Ah receptor expression in embryonic palate exposed to TCDD.

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TCDD is teratogenic to C57BL/6N mouse embryos, inducing hydronephrosis and cleft palate at doses that are not overtly maternally toxic[1,2]. In the developing palate, the medial epithelium normally loses the peridermal cell layer and the basal cells transform to fibroblasts or migrate from the midline seam. TCDD stimulates differentiation of the medial epithelial cells, altering proliferation, and results in formation of a stratified, squamous epithelium[3]. The TCDD-induced altered fate of the palatal cells correlates with changed expression of glucocorticoid receptors[4], EGF receptor, and the growth factors EGF, TGFα, and TGFβ[5]. The mechanism of TCDD-induced clefting appears to involve regulation of genes that control growth and differentiation. The mediator of TCDD activity is the Ah receptor[6,7]. This study examines the expression of the Ah receptor in the gestation day (GD) 14 fusing embryonic palate and characterizes the response to TCDD exposure.

METHODS. TCDD in corn oil was administered by gavage to pregnant mice on GD 10 at 24 μg/kg (5 ml/kg) or daily from GD 10-13 at 3 μg/kg/day and controls received corn oil alone. Fetuses were collected on GD 14 and prepared for either immunohistochemistry, in situ hybridization, or total RNA preparation for Northern blotting. The mRNA for Ah receptor was localized by in situ hybridization of palatal sections. Riboprobe was prepared (^{35}S-RNA antisense) using the cAh1 probe which includes 1.8 Kb of the amino terminal DNA sequence (kindly provided by C. Bradfield[8]). Northern blots were probed with ^{32}P-DNA prepared by random primer methods from this same cDNA sequence. Total RNA was prepared from dissected mid-craniofacial tissues of control and TCDD-exposed litters. Total RNA from 20-30 embryos and 2-3 litters was pooled for each sample and 30 μg RNA were loaded per lane. Hybridization to GAPDH was performed to adjust for differences of loading between lanes and blots. The density of hybridization for TCDD-exposed samples was measured

densitometrically and expressed relative to GAPDH and as percent of control. The Ah receptor was localized immunohistochemically with purified monoclonal antibody raised against N-terminal peptide sequence[9]. The intensity of immunostaining was measured densitometrically with the Olympus Cue 2 System and data were statistically analyzed by ANOVA.

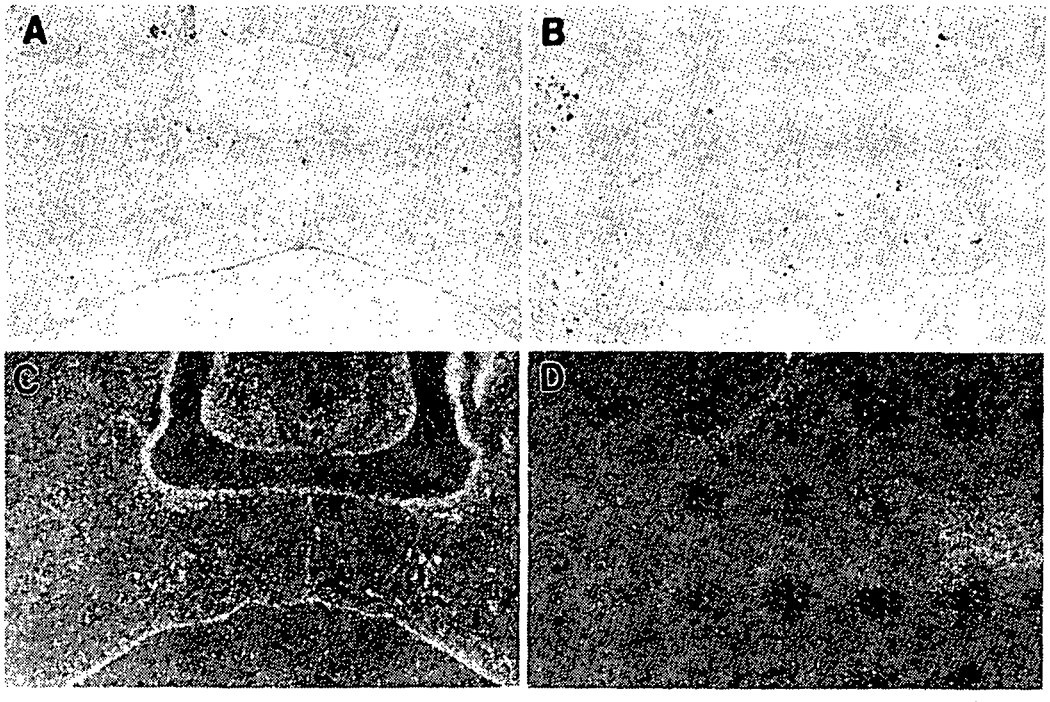
RESULTS. Ah receptor was expressed in palatal epithelial and mesenchymal cells. In the control tissues nuclear localization was predominant in oral epithelia with cytoplasmic and nuclear staining of medial and nasal epithelia. The mesenchymal cells had a perinuclear pattern except in regions of bone formation where strong nuclear localization occurred. In controls the density of staining (Table 1) was significantly different between epithelial and mesenchymal cells ($p<.001$) and between epithelia oral > medial > nasal intensity ($p<.001$). When examining the trend across all palatal regions, TCDD significantly decreased Ah receptor expression ($p<.001$) with either the low or high dose (Fig. 1A & B). Comparison of specific cell types showed significantly decreased expression in nasal epithelia and mesenchyme at the high dose, and decreased levels in oral epithelia and mesenchyme at the low dose. The down-regulation of Ah receptor by TCDD correlated with lower expression of mRNA as detected using in situ localization (Fig. 1C & D) and Northern blots (Fig. 2). The mRNA localized to the epithelial cells in controls with strong hybridization to lateral bone-forming mesenchyme. TCDD exposure (24 μg/kg GD 10) strongly reduced localization over all cell types. On Northern blots a 5.4 Kb band hybridized to the probe and this was reduced to 34% of control in the

