

## The Molecular Basis of Retinoic Acid Induced Night Blindness

Wing C. Law and Robert R. Rando\*

Department of Biological Chemistry and Molecular Pharmacology  
Harvard Medical School, Boston, MA 02115

Received April 28, 1989

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**Summary** Drugs which affect the processing of vitamin A in the retina or pigment epithelium can cause ocular toxicity. It is shown here that the retinoic acids, which are used in the treatment of skin disorders and which cause night blindness, inhibit the ocular retinol dehydrogenases in an *in vitro* system. This is shown to lead to a decrease in the formation of the visual chromophore 11-*cis*-retinal, thus explaining why night blindness might occur. © 1989 Academic Press, Inc.

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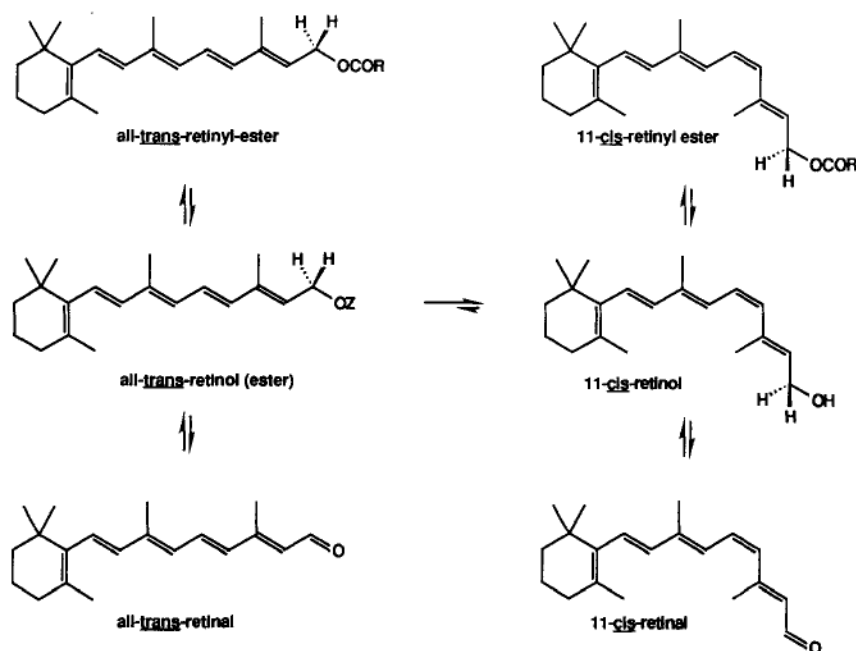
A significant number of commonly used drugs have deleterious effects on vision. Of this group a certain number cause night blindness (nyctalopia). It would not be unreasonable to surmise that at least some of these drugs affect the processing of vitamin A in the retina and pigment epithelium. For example, the hydrophobic aromatic amine based drugs which cause night blindness do so by catalyzing the exothermic isomerization of the visual chromophore 11-*cis*-retinal into all-*trans*-retinal *in vivo*, inhibiting pigment regeneration (1,2).

Drugs which inhibit enzymes of the classical visual cycle (Scheme 1) would also be expected to produce night blindness and other ocular disorders, since they reduce the flow of retinoids through the system and hence reduce the production of the visual chromophore 11-*cis*-retinal. This latter retinoid is only synthesized in the visual system in nature and is, thus, not a normal dietary constituent. Although none of the enzymes of the visual cycle has been purified, reliable assays are available to measure their activities and to determine the effects drugs might have on them. For example, the ester synthetase(s) and the retinol dehydrogenases are readily assayed (1).

The recent discovery of an enzyme system capable of isomerizing free all-*trans*-retinol into its 11-*cis* congener completes the visual cycle (Scheme 1) and also provides an assay for this important enzyme (3,4). Inhibitors of this enzyme would certainly be expected to produce visual deficits, as this enzyme is rate limiting in the biosynthesis of 11-*cis*-retinoids (3,4). This article explores the *in vitro* effects on the enzymes of the visual cycle of two clinically used drugs which are known to produce visual toxicities. All-*trans* and 13-*cis*-retinoic acids are important drugs in the treatment of Acne and other skin diseases, and both molecules can cause night blindness (5). It is shown here that these retinoids appear to powerfully inhibit the ocular retinol dehydrogenases, thus hindering the formation of 11-*cis*-retinal from its 11-*cis*-retinol precursor. This inhibition

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\*To whom all correspondence should be addressed: Department of Biological Chemistry and Molecular Pharmacology, 250 Longwood Ave., Harvard Medical School, Boston, MA 02115.



Scheme 1. The Visual Cycle.

results in a decrease in rhodopsin formation and, hence, would be expected to lead to night blindness. The retinoic acids have no measurable effect on the isomerase and ester synthetase activities. Thus, we will present here a molecular explanation for a toxic side effect of important clinically used drugs.

### Materials and Methods

*Rana pipiens* frogs (3-3.5 in. size) were from Sullivan's Amphibians, Nashville, TN. [11,12<sup>3</sup>H]-all-*trans*-Retinol (29 Ci/mmol) was purchased from New England Nuclear. The retinoic acids were products of the Sigma Chemical Co.

