0022-3565/93/2671-0308$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Copyright © 1993 by The American Society for Pharmacology and Experimental Therapeutics
Vol. 267, No. 1
Printed in U.S.A.

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Polychlorinated Biphenyl-Induced Immune Suppression: Castration, but Not Adrenalectomy or RU 38486 Treatment, Partially Restores the Suppressed Cytotoxic T Lymphocyte Response to Alloantigen[1]

GREGORY K. DE KREY, LINDA BAECHER-STEPPAN, JAMES A. DEYO, BRADFORD SMITH and NANCY I. KERKVLIET

College of Veterinary Medicine (G.K.D., L.B.-S., J.A.D., B.S., N.I.K.), Department of Agricultural Chemistry (N.I.K.) and Environmental Health Sciences Center (G.K.D., N.I.K.), Oregon State University, Corvallis, Oregon

Accepted for publication June 7, 1993

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

ABSTRACT

The cytotoxic T lymphocyte (CTL) response to allogeneic P815 tumor in C57Bl/6 mice is dose-dependently suppressed after treatment with 3,3′,4,4′,5,5′-hexachlorobiphenyl (HxCB). Elevation of plasma corticosterone (CS) is also observed coincident with CTL suppression. Because immune suppression is inducible by glucocorticoid administration, the role of elevated CS was investigated as an indirect mechanism of HxCB-induced immunotoxicity. In multiple experiments, HxCB treatment (10 mg/kg b.w.) consistently reduced CTL activity by 70 to 85% in male mice. Adrenalectomy failed to alter the suppression of CTL activity by HxCB. However, the mortality rate was high (≥70%) in these experiments and plasma CS elevation persisted in HxCB-treated adrenalectomy survivors. Therefore, the use of adrenalectomized mice was inadequate to determine whether CS elevation leads to CTL suppression after HxCB treatment. Daily administration of the glucocorticoid receptor antagonist 17-β-hydroxy-11-β-(4-dimethylaminophenyl)-17-α-(propanyl)-estra-4,9-dien-3-one (RU 38486) (150 mg/kg b.w., p.o.) also failed to alter the suppression of CTL activity in HxCB-treated mice; however, spleen cellularity was significantly increased, suggesting functional GCR antagonism. Male mice were more sensitive to HxCB-induced CTL suppression than female mice, and HxCB-induced plasma CS elevation was greater in male mice. Castration failed to reduce the elevation of plasma CS in HxCB-treated male mice. However, castration partially alleviated CTL suppression in HxCB-treated male mice. Taken together, these data suggest that 1) glucocorticoid receptor antagonism does not alleviate HxCB-induced CTL suppression, 2) suppression of CTL may be enhanced in male mice by HxCB effects in the testes or by an HxCB-induced altered sensitivity of the immune system to testes-specific factors.

PCBs are a member of a large group of structurally related HAH that are ubiquitous environmental contaminants with wide-ranging toxicologic potential for both man and wildlife (McFarland and Clarke, 1989). Concern for these compounds revolves around their toxic potency which is dependent on isomer, exposure route and the species studied (Vickers *et al.*, 1985). Toxic potency has also been shown to segregate with binding affinity for the cytosolic AhR (Silkworth and Grabstein, 1982; Silkworth and Antrim, 1985; Kerkvliet *et al.*, 1990), the affinity for which is determined by the chlorine substitution pattern of each isomer (Bandiera *et al.*, 1982). The prototype AhR ligand, and the most potent HAH is TCDD; HxCB is among the most potent of the PCB.

Immune suppression is a hallmark of HAH toxicity in many animal species (Poland and Knutson, 1982). Although the effects of HAH on immune function have been widely studied (reviewed by Vos and Luster, 1989), the mechanism(s) for the immunotoxic effects have yet to be resolved. Past studies have shown that HAH suppress CTL responses to tumor allograft (Clark *et al.*, 1981; Kerkvliet and Baecher-Steppan, 1988a). The potency of suppression *in vivo* correlated directly with the binding affinity of HAH congeners for the AhR (Clark *et al.*, 1983; Kerkvliet *et al.*, 1990). However, neither TCDD (Clark *et al.*, 1981) nor HxCB (Kerkvliet and Baecher-Steppan, 1988b), which are both highly immunosuppressive *in vivo*, directly

Received for publication November 2, 1992.
[1] This work was supported by National Institute of Environmental Health Sciences Grants ES00040 and ES03966.

ABBREVIATIONS: PCB, polychlorinated biphenyl; HAH, halogenated aromatic hydrocarbons; AhR, aromatic hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; HxCB, 3,3′,4,4′,5,5′-hexachlorobiphenyl; CTL, cytotoxic T lymphocyte; CS, corticosterone; GC, glucocorticoids; GCR, glucocorticoid receptor; ADX, adrenalectomized/adrenalectomy; ODX, castrated/castration; RU 38486, 17-β-hydroxy-11-β-(4-dimethylaminophenyl)-17-α-(propanyl)-estra-4,9-dien-3-one; CTX, cytotoxicity; E/T, effector cell:tumor cell; AUCC, area under the cytotoxicity curve; TT, testosterone.

aspet

PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS JPET aspet

altered T cell responses when added to mixed lymphocyte cultures. These results suggest that an indirect mechanism may be responsible for the suppression of CTL function observed *in vivo*.

Coincident with suppression of the CTL response by PCB *in vivo* is the elevation of plasma CS levels (Kerkvliet *et al.*, 1990). The degree of CS elevation correlates with the dose and binding affinity of PCB congeners for the AhR. Elevations of circulating GC levels after treatment with other HAH have also been reported (Sanders *et al.*, 1977, 1974; Gorski *et al.*, 1988a; Jones *et al.*, 1987; DiBartolomeis *et al.*, 1987). Deviations from normal levels of GC by PCB may have significance to their immunotoxicity because GC affect macrophages, as well as B and T cells (del Rey *et al.*, 1984; Bradley and Mishell, 1982; Schechter and Feldman, 1977). Importantly, development of CTL activity has been shown to be sensitive to suppression by GC (Gillis *et al.*, 1979; Schleimer *et al.*, 1984).

In the studies reported here, several methods to regulate GC were used to examine the relationship between elevated plasma CS levels and suppression of the CTL response in HxCB-treated mice. ADX and ODX were used in attempts to reduce CS production. RU 38486 treatment was used in an attempt to block the effects of elevated CS at the receptor level.

