Genetic Evidence That Retinaldehyde Dehydrogenase Raldh1 (Adh1a1) Functions Downstream of Alcohol Dehydrogenase Adh1 in Metabolism of Retinol to Retinoic Acid

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Andrei Molotkov and Gregg Duester‡
From the OncoDevelopmental Biology Program, Burnham Institute, La Jolla, California 92037

Vitamin A (retinol) is a nutrient that is essential for developmental regulation but toxic in large amounts. Previous genetic studies have revealed that alcohol dehydrogenase Adh1 is required for efficient clearance of excess retinol to prevent toxicity, thus demonstrating that the mechanism involves oxidation of excess retinol to retinoic acid (RA). Whereas Adh1 plays a dominant role in the first step of the clearance pathway (oxidation of retinol to retinaldehyde), it is unknown what controls the second step (oxidation of retinaldehyde to RA). We now present genetic evidence that aldehyde dehydrogenase Adh1a1, also known as retinaldehyde dehydrogenase Raldh1, plays a dominant role in the second step of retinol clearance in adult mice. Serum RA levels following a 50 mg/kg dose of retinol were reduced 72% in Raldh1<sup>−/−</sup> mice and 82% in Adh1<sup>−/−</sup> mice. This represented reductions in RA synthesis of 77–78% for each mutant after corrections for altered RA degradation in each. After retinol dosing, serum retinaldehyde was increased 2.5-fold in Raldh1<sup>−/−</sup> mice (indicating defective retinaldehyde clearance) and decreased 3-fold in Adh1<sup>−/−</sup> mice (indicating defective retinaldehyde synthesis). Serum retinol clearance following retinol administration was decreased 7% in Raldh1<sup>−/−</sup> mice and 69% in Adh1<sup>−/−</sup> mice. LI<sub>50</sub> studies indicated a small increase in retinol toxicity in Raldh1<sup>−/−</sup> mice and a large increase in Adh1<sup>−/−</sup> mice. These observations demonstrate that Raldh1 functions downstream of Adh1 in the oxidative metabolism of excess retinol and that toxicity correlates primarily with accumulating retinaldehyde rather than retinaldehyde.

Retinoic acid (RA)<sup>3</sup> is a metabolic derivative of vitamin A (retinol) that regulates developmental pathways in chordate animals (1). RA functions in the control of gene expression by serving as a ligand for several nuclear RA receptors and retinoid X receptors (2). Whereas <i>in vitro</i> studies suggested that all-trans-RA (a ligand for RA receptors) and its isomer 9-cis-RA (a ligand for retinoid X receptors) might both regulate gene expression (3), <i>in vivo</i> studies indicate that only all-trans-RA is needed physiologically to correct a lethal genetic defect in RA synthesis (4). Retinoid metabolic enzymes ensure that sufficient all-trans-RA is produced from retinol to control RA signaling in target tissues (5). Also, mechanisms exist to prevent excessive accumulation of RA and retinol as high levels of either of them are teratogenic during development or toxic to adult animals (6–9).

Retinol can be considered a pro-ligand for RA signaling that is activated to a ligand when its alcohol group is sequentially oxidized to first an aldehyde (to produce retinaldehyde) and then to a carboxylic acid (to produce RA). Enzymes that can activate retinol to RA include cytosolic alcohol dehydrogenases (ADH) (10–13) and microsomal short-chain dehydrogenases/reductases (14–17) that oxidize retinol to retinaldehyde as well as cytosolic aldehyde dehydrogenases (ALDH), also known as retinaldehyde dehydrogenases (RALDH), that oxidize retinaldehyde to RA (18–22). Various cytochrome P450 (CYP) enzymes deactivate RA by oxidation to more polar metabolites such as 4-oxo-RA that are more easily excreted (23–25). The combined activity of RA-synthesizing and RA-degrading enzymes determines the steady-state level of RA in target tissues for signaling plus ensures that excess retinol or RA is metabolized to excretable forms.

