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Heart as a target organ in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: Decreased β-adrenergic responsiveness and evidence of increased intracellular calcium

(isoproterenol/force frequency/guinea pig papillary muscle/polyhalogenated aromatic hydrocarbons)

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ABSTRACT **The heart has not been regarded as a major target organ of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity notwithstanding that lethal cardiac dysfunction can occur in the absence of histopathological changes. To assess possible TCDD cardiotoxicity, we studied the effect of TCDD five days after treatment (10 μg/kg of body weight; single dose given i.p. in corn oil) on the contractility of guinea pig right ventricular papillary muscle. Controls were treated with corn oil. TCDD treatment significantly decreased β-adrenergic responsiveness. In papillary muscles from TCDD-treated guinea pigs, the positive inotropic effect of isoproterenol (0.03–0.3 μM) was decreased by a mean of 65% ($P < 0.001$), and the enhancement in the velocity of relaxation was 60% less than in the controls ($P < 0.05$). On the other hand, TCDD treatment did not alter the positive inotropic effect of lower concentrations of isoproterenol (0.1–10 nM). After TCDD, responsiveness to low-frequency stimulation (0.1 and 0.25 Hz) was enhanced, responsiveness to increases in extracellular Ca^{2+} concentration was attenuated, and isoproterenol-elicited aftercontractions in K^+-depolarized preparations were increased in magnitude. Collectively, the latter findings suggest that in addition to decreasing β-adrenergic responsiveness, TCDD increases the intracellular Ca^{2+} concentration in papillary muscle. Finally, slow Ca^{2+} channels were not blocked after TCDD treatment, inasmuch as isoproterenol restored contractility equally effectively in K^+-depolarized TCDD-treated and control papillary muscles. Our findings indicate that TCDD causes a specific pattern of cardiac dysfunction in a mammalian species, selectively augmenting or decreasing different cardiac responses. The cardiac changes are consistent with reported membrane effects of TCDD; further, they suggest that the heart may be a major target organ for TCDD toxicity.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype of a group of chemically related, highly toxic environmental chemicals. Binding by TCDD and other toxic polyhalogenated biphenyls to specific cytosolic and nuclear receptors leads to induction of a group of enzymes, the best-characterized of which are specific isozymes of cytochrome P-450. These chemicals also elicit a symptom complex characterized by weight loss, thymic involution, tumor promotion, edema, increased mortality, and in some species, keratinocyte proliferation (1). Neither the biochemical basis for the toxic changes nor the cause of death is understood at the present time. For example, although the same structure–activity relationships pertain for production of toxicity and P-448 isozyme induction, P-448 induction *per se* cannot account for the toxic changes (1, 2). Histopathologic changes found in thymus and liver, the main organs exhibiting cytologic damage, have been insufficient to explain the increased mortality (3). Characteristically, animals die "suddenly without any other signs of toxicity" (4).

Although there have been occasional reports of cardiac involvement (4–7) after TCDD exposure, the heart has not been considered a major target organ of polyhalogenated hydrocarbon toxicity. Cardiac contractile malfunction can, however, lead to death without accompanying histologic changes.

Previous work using a chicken embryo model showed that treatment with toxic polychlorinated biphenyls (but not with nontoxic congeners) decreased the contractile response of cardiac ventricular muscle to β-adrenergic stimulation (8). To ascertain whether TCDD affects mammalian cardiac contractile function, and accordingly whether the heart may be a target organ of polyhalogenated hydrocarbon toxicity across species, we examined the effect of TCDD treatment on the contractile responses of guinea pig papillary muscles to various stimuli including isoproterenol and calcium. The results show that there is a striking decrease in β-adrenergic responsiveness of TCDD-treated guinea pig papillary muscle and suggest that in addition to this defect, TCDD may increase the intracellular calcium content of guinea pig papillary muscles.