Methods

Animals and Treatments

Animals. Male or female C57Bl/6 mice, 7 to 11 weeks of age, were used in all experiments. Mice were obtained from Jackson Laboratories (Bar Harbor, ME), Bantin and Kingman (Freemont, CA) or Taconic Farms (Germantown, NY). Animals were housed in front of a sterile laminar flow device and acclimated for a minimum of 7 days before experimentation. Animal rooms were maintained with a 12-hr light/dark cycle (fluorescent, 7:30 A.M. lights on) and constant temperature [79 ± 1 for ADX, 72 ± 1 for others (°F)] and 50% humidity. Animals were housed with a maximum population of four per cage in polycarbonate shoebox cages which were randomly assigned to positions in a cage rack. Animals were provided with Bed-O-Cob bedding (The Andersons, Maumee, OH), Wayne Rodent Blox (Harlan Sprague Dawley Co., Bartonville, IL) and drinking water *ad libitum* (0.9% saline for ADX animals, tap water for all others). Unless otherwise indicated, mice were surgically modified in our laboratory while under ketamine and xylazine anesthesia. After ADX, animals were rested a minimum of 3 days before HxCB treatment. After ODX, animals were rested a minimum of 7 days before HxCB treatment.

HxCB treatment. Environmental standard grade (99% purity) HxCB was obtained from Ultrascientific (Hope, RI). HxCB was dissolved in acetone and mixed with peanut oil (Nabisco Brands Inc., East Hanover, NJ); the acetone was evaporated under a stream of nitrogen for a final concentration of 1.0 mg/ml. Animals were given 0 or 10 mg/kg of HxCB (0.1 ml/10 g b.w.) by gavage 1 day before P815 injection. HxCB (10 mg/kg) was a dose previously shown to cause reproducible and statistically significant suppression of CTL activity (Kerkvliet and Baecher-Steppan, 1988a).

P815 injection. The P815 mastocytoma cell line was propagated in ascites form by weekly passage in DBA mice, the strain of origin. C57Bl/6 mice (H-2^b) were inoculated with 1.0×10^7 of viable P815 cells (H-2^d) by i.p. injection in a 0.5-ml volume of Hank's balanced salt solution.

Experimental design. Unless otherwise indicated, the time course for experimentation is given relative to HxCB treatment as follows: HxCB treatment on day 0; P815 injection on day 1; termination on day 11. Experiments were terminated by euthanizing all animals in the morning (8:30–11:00 A.M.), the low period in the murine diurnal CS rhythm (Shimizu *et al.*, 1983). In some experiments animals were killed by cervical dislocation followed by decapitation, and drained blood was collected. In most experiments, animals were killed by an overdose of CO_2 and blood was collected by heart puncture. Death due to an overdose of CO_2 was rapid (approximately 20 sec) and did not cause a detectable elevation of CS. Serum or EDTA-treated plasma was stored at −20°C until analyzed. Male mice were used in all experiments except where indicated.

RU 38486 treatment. RU 38486 was received as a generous gift from Roussel-Uclaf (Romainville, France). RU 38486 was suspended in 0.25% carboxymethyl cellulose and 0.2% Tween 80 and given orally twice daily (0.1 ml/10 g b.w.) for a total dose of 150 mg/kg/day. Two separate treatment regimens were conducted in different experiments: 1) administration for 10 days beginning on the day of HxCB treatment; 2) administration for three days beginning on day 3, 5 or 7 post-P815 injection. Controls received placebo (carboxymethyl cellulose/Tween 80) by the same treatment regimen.

Assays

CTL activity. Splenic CTL activity was measured in a chromium-51 release assay as described previously (Kerkvliet and Baecher-Steppan, 1988a). The percent CTX at each E/T ratio was calculated by the equation:

$$\%CTX = \frac{cpm_i - cpm_{ni}}{cpm_{mr} - cpm_{ni}} \times 100,$$

where CPM_i = cpm using spleen cells from P815-injected animals, CPM_{ni} = cpm using spleen cells from nonP815-injected animals and CPM_{mr} = the maximum cpm released from cultures incubated with sodium dodecyl sulfate. E/T ratios of 3.7:1, 11:1, 33:1 and 100:1 were used. In order to compare the overall cytotoxic potential, the AUCC was calculated for each animal using the trapezoidal rule and log(E/T) ratios from 3.7:1 to 100:1. AUCC is given as units %CTX · log(E/T).

CS and TT. Serum or plasma CS or TT levels were determined using [^{125}I]-coupled double antibody radioimmunoassay kits (ICN Biochemicals, Carson, CA, for CS; Diagnostic Products Corp., Los Angeles, CA, for TT). Protocols optimized by the manufacturers were used. Standard curves were generated with each assay. The lower limits of detection for each assay were 25 ng of CS/ml and 0.05 ng of TT/ml. The maximum interassay coefficients of variance for internal controls were 15.7% (CS) and 12.9% (TT) as determined by the manufacturers. The maximum intra-assay variations were 8.8% (CS) and 17.7% (TT). For statistical purposes, samples with analyte levels below the lower limit of detection were assigned the limit value.

Statistical Analysis

Statistical analyses were performed using the SAS statistical software database (version 6.03, SAS Institute Inc., Cary, NC) for the IBM personal computer. Significant treatment effects were determined by analysis of variance using the General Linear Models (GLM) procedure of SAS. Comparisons between two means were performed using *t* tests (TTEST of SAS). Comparisons between more than two means were performed using least significance difference multiple comparison *t* tests (GLM of SAS). Regression analysis was performed using the REG procedure of SAS. Values of $P \leq .05$ were considered statistically significant.

Results

Coincident effects of HxCB over time after P815 injection. The effects of HxCB on CS levels and CTL activity are shown in figures 1, A and B, respectively. Significant CS elevation was evident in HxCB-treated mice 3 days after P815 injection; the degree of CS elevation increased through day 9. P815 injection alone did not alter CS levels on any day measured. In other experiments, mice treated with HxCB alone (10 mg/kg) had significantly elevated CS levels only on day 4 (data

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

JPET
PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
aspet

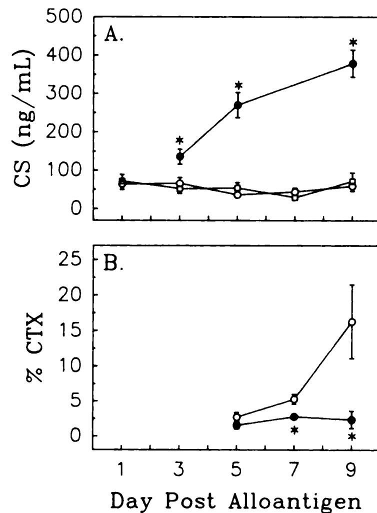

Fig. 1. Coincident effects of HxCB treatment on plasma CS levels and splenic CTL activity. Data are presented as mean ± S.E. for 6 to 10 male mice per group. Animals were injected with Hanks' balanced salt solution or P815 cells 1 day after treatment with 0 or 10 mg/kg HxCB. Animals were killed on days indicated. For statistical purposes (panel A), plasma samples with CS levels below the lower limit of detection (25 ng/ml) were given the value 25 ng/ml. Percent CTX data (panel B) is given for an E/T ratio of 33:1. Data for double vehicle-treated animals are provided as normal reference points (open squares). Open circles indicate vehicle-treated, P815-injected animals and closed circles indicate HxCB-treated, P815-injected animals. *Indicates a significant difference between treatment groups for P815-injected animals at $P < .05$.

