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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Induces Vascular Dysfunction That is Dependent on Perivascular Adipose and Cytochrome P4501A1 Expression

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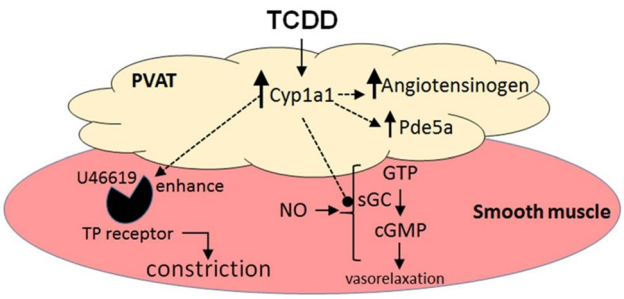
Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is associated with hypertension in humans and animals, and studies suggest that cytochrome P4501A1 (Cyp1a1) induction and vascular dysfunction may contribute. We investigated the role of perivascular adipose tissue (PVAT) and Cyp1a1 in TCDD-induced vascular dysfunction. Cyp1a1 wild-type (WT) and knockout (KO) male mice were fed a dough pill containing 1,4-*p*-dioxane (TCDD vehicle control) on days 0 and 7, or 1000 ng/kg TCDD on day 0 and 250 ng/kg TCDD on day 7. mRNA expression of Cyp1a1 was assessed on days 3, 7, and 14, and of Cyp1b1, 1a2, angiotensinogen, and phosphodiesterase 5a on day 14. Dose-dependent vasoconstriction to a thromboxane A_2 mimetic (U46619), and vasorelaxation to acetylcholine and a nitric oxide donor (S-nitroso-N-acetyl-DL-penicillamine, SNAP), were investigated in the aorta with and without PVAT. Cyp1a1 and 1a2 mRNA was induced in aorta of WT mice only with PVAT, and Cyp1a1 induction was sustained through day 14. TCDD significantly enhanced constriction to U46619 in WT mice and inhibited relaxation to both acetylcholine and SNAP, but only in the presence of PVAT. The effects of TCDD on U46619 constriction and SNAP relaxation were not observed in Cyp1a1 KO mice. Finally, in aorta + PVAT of WT mice TCDD significantly induced expression of angiotensinogen and phosphodiesterase 5a both of which could contribute to the TCDD-induced vascular dysfunction. These data establish PVAT as a TCDD target which is critically involved in mediating vascular dysfunction.

Graphical Abstract

TCDD enhances vasoconstriction via the thromboxane/prostanoid (TP) receptor and inhibits vasorelaxation via nitric oxide (NO) signaling. This TCDD-induced vascular dysfunction requires perivascular adipose (PVAT) and cytochrome P4501a1 (CYP1a1) induction.



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Keywords Angiotensinogen · Cytochrome P4501A1 · Perivascular adipose tissue (PVAT) · 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) · Vascular dysfunction · Nitric oxide

Introduction

Studies in both animal models and humans have shown that exposure to the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is associated with hypertension. For example, Vietnam veterans were exposed to TCDD as a contaminant of the defoliant Agent Orange, which was sprayed extensively by the Army Chemical Corps (ACC) in ground combat areas of South Vietnam. A recent study reports a significant association between herbicide spraying and the risk of hypertension with ACC sprayers serving in Vietnam exhibiting the greatest risk of hypertension (adjusted odds ratio = 2.21, 95% confidence interval, 1.76–2.77) [6]. This observation is consistent with previous studies linking spraying herbicides in Vietnam with hypertension [13]. Similarly, chronic exposure of mice to low levels of TCDD that accumulate over time results in significant increases in blood pressure, reaching a plateau 20 mmHg above unexposed levels within a month after exposure begins [18].

The specific mechanism by which TCDD mediates increases in blood pressure has not been fully elucidated. We have shown that TCDD-induced vascular dysfunction and hypertension require cytochrome P4501a1 (Cyp1a1) [17, 18]. TCDD induces hypertension in Cyp1a1 wild-type (WT) mice, but not Cyp1a1 knockout (KO) mice, and this is associated with aortic endothelial dysfunction, reflected by a loss of acetylcholine-mediated, nitric oxide (NO)-dependent vasorelaxation. Further, the TCDD-induced endothelial dysfunction is associated with increases in oxidative stress and TCDD-induced oxidative stress in endothelial cells requires Cyp1a1 expression. Despite these observations, the sustained increase in blood pressure in TCDD-exposed mice is not associated with activation of common hypertension-associated pathways, including the systemic renin–angiotensin system (RAS) or sympathetic nervous system [18]. Further, the vascular dysfunction occurred after gradual accumulation of TCDD over 30 days of exposure. It is unknown if rapid sustained induction of Cyp1a1, as might occur with a therapeutic drug that activates the aryl hydrocarbon receptor [21], would result in the same pattern of vascular dysfunction.