MATERIALS AND METHODS

Animals, Treatment, and Preparation of Tissues. TCDD (provided by A. Poland, McArdle Cancer Laboratory, Madison, WI) was dissolved in 0.1 ml of acetone and diluted in corn oil to 25 μg/ml, after which the acetone was evaporated with a stream of nitrogen. Corn oil with acetone alone was treated comparably. Male Hartley guinea pigs (300–350 g) were injected i.p. with TCDD (10 μg/kg of body weight) in corn oil or with an equivalent volume of corn oil alone (controls). Guinea pigs were maintained on Purina guinea pig chow (no. 5025) and water ad libitum for 5 days. They were weighed at the beginning and end of that period. After 5 days they were lightly anesthetized by exposure to CO_2 vapor and then killed by a sharp blow to the base of the skull. Livers and thymus glands were removed and weighed. Hearts were excised and immediately transferred to a dish containing a modified Tyrode's solution at pH 7.4 (149.3 mM Na^+/5.4 mM K^+/1.8 mM Ca^{2+}/1.05 mM Mg^{2+}/0.4 mM $H_2PO_4^-$/148.1 mM Cl^-/11.9 mM HCO_3^-/10 mM glucose) aerated with 95% O_2 and 5% CO_2. The right ventricular papillary muscle was dissected, after which the rest of the heart was weighed.

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; $[Ca^{2+}]_i$, intracellular Ca^{2+}; $[Ca^{2+}]_o$, extracellular Ca^{2+}.

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Papillary Muscle Preparations. Each papillary muscle was suspended vertically in a double-walled tissue chamber containing 2.5 ml of aerated Tyrode's solution maintained at 30°C. The tendinous end of the papillary muscle was connected with a thread to an isometric force-displacement transducer (Grass FT03C). The preparation was paced at a basal frequency of 1 Hz using square-wave pulses (duration, 5 msec; voltage, 1.5 times threshold) delivered by two coiled platinum electrodes. The electrodes were connected to a stimulator (Grass, model S88) via a stimulus-isolation unit (Grass, model SIU5). Changes in tension were displayed on a polygraph (Grass, model 7D). Resting tension was adjusted to 0.5 g and the muscles were allowed to equilibrate for 1 hr before further experimental procedures.

Measurement of Contractile Responses. The effect of isoproterenol on contractility of the papillary muscles was determined, as described by Van Rossum (9), from 0.1 nM to 0.3 μM isoproterenol [(−)-isoproterenol bitartrate (Sigma) dissolved in Tyrode's solution containing ascorbic acid at 1 mg/ml]. Isoproterenol was added to the tissue bath in cumulative increments of 0.5 logarithmic units after the increase in contraction from the previous dose reached a plateau. Peak developed tension was measured before and after each dose of isoproterenol. A full dose–response curve was constructed for each preparation. The time from peak tension to half relaxation (in msec) was measured from high-speed tracings taken before and after isoproterenol; values for three successive contractions were averaged for each preparation.

The contractile response to increasing stimulus frequency, the force-frequency response, was measured by stepwise stimulation of the muscle between 0.1 and 1 Hz. Frequency was increased when the developed tension reached a plateau (10). Force-frequency responses were measured after equilibrium and before the addition of isoproterenol.

The contractile responses to changes in Ca^{2+} concentration between 1.8 mM and 7.7 mM were evaluated by adding $CaCl_2$ to the bathing medium in increments of 0.4–0.8 mM.

Depolarization with Potassium. After examining the response to isoproterenol, some papillary muscles were washed free of isoproterenol and reequilibrated in Tyrode's solution for 30 min. The bathing medium was then changed to Tyrode's solution containing 22 mM K^+ in which osmolarity was maintained by lowering the sodium content (11). Stimulation frequency was reduced to 0.2 Hz, intensity was increased 2-fold, and stimulus duration was held at 5 msec. The time required for contractions to stop was measured. After about 10 min in high-K^+ medium, isoproterenol was added at concentrations that had elicited maximum responses at normal extracellular K^+ concentration to learn whether isoproterenol would restore contractility in the K^+-depolarized preparations.

