activated whenever one or more protein-tyrosine kinases are activated (48).

Further research is needed to answer these questions. The phenomenon of activation of c-*erb-A* expression and protein-tyrosine kinases by TCDD alone appears to merit close attention in view of the recent realization of the importance of these biochemical pathways in cellular modulation and the extreme toxicities of TCDD itself.

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