TCDD is highly lipophilic and accumulates in adipose tissue [7]. Studies show that TCDD significantly alters the transcriptome of rat white adipose tissue in vivo, impairs insulin signaling in cultured differentiated mouse adipocytes, and induces proinflammatory gene expression in human multipotent adipose-derived stem cells in vitro and in mouse adipose tissue in vivo [11, 15, 23]. Thus, adipose tissue is a target of TCDD-induced changes in gene expression and toxicity [12]. However, no studies have investigated the effects of TCDD on adipose tissue surrounding blood vessels, termed perivascular adipose tissue or PVAT.

The importance of PVAT to vascular function and cardiovascular disease, including hypertension, has become increasingly appreciated [3]. PVAT can secrete adipokines, chemokines, and vasoactive substances that have autocrine and paracrine function. Importantly, PVAT is a major source of RAS components that act locally to regulate vascular function and blood pressure [5, 33], and dysfunctional PVAT has been suggested to contribute to hypertension [1]. The impact of TCDD on PVAT-dependent regulation of vascular function has never been investigated. Our objectives were to determine the degree to which TCDD induced cytochrome P450 s in blood vessels with and without PVAT, and how PVAT and Cyp1a1 influence TCDD-induced vascular dysfunction. Further, we wanted to determine whether rapid sustained induction of Cyp1a1 would result in a similar manner of vascular dysfunction as observed with gradual increases in expression.

Materials and Methods

Chemicals

9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α (U46619) was purchased from Cayman Chemical (Ann Arbor, MI). Potassium chloride (KCl), acetylcholine, *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), and all ingredients of physiological saline were purchased from Sigma-Aldrich (St Louis, MO). TCDD was a gift from Dr. Richard E. Peterson (University of Wisconsin-Madison).

Animals

Cyp1a1 WT and KO male mice backcrossed > 10 generations on C57Bl6/J background were housed in a temperature-controlled environment and fed standard mouse chow. At 8 weeks of age, mice were randomly assigned to receive dough pills (Bio-Serv, Frenchtown, NJ) containing vehicle control (1,4-*p*-dioxane) or 1000 ng/kg TCDD loading dose on day 0, and vehicle control or 250 ng/kg TCDD maintenance dose on day 7 [30]. The goal of this dosing regimen was to achieve a rapid and sustained induction of Cyp1a1. On days 3, 7, and 14 mice were anesthetized ($n = 4$–6) with

a single intraperitoneal injection of ketamine/xylazine (80/4 mg/kg) and euthanized by exsanguination. Heart, liver, and abdominal aorta with and without PVAT were weighed, flash frozen, and stored at − 80° for RNA analysis. Only male mice were used in order to compare this new dosing regimen with our earlier studies using lower doses and gradual accumulation [17, 18]. All animal protocols were approved by the University of New Mexico Animal Care and Use Committee (No. 100849) and the investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Analysis of Aortic Vasoreactivity

On day 14 mice were euthanized, and thoracic aortas were placed into ice-cold physiological saline (130 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO4, 1.17 mM $MgSO_4$, 14.9 mM $NaHCO_3$, 5.5 mM glucose, 26 mM $CaNa_2EDTA$, 1.8 mM $CaCl_2$, pH 7.4) and cut into four rings of approximately 3 mm in length ($n = 6$–11). PVAT was left intact surrounding the adventitia from two rings and was removed from the other two rings. All four rings were then mounted in a wire myograph (Radnoti Glass Technology Inc., Monrovia, CA) attached to a force transducer (Grass Technologies, West Warwick, RI) in physiological saline at 37° C and bubbled with 20% O_2, 5% CO_2, and balanced N. Vessel viability was confirmed by constriction to 100 mM KCl. After a 30 min wash and phenylephrine (10^{-5} M) constriction, endothelial viability was confirmed by acetylcholine-mediated dilation (10^{-5} M). Following these checks, dose-dependent vasoconstriction to U46619 ($10^{-9.2}$–$10^{-8.6}$ M) was assessed followed by dose-dependent vasorelaxation to either acetylcholine (10^{-9}–10^{-5} M) or SNAP (10^{-8}–10^{-5} M).

Analysis of Gene Expression

Total RNA was isolated from the aorta ± PVAT, heart, and liver using RNeasy kit (Qiagen, GmbH, Germany). cDNA was synthesized using iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) with the supplied random primers and 250 ng RNA. PCR amplification was performed using an iCycler (Bio-Rad Laboratories) with a reaction mixture comprised of iQ SYBR Green Supermix (Bio-Rad Laboratories) with 500 nM Cyp1a1, Cyp1a2, Cyp1b1, angiotensinogen, phosphodiesterase 5a, or glyceraldehyde 3-phosphate dehydrogenase (reference gene) primers (Sigma-Aldrich, KiCqStartSYBR Green Primers, Supplementary Table 1) and 250 pg cDNA/µl. Cycle threshold data for both the target and reference gene were used to calculate mean normalized expression as previously described, using the $\Delta\,\Delta^{-Ct}$ approach [20].