Statistical Evaluation of Data. Data were analyzed by two-tailed *t* tests where appropriate. Dose–response curves were evaluated by two-way analyses of variance. Post hoc comparisons were made with the two-tailed *t* test using the error term from the antecedent analysis of variance.

RESULTS

Response to Isoproterenol. Mean tension developed by papillary muscles from control guinea pigs before addition of isoproterenol was the same as that of muscles from TCDD-treated guinea pigs [mean ± SEM; 444 ± 57 mg for controls (n = 8); 474 ± 76 mg for TCDD-treated (n = 11)]. The contractile responses to isoproterenol are shown in Fig. 1. Analysis of variance indicated that TCDD had a significant effect on the response to isoproterenol, [$F(1,99) = 10.74$, $P < 0.002$]. The response was less in the TCDD-treated muscles than in the controls at the higher concentrations of isoproterenol ($P < 0.01$ at 0.03–0.3 μM isoproterenol) but was not significantly changed at lower concentrations of isoproterenol (0.1–10 nM). Thus TCDD treatment decreased β-adrenergic responsiveness at high but not at low concentrations of isoproterenol.

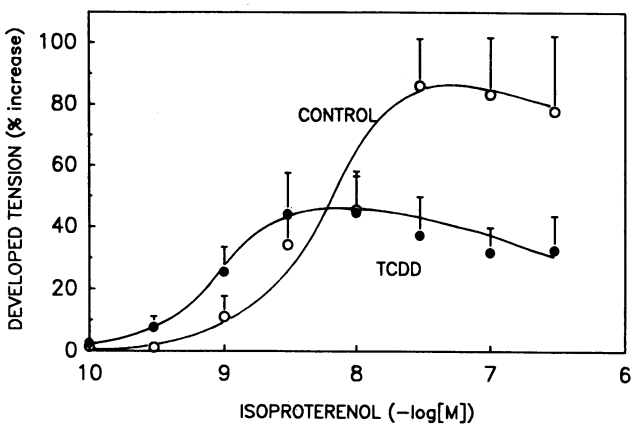

FIG. 1. Dose–response curves for effects of isoproterenol on contractile tension developed by guinea pig papillary muscles. Papillary muscles were removed from right ventricles of male guinea pigs 5 days after i.p. injection of either TCDD (10 μg/kg) in corn oil (●) or an equivalent volume of corn oil (control, ○). Preparations were electrically stimulated at 1 Hz in normal Tyrode's solution. Isoproterenol was added in cumulative increments of 0.5 logarithmic units. Results are expressed as the percent increase in tension developed over the basal tension prior to the addition of isoproterenol, which was considered as 100%. Basal tension (mean ± SEM) was 444 ± 57 mg for controls (n = 8 animals) and 474 ± 76 mg for the TCDD-treated papillary muscles (n = 11 animals) (not significant). Values shown are means; error bars represent 1 SEM.

Relaxation Time for the Contractile Response. β-Adrenergic stimulation is known to shorten the relaxation time for cardiac muscle contraction (12). Isoproterenol shortened the relaxation time in both groups but did so less in the TCDD-treated papillary muscles than in the controls (18% in the TCDD-treated and 30% in the controls, respectively, $P < 0.05$; Table 1). Thus, papillary muscles from TCDD-treated guinea pigs responded less than the controls to the β-adrenergic-mediated decrease in relaxation time as well as to the β-adrenergic-mediated positive inotropic effect.