MEAN AH RECEPTOR IMMUNOHISTOCHEMICAL SCORES[1]

GROUP	EPITHELIAL REGION			MESENCHYMAL REGION			
	O	M	N	O	M	N	C
CONTROL	21.5 ±0.7	17.5 ±0.8	14.6 ±0.3	13.4 ±0.2	12.9 ±0.2	14.6 ±0.2	15.4 ±0.3
TCDD 3 μg/kg (day 10-13)	17.3[a] ±1.2	15.6 ±1.4	13.4 ±0.6	12.1[a] ±0.4	12.2 ±0.4	13.5 ±0.4	14.3 ±0.5
24 μg/kg (day 10 only)	19.6 ±1.3	16.5 ±1.5	12.2[b] ±0.6	12.3 ±0.4	11.7 ±0.4	12.0[c] ±0.4	13.8 ±0.5

[1]Mean ± S.E.; [a]$P<.05$, [b]$P<.01$, [c]$P<.001$ vs Control; N = 12 for all groups. O = oral, M = medial, N = nasal, C = chondrogenic. Trend across all regions: Control > TCDD 3μg/kg and TCDD 24 μg/kg $P<.001$.

1

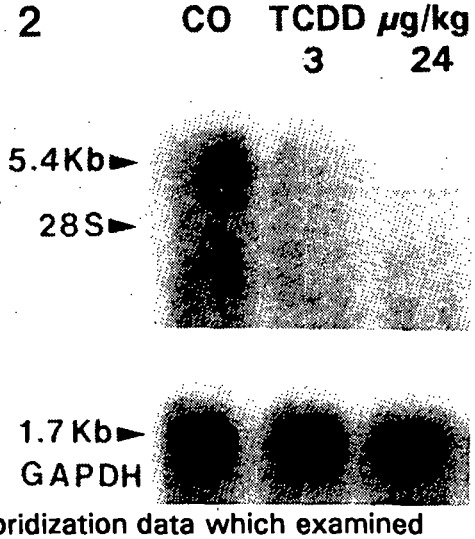


AH RECEPTOR mRNA EXPRESSION

GROUP	N	DENSITY[A]
Control	5	100%
TCDD 3 μg/kg	4	101% ±11
TCDD 24 μg/kg	5	34% ±3

[A]Mean ± SE. Expressed as % control corrected for GAPDH density. N = #samples.

2



high dose group. In contrast to in situ hybridization data which examined localized expression, this data represents the total average expression over the entire mid-craniofacial region. Although a decrease in mRNA was not seen after the low dose in the Northerns, in situ revealed moderate decreases localized to the palatal shelves (data not shown).

CONCLUSIONS The Ah receptor is expressed in embryonic palatal cells and the level of expression appeared to vary between epithelial and mesenchymal cell types. The state of differentiation may also influence the level of expression as differentiating chondrogenic mesenchymal cells expressed Ah receptors at high levels. Ah receptor localization was nuclear in most cell types, but cytoplasmic expression was also seen in some cells. TCDD exposure of C57BL/6N embryos reduced the expression of the Ah receptor in the palatal cells. The down-regulation appeared to be at a transcriptional level as the mRNA in the tissue was also decreased. The single high dose exposure on GD 10 has been shown to induce >90% incidence of cleft palate[3] while exposure to 3 μg/kg/day for 4 days does not significantly increase clefting[5]. Even though the lower dose did not induce cleft palate, there are effects of TCDD on Ah receptor expression. This was also observed for effects of low dose TCDD on growth factor expression[4]. There are detectable cellular and biochemical responses to low levels of TCDD which are not sufficient to result in gross morphological abnormalities.

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