Statistics

Data are expressed as mean ± SEM. mRNA expression data were analyzed by *t* test for treatment differences in WT mice, and by two-way ANOVA with post hoc Holm-Sidak comparisons for treatment- and genotype-related differences between WT and KO mice. To account for potential differences in aortic ring size/responsiveness, U46619 constriction data in mg were expressed as a percentage, relative to the constriction of KCl. Dose–response vasoconstriction and vasorelaxation data within genotype were analyzed by two-way (dose and treatment) repeated measures ANOVA with post hoc Holm-Sidak comparisons. In addition, dose–response vasoconstriction and vasorelaxation data within treatment were analyzed by two-way (dose and genotype) repeated measures ANOVA with post hoc Holm-Sidak comparisons. A $p < 0.05$ was considered statistically significant in all cases.

Results

Cytochrome P450 mRNA Induction

It has been demonstrated previously that gradual accumulation of TCDD induces vascular dysfunction that is mediated, in part, by Cyp1a1 [18]. We assessed the degree to which TCDD-induced Cyp1a1, Cyp1a2, and Cyp1b1 mRNA expression was associated with aortic PVAT and whether our dosing model resulted in Cyp1a1 induction that was sustained through day 14 of the study. Surprisingly, Cyp1a1 and Cyp1a2 mRNA expression was undetectable in aorta cleaned of PVAT in both control and TCDD-treated mice, but was detectable in control mice and significantly induced by TCDD in aorta with PVAT, while Cyp1b1 mRNA was detected in aorta ± PVAT, but not induced by TCDD (Fig. 1a, b). The lack of detectable Cyp1a1 expression in aorta without PVAT may have resulted from endothelial cells contributing only a small amount to the RNA pool as compared to smooth muscle [34]. In addition, the highest degree of Cyp1a1 mRNA induction occurred on day 3, declined by 25–30% by day 7, and plateaued through day 14 in both the heart and aorta with PVAT (Fig. 1c).

Effects of TCDD on Body and Organ Weights

To determine if the 14-day exposure to TCDD resulted in overt toxicity, body, heart and liver weights were measured. Two-way ANOVA revealed that TCDD treatment and Cyp1a1 KO genotype were both associated with reduced absolute heart weights, compared to control and WT genotype (Table 1). These differences were not observed when

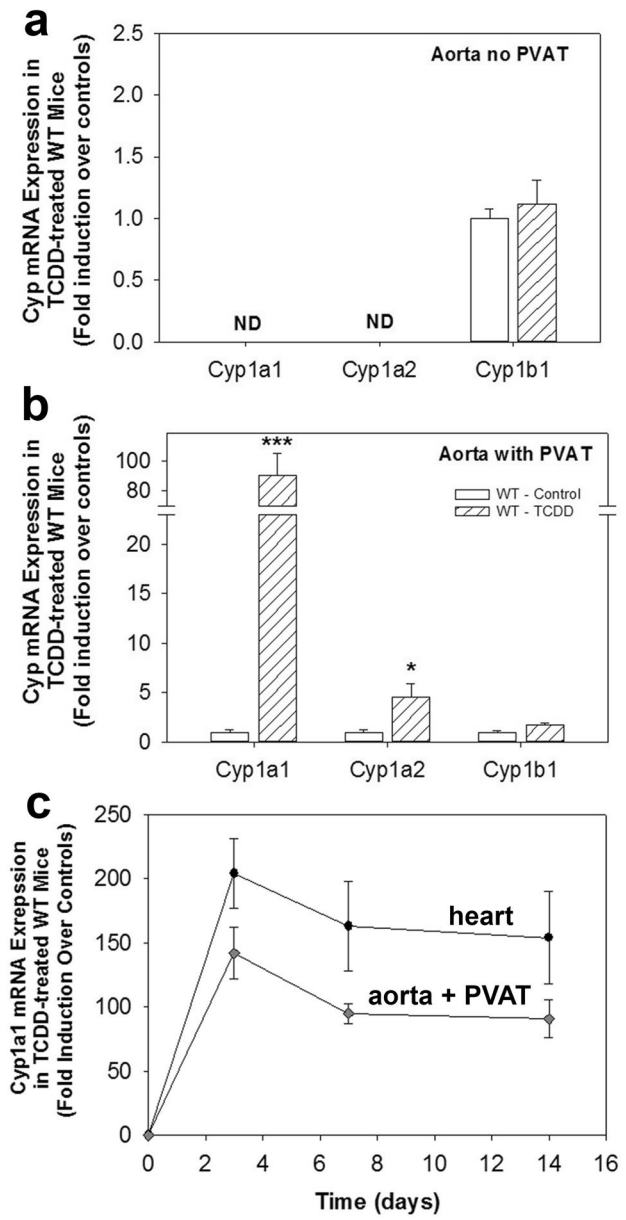

Fig. 1 Cyp1a1, Cyp1a2, Cyp1b1 mRNA expression in control and TCDD-treated WT mice. **a** Cyp mRNA expression in aorta lacking PVAT on day 14. **b** Cyp mRNA expression in aorta with PVAT on day 14. **c** Time course of Cyp1a1 mRNA expression in heart and aorta plus PVAT-treated mice. WT mice were treated with vehicle control or a loading dose of TCDD (1000 ng/kg) on day 0, and vehicle control or a maintenance dose (250 ng/kg) on day 7. mRNA expression between control and TCDD-treated WT mice was analyzed by *t*-test. ***$p<0.001$, *$p<0.05$. **a**, **b** $n=6$/group; **c** $n=4$/group on days 3 and 7, and $n=6$/group day 14

heart/body weight ratios were calculated. No other statistically significant changes were found related to body and organ weights.