Effect of Increasing Stimulation Frequency on Contractile Force. Fig. 2 compares the effect of increasing stimulation frequency on developed tension in papillary muscles from control and TCDD-treated guinea pigs. Analysis of variance showed a significant effect of TCDD treatment [$F(1,24) = 13.5$, $P < 0.001$]. The contractile responses at frequencies of

Table 1. Effect of TCDD treatment on the relaxation time for papillary muscle contraction after isoproterenol

Treatment*	Time for half relaxation, msec: Before isoproterenol	Time for half relaxation, msec: After isoproterenol†	Decrease in half relaxation time, msec
Control	141 ± 4	98 ± 4‡	43 ± 5
TCDD	125 ± 8	103 ± 7‡	22 ± 7§

All values are means ± SEM.
*Guinea pigs were injected with corn oil (control; n = 5 animals) or with TCDD in corn oil (n = 7 animals) 5 days before the papillary muscles were isolated.
†The record was read at the dose of isoproterenol giving the maximal contractile response for each preparation as determined in the experiments shown in Fig. 1.
‡Significantly different from value before addition of isoproterenol; $P < 0.001$ for the control group and $P < 0.05$ for the TCDD-treated group.
§Significantly different from control group; $P < 0.05$.

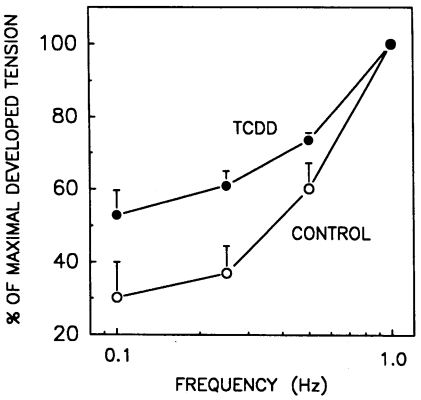

FIG. 2. Force-frequency response for papillary muscles from TCDD-treated (●) and corn oil-treated (○) guinea pigs. Guinea pigs were treated as described in the legend to Fig. 1. Papillary muscles were stimulated at 0.1, 0.25, 0.5, and 1.0 Hz in a stepwise manner. The stimulation frequency was successively increased after the developed tension at the prior frequency had reached a plateau. The tension developed at 1 Hz was defined as maximal (100%), and the results are expressed as mean percent of that maximal tension. Mean developed tension at 1 Hz (±SEM) was 482 ± 83 mg for the control papillary muscles (n = 4) and 496 ± 109 mg for the TCDD-treated group (n = 4) (not significant). Values shown are means; error bars represent 1 SEM.

0.1 and 0.25 Hz were significantly greater for TCDD-treated preparations than for controls ($P < 0.01$).

Response to Calcium. Fig. 3 shows the effect of increasing the Ca^{2+} concentration in the bathing medium ($[Ca^{2+}]_o$) from 1.8 mM, the concentration in the standard Tyrode's medium, to 7.7 mM. Initial developed tensions did not differ significantly in the corn oil and TCDD groups [mean developed tension ± SEM: 581 ± 123 mg for controls (n = 4); 660 ± 210 mg for TCDD-treated (n = 4)], but the response to Ca^{2+} was decreased in the TCDD-treated group. Analysis of variance showed that the decreased response of the TCDD-treated tissues to Ca^{2+} was significant [$F(1,48) = 13.3$, $P < 0.001$]. Also in the TCDD-treated muscles, dose-related increases in contractility were observed over a narrower range of $[Ca^{2+}]_o$ (1.8–3.6 mM) than in the controls (1.8 mM–5.4 mM).