Within the enzyme families listed above, genetic studies are beginning to reveal the identity of individual members that function in retinoid metabolism <i>in vivo</i>. Adh1<sup>−/−</sup> mice do not exhibit defects in growth or survival but display greatly reduced metabolism of a dose of retinol to RA and increased retinol toxicity (26). ADH1 possesses high activity for oxidation of retinol to retinaldehyde, which is important for clearance of retinol to prevent its toxic buildup. Adh3<sup>−/−</sup> mice display a small reduction in metabolism of a dose of retinol to RA, but more notably they suffer defects in postnatal growth and survival (27). ADH3 possesses low activity for oxidation of retinol to retinaldehyde, but this is evidently useful for synthesis of RA needed for RA signaling during development. Further studies have shown that ADH1 (expressed at high levels in liver) is the primary retinol-oxidizing enzyme responsible for the clearance of excess retinol to prevent toxicity, whereas ADH3 (expressed ubiquitously) plays a secondary role in this process (28). Adh4<sup>−/−</sup> mice do not display a significant reduction in metabolism of a dose of retinol to RA but are much more sensitive to defects in postnatal growth and survival during vitamin A deficiency (26). ADH4 has high activity for oxidation of retinol to retinaldehyde, which is apparently needed to produce sufficient RA for developmental RA signaling when retinol levels are low. An analysis of Rh<sub>5</sub><sup>−/−</sup> mice has shown that the short-chain dehydrogenase/reductase enzyme RDH5 participates in oxidation of 11-cis-retinol to 11-cis-retinaldehyde for...
production of the visual pigment, but there is no evidence reported that RDH5 plays an in vivo role in RA synthesis (29, 30). Raldh2\(^{-/-}\) (Adh1a2\(^{-/-}\)) mice have severe embryonic developmental defects and greatly reduced embryonic RA, indicating that RALDH2 (ALDH1A2) plays an important in vivo role in oxidation of retinaldehyde to RA to activate RA signaling during development (31, 32). Cyp26a1\(^{-/-}\) mice display abnormal embryonic RA levels and embryonic defects similar to those observed during RA teratogenesis, suggesting that the ability of CYP26A1 to oxidize RA to polar metabolites including 4-oxo-RA protects against excessive accumulation of RA during development (33, 34). Further genetic studies have shown that the oxidative derivatives of RA produced by CYP26A1 are not needed for RA signaling during development but are degradation products (35). These genetic studies indicate that retinoid-oxidizing enzymes may perform two different functions: 1) establishment and maintenance of correct RA levels for developmental RA signaling, or 2) clearance of excess retinol to prevent toxicity in adult animals. In the first case, the RA produced is used directly to regulate developmental events, but in the second case, the RA produced is an intermediate in the degradation process to prevent retinol toxicity. With regard to synthesis and degradation of RA for developmental signaling, physiological roles for ADH3, ADH4, RALDH2, and CYP26A1 have been established, thus covering all of the three oxidation steps. As for prevention of retinol toxicity in adult animals, a physiological role for ADH1 in the first step has been established, but enzymes involved physiologically in the subsequent steps have not been identified.

RALDH1 (ALDH1A1) was the first member of the ALDH family found to possess in vitro activity for oxidation of retinaldehyde to RA (18, 20). In addition, introduction of mouse RALDH1 mRNA into frog embryos has been found to stimulate RA synthesis in vivo (36). RALDH1 is highly expressed in the embryonic and adult dorsal retina as well as in adult liver in mouse (37), chicken (38, 39), and frog (40). However, we have reported that Raldh1\(^{-/-}\) (Adh1a1\(^{-/-}\)) mice do not exhibit obvious developmental defects in the retina or other tissues (41). RALDH1 is much less abundant than RALDH2 in embryonic tissues; however, it is much more abundant than RALDH2 in adult mouse tissues including liver (42), suggesting that RALDH1 may play a role in adult retinoid metabolism similar to ADH1. Here we have examined retinoid metabolism in Raldh1\(^{-/-}\) mice following a dose of retinol and compared these results with previous studies on Adh1\(^{-/-}\) mice (43) as well as new studies presented here on Adh1\(^{-/-}\) mice. Our results indicate that RALDH1 plays a role in oxidation of retinaldehyde to RA for clearance of excess retinol in adult mice, thus acting downstream of ADH1.