Table 1 Body and organ weights of 10-wk-old wild-type (WT) and Cyp1a1 knockout (KO) mice exposed to control or TCDD for 14 days

Weight[a]	WT		Cyp1a1 KO	
	Control	TCDD	Control	TCDD
Body (g)	27.9 ± 0.6	25.7 ± 0.6	25.5 ± 0.6	25.7 ± 0.6
Heart[d]	113 ± 2[b] (0.41 ± 0.01)[c]	106 ± 2* (0.41 ± 0.01)	105 ± 2* (0.41 ± 0.01)	103 ± 2* (0.40 ± 0.01)
Liver	1442 ± 62[b] (5.4 ± 0.1)[c]	1461 ± 62 (5.7 ± 0.1)	1394 ± 56 (5.5 ± 0.1)	1428 ± 56 (5.6 ± 0.1)

[a]Values are expressed as mean ± SEM, n = 8-10

[b]Absolute organ weight (mg)

[c]Relative organ weight (%; organ weight (g)/body weight (g)) × 100

[d]Two-way ANOVA, treatment $p=0.03$, genotype $p=0.03$

*$p<0.05$ versus WT control via post hoc Holm-Sidak comparison

Effects of TCDD on Aortic Vasoconstriction

The effects of TCDD, and influence of Cyp1a1 genotype and PVAT, on aortic constriction responses to a thromboxane A_2 mimetic, U46619, were assessed. TCDD had no effect on the dose-dependent constriction of U46619 in the absence of PVAT, but significantly increased U46619 constriction in the presence of PVAT in WT mice at the three highest U46619 doses (Fig. 2a, b). Notably, genetic deletion of Cyp1a1 eliminated the TCDD-induced, PVAT-dependent enhancement of U46619 constriction and reduced U46619 constriction at selected doses with and without PVAT (Fig. 2c, d).

Effects of TCDD on Aortic Vasorelaxation

The effects of TCDD, and influence of Cyp1a1 genotype and PVAT, on acetylcholine relaxation following U46619 constriction were assessed. TCDD had no effect on the dose-dependent relaxation of acetylcholine in the absence of PVAT, but significantly reduced acetylcholine relaxation in the presence of PVAT (Fig. 3a, b). In addition, acetylcholine relaxation of the aorta following U46619 constriction was reduced slightly in absence of Cyp1a1 without PVAT in the control group and was nearly eliminated with PVAT in both the control and TCDD groups (Fig. 3c, d).

The NO donor, SNAP, was used to determine if signaling downstream of NO was impaired by TCDD or Cyp1a1 deletion. TCDD had no effect on the dose-dependent relaxation of SNAP in the absence of PVAT, but significantly reduced SNAP relaxation in the presence of PVAT in WT mice (Fig. 4a, b). Notably, genetic deletion of Cyp1a1 eliminated the TCDD-induced, PVAT-dependent impairment of SNAP-mediated relaxation (Fig. 4c, d).