TABLE 1

Effects of HxCB treatment and ADX in P815-injected mice

Values represent mean ± S.E. for five to seven male mice. Animals were ADX or sham operated 3 days before HxCB treatment. All animals were injected with P815 1 day after HxCB treatment. Survivors were killed 10 days after HxCB treatment.

ADX	HxCB	Mortality[a]	Body Weight Change[b]	AUCC[c]	Corticosterone
	mg/kg	%	g		ng/ml
−	0	0	3.1 ± 0.3[d]	73.7 ± 1.6[d]	71 ± 16[d]
+	0	10	1.2 ± 0.5[e]	86.8 ± 6.1[d]	77 ± 12[d]
−	10	0	−1.0 ± 0.8[f]	15.7 ± 3.8[e]	551 ± 111[e]
+	10	77	−1.5 ± 0.6[f]	25.4 ± 4.8[e]	369 ± 61[e]

[a] Mortality given as the percent of initial group size of 5 to 30 animals.
[b] Body weight change was computed as the difference in weights on the day of surgery and the day of necropsy. The mean initial body weight (±S.D.) was 21.9 ± 1.6 g and there was no difference between groups.
[c] Area under the cytotoxicity curve (AUCC) is given for E/T ratios of 4:1, 11:1, 33:1 and 100:1.
[d–f] Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison t tests.

not shown). CTL activity in HxCB-treated mice was significantly suppressed compared with vehicle-treated mice at all time points after day 5.

Influence of ADX on HxCB effects. Experiments utilizing ADX mice were complicated by a high rate of mortality among HxCB-treated ADX mice. Results shown in table 1 are representative of several experiments. A mean time to death of 7 days after P815 injection was consistently observed for HxCB-treated ADX mice; the time to death correlated with the time of maximum tumor burden (data not shown). As observed in surviving mice, ADX had no effect on HxCB-induced suppression of CTL activity or elevation of CS levels (table 1).

Effects in RU 38486-treated mice. Treatment with RU 38486 twice daily during the 10 days after HxCB treatment did not alter body weight gain or spleen cellularity (table 2), plasma CS levels (fig. 2) or CTL activity (fig. 3) in vehicle-treated/P815-injected mice. In contrast, RU 38486 treatment significantly attenuated some of the effects of HxCB treatment: body weight loss was reduced 50% and spleen cellularity was increased 144% when compared to HxCB-treated mice given placebo twice daily (table 2). Interestingly, the elevation of plasma CS levels by HxCB was significantly enhanced (2.6-fold) by RU 38486 treatment (fig. 2). The degree of HxCB-induced CTL suppression was significantly enhanced by RU 38486 treatment when measured as AUCC (from 72% suppressed to 84% suppressed), although %CTX was significantly different only at the 11:1 E/T ratio (fig. 3).

Administration of RU 38486 to vehicle-treated mice for 3-day periods from day 3 through day 9 post-P815 injection did not alter body weight gain or spleen cellularity (table 3). However, RU 38486 treatment of vehicle-treated mice did lead to enhancement of splenic CTL activity when RU 38486 was given on days 7 to 9 (table 3 and fig. 4). Administration of RU 38486 to HxCB-treated mice on days 3 to 5 or days 7 to 9 had no effect on body weight gain, splenic CTL activity (table 3 and fig. 4) or the HxCB-induced elevation of plasma CS (data

TABLE 2

Effects of HxCB and 10 days of RU 38486 treatment in P815-injected mice

Values represent mean ± S.E. for six to seven male mice given HxCB 1 day before injection with P815 cells; RU 38486 was given twice daily from the day of HxCB treatment to the day of necropsy (day 10 post-HxCB treatment).

RU 38486	HxCB	Body Weight Change[a]	Spleen Cells
	mg/kg	g	$\times 10^6$
−	0	1.8 ± 0.2[b]	167.4 ± 7.9[b]
+	0	1.9 ± 0.4[b]	174.1 ± 6.2[b]
−	10	−2.4 ± 0.2[c]	87.4 ± 2.3[c]
+	10	−1.2 ± 0.6[d]	125.6 ± 6.0[d]

[a] Body weight change was computed as the difference in weights on the day of HxCB treatment and the day of necropsy. The mean initial body weight (±S.D.) was 22.9 ± 1.6 g and there was no difference between groups.
[b–d] Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison t tests.

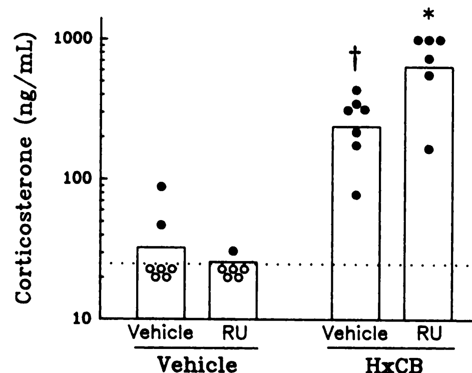

Fig. 2. The effects of RU 38486 administration for 10 days on plasma CS levels in HxCB-treated mice. Mice were treated as described in table 2. Data for plasma CS in male mice are presented as geometric means (bars) with individual animal data indicated by closed or open circles if above or below the lower limit of detection (20 ng/ml, dotted line), respectively. For statistical purposes, samples with levels below the lower limit of detection were given the value 20 ng/ml. †Indicates a significant HxCB effect between placebo-treated mice at $P < .05$. *Indicates a significant RU 38486 effect between HxCB-treated mice at $P < .05$.

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

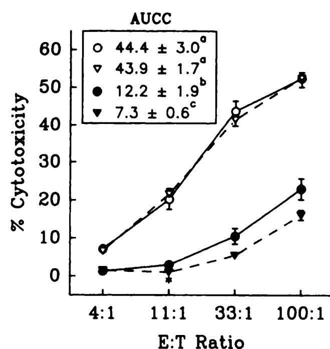

Fig. 3. The effects of RU 38486 administration for 10 days on CTL activity in HxCB-treated mice. Animals were treated as described in table 2. Symbols indicate double vehicle-treated animals (open circles), HxCB-treated/vehicle-treated animals (closed circles), vehicle-treated/RU 38486-treated animals (open triangles) and HxCB-treated/RU 38486-treated animals (closed triangles). The %CTX, at each E/T ratio, and AUCC for HxCB-treated mice were significantly less than in vehicle-treated mice ($P < .05$). *Indicates a significant RU 38486 effect at the indicated E/T ratio between HxCB-treated mice at $P < .05$. Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison *t* tests.