**EXPERIMENTAL PROCEDURES**

**Animals—** Raldh1\(^{-/-}\) mice were generated as described previously using a gene replacement targeting vector that removed exon 11 from the Raldh1a1 (Adh1a1) gene (41). The wild-type and Adh1\(^{-/-}\) mice used here have been described previously (43, 44). All of the mice examined were matched for age, weight, and sex.

**Quantitation of Serum Retinoidic Acid and Retinol—** Retinoids were administered orally essentially as described previously (45). All-trans-retinol or all-trans-RA (Sigma) were dissolved in acetone-Tween 20-water (0.25:5.4:75 v/v/v) and were administered by oral injection at a dose of 50 mg/kg for retinol or 10 mg/kg for RA. At several time points following retinoid injection, blood was collected and stored at \(-20\) \({}^\circ\)C. Retinoids were extracted from 0.2 ml of serum under darkened conditions, and all-trans-retinol and all-trans-RA were quantitated by reversed-phase HPLC using a MICROSORB-MVTM 100 C18 column as described previously (45).

**Quantitation of Serum Retinaldehyde—** All-trans-retinaldehyde was dissolved in acetone-Tween 20-water (0.25:5.4:75 v/v/v) and administered by oral injection at a dose of 100 mg/kg. Blood was collected at 1 h after injection and processed immediately as below. All of the extraction and analytical procedures were carried out in a darkened room to protect retinoids from exposure to light. All-trans-retinaldehyde was quantitated after conversion to the more stable oxime as described previously (46). Serum (0.2 ml) was mixed with 0.05 ml of 1 M hydroxylamine (pH 6.8) and 0.2 ml of methanol. After a 30-min incubation at room temperature, the mixture was extracted twice with 1 ml of hexane. Hexane layers were collected, combined, and evaporated under vacuum. The residue was dissolved in 0.1 ml of water, and 0.1 ml of resulting mixture was injected into the HPLC.

Normal-phase HPLC analysis was performed on a Waters 2695 HPLC system using a MICROSORB-MVTM 100 Silica column (250 \(\times\) 4.6 mm, Varian) at a flow rate of 2 ml/min and column temperature of 35 °C. Mobile phase consisted of 92% hexane and 8% ethyl acetate. Detection of retinoids was performed using a photodiode array detector (Waters model 200), which collected spectra between 200 and 450 nm. A standard solution of all-trans-retinaldehyde oximes (syn- and anti-oximes) was prepared by treatment of all-trans-retinaldehyde (Sigma) with 1 M hydroxylamine (pH 6.8) as above. This standard solution was used to identify the peaks and obtain the calibration curves. Characteristic peak spectra and retention times were used to identify all-trans-retinaldehyde, and quantitation of peak areas was calculated at \(\lambda_{max}\) using Waters Millennium Chromatography Manager software.

**Retinol LD50 Determination—** Mice were given oral doses of retinol to determine the lethal dose as reported previously (26). For all of the strains of the mice examined, male 14-week-old mice were used. All-trans-retinol (Sigma) was dissolved in corn oil and administered by gavage at doses ranging from 0.5 to 3.5 g/kg (the volume of the dose was 0.2 ml/10 g body weight). Lethality was monitored daily for 14 days after retinol administration. The methods of Litchfield and Wilcoxon (47) were used to calculate the doses resulting in the death of 16 (LD16), 50 (LD50), or 95% (LD95) of the mice by day 14 plus the 95% confidence limits for the LD50 dose.