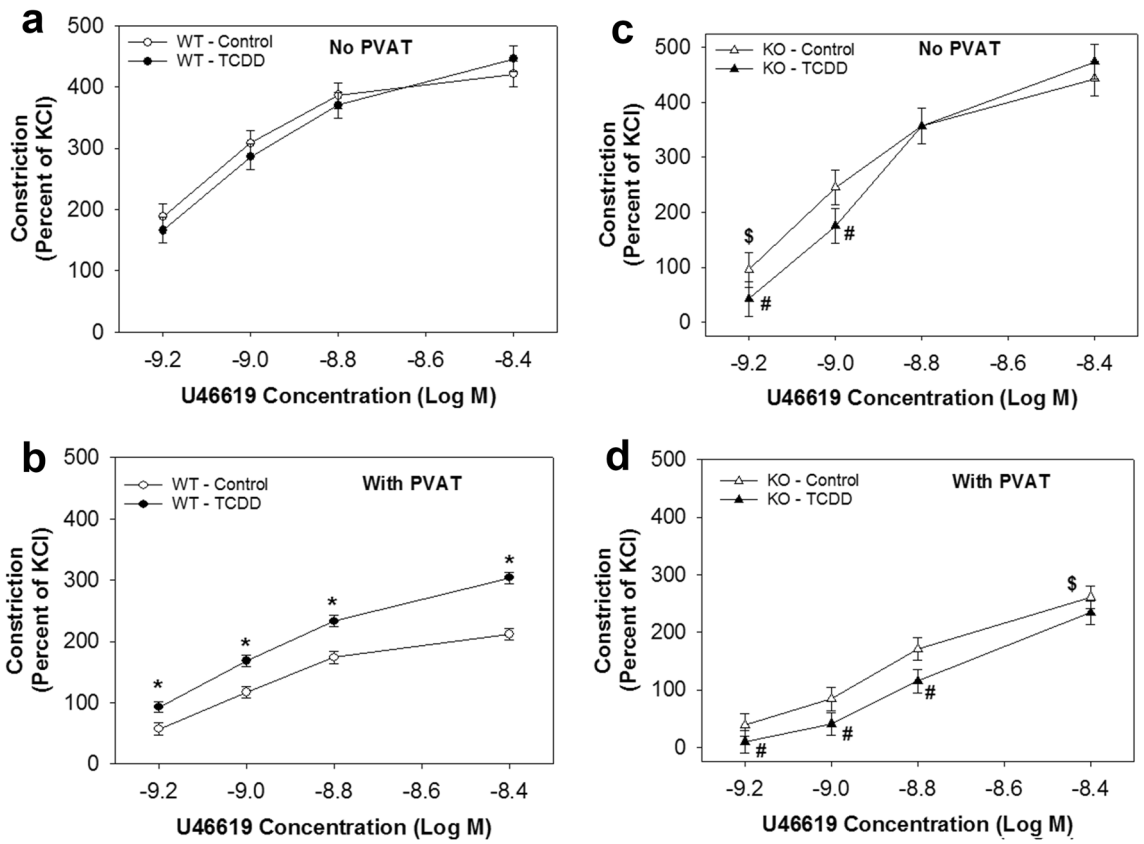

Fig. 2 U46619 dose-dependent constriction, expressed as a percentage of KCl constriction, in aorta of wild-type (**a**, **b**) and Cyp1a1 knockout mice (**c**, **d**) with and without PVAT. Dose–response data were analyzed within genotype by two-way (dose and treatment) repeated measures (RM) ANOVA and within treatment by two-way (dose and genotype) RM ANOVA followed by post hoc Holm-Sidak comparisons. **a**, **c**, **d** There was a significant dose effect ($p < 0.001$), but no treatment effect, in U46619 constriction in WT and KO mice without PVAT, and KO mice with PVAT. **b** There was a significant dose ($p < 0.001$) and treatment–dose interaction ($p < 0.029$) in U46619 constriction in WT mice with PVAT. **a**–**d** There was a significant genotype–dose interaction in KO mice without PVAT ($p < 0.001$) and with PVAT ($p < 0.02$) in both the control and TCDD groups. *$p < 0.05$ WT TCDD versus WT control; \$$p < 0.05$ KO control versus WT control; #$p < 0.05$ KO TCDD versus WT TCDD ($n = 11$/group)

Effects of TCDD on PVAT mRNA Expression

Angiotensin II inhibits NO signaling by inhibiting soluble guanylyl cyclase (sGC) and inducing phosphodiesterase 5a, which inhibits cGMP synthesis and promotes cGMP degradation, respectively. Since TCDD impaired SNAP relaxation, suggesting that NO signaling was inhibited, we investigated the gene expression of angiotensinogen and phosphodiesterase 5a. TCDD resulted in significant induction of angiotensinogen and phosphodiesterase 5a in aorta with PVAT in WT mice, but not in the absence of PVAT and not in Cyp1a1 KO mice (Fig. 5a, b). In addition, TCDD failed to induce angiotensinogen mRNA in the liver (data not shown).

Discussion

This study reveals for the first time that PVAT is a transcriptional target of TCDD and is required to mediate TCDD-induced vascular dysfunction, characterized by increases in constriction to a thromboxane A_2 mimetic and decreases in relaxation downstream of NO synthesis. Further, the significant induction of the rate-limiting substrate for the RAS pathway, angiotensinogen, in PVAT by TCDD suggests a potential link between TCDD-induced vascular dysfunction and ultimately hypertension. Finally, TCDD-induced expression of Cyp1a1 contributes to both of these responses, but the specific mechanisms mediating the Cyp1a1 contribution remain to be elucidated.

Thromboxane A_2 is a cyclooxygenase-1-dependent metabolite of arachidonic acid, an agonist of the thromboxane/prostanoid (TP) receptor, and a potent vasoconstrictor.