TABLE 3

Effects of HxCB and 3 days of RU 38486 treatment in P815-injected mice

Values represent mean ± S.E. for four to seven male mice given HxCB 1 day before injection with P815 cells; RU 38486 was given twice daily for 3 days as indicated. All animals were killed on day 11 post-HxCB treatment.

RU 38486	HxCB	Body Weight Change[a]	Spleen Cells	AUCC[b]
mg/kg/day	*mg/kg*	*g*	$\times 10^6$	
A. RU38486 given on days 3–5 postantigen injection				
0	0	2.6 ± 0.2[d]	149.2 ± 7.5[d]	49.2 ± 2.9[d]
150	0	2.6 ± 0.3[d]	142.7 ± 5.6[d]	50.1 ± 1.9[d]
0	10	−2.0 ± 0.8[e]	77.7 ± 4.0[e]	19.0 ± 2.9[e]
150	10	−0.4 ± 0.8[e]	98.2 ± 6.3[e]	18.2 ± 2.9[e]
B. RU38486 given on days 5–7 postantigen injection				
0	0	1.6 ± 0.2[d]	132.7 ± 4.3[d]	55.5 ± 1.7[d]
150	0	1.7 ± 0.4[d]	154.6 ± 6.8[d]	51.9 ± 1.5[d]
0	10	−1.7 ± 0.6[e]	81.8 ± 10.8[e]	20.1 ± 2.8[e]
150	10	N.D.[c]	N.D.	N.D.
C. RU38486 given on days 7–9 postantigen injection				
0	0	2.3 ± 0.2[d]	158.6 ± 5.9[d]	47.0 ± 1.2[d]
150	0	2.2 ± 0.2[d]	159.4 ± 9.5[d]	52.5 ± 1.4[e]
0	10	−1.2 ± 0.4[e]	86.1 ± 6.3[e]	18.8 ± 3.2[f]
150	10	−0.8 ± 0.3[e]	102.2 ± 4.9[e]	18.2 ± 3.4[f]

[a] *Body weight change was computed as the difference in weights on the day of HxCB treatment and the day of necropsy. The mean initial body weight (±S.D.) was 21.2 ± 1.4 g and there was no difference between days or groups.*

[b] *Area under the cytotoxicity curve (AUCC) is given for E/T ratios of 4:1, 11:1, 33:1 and 100:1.*

[c] *N.D. indicates not determined because all animals died before necropsy. The mean time to death was 7 days post-P815 injection.*

[d–f] *Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison t tests.*

not shown). Administration of RU 38486 to HxCB-treated mice on days 7 to 9 appeared to increase spleen cellularity (not significant; table 3) and did significantly increase spleen weight when measured as a percent of body weight (data not shown). Effects of RU 38486 treatment on days 5 to 7 post-P815 injection could not be assessed in HxCB-treated mice because of a 100% mortality rate (table 3). In addition, two out of six mice that were given RU 38486 on days 3 to 5 post-P815 injection died.

Effects of HxCB in male and female mice. Compared with male mice, female mice gained less body weight and had lower spleen cellularity after P815 injection (table 4). CTL activity in female mice was significantly lower than in male mice (fig. 5). In HxCB-treated mice, males were more sensitive to immune suppression than females; CTL activity (AUCC) was reduced 68% in males and 48% in females (fig. 5). HxCB treatment reduced spleen cellularity 50% in males and 17% in females (table 4). HxCB treatment led to a loss of body weight

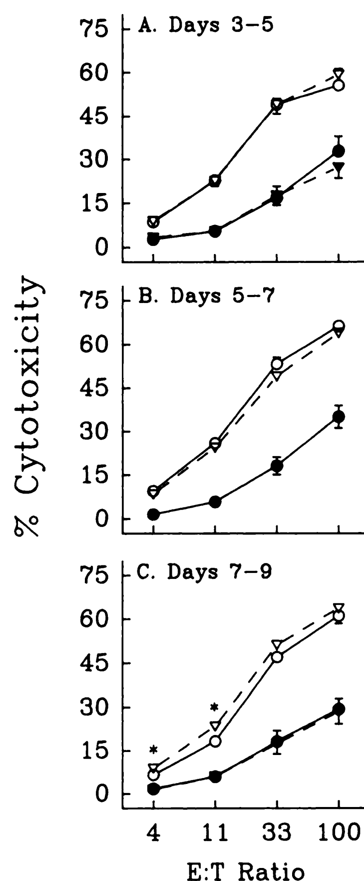

Fig. 4. The effects of RU 38486 administration for 3 days on CTL activity in HxCB-treated mice. Animals were treated as described in table 3. Symbols indicate vehicle-treated/placebo-treated animals (open circles), HxCB-treated/placebo-treated animals (closed circles), vehicle-treated/RU 38486-treated animals (open triangles) and HxCB-treated/RU 38486-treated animals (closed triangles). At each E/T ratio, the %CTX for HxCB-treated mice was significantly less than in vehicle-treated mice ($P < .05$). *Indicates a significant RU 38486 effect at an individual E/T ratio between vehicle-treated mice at $P < .05$. RU 38486 did not significantly alter CTL activity in HxCB-treated mice.

TABLE 4

Effects of HxCB treatment in male and female P815-injected mice

Values represent mean ± S.E. for five to eight animals. All animals were injected with P815 cells 1 day after HxCB treatment. All animals were killed 11 days after HxCB treatment.

Sex	HxCB	Body Weight Change[a]	Spleen Cells
	mg/kg	*g*	$\times 10^6$
Male	0	3.6 ± 0.3[b]	122.2 ± 5.1[b]
Male	10	−0.5 ± 0.4[c]	62.1 ± 1.9[c]
Female	0	2.1 ± 0.2[d]	106.6 ± 7.3[d]
Female	10	1.6 ± 0.3[d]	88.1 ± 2.7[e]

[a] Body weight change was computed as the difference in weights on the day of HxCB treatment and the day of necropsy. The mean initial body weight (±S.D.) for male and female mice was 18.8 ± 1.4 and 16.2 ± 0.7 g, respectively.

[b–e] Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison *t* tests.

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

JPET
PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
aspet

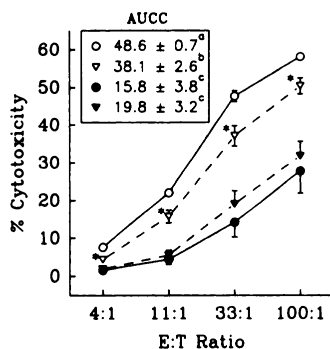

Fig. 5. The effects of HxCB treatment on CTL activity in male and female mice. Animals were treated as described in table 4. Symbols indicate vehicle-treated male animals (open circles), HxCB-treated male animals (closed circles), vehicle-treated female animals (open triangles) and HxCB-treated female animals (closed triangles). At each E/T ratio, the %CTX for HxCB-treated mice was significantly less than in vehicle-treated mice ($P < .05$). *Indicates a significant sex effect at individual E/T ratios between vehicle-treated mice ($P < .05$). Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison *t* tests.