**Data Analysis—** Quantitation of RA synthesis or degradation over time was performed by determining the area under the curve (AUC). Statistical significance was determined for raw data using the unpaired Student’s t test (Statistica version 5.0).

**RESULTS**

**Growth of Raldh1\(^{-/-}\) Mice—** We previously reported that Raldh1\(^{-/-}\) mice were obtained at the normal Mendelian ratio and appeared to be viable and fertile without obvious defects in growth or survival (41). Western blot analysis using a previously described mouse RALDH1 antibody (42) demonstrated that RALDH1 protein is detectable in Raldh1\(^{-/-}\) liver, lung, and testes while being abundant in all of these tissues from wild-type mice (41). This indicates that a null mutation was generated. We have obtained no evidence as of yet that Raldh1 is essential for development, contrasting with the results obtained from null mutants of Raldh2, which result in embryonic lethality at mid-gestation (31, 32).

**Metabolism of Retinol to RA in Raldh1\(^{-/-}\) and Adh1\(^{-/-}\) Mice—** RA is normally very low in serum, being reported at \(-4\) ng/ml in mouse (48) and 0.5 ng/ml in rat (49). However, treatment of mice with retinol results in a temporary increase of serum RA as retinol is metabolized to RA and then further metabolized to 4-oxo-RA (43, 45). Here, mice were treated orally with a single dose of retinol (50 mg/kg) and metabolism to RA was followed over time by quantitation of serum RA. Our previous studies indicated peak RA concentrations of 1170 ng/ml for wild-type mice and 270 ng/ml for Adh1\(^{-/-}\) mice (43). In comparison, Raldh1\(^{-/-}\) mice exhibited a peak RA concentration of 390 ng/ml (Fig. 1). A comparison of AUC values for wild-type mice and the two mutant strains demonstrated that Raldh1 deficiency results in a 72% reduction in serum RA detectable during the 4-h period following retinol administration, whereas Adh1 deficiency results in an 82% reduction in serum RA (Table I). These findings suggest that RALDH1 and ADH1 are both needed to efficiently metabolize excess retinol to RA.

**RA Degradation in Raldh1\(^{-/-}\) and Adh1\(^{-/-}\) Mice—** We examined whether the observed decrease in serum RA detectable in Raldh1\(^{-/-}\) mice after retinol administration is due to a-
decreases in RA synthesis. Following a 50 mg/kg oral dose of retinol, all-trans-RA was quantitated in the serum of wild-type (WT) mice, Raldh1−/− mice, and Adh1−/− mice over a 4-h period. All of the values are mean ± S.E. (n = 3). The data for wild-type and Adh1−/− mice were reported previously (43).

**TABLE I**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>All-trans-retinoic acid AUC</th>
<th>∆AUC%</th>
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<tr>
<td>RA synthesis&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) wild-type</td>
<td>Retinol (50 mg/kg)</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td>2) Raldh1−/−</td>
<td>Retinol (50 mg/kg)</td>
<td>0.83</td>
<td>−72</td>
</tr>
<tr>
<td>3) Adh1−/−</td>
<td>Retinol (50 mg/kg)</td>
<td>0.52</td>
<td>−82</td>
</tr>
<tr>
<td>RA degradation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) wild-type</td>
<td>RA (10 mg/kg)</td>
<td>4.90</td>
<td></td>
</tr>
<tr>
<td>2) Raldh1−/−</td>
<td>RA (10 mg/kg)</td>
<td>6.05</td>
<td>+23</td>
</tr>
<tr>
<td>3) Adh1−/−</td>
<td>RA (10 mg/kg)</td>
<td>3.65</td>
<td>−26</td>
</tr>
</tbody>
</table>

<sup>a</sup> [AUC (2 or 3) − AUC (1)] × 100 − AUC (1).
<sup>b</sup> Values were calculated from data shown in Fig. 1.
<sup>c</sup> Values were calculated from data shown in Fig. 2.
<sup>d</sup> Values were calculated from data shown in Fig. 3.