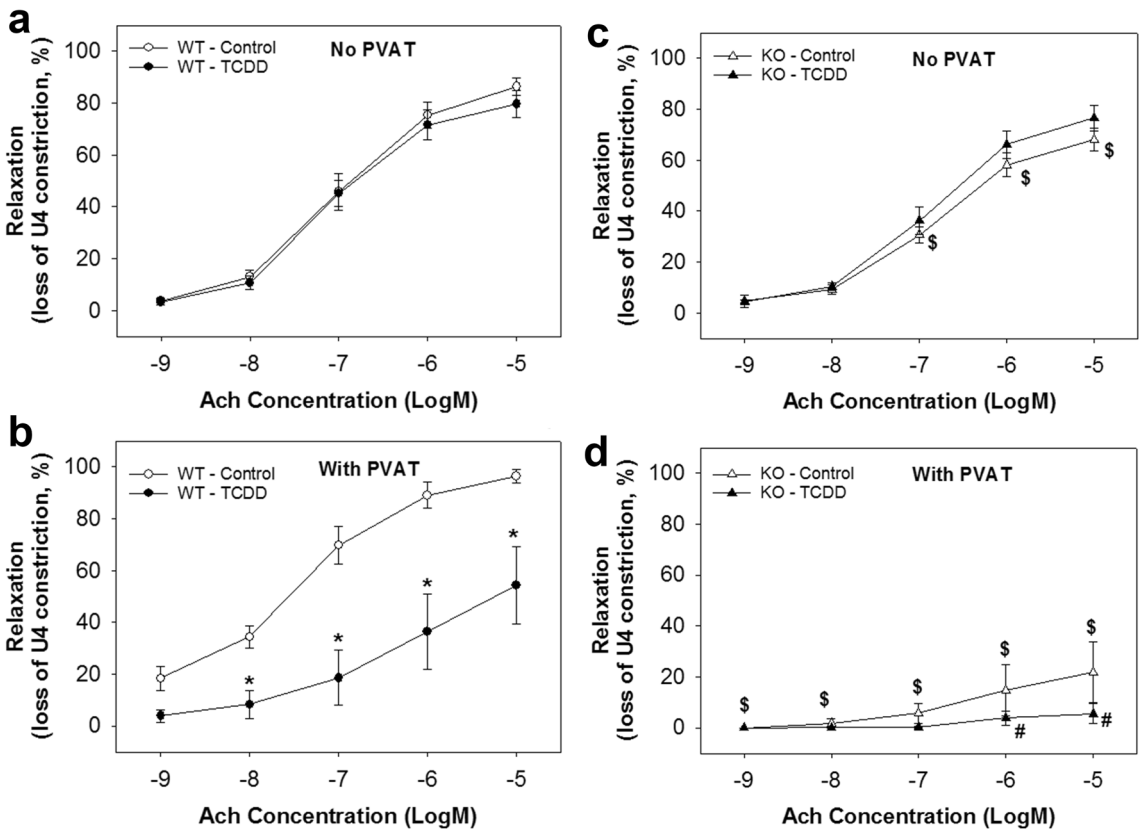

Fig. 3 Acetylcholine (Ach) dose-dependent relaxation of aorta in wild-type (**a**, **b**) and Cyp1a1 knockout mice (**c**, **d**) with and without PVAT. Dose–response data were analyzed within genotype by two-way (dose and treatment) RM ANOVA and within treatment by two-way (dose and genotype) RM ANOVA followed by post hoc Holm-Sidak comparisons. **a**, **c** There was a significant dose effect ($p < 0.001$), but no treatment effect, in Ach relaxation in WT and KO mice without PVAT. **b** There was a significant treatment ($p < 0.001$), dose ($p < 0.001$) and treatment–dose interaction ($p < 0.001$) in Ach relaxation in WT mice with PVAT. **d** There was no significant effect of dose or treatment in Ach relaxation in KO mice with PVAT. **a**, **c** There was a significant genotype effect in the control group without PVAT. **b**, **d** There was a significant genotype effect in both control and TCDD groups ($p < 0.001$) with PVAT. *$p < 0.05$ WT TCDD versus WT control, \$$p < 0.05$ KO control versus WT control, #$p < 0.05$ KO TCDD versus WT TCDD ($n = 5$–6/group)

Thromboxane A_2 biosynthesis and TP receptor expression are both elevated in cardiovascular disease and are pathophysiological mediators of hypertension [29]. Further, cyclooxygenase-generated, PVAT-derived contracting factors are novel regulators of arterial vasoconstriction [22]. In our study, TCDD enhanced U46619 constriction in the presence of PVAT. Thus, TCDD may increase the synthesis of thromboxane A_2 or other isoprostanes from PVAT that agonize the TP receptor, contributing to the enhanced constriction response. This would be consistent with previous work showing that TCDD enhances the constriction response to arachidonic acid via the TP receptor ([31] Fig. 1c) and increases hepatic and plasma expression of the TP receptor agonist, 20-hydroxyeicosatetraenoic acid (20-HETE) [31].

It is also possible that TCDD sensitizes the TP receptor signaling pathway downstream of agonist stimulation. The TCDD-enhanced U46619 constriction required the induction of Cyp1a1. Sustained induction of Cyp1a1 increases reactive oxygen species (ROS) [19, 25], which have been demonstrated to stabilize the TP receptor [29]. Thus, future studies investigating the role of the TP receptor in TCDD-induced vascular dysfunction and hypertension would be warranted.

The results from this study also show that TCDD-induced vascular dysfunction is associated with decreases in acetylcholine- and NO-mediated vasorelaxation. This is consistent with an earlier study where TCDD accumulated gradually over time, inhibiting acetylcholine-mediated vasorelaxation after 30 days of exposure [17]. In the mouse aorta, acetylcholine stimulates endothelial NO synthase (eNOS) increasing NO biosynthesis. NO diffuses to the smooth muscle where it activates sGC, increases the accumulation of cGMP, and mediates vasorelaxation. Inhibition of acetylcholine-stimulated vasorelaxation could