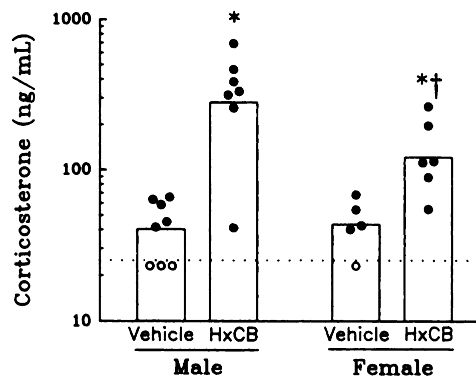

Fig. 6. Differential effects of HxCB on plasma CS levels in male and female mice. Animals were treated as described in table 4. Bars indicate the geometric mean with individual animal data indicated by closed or open circles if above or below the lower limit of detection (25 ng/ml, dotted line), respectively. For statistical purposes, samples with levels below the lower limit of detection were given the value of 25 ng/ml. †Indicates a significant sex effect between HxCB-treated mice at $P < .05$. *Indicates a significant HxCB effect between male or female mice at $P < .05$.

in male mice, whereas the gain in body weight was reduced by HxCB treatment in female mice (table 4). Plasma CS levels were significantly increased by HxCB treatment in both male and female mice, but the degree of elevation in male mice was greater than that observed in female mice (fig. 6).

Effects of HxCB in ODX male mice. As shown in table 5, ODX of P815-injected mice significantly increased spleen cellularity (114%), but had no effect on CTL activity (fig. 7). In contrast, ODX significantly enhanced CTL activity by 2.4-fold, as measured by AUCC, in HxCB-treated mice (fig. 7). However, CTL suppression by HxCB was not fully eliminated by ODX. In addition, ODX reduced the loss of body weight in HxCB-treated mice (table 5). HxCB treatment significantly reduced plasma TT levels by 80%, whereas ODX reduced plasma TT levels to nearly undetectable levels in both vehicle- and HxCB-treated mice (fig. 8A). ODX did not alter the HxCB-induced elevation of plasma CS (fig. 8B).

Regression analysis of CTL activity *vs.* CS levels. Data

TABLE 5

Effects of ODX and HxCB treatment in P815-injected mice

Values represent mean ± S.E. for five to eight male mice castrated or sham operated 7 days before treatment with HxCB. All animals were injected with P815 cells 1 day after HxCB treatment. All animals were killed 11 days after HxCB treatment.

ODX	HxCB	Body Weight Change[a]	Spleen Cells
	mg/kg	*g*	$\times 10^{6}$
−	0	1.8 ± 0.2[b,c]	135.0 ± 6.7[b]
+	0	2.9 ± 0.2[b]	154.1 ± 7.5[c]
−	10	−1.8 ± 0.7[c]	73.6 ± 4.2[d]
+	10	−0.7 ± 0.3[b]	78.9 ± 5.8[d]

[a] Body weight change was computed as the difference in weights on the day of HxCB treatment and the day of necropsy. The mean initial body weight (±S.D.) for sham and ODX mice was 24.7 ± 1.2 and 23.0 ± 1.5 g, respectively.

[b-d] Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison *t* tests.

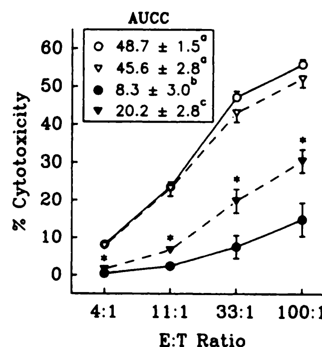

Fig. 7. The effects of ODX on CTL activity in HxCB-treated mice. Animals were treated as described in table 5. Symbols indicate vehicle-treated/sham-operated animals (open circles), HxCB-treated/sham-operated animals (closed circles), vehicle-treated/ODX animals (open triangles) and HxCB-treated/ODX animals (closed triangles). At each E/T ratio, the %CTX for HxCB-treated mice was significantly less than in vehicle-treated mice ($P < .05$). *Indicates a significant ODX effect at individual E/T ratios between HxCB-treated animals ($P < .06$). Values with different superscripts are significantly different ($P < .05$) as determined by analysis of variance followed by multiple comparison *t* tests.

were compiled from individual experiments and subjected to regression analysis to determine whether CTL suppression was correlated with increased CS levels. Only data from HxCB-treated mice were analyzed as CS levels in many control samples were measured at or below the lower limit of detection. As shown in figure 9, no significant correlation was found between CTL activity (AUCC) and CS level in HxCB-treated mice.

Discussion

Reduced CTL activity and elevation of plasma CS occurred coincidentally in HxCB-treated mice over the time course of CTL development after injection with P815 cells. Kerkvliet and Baecher-Steppan (1988a) showed that the kinetics of CTL generation was not altered by HxCB treatment in P815-injected mice, with the peak day of splenic CTL activity occurring on day 10 post-P815 injection. They also showed that the growth of peritoneal P815 cells was not altered by HxCB treatment, suggesting that CTL suppression is not due to a reduction of the antigen challenge in HxCB-treated mice. Recently, we have also shown that the expression of class I antigens (H-2D[d]) on peritoneal P815 cells, taken from C57Bl/6 mice, was not altered by HxCB treatment (unpublished data). Thus, the coincident nature of CS elevation and immune suppression suggested a

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

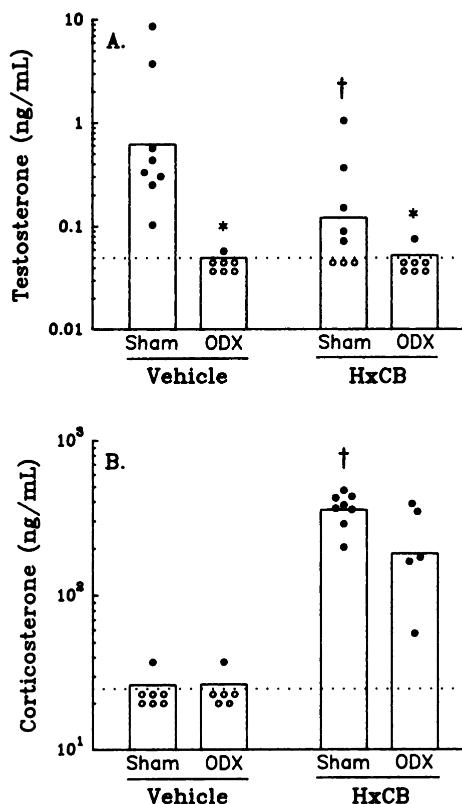

Fig. 8. The effects of ODX on plasma CS and TT levels in HxCB-treated mice. Animals were treated as described in table 5. Bars indicate the geometric mean with individual animal data indicated by closed or open circles if above or below the lower limit of detection (dotted lines), respectively. For statistical purposes, samples with levels below the lower limit of detection were given the value of 0.05 ng/ml (TT) or 25 ng/ml (CS). †Indicates a significant HxCB effect between sham-operated mice at P < .05. *Indicates a significant ODX effect between vehicle- or HxCB-treated mice at P < .05.