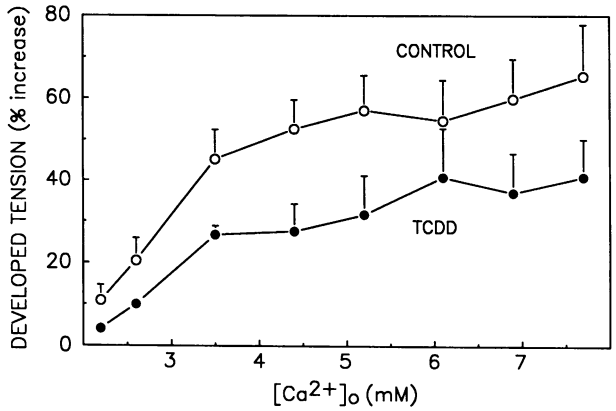

FIG. 3. Concentration–response curves for effect of $[Ca^{2+}]_o$ on contractility of papillary muscles from TCDD-treated (●) and corn oil-treated (○) guinea pigs. Papillary muscles were from guinea pigs treated with TCDD or corn oil as described for Fig. 1. Ca^{2+} was added to the oxygenated Tyrode's solution in cumulative increments. Results are expressed as the mean percent increase in tension in the presence of added Ca^{2+} over the basal tension at the starting Ca^{2+} concentration of 1.8 mM. Mean basal tension at 1.8 mM Ca^{2+} (±SEM) was 581 ± 123 mg for controls (n = 4) and 660 ± 210 mg for the TCDD-treated group (n = 4) (not significant). Values shown are means; error bars represent 1 SEM.

Response to High Potassium. In medium of high K^+ concentration the cardiac membrane is depolarized and fast Na^+-, K^+-, and Ca^{2+}-channel activities are eliminated. Stimulation of contraction after depolarization in high K^+ reflects only slow Ca^{2+}-channel activity, and the ability of drugs to prevent isoproterenol from restoring contractility in cardiac muscle depolarized by high K^+ is used as an index of Ca^{2+}-channel-blocking activity (13).

When papillary muscles from corn oil- and TCDD-treated guinea pigs were exposed to Tyrode's solution containing 22 mM K^+ (high-K^+ medium), the time to complete cessation of contraction was 84% longer in the TCDD-treated preparations [mean time ± SEM: 562 ± 92.7 sec for the TCDD group (n = 5); 305 ± 103 sec for the controls (n = 5); P = 0.1]. A sixth papillary muscle preparation from a TCDD-treated guinea pig did not stop contracting even after 30 min. Thus TCDD treatment appeared to make the papillary muscles more resistant to depolarization by high K^+.

TCDD did not prevent isoproterenol from restoring contractility in high-K^+ medium. Thus isoproterenol restored contractility in all of the control and TCDD-treated preparations, indicating that TCDD does not act as a Ca^{2+}-channel blocker.

Although contractility was restored by isoproterenol in both control and TCDD-treated tissues, the magnitude of the aftercontractions differed in the two groups. The mean aftercontraction ± SEM was 16 ± 12 mg for the controls and 103 ± 38 mg for the TCDD-treated papillary muscles (n = 4, P = 0.07).

Other Evidence of TCDD Toxicity. Before treatment the body weights of the two groups did not differ significantly (Table 2). Five days after treatment, 14 of the 15 TCDD-treated guinea pigs lost weight (range, 35–131 g); one gained only 5 g. All of the controls gained weight (range, 22–76 g). Other than weight loss the TCDD-treated animals did not exhibit any gross evidence of toxicity. Liver and heart weights were decreased after TCDD treatment, but when expressed per gram of body weight, liver weights were the same in both groups and heart weights were slightly greater in the TCDD-treated group. Thymus weights were decreased significantly both by absolute measurement and per gram of body weight in the TCDD-treated group. Thus the cardiac effects were observed in animals exhibiting the characteristic major signs of TCDD toxicity: body weight loss and a selective decrease of thymic weight.

DISCUSSION

The findings that papillary muscles from TCDD-treated guinea pigs exhibit reduced contractile responsiveness to stimulation by isoproterenol at concentrations >10 nM and that isoproterenol reduced the relaxation time in TCDD-treated papillary muscles less than in controls indicate that cardiac β-adrenergic responsiveness is decreased after TCDD treatment.