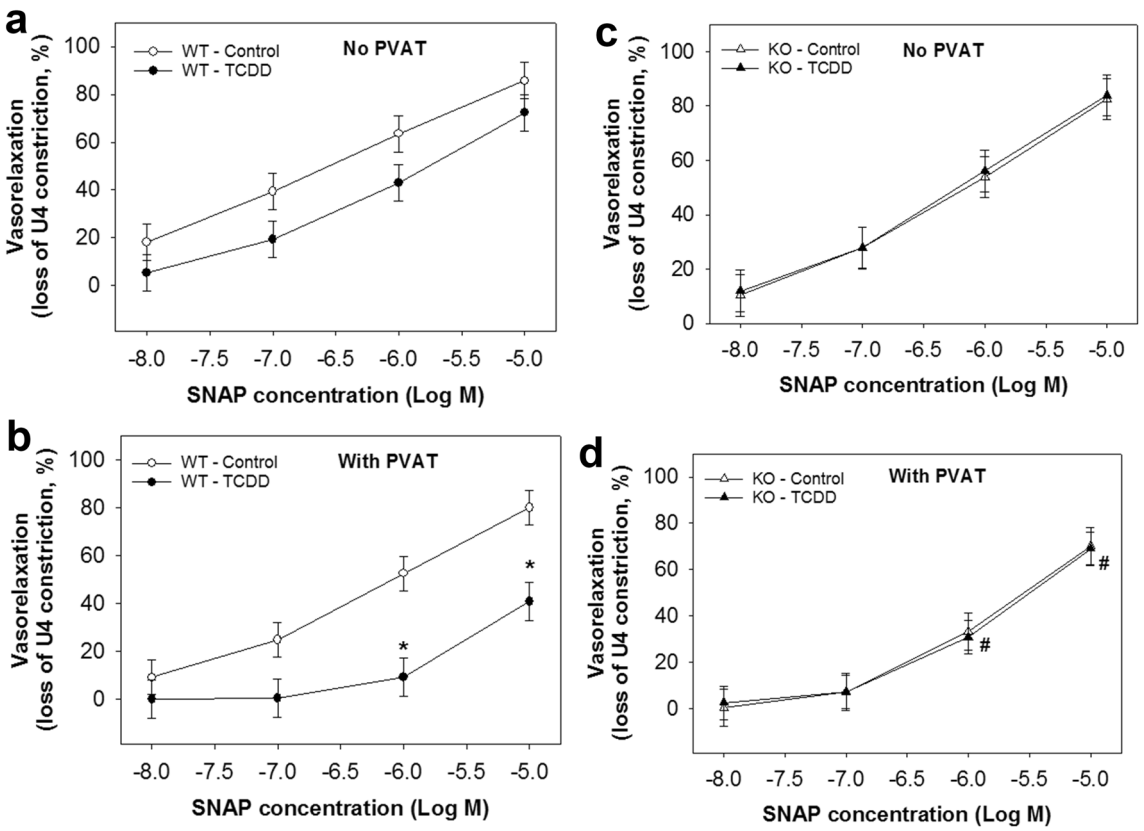

Fig. 4 SNAP dose-dependent relaxation of aorta in wild-type (**a**, **b**) and Cyp1a1 knockout mice (**c**, **d**) with and without PVAT. Dose–response data were analyzed by two-way repeated measures ANOVA followed by post hoc Holm-Sidak comparisons. **a**, **c**, **d** There was a significant dose effect ($p < 0.001$), but no treatment effect, in U46619SNAP relaxation in WT and KO mice without PVAT, and KO mice with PVAT. **b** There was a significant treatment ($p = 0.012$), dose ($p < 0.001$) and treatment–dose interaction ($p < 0.001$) in SNAP relaxation in WT mice with PVAT. **a**, **c** There was no effect of genotype on SNAP relaxation without PVAT. **b**, **d** There was a significant effect of genotype with PVAT in the TCDD-treated group ($p < 0.001$). *$p < 0.05$ WT TCDD versus WT control, #$p < 0.05$ KO TCDD versus WT TCDD ($n = 5$–6/group)

result from decreased NO synthesis, bioavailability, signaling, or a combination of these. In addition, the degree to which TCDD reduced vasorelaxation by the NO donor ($25 \pm 7\%$ of control) was not significantly different than the degree to which TCDD reduced acetylcholine vasorelaxation ($37 \pm 15\%$ of control), suggesting that the primary target of TCDD inhibition is downstream of NO synthesis and involves NO signaling.

Possible targets in PVAT that could influence NO bioavailability and the NO signaling pathway include ROS, sGC, angiotensinogen, and phosphodiesterase 5a. All of these potential targets have been implicated in PVAT-dependent vascular dysfunction [8]. Increases in ROS, such as super oxide anion, can scavenge NO-generating peroxynitrite. Induction of Cyp1a1 by TCDD is associated with increases in ROS, and this could contribute to a decrease in acetylcholine-mediated vasorelaxation reducing NO bioavailability [19, 27]. Increases in ROS can also reduce expression and activity of sGC [9].