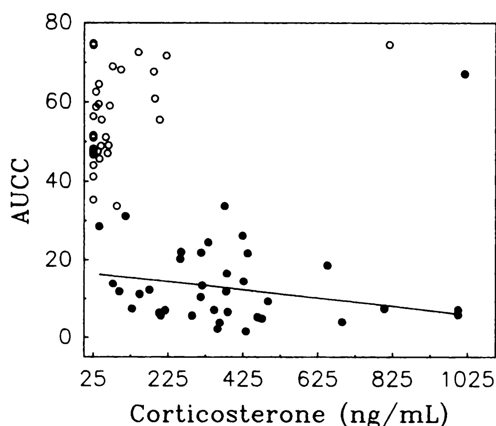

Fig. 9. The lack of correlation between plasma CS levels and CTL suppression. CTL activity (AUCC) is presented *vs.* plasma CS data compiled from five separate experiments. Animals were injected with P815 cells 1 day after treatment with HxCB at 0 or 10 mg/kg b.w. All animals were killed 11 days after HxCB treatment. The equation of the regression line for data from HxCB-treated animals is $y = -0.0107 \times x + 16.7865$ ($r^2 = 0.0791$). The slope is not significantly different from 0.

possible cause-effect relationship between them. In the studies reported here, attempts were made to reduce plasma CS levels in HxCB-treated mice to determine the impact on the CTL response.

The use of ADX mice was inadequate to determine whether CS elevation led to CTL suppression after HxCB treatment. Studies using ADX animals were complicated by a high rate of mortality (≥70%) among HxCB-treated animals, potentially caused by hypoglycemia. Although glucose levels of moribund HxCB-treated ADX animals were not routinely measured, the few animals that were sampled had extremely low plasma levels (≤40 mg/dl). In addition, hypoglycemia has been reported in TCDD-treated rats (Potter *et al.*, 1983; Gorski and Rozman, 1987). The likely mechanism responsible for hypoglycemia is reduced gluconeogenesis. GC stimulate gluconeogenesis and reduce glucose utilization in the periphery, two compensatory mechanisms that would be reduced in ADX animals. Further, HAH have been shown to reduce the activities of gluconeogenesis enzymes in rats (Messner *et al.*, 1976; Hsia and Kreamer, 1985; Weber *et al.*, 1991). Gorski *et al.* (1988b) showed that ADX increased the mortality rate and reduced the mean time to death in TCDD-treated rats. The dose of HxCB used here (10 mg/kg), when given alone, was nonlethal for P815-naive male C57Bl/6 mice; the survival time exceeded 84 days for unaltered mice and 21 days for ADX mice (data not shown). Therefore, it seems likely that the energy demands of rapidly proliferating tumor cells placed a lethal burden on the energy production capacity of HxCB-treated ADX mice. Interestingly, HxCB-treated ADX survivors had unexpectedly elevated plasma CS levels. This was potentially due to accessory-adrenal production of CS (Dunn, 1970; Hummel, 1958) as no adrenal glands were found in these animals at necropsy. Survival appeared to depend on the bioavailability of CS as supplementation with exogenous CS (2 mg/kg/day) reduced the mortality rate of HxCB-treated ADX mice (data not shown). The survival requirement for CS is supported by the observation that HxCB-treated mice had a 30% mortality rate if given RU 38486 on days 3 to 5 post-P815 injection, and a 100% mortality rate if given RU 38486 on days 5 to 7. In the latter group, the mean time to death was 7 days post-P815 injection which corresponds with the day of maximal tumor burden (Kerkvliet and Baecher-Steppan, 1988a). The lower mortality rate in HxCB-treated animals that received RU 38486 on days 3 to 5 may be due to a lower tumor burden. Rapid clearance of tumor from the peritoneal cavity occurs after day 7 post-P815 injection (Kerkvliet and Baecher-Steppan, 1988a), an event that may account for the lack of death observed in animals given RU 38486 on days 7 to 9.

Because of the inability to eliminate CS elevation in HxCB-treated mice *via* ADX, attempts were made to antagonize the physiologic effects of CS elevation by administering RU 38486, a competitive receptor antagonist of GC and progesteroids (Philibert, 1984). Preliminary experiments showed that thymic involution induced by 50 mg/kg of corticosterone-21-acetate i.p. was blocked by 24-hr pretreatment with RU 38486 at 150 mg/kg p.o. (data not shown). Therefore, this dose of RU 38486 was used in subsequent studies with HxCB. It was hypothesized that HxCB-induced CTL suppression was due to elevated CS and would be attenuated by daily treatment with RU 38486 after HxCB exposure. To the contrary, when CTL activity was analyzed as AUCC, a small but significant enhancement of the HxCB-induced CTL suppression was observed. The mechanism

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009
JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS JPET aspet

for this increased suppression by RU 38486 is unknown. It is unlikely that the observed hyperelevation of plasma CS would contribute to CTL suppression if RU 38486 were functioning as an effective GC antagonist. The efficacy of RU 38486 in HxCB-treated mice was suggested by its effects on body weight gain and spleen cellularity, effects that were not evident in RU 38486-treated control mice having normal CS levels. However, it is possible that the efficacy of RU 38486 is diminished after prolonged treatment in mice (Dr. S. Pruet, personal communication). Therefore, an additional study was conducted in which RU 38486 treatments were limited to durations of 3 days during the period in which CS elevation is known to occur in HxCB-treated/P815-injected mice. Administration of RU 38486 did not alter the HxCB-induced suppression of CTL activity when given on days 3 to 5 or days 7 to 9 post-P815 injection. These results argue against the hypothesis that CS elevation is responsible for CTL suppression in HxCB-treated mice. The 100% lethality of RU 38486, when given to HxCB-treated mice on days 5 to 7 post-P815 injection, further suggests its efficacy as a GC antagonist in this model. When compared to the lack of lethality in HxCB-treated mice given RU 38486 for 10 days, these data suggest that the efficacy of RU 38486 as a GC antagonist is reduced with prolonged treatment.