Clearance of Retinol in Raldh1−/− and Adh1−/− Mice—The normal concentration of retinol in mouse serum is 0.3 μg/ml (26). Our previous investigations have shown that serum retinol levels measured 2 h after retinol administration (50 mg/kg) are increased to 1.6 μg/ml in wild-type mice and to 3.1 μg/ml in Adh1−/− mice, suggesting slower retinol clearance in Adh1−/− mice (43). Here, retinol clearance was measured over a 4-h period for wild-type, Raldh1−/−, and Adh1−/− mice treated with 50 mg/kg retinol (Fig. 3). A comparison of AUC values for wild-type mice and the two mutant strains indicates that Raldh1 deficiency results in a 7% increase in serum retinol detectable during the 4-h period following retinol administration, whereas Adh1 deficiency results in a 69% increase (Table I). This demonstrates that Raldh1−/− mice have a small decrease in retinol clearance, whereas Adh1−/− mice have a relatively large decrease in retinol clearance.

Appearance of Retinaldehyde Following Retinol Administration—As the equilibrium of the reversible retinol/retinaldehyde interconversion by ADH favors retinaldehyde reduction (51) and as further oxidation of retinaldehyde to RA by ALDH is functions in the visual cycle (53). Here, we found that retinaldehyde was undetectable in serum of wild-type, Raldh1−/−, and Adh1−/− mice prior to retinol treatment (the limit of detection was 1 ng/ml). One hour after retinol administration (100 mg/kg), serum retinaldehyde was detected at 60 ng/ml in wild-type mice, 150 ng/ml in Raldh1−/− mice, and 20 ng/ml in Adh1−/− mice (Fig. 4). Thus, during clearance of excess retinol, deficiency of Raldh1 leads to excessive accumulation of retinaldehyde, whereas deficiency of Adh1 leads to a reduction in retinaldehyde accumulation.

**Retinol Toxicity in Raldh1−/− and Adh1−/− Mice**—We previously reported retinol LD50 values for wild-type mice (2.72 g/kg) and Adh1−/− mice (0.9 g/kg), demonstrating that Adh1 plays a large role in reducing retinol toxicity (26). Here, the retinol LD50 value for Raldh1−/− mice was found to be 2.0 g/kg, close to the border of being statistically significant when considering the confidence limits (Table II). These findings suggest that Raldh1 may provide some protection against retinol toxicity but that Adh1 plays a larger role.
Raldh1 Acts Downstream of Adh1 in Retinoid Metabolism

versibly oxidizing retinaldehyde to RA. This function distinguishes Raldh1 from Raldh2, which clearly plays a role in the production of RA for developmental RA signaling (31, 32). It remains to be determined whether Raldh1 functions in any aspects of RA synthesis needed for RA signaling in either adult or developing animals in addition to its function in retinol turnover described here.

The genetic studies presented here have demonstrated that RALDH1 metabolizes large quantities of retinaldehyde to RA in adult mice as part of the oxidative pathway to remove toxic levels of retinol initiated by ADH1. Although acute retinol doses of 50–100 mg/kg were needed to effectively quantitate the RA and retinaldehyde produced in Raldh1−/− mice, this level of retinol would not be encountered in the normal diet of animals in the wild. However, our previous results demonstrating decreased postnatal survival and increased liver toxicity in Adh1−/− mice treated chronically with a retinol-supplemented diet containing 10-fold higher retinol are applicable to physiological conditions for some animals (such as carnivores) as the amount of retinol ingested could be obtained by natural diets rich in vitamin A (i.e. liver) (26). Thus, we hypothesize that in some animals ADH1 and RALDH1 are useful for prevention of chronic accumulation of dietary retinol.