In addition, angiotensin II can upregulate expression of phosphodiesterase 5a which degrades cGMP and attenuates NO signaling in vascular smooth muscle [14]. TCDD significantly induced angiotensinogen and phosphodiesterase 5a mRNA expression in aorta plus PVAT. Further, this induction required Cyp1a1 as demonstrated by the failure of TCDD to induce angiotensinogen in PVAT of Cyp1a1 KO mice. The mechanism by which Cyp1a1 leads to angiotensinogen induction in adipose is unknown. Fatty acids and activators of peroxisome proliferator activated receptor-gamma (PPARγ) stimulate angiotensinogen expression in adipocytes at the transcriptional level [24]. In addition, the omega-6 polyunsaturated fatty acid, arachidonic acid, stimulates angiotensinogen gene expression from adipocytes, and it has been proposed that this regulation may be mediated by the arachidonic acid metabolites, some of which are PPARγ

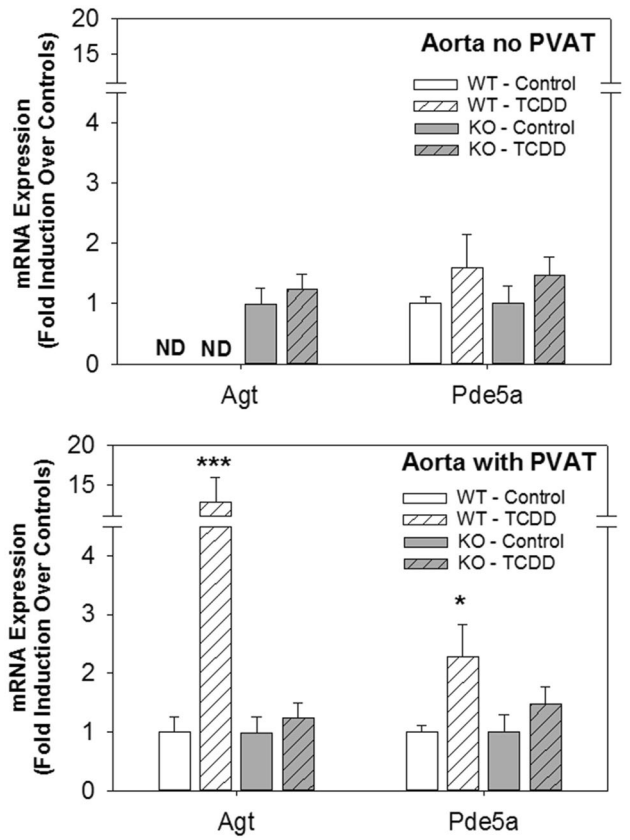

Fig. 5 mRNA expression of angiotensinogen (Agt) and phosphodiesterase 5a (Pde5a) in aorta with and with PVAT of wild-type and Cyp1a1 knockout mice treated with control or TCDD. Data were analyzed by two-way (treatment and genotype) ANOVA followed by post hoc Holm-Sidak comparisons. **a** In aortas without PVAT from WT and KO mice, Agt mRNA expression was not detected, and there was no treatment or genotype effect on Pde5a mRNA expression. **b** In aortas with PVAT, there was a significant treatment- ($p < 0.008$) and genotype-dependent difference ($p < 0.002$), and a treatment–geno type interaction ($p < 0.03$) in Agt mRNA expression; and a significant treatment- ($p < 0.03$) and genotype-dependent difference ($p < 0.003$) in Pde5a mRNA expression. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus WT control, $^{\#\#}p < 0.01$ versus WT TCDD treated ($n = 5$–6)

agonists [28]. Cyp1a1 can metabolize arachidonic acid to epoxides, and mono- and dihydroxy-metabolites [16, 26], and TCDD treatment of mice increases these metabolites in many organs [4, 10, 32]. It is possible that Cyp1a1 induction leads to increased arachidonic acid metabolism in adipose that generate PPARγ agonists.

Finally, our data also suggest dichotomous roles for basal Cyp1a1 versus TCDD-induced Cyp1a1 in PVAT on NO-mediated vasorelaxation in the aorta. Genetic deletion of Cyp1a1 resulted in significant loss of acetylcholine-mediated relaxation in the presence of PVAT (Fig. 3c vs. d). In contrast, genetic deletion of Cyp1a1 failed to affect NO-mediated vasorelaxation by the NO donor SNAP with or without PVAT (Fig. 4c vs. d). Taken together these data suggest that basal Cyp1a1 in PVAT is required to mediate vasorelaxation upstream of NO signaling, but is not required for vasorelaxation downstream of NO synthesis. This interpretation is consistent with previous studies showing Cyp1a1 KO mice exhibit significantly reduced acetylcholine-mediated, NO-dependent vasodilation in resistance arterioles [2]. In contrast, TCDD treatment inhibited vasorelaxation downstream of NO synthesis in the presence of PVAT, and this was eliminated by genetic deletion of Cyp1a1. These data suggest that TCDD-induced Cyp1a1 in PVAT is detrimental to vasorelaxation downstream of NO synthesis.

In conclusion, the results of this study show that PVAT is a transcriptional target of TCDD and is required to mediate TCDD-induced vascular dysfunction that includes enhanced vasoconstriction via the TP receptor and inhibited vasorelaxation via NO signaling. Given the essential role of PVAT in regulating vascular function, it is possible that the ability of other AhR agonists to induce vascular dysfunction may be dependent, in part, on their ability to partition into adipose.

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