Previous studies in our laboratory have shown that male mice are more sensitive to HxCB-induced suppression of the CTL response when compared to female mice (Kerkvliet and Baecher-Steppan, 1988a). In addition, as shown here, HxCB treatment led to a greater elevation of plasma CS in male mice compared to female mice. It was hypothesized that the higher CS levels of male mice might explain the greater CTL suppression observed. Further, studies by Mebus *et al.* (1987) have shown that treatment with TCDD can affect the metabolism of steroids in the testes of rats. Abnormal CS production in the testes of HxCB-treated mice might result in the significantly higher CS levels observed in male mice. Therefore, ODX male mice were tested for their sensitivity to HxCB. ODX did not eliminate the HxCB-induced elevation of plasma CS; therefore, the testes are not of significant concern as a site of CS production in this model. However, unlike ADX or RU 38486 treatment, ODX significantly enhanced CTL activity in HxCB-treated mice. This may be attributable to the elimination of testes-specific factors. TT has been shown to suppress immune function (Hirasawa and Enosawa, 1990), and ODX has been shown to increase both lymphoid organ weights and antigen-specific immune responses (Castro, 1974a,b). However, because ODX in vehicle-treated mice did not enhance the CTL response, the data suggest that HxCB may alter the sensitivity of the immune system to testes-derived factor(s), and/or that HxCB treatment alters the production of testes-specific immunomodulatory factor(s). In the latter case, TT alone is not a likely candidate because circulating levels were decreased in HxCB-treated mice. HxCB effects on the testes could explain the higher sensitivity of male mice to HxCB-induced immune suppression. However, the basis of the remaining HxCB-induced CTL suppression in female and ODX male mice remains unknown.

After treatment with various HAH, both rats and mice have been shown to have elevated circulating glucocorticoid levels (Sanders *et al.*, 1974, 1977; Gorski *et al.*, 1988a). DiBartolomeis *et al.* (1987) and Jones *et al.* (1987) have suggested that morning CS elevation in TCDD-treated rats is caused by a dysregulation of the normal diurnal CS rhythm. In contrast, Dunn *et al.* (1983) showed that Aroclor 1254 did not alter the diurnal rhythm of CS in mice. Results in our laboratory have indicated that, whereas the normal evening CS elevation was significantly enhanced by HxCB treatment, the diurnal CS cycle of mice was unaffected 6 days after HxCB treatment. This was observed for both P815-injected and -naive mice (unpublished results). Therefore, morning CS elevation in HxCB-treated, P815-injected mice is most likely due either to augmented production or reduced clearance of CS. Evidense to support the latter cause has been observed in this laboratory: CS supplementation (2 mg/kg/day) of P815-injected ADX mice led to higher plasma CS levels after HxCB treatment *vs.* vehicle treatment.

Recently, it has been reported that treatment of rats with HAH leads to a reduction in the binding capacity of GCR in both liver (Lin *et al.*, 1991; Ryan *et al.*, 1989; Sunahara *et al.*, 1989) and skeletal muscle (Max and Silbergeld, 1987). A desensitization of the hypothalamus to the negative feedback of CS, *via* a reduced GCR binding capacity, could conceptually explain higher plasma CS levels after HxCB exposure. Such an effect might be enhanced by receptor antagonists leading to even higher CS levels, as was shown here after RU 38486 treatment. Further, if a reduction in the binding capacity of GCR is a generalized effect of HxCB treatment in all tissues, the degree of CS elevation observed here for HxCB-treated mice may have less of an impact on immune function, or other physiological processes, than an elevation of equal magnitude in untreated mice.

Acknowledgments

The authors would like to thank Dr. Karen Timm, Julie Oughton and Annette Youngberg for their technical assistance and Paula Herd for her assistance with this manuscript.

References

Bandiera, S., Safe, S. and Okey, A. B.: Binding of polychlorinated biphenyls classified as either phenylbarbitone-, 3-methylcholanthrene-, or mixed-type inducers to cytosolic Ah receptor. Chem-Biol. Interact. **39:** 259–277, 1982.

Bradley, L. M. and Mishell, R. I.: Selective protection of murine thymic helper cells from glucocorticosteroid inhibition by macrophage-derived mediators. Cell. Immunol. **73:** 115–127, 1982.

Castro, J. E.: Orchidectomy and the immune response I. Effect of orchidectomy on lymphoid tissues of mice. Proc. R. Soc. Lond. B. Biol. Sci. **185:** 425–436, 1974a.

Castro, J. E.: Orchidectomy and the immune response II. Response of orchidectomized mice to antigen. Proc. R. Soc. Lond. B. Biol. Sci. **185:** 437–451, 1974b.

Clark, D. A., Gauldie, J., Szewczuk, M. R. and Sweeney, G.: Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Proc. Soc. Exp. Biol. Med. **168:** 290–299, 1981.

Clark, D. A., Sweeney, G., Safe, S., Hancock, E., Kilburn, D. G. and Gauldie, J.: Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. Immunopharmacology **6:** 143–153, 1983.

del Rey, A., Besedofsky, H. and Sorkin, E.: Endogenous blood levels of corticosterone control the immunologic cell mass and B cell activity in mice. J. Immunol. **133:** 572–575, 1984.

DiBartolomeis, M. J., Moore, R. W., Peterson, R. E., Christian, B. J. and Jeffcoate, C. R.: Altered regulation of adrenal steroidogenesis in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. Biochem. Pharmacol. **36:** 59–67, 1987.

Dunn, J. D., Carter, J. W. and Henderson, D. A.: Effect of polychlorinated biphenyls (Aroclor 1254) on rhythmic pituitary-adrenal function. Bull. Environ. Contam. Toxicol. **31:** 322–325, 1983.

Dunn, T. B.: Normal and pathologic anatomy of the adrenal gland of the mouse, including neoplasms. J. Natl. Cancer Inst. **44:** 1323–1389, 1970.

Gillis, S., Crabtree, G. R. and Smith, K. A.: Glucocorticoid induced inhibition of T cell growth factor production. II. The effect on the in vitro generation of cytolytic T cells. J. Immunol. **123:** 1632–1638, 1979.

Gorski, J. R. and Rozman, K.: Dose-response and time course of hypothyroxinemia and hypoinsulinemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. Toxicology **44:** 297–307, 1987.

Gorski, J. R., Muzi, G., Weber, L. W., Pereira, P. W., Iatropoulos, M. J. and Rozman, K.: Elevated plasma corticosterone levels and histopathology of the adrenals and thymuses in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. Toxicology **53:** 19–32, 1988a.

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009

GORSKI, J. R., ROZMAN, T., GREIM, H. AND ROZMAN, K.: Corticosterone modulates acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in male Sprague-Dawley rats. Fundam. Appl. Toxicol. **11:** 494–502, 1988b.

HIRASAWA, K. AND ENOSAWA, S.: Effects of sex steroid hormones on sex-associated differences in the survival time of allogeneic skin grafts in rats. Transplantation (Balto.) **50:** 637–641, 1990.