We had demonstrated earlier by analysis of Adh1−/− mice and wild-type mice treated with ethanol (an effective competitive inhibitor of ADH1-catalyzed retinol oxidation) that ADH1 is responsible for most of the metabolism of retinol to RA during the 4-h period following retinol administration (43). As calculated here, ADH1 is responsible for 82% of the AUC RA before correction for an apparent 26% increase in RA degradation in Adh1−/− mice and 78% after correction for increased RA degradation (AUCuncorrected (0.52) × 1.26 = AUCcorrected (0.65)). Under the same experimental conditions, we show here that RALDH1 is responsible for 72% of the AUC RA before correction for an apparent 23% decrease in RA degradation in Raldh1−/− mice and 77% after correction for decreased RA degradation (AUCuncorrected (0.83) × 1.23 = AUCcorrected (0.67)). The effects of Adh1 or Raldh1 null mutations on RA degradation are relatively small, demonstrating that the observed reductions in serum RA following administration of retinol to Adh1−/− and Raldh1−/− mice are mostly the result of reduced RA synthesis. As the corrected AUC values show that each null mutant reduces RA synthesis by approximately the same amount (77–78%), this establishes quite conclusively that they are effecting the same metabolic pathway. In addition, the observation of 3-fold reduced serum retinaldehyde levels in Adh1−/− mice following retinol treatment provides evidence that ADH1 is the dominant enzyme-catalyzing oxidation of excess retinol to retinaldehyde. Likewise, the observation of 2.5-fold higher serum retinaldehyde levels in Raldh1−/− mice following retinol treatment provides evidence that RALDH1 is the dominant enzyme-catalyzing oxidation of excess retinaldehyde to RA. Thus, a loss of RALDH1 results in accumulation of retinaldehyde produced by ADH1.

We have previously shown that RA produced following a dose of retinol is not the primary toxic moiety in adult animals, because Adh1−/− mice have less RA production while exhibiting increased toxicity (26). This finding suggests that retinol may be the primary toxin, although the intermediate retinaldehyde should be considered. The LD50 value for Adh1−/− mice was reduced 3-fold compared with wild-type mice, whereas the LD50 value for Raldh1−/− mice was reduced 1.4-fold. Thus, ADH1 plays a more important role than RALDH1 in protection against retinol toxicity. Following a dose of retinol, we observed excessive accumulation of serum retinol in Adh1−/− mice, whereas accumulation of retinol in Raldh1−/− mice was only

![Graph showing serum retinol clearance in Raldh1−/− and Adh1−/− mice following a dose of retinol.](image)

**FIG. 3. Serum retinol clearance in Raldh1−/− and Adh1−/− mice following a dose of retinol.** All-trans-retinol was quantitated in serum of wild-type (WT), Raldh1−/−, and Adh1−/− mice over a 4-h period after a 50 mg/kg dose of retinol. All of the values are mean ± S.E. (n = 3).<ref>

![Table II: Effect of Raldh1 or Adh1 deficiency on retinol lethal dose](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>LD50</th>
<th>LD90</th>
<th>LD100</th>
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</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>15</td>
<td>2.31</td>
<td>2.72 (2.32/3.18)</td>
<td>2.25</td>
</tr>
<tr>
<td>Raldh1−/−</td>
<td>16</td>
<td>1.55</td>
<td>2.00 (1.61/2.48)</td>
<td>1.97</td>
</tr>
<tr>
<td>Adh1−/−</td>
<td>12</td>
<td>0.73</td>
<td>0.90 (0.65/1.25)</td>
<td>1.12</td>
</tr>
</tbody>
</table>