**Isomerase Preparations and Assays.** The detailed procedures for the preparation of the 600  $\times$  g supernatant isomerase containing membranes from the frog (*R. pipiens*) retina/pigment epithelium have been previously described in detail (3,6). The incubations were performed in 50 mM sodium phosphate buffer (pH 7.2). The detailed methods used for performing the isomerase assays have been published (3,6), as have the methods used for the HPLC separation and analysis of retinoids (3,6). Under the above experimental conditions, generally 10-15% of the radioactivity was recovered as the retinols, 10-15% as the retinals, and 70-80% as the retinyl palmitates. The 11-*cis*-retinoids generally account for over 30% of the retinal pool, over 20% of the retinol pool, and over 10% of the retinyl palmitate pool.

### Results

#### Inhibition of the Retinol Dehydrogenases by the Retinoic Acids

The retinoic acids (13-*cis*-, all-*trans*-) or isotretinoin and tretinoin respectively, are important drugs in the treatment of serious forms of Acne, but these drugs and their derivatives have been reported to cause night blindness (5). We have previously shown that a membrane preparation from the amphibian retina/pigment epithelium exhibits all of the reactions of the visual cycle (3,6). This *in vitro* 11-*cis*-retinoid biosynthetic system provides an ideal entry into the issue

of whether or not these drugs produce their undesired visual side effects by suppressing a reaction in the visual cycle. As shown in Table 1, when the 600 x g supernatant of amphibian retina/pigment epithelium was incubated with  $^3\text{H}$ -all-trans-retinol in the presence or absence of all-trans-retinoic acid, the amount of 11-cis-retinal formation strongly decreased with the increasing amount of all-trans-retinoic acid, while the formation of 11-cis-retinol and 11-cis-retinyl palmitate remained approximately the same. These data indicate that all-trans-retinoic acid does not affect either isomerase or ester synthetase activities but does inhibit 11-cis-retinol dehydrogenase. The fact that a significant decrease in the formation of all-trans-retinal was not observed does not necessarily mean that the drug is specific for the 11-cis-retinol dehydrogenase as opposed to its all-trans congener. It must be remembered that 11-cis-retinol forms very slowly with time and therefore a kinetic effect of a drug on its processing will be more apparent than on all-trans-retinol (6). That all-trans-retinoic acid can inhibit all-trans retinol dehydrogenase was shown by using  $^3\text{H}$ -all-trans-retinal as the substrate (Table 1). In the presence of all-trans-retinoic acid, the reduction of all-trans-retinal decreased significantly (Table 1). Although the all-trans-retinal which was reduced was processed to form 11-cis-retinol and 11-cis-retinyl palmitate, no significant formation of 11-cis-retinal was observed. When 13-cis-retinoic acid was used as the inhibitor, the formation of both 11-cis-retinal and all-trans-retinal decreased with increasing concentrations of 13-cis-retinoic acid. The retinal pool dropped from approximately 13% to approximately 5% of the total, but the ratio between 11-cis and all-trans did not change (Table 2). The formation of 11-cis-retinol and 11-cis-retinyl palmitate was not negatively affected (Table 2). These data indicate that

**Table 1. Effect of all-trans-Retinoic Acid on the Formation of 11-cis-Retinoids from all-trans-Retinoids in Frog Retina/Pigment Epithelium 600 x g supernatant<sup>a,b</sup>**

| Substrate      | all- <u>trans</u><br>Retinoic<br>Acid | Retinol         |           | Retinal         |           | Retinyl palmitate |           |
|----------------|---------------------------------------|-----------------|-----------|-----------------|-----------|-------------------|-----------|
|                |                                       | %11- <u>cis</u> | %recovery | %11- <u>cis</u> | %recovery | 11- <u>cis</u>    | %recovery |
| Retinol (-enz) | 0                                     | 0.5             | >99       | /               | <1        | /                 | <1        |
| Retinol (+enz) | 0                                     | 20.1±1.0        | 11.4±0.4  | 47.8±0.4        | 10.0±0.6  | 5.1±1.1           | 78.6±1.1  |
| Retinol (+enz) | 3μM                                   | 23.9±0.2        | 11.8±1.0  | 21.6±0.8        | 11.1±1.3  | 5.1±0.3           | 77.2±2.2  |
| Retinol (+enz) | 10μM                                  | 19.9±0.7        | 17.4±2.4  | 14.7±1.0        | 14.9±0.1  | 4.2±0.0           | 68.4±1.7  |
| Retinol (+enz) | 30μM                                  | 15.4±0.3        | 23.0±5.7  | 14.8±0.7        | 9.3±0.4   | 4.4±0.2           | 67.2±6.1  |
| Retinal (-enz) | 0                                     | /               | <1        | 2.6             | >99       | /                 | <1        |
| Retinal (+enz) | 0                                     | 25.3±0.2        | 12.2±0.2  | 41.6±4.7        | 16.5±2.0  | 8.5±0.9           | 71.4±0.9  |
| Retinal (+enz) | 10μM                                  | 23.6±1.8        | 11.6±0.5  | 4.5±0.2         | 43.5±1.0  | 7.5±0.3           | 44.9±1.5  |

<sup>a</sup>the incubation mixture included 300μl of the 600 x g supernatants, 1 μCi of  $^3\text{H}$ -all-trans retinol (60 Ci/mmol) or 0.2 μCi of  $^3\text{H}$ -all-trans-retinal, 15 μl of 10% BSA, and the indicated amount of all-trans-retinoic acid. The incubations were carried out at room temperature for 3 h. <sup>b</sup>the distribution of retinoids was determined as previously published (3) and all values are mean±SD.