The findings are consistent with recent evidence of β-adrenergic desensitization in cultured keratinocytes exposed to TCDD (14). An earlier report that benzo[a]pyrene applied to skin of mice decreased the cAMP response to isoproterenol in skin suggested that other cytochrome P-448 inducers might impair β-adrenergic responsiveness (15). The results reported here suggest that pervasive impairment of β-adrenergic responsiveness may be a characteristic effect of TCDD and other P-448-type inducers.

Decreased β-adrenergic responsiveness could be due to decreased β-adrenergic receptor number or affinity, possibly secondary to prior exposure of the heart to β-adrenergic stimulants (e.g., catecholamines), or by uncoupling of β-adrenergic receptors from adenylate cyclase. While increases in catecholamines have not been reported after

Table 2. Body and organ weights of control and TCDD-treated guinea pigs

Treatment*	*n*	Body weight, g		Change in body weight, g	Organ weight, g†		
		Before treatment	After treatment		Thymus	Liver	Heart
Control	12	325 ± 13	358 ± 12‡	+33 ± 4‡	0.522 ± 0.047 (0.146 ± 0.009)	13.4 ± 1.0 (3.7 ± 0.2)	0.948 ± 0.030 (0.26 ± 0.01)
TCDD	15	332 ± 14	263 ± 10‡	−69 ± 11‡	0.284 ± 0.026§ (0.107 ± 0.008)¶	10.7 ± 4‖ (4.1 ± 0.1)	0.848 ± 0.039** (0.322 ± 0.008)§

All values are means ± SEM.
*Guinea pigs received a single i.p. dose of TCDD (10 μg/kg) in corn oil. Controls received corn oil alone. Animals were killed 5 days later.
†Values in parentheses are (organ weight/body weight) × 100.
‡$P < 0.001$ for before and after treatment.
§$P < 0.001$, for TCDD vs. control.
¶$P < 0.01$, for TCDD vs. control.
‖$P < 0.05$, for TCDD vs. control.
**$P = 0.05$, for TCDD vs. control.

TCDD exposure, defects in both β-adrenergic receptor number and coupling were reported in keratinocytes exposed to TCDD (14). Phorbol esters, tumor promoters like TCDD, also cause β-adrenergic desensitization (16), an effect attributed to receptor–adenylate cyclase uncoupling (17). TCDD has been found to increase protein kinase C in rat and guinea pig liver (18) suggesting that phorbol esters and TCDD may decrease cAMP-mediated β-adrenergic responses by similar mechanisms.

TCDD-treated papillary muscles also had effects other than decreased β-adrenergic responsiveness. Thus, it is noteworthy that the response to low concentrations of isoproterenol was not decreased by TCDD treatment, although decreased responses at both low and high concentrations would be expected if only β-adrenergic responsiveness were disturbed. Further, the force-frequency response was greater in TCDD-treated tissues than in controls; this also would not be expected as a consequence of impaired β-adrenergic responsiveness alone. Increasing the frequency of stimulation increases first Na^+ and then Ca^{2+} influx into the cells (19). The resulting increases in Ca^{2+} release from the sarcoplasmic reticulum lead to increased contractility (20). The increased response of TCDD-treated papillary muscles to stimulation at low frequency, and their unimpaired responsiveness to stimulation by low but not higher concentrations of isoproterenol, suggests that in addition to decreasing β-adrenergic responsiveness, TCDD treatment may increase the Ca^{2+} content of papillary muscle cells.

The hypothesis that intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is increased by TCDD treatment is supported by our other findings. Thus, if TCDD treatment increased $[Ca^{2+}]_i$, the treated tissues might be better able to respond to small increases in $[Ca^{2+}]_i$ than controls, compatible with the enhanced responses to 0.1 and 0.25 Hz (Fig. 2), but be more refractory than controls to larger increases in Ca^{2+}, as was in fact observed in hearts stimulated at 1 Hz and exposed to further increments in the $[Ca^{2+}]_o$ (Fig. 3). Further, our finding that aftercontractions were larger in TCDD-treated muscles than in the controls after restoration of contractility in high-K^+ medium would also be expected as a consequence of "Ca^{2+} overload" leading to spontaneous cycles of Ca^{2+} uptake and release by the sarcoplasmic reticulum and to arrhythmogenic effects (21). It was of interest in that regard that spontaneous (automatic) activity appeared in 3 of 16 TCDD-treated preparations (in 2 at high concentrations of isoproterenol and in 1 from the beginning of the experiment) but was not seen in any of 14 controls.