*a The 95% confidence limits for LD50 are in parentheses.*

**DISCUSSION**

Earlier studies provided no evidence that Raldh1 is needed to produce RA for developmental signaling pathways (41). However, the results reported here establish that Raldh1 plays a role in retinol clearance in vivo. Evidence is provided that Raldh1 functions downstream of Adh1 in the oxidative clearance of excess retinol through metabolism to RA. As the oxidation of retinol to retinaldehyde by ADH1 is a reversible reaction (10, 51, 54), efficient clearance of retinol requires that retinaldehyde be metabolized further and RALDH1 does this by irreversibly oxidizing retinaldehyde to RA. This function distinguishes Raldh1 from Raldh2, which clearly plays a role in the production of RA for developmental RA signaling (31, 32). It remains to be determined whether Raldh1 functions in any aspects of RA synthesis needed for RA signaling in either adult or developing animals in addition to its function in retinol turnover described here.

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However, serum retinaldehyde levels were lower in Adh1−/− mice while toxicity was much higher, providing evidence that excessive accumulation of retinol rather than retinaldehyde is the major factor contributing to toxicity after retinol administration.

Our findings indicate that RALDH1-independent mechanisms for retinaldehyde disposition must exist since a large blockage in the further disposition of retinaldehyde would have resulted in a large accumulation of retinol due to the irreversible nature of the retinol/retinaldehyde reaction catalyzed by ADH1. We observed that RA was produced at a slower rate in the absence of RALDH1, suggesting that another enzyme can oxidize retinaldehyde to RA in adult mice. This slower rate of RA production may have been sufficient to allow Raldh1−/− mice to escape major retinol toxicity, but it is possible that retinaldehyde was also metabolized in other fashions. In any event, our results show that the critical step in prevention of retinol toxicity lies in the oxidative metabolism of retinol to retinaldehyde by ADH1, which evidently produces less toxicity than disposition of retinol through other pathways. Other pathways may include P450s, which are known to metabolize retinol to 4-hydroxyretinol (23). However, metabolism of this sort would actually contribute further to toxicity as P450-mediated metabolism requires molecular oxygen and produces oxygen free radicals that can cause liver damage (55, 56). Metabolism of retinol by ADH (and retinaldehyde by ALDH) does not directly produce oxygen free radicals because it occurs via dehydrogenation with the cofactor NAD. Another pathway of retinol disposition is glucuronidation to form retinyl glucuronides (57). However, excessive production of retinyl glucuronides may result in toxicity as this has been demonstrated to greatly reduce the amount of uridine diphosphoglucuronic acid substrate available to perform glucuronidation reactions of other compounds to facilitate their excretion (58).

There are several additional similarities between Adh1 and Raldh1 that provide further evidence that they are functioning in the same metabolic pathway. Similar to Raldh1−/− mice, Adh1−/− mice also do not have noticeable defects in development, postnatal viability, or fertility (26). This points to a function for both ADH1 and RALDH1 in some aspect of adult maintenance, perhaps protection against toxic alcohols or aldehydes. ADH1 is the most abundant ADH protein in mouse liver (59–61), and RALDH1 protein is also quite abundant in mouse liver (42), suggesting that a major function for both enzymes is the removal of toxic substances in the liver. ADH1 and RALDH1 proteins are both easily detectable in a variety of adult tissues (liver, intestine, adrenal, uterus, and ovary), suggesting that they may function in common metabolic pathways in several adult tissues. ADH1 and RALDH1 can use many corresponding alcohol/alddehyde pairs as substrates including retinol/retinaldehyde, ethanol/acetaldheyde, propanol/propi- onaldehyde, hexanol/hexanal, and 4-hydroxynonenal/4-hydroxynonenal (10, 59, 62). These observations indicate that ADH1 and RALDH1 are likely to cooperate in the oxidative clearance of many toxic aliphatic alcohols and aldehydes through reversible equilibration of the alcohol/alddehyde pair by ADH1 followed by irreversible conversion to the carboxylic acid by RALDH1.

Acknowledgment—We thank the Burnham Institute Mouse Genetics Facility for help in generation of Raldh1−/− mice.
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