**Table 2. Effect of 13-*cis*-Retinoic Acid on the Formation of 11-*cis*-Retinoids from all-*trans*-Retinoids in Frog Retina/Pigment Epithelium 600 x g supernatant a,b**

| Substrate     | 13- <i>cis</i><br>Retinoic<br>Acid | Retinol         |                | Retinal         |                | Retinyl palmitate |                |
|---------------|------------------------------------|-----------------|----------------|-----------------|----------------|-------------------|----------------|
|               |                                    | %11- <i>cis</i> | % <i>recov</i> | %11- <i>cis</i> | % <i>recov</i> | %11- <i>cis</i>   | % <i>recov</i> |
| Retinol       | 0                                  | /               | <1             | <1              | >99            | /                 |                |
| Retinol(+enz) | 0                                  | 29.8±1.1        | 8.4±0.3        | 75.1±1.3        | 13.1±0.1       | 10.4±0.5          | 78.5±0.3       |
| Retinol(+enz) | 3μM                                | 37.8±0.1        | 12.3±0.1       | 72.3±1.2        | 8.7±0.4        | 23.4±0.5          | 79.0±0.6       |
| Retinol(+enz) | 30μM                               | 34.9±0.6        | 13.9±0.1       | 71.4±1.8        | 5.4±0.2        | 24.6±0.7          | 80.8±0.2       |

<sup>a</sup>the incubations were conducted as in Table except that 13-*cis*-retinoic acid was added as an inhibitor. The incubations were carried out at room temperature for 3 hours. <sup>b</sup>all values are mean±SD.

13-*cis*-retinoic acid does not inhibit the isomerase or ester synthetase, but does inhibit the retinol dehydrogenases.

### Discussion

There are many factors that can induce night blindness. The absence of vitamin A or its precursor  $\beta$ -carotene in the diet remains the most important cause of this disease (7). However, drugs can also induce this disease and it is important to uncover the mechanisms by which they can bring it about, so that effective therapies can be designed. One way that a drug might affect vision is to inhibit one or more of the enzymes of the visual cycle in such a way as to deprive the photoreceptors of their chromophore, 11-*cis*-retinal. A mechanism of this type could produce an effect similar to that found in vitamin A deficiency.

The classical visual cycle is comprised of those reactions involved in the processing of retinoids in the eye (Scheme 1). The inhibition of any of the reactions in the cycle could lead to a deficiency of 11-*cis*-retinal with a concomitant decrease in rhodopsin formation. As an example of this, both all-*trans* and 13-*cis*-retinoic acid proved to be quite effective at inhibiting the ocular all-*trans*-retinol and 11-*cis*-retinol dehydrogenases. It should be noted that in order to accumulate significant 11-*cis*-retinoids, the inhibition assays had to be run over a long period of time. The fact that inhibition of the dehydrogenases was still observed suggests that the retinoic acids are powerful inhibitors of these enzymes. Under the conditions of the assays it appeared that 11-*cis*-retinol dehydrogenase might have been more effectively inhibited by all-*trans*-retinoic acid. The results also showed that the isomerization reaction, which occurs via the conversion of free all-*trans*-retinol to 11-*cis*-retinol, is not inhibited by the retinoic acids, nor is the ester synthetase.

Although the work described here was performed on the intact visual cycle of the amphibian, it is likely also to be relevant to the mammalian situation, because a retinoic acid of unspecified stereochemistry has been shown to be inhibitory of the detergent solubilized retinol dehydrogenase from bovine retinal rod outer segments (8). This latter observation is consistent with the known sensitivity of alcohol dehydrogenases by long chain fatty acids (9). In addition,

the visual cycle retinol dehydrogenases of mammals tend to be more readily affected by alcohol dehydrogenase inhibitors than those from amphibians (1).

That inhibitors of the retinol dehydrogenases can prevent rhodopsin regeneration is clear from published studies on the effects of well characterized alcohol dehydrogenase inhibitors on rhodopsin regeneration in mammals (10,11). These drugs have clear inhibitory effects on pigment regeneration which can most easily be ascribed to an inhibition of 11-cis-retinol dehydrogenase. This would prevent the formation of 11-cis-retinal, thus decreasing the rate of pigment regeneration (10,11). Although we have not studied the synthetic retinoid analogs used in clinical medicine (such as fenretinide) which also cause night blindness, the expectation would be that these drugs would act similarly to the retinoic acids (12).

### **Acknowledgment**

This work was supported by a grant from the U.S. Public Health Service, Research Grant EY04096 from the National Institutes of Health.

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