The Ca^{2+} content of the tissues could be increased by several mechanisms. First, α-adrenergic stimulation could increase $[Ca^{2+}]_i$ by sequentially increasing phosphatidylinositol turnover and diacylglycerol and *myo*-inositol-3-phosphate production, which in turn leads to an increased release of intracellular Ca^{2+}. Ca^{2+} so released, together with diacylglycerol, can activate protein kinase C (22). This mechanism could lead to a decrease in cAMP (23), as well as to the observed decrease in β-adrenergic responsiveness and to an increase in $[Ca^{2+}]_i$. The reported increase in protein kinase C activity after TCDD treatment (18), albeit in a different system, supports this hypothesis.

A second possibility is that the cardiac Na^+,K^+-ATPase could be inhibited, leading to impaired function of the Na^+/K^+ pump, an increase in the intracellular concentration of Na^+ and a net accumulation of intracellular Ca^{2+} through Na^+/Ca^{2+} exchange mechanisms (24). Thus an inhibition of the pump could lead to all of our findings that indicate an increased Ca^{2+} content (increased force-frequency response, decreased contractile response to Ca^{2+}, and an increased aftercontraction). Inhibiting the Na^+,K^+-ATPase would also decrease the intracellular concentration of K^+, which in turn might be expected to increase the time required for cessation of contraction in high-K^+ medium, another observed effect of TCDD treatment.

Precedent for postulating a decrease in Na^+,K^+-ATPase activity in TCDD-treated tissues comes from evidence that TCDD treatment decreases rat liver Na^+,K^+-ATPase activity (25). Further, it has been reported that cytochrome P-450 of renal medullary cells metabolizes arachidonic acid to a product that inhibits Na^+,K^+-ATPase (26). Our findings suggest that TCDD may stimulate production of such an inhibitor. Evidence that TCDD treatment stimulates hepatic production of NADPH-dependent arachidonic acid metabolites, including a metabolite with the HPLC retention time of the reported Na^+,K^+-ATPase inhibitor (A.B.R., unpublished data), supports this hypothesis.

We do not know to what extent the cardiac findings described above are related to body weight loss in TCDD-treated animals. Cardiac ventricular muscle of chicken embryos treated with toxic polychlorinated biphenyls had decreased contractile responsiveness to norepinephrine without any decrease in body or heart weight (8). TCDD treatment similarly decreased the contractile response to isoproterenol in hearts of chicken embryos, again without any changes in body weight (unpublished data), suggesting that depressed β-adrenergic cardiac responses can occur independently of weight loss. That the initial developed tension of papillary muscles prior to stimulation was the same for the TCDD-treated and control guinea pigs also suggests that weight loss *per se* did not impair basal contractile function. The findings that TCDD-treated tissues showed a decreased response to some stimuli and an increased or unchanged response to others suggest further that TCDD has

specific effects on cardiac metabolic functions (e.g., TCDD decreased β-adrenergic responsiveness but did not block slow Ca^{2+}-channel activity).

The present results suggest that TCDD treatment impairs the capacity of guinea pig papillary muscle to contract when subjected to β-adrenergic and Ca^{2+} stimulation. The cardiac responses to such stimuli are homeostatic mechanisms needed to cope with stress. Our findings suggest that the heart is a target organ for TCDD toxicity and that TCDD may lead to a decreased capacity for positive inotropic responses when needed. Further, the changes described could predispose TCDD-treated animals to cardiac arrhythmias and sudden death.

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