HSIA, M. T. S. AND KREAMER, B. L.: Delayed wasting syndrome and alterations of liver gluconeogenic enzymes in rats exposed to the TCDD congener 3,3′,4,4′-tetrachloroazoxybenzene. Toxicol. Lett. (Amst.) **25:** 247–258, 1985.

HUMMEL, K. P.: Accessory adrenal cortical nodules in the mouse. Anat. Rec. **132:** 281–295, 1958.

JONES, M. K., WEISENBURGER, W. P., SIPES, I. G. AND RUSSELL, D. H.: Circadian alterations in prolactin, corticosterone, and thyroid hormone levels and down-regulation of prolactin receptor activity by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. **87:** 337–350, 1987.

KERKVLIET, N. I., BAECHER-STEPPAN, L., SMITH, B. B., YOUNGBERG, J. A., HENDERSON, M. C. AND BUHLER, D. R.: Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): Structure-activity relationships and effects in C57Bl/6 mice congenic at the Ah locus. Fundam. Appl. Toxicol. **14:** 532–541, 1990.

KERKVLIET, N. I., BAECHER-STEPPAN, L.: Suppression of allograft immunity by 3,4,5,3′,4′,5′-hexachlorobiphenyl. I. Effects of exposure on tumor rejection and cytotoxic T cell activity in vivo. Immunopharmacology **16:** 1–12, 1988a.

KERKVLIET, N. I. AND BAECHER-STEPPAN, L.: Suppression of allograft immunity by 3,4,5,3′,4′,5′-hexachlorobiphenyl. II. Effects of exposure on mixed lymphocyte reactivity and induction of suppressor cell activity in vitro. Immunopharmacology **16:** 13–23, 1988b.

LIN, F. H., STOHS, S. J., BIRNBAUM, L. S., CLARK, G., LUCIER, G. W., AND GOLDSTEIN, J. A.: The effects of 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah nonresponsive C57Bl/6 mice. Toxicol. Appl. Pharmacol. **108:** 129–166, 1991.

MAX, S. R. AND SILBERGELD, E. K.: Skeletal muscle glucocorticoid receptor and glutamine synthetase activity in the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. **87:** 523–527, 1987.

MCFARLAND, V. A. AND CLARKE, J. U.: Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. Environ. Health Perspect. **81:** 225–239, 1989.

MEBUS, C. A., REDDY, V. R. AND PIPER, W. N.: Depression of rat testicular 17-hydroxylase and 17,20-lyase after administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Biochem. Pharmacol. **36:** 727–731, 1987.

MESSNER, B., BERNDT, J. AND STILL, J.: Inhibition of PEP-carboxykinase in rat liver by polychlorinated biphenyl. Nature (Lond.) **263:** 599–600, 1976.

PHILIBERT, D.: RU 38486: An original multifaceted antihormone in vivo. *In* Adrenal Steroid Antagonism, ed. by M. K. Agarwal, pp. 77–101, Walter de Gruyter & Co., New York, 1984.

POLAND, A. AND KNUTSON, J. C.: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. **22:** 517–554, 1982.

POTTER, C. L., SIPES, I. G. AND RUSSELL, D. H.: Hypothyroxinemia and hypothermia in rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administration. Toxicol. Appl. Pharmacol. **69:** 89–95, 1983.

RYAN, R. P., SUNAHARA, G. I., LUCIER, G. W., BIRNBAUM, L. S. AND NELSON, K. G.: Decreased ligand binding to the hepatic glucocorticoid and epidermal growth factor receptors after 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran treatment of pregnant mice. Toxicol. Appl. Pharmacol. **98:** 454–464, 1989.

SANDERS, O. T., KIRKPATRICK, R. L. AND SCANLON, P. E.: Polychlorinated biphenyls and nutritional restriction: Their effects and interactions on endocrine and reproductive characteristics of male white mice. Toxicol. Appl. Pharmacol. **40:** 91–98, 1977.

SANDERS, O. T., ZEPP, R. L. AND KIRKPATRICK, R. L.: Effect of PCB ingestion on sleep times, organ weights, food consumption, serum corticosterone and survival of albino mice. Bull. Environ. Contam. Toxicol. **12:** 394–399, 1974.

SCHECHTER, B. AND FELDMAN, M.: Hydrocortisone affects tumor growth by eliminating precursors of suppressor cells. J. Immunol. **119:** 1563–1568, 1977.

SCHLEIMER, R. P., JACQUES, A., SHIN, H. S., LICHTENSTEIN, L. M. AND PLAUT, M.: Inhibition of T cell-mediated cytotoxicity by anti-inflammatory steroids. J. Immunol. **132:** 266–271, 1984.

SHIMIZU, K., AMAGAYA, S. AND OGIHARA, Y.: Analysis of corticosterone in the serum of mice and rats using high-performance liquid chromatography. J. Chromatogr. **272:** 170–175, 1983.

SILKWORTH, J. B. AND GRABSTEIN, E. M.: Polychlorinated biphenyl immunotoxicity: Dependence on isomer planarity and the Ah gene complex. Toxicol. Appl. Pharmacol. **65:** 109–115, 1982.

SILKWORTH, J. B. AND ANTRIM, L.: Relationship between Ah receptor-mediated polychlorinated biphenyl (PCB)-induced humoral immunosuppression and thymic atrophy. J. Pharmacol. Exp. Ther. **235:** 606–611, 1985.

SUNAHARA, G. I., LUCIER, G. W., MCCOY, Z., BREZNICK, E. H. AND SANCHEZ, E. R.: Characterization of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin mediated decrease in dexamethasone binding to rat hepatic cytosolic glucocorticoid receptor. Mol. Pharmacol. **36:** 239–247, 1989.

VICKERS, A. E. M., SLOOP, T. C., LUCIER, G. W.: Mechanism of action of toxic halogenated aromatics. Environ. Health Perspect. **59:** 121–128, 1985.

VOS, J. G. AND LUSTER, M. I.: Immune alterations. *In* Halogenated Biphenyls, Terphenyls, Napthalenes, Dibenzodioxins and Related Products, eds. by R. D. Kimbrough and A. A. Jensen, pp. 295–322, Elsevier Science Publishers B.V., Amsterdam, 1989.

WEBER, L. W. D., LEBOFSKY, M., STAHL, B. U., GORSKI, J. R., MUZI, G. AND ROZMAN, K.: Reduced activities of key enzymes of gluconeogenesis as possible cause of acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicology **66:** 133–144, 1991.

Send reprint requests to: Nancy I. Kerkvliet, Ph.D., Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

Downloaded from jpet.aspetjournals.org at Univ of Northern Colorado Michener Lib/Serials Record Div ES on July 2, 2009