THE EFFECTS OF LIGHT AND DARK ADAPTATION ON THE LEVELS OF CYCLIC NUCLEOTIDES IN RETINAS OF MICE HETEROZYGOUS FOR A GENE FOR PHOTORECEPTOR DYSTROPHY

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SUMMARY

Levels of cyclic AMP and cyclic GMP were measured in light and dark adapted retinas from normal mice (++/++) and from mice heterozygous for the photoreceptor dystrophy gene (rdle/++). In light adapted retinas cyclic GMP levels were 40% lower in the heterozygotes than in the normals, whereas cyclic AMP levels were the same. Dark adaptation elevated cyclic GMP levels 120% in both groups and also elevated cyclic AMP levels 90% in the ++/++ and 45% in the heterozygotes. These data suggest that animals heterozygous for photoreceptor dystrophy have an abnormality of their retinal cyclic GMP system.

INTRODUCTION

Studies of mice with an autosomal recessively inherited photoreceptor dystrophy (rd) have led to the suggestion that this disorder might be due to an abnormally elevated level of cyclic GMP resulting from an inherited deficiency of photoreceptor phosphodiesterase (1-3). In carrying out related studies in a population of mice in which the rd gene was segregating, we observed that animals heterozygous for this gene had retinal levels of cyclic GMP which were significantly different from both animals lacking rd and those homozygous for rd. We report these data here. In addition, the effect of *in vivo*

light and dark adaptation on retinal cyclic AMP and cyclic GMP levels is described for these animals.

METHODS

Using previously published methods (4), retinas were obtained from light- or dark-adapted, 30-day-old, black, pigmented mice of the normal C57BL (6)J (++/++) strain or from congenic mice which were either heterozygous or homozygous for the closely linked recessive genes, rd and le. The former recessive in the homozygous state results in an early onset photoreceptor dystrophy; the latter recessive in the homozygous state results in light ears and tails, thus permitting recognition of the dystrophic animals in litters. Crosses employed included ++/rdle x rdle/rdle backcrosses to yield heterozygotes and dystrophics and ++/rdle x ++/rdle to yield (F_2) dystrophics and a mixture of phenotypically normal appearing heterozygotes and homozygotes in the predicted 1:2:1 ratio. The readily-recognized, dystrophic animals had levels of cyclic GMP which were but a few percent of control values. They were discarded and are not part of this report.

Dark adaptation was for 60 to 90 min. Eyes were rapidly removed after decapitation in dim red light (selected GE ruby glass bulbs, BAS-115V) and transferred to ice-cold physiological saline where retinas were obtained by dissection under dim red light (6 volt microscope lamp with a Tiffen #70 filter, operated at 2 volts). Light adaptation consisted of 60 to 90 min. of exposure of the animals to 190 ft-cd of white light with similar dissection in light from the microscope lamp at 2 volts but without the red filter. Retinas were frozen in liquid N_2. Decapitation to freezing times averaged 142 $\pm$ 23 s.d. sec. and 253 $\pm$ 34 s.d. sec. for the first and second retinas in white light and 182 $\pm$ 30 s.d. sec. and 352 $\pm$ 29 s.d. sec. for red light. Thus, it required about 60 additional sec. at 0° C. to process a retina in red light. On the average, cyclic AMP levels were slightly higher and cyclic GMP levels slightly lower in the second eye, but these differences were not statistically significant ($p>0.05$).

The frozen retinas were individually placed in 7 x 70 mm test tubes at -20°, and to each, 200 µl of 10% ice-cold trichloroacetic acid was added. The tissue was finely dispersed with a ground-glass pestle, and the mixture was centrifuged at 17,000 x g for 15 min. at 4°. A 180 µl aliquot of the clear supernatant fluid was transferred to a 10 x 75 mm test tube and was washed 4 times with 4 vols. of water-saturated ethyl ether. This acid-free solution was dried under a stream of N_2, and the residue was dissolved in 250 µl of 50 mM Na-acetate buffer, pH 6.0. A 100 µl aliquot of this solution was transferred to another 10 x 75 mm tube, and 2 µl of triethylamine and 1 µl of acetic anhydride was added in sequence to acetylate cyclic nucleotides (6). Both cyclic AMP and cyclic GMP were measured by radioimmunoassays (5,6). The trichloracetic acid precipates were dissolved in 1 N NaOH, and the protein content of these solutions was measured according to the method of Lowry *et al.* (7).

RESULTS

The cyclic AMP and cyclic GMP concentrations in light-

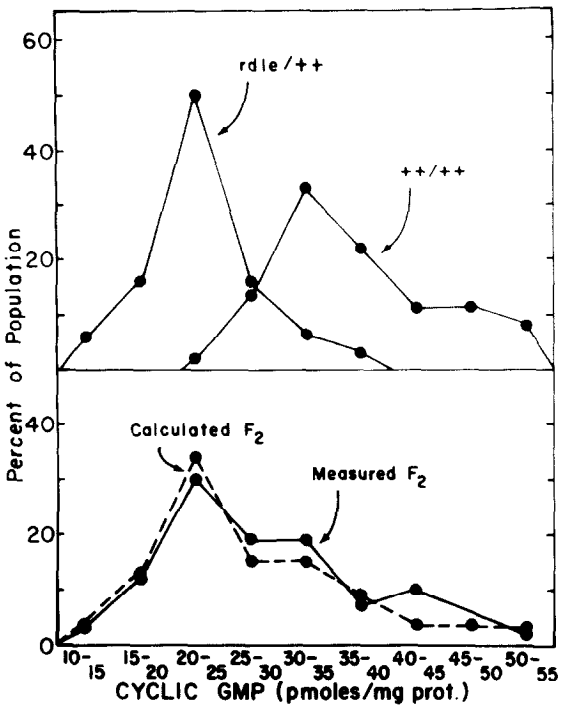

FIG. 1 Concentration distribution of cyclic GMP in light-adapted retinas from ++/++ mice (n=54); from mice heterozygous for the photoreceptor dystrophy gene (rdle/++) (n=30); and from mixed litters of mice obtained by crossing ++/rdle x ++/rdle (measured F_2) (n=60). All mice homozygous for the rdle genes have been omitted from the F_2 group; therefore, this group contains only normals and heterozygotes (1:2). Each point represents the percent of the population containing the designated concentration of cyclic GMP. The calculated F_2 values were estimated from measured concentrations of cyclic GMP in the rdle/++ and ++/++ populations and represent a hypothetical 2:1 mixture of these animals.

adapted retinas from ++/++ animals were 8.5 ± 0.2 and 37.3 ± 1.1 pmoles/mg prot (n = 54), respectively. These values, obtained in 1 month-old animals, were highly similar to those previously found by us in 3 month-old animals.

The average concentration of cyclic AMP in light-adapted retinas from dark-eared (non-dystrophic), rdle/++ animals was the same (8.0 ± 0.2) as that in ++/++ mice, but the average cyclic GMP concentration was 40% lower. A plot of the cyclic GMP concentra-

TABLE 1

EFFECT OF ADAPTATIONAL STATE ON RETINAL CYCLIC NUCLEOTIDE LEVELS IN ++/++ AND ++/rdle MICE

	Adapted and Isolated in: Light	Dark
	Cyclic GMP (pmoles/mg prot)	
++/++	37.3 ± 1.1 (54)	82.0 ± 3.6 (12)
++/rdle	22.2 ± 0.9 (30)	49.2 ± 2.1 (12)
	Cyclic AMP (pmoles/mg prot)	
++/++	8.5 ± 0.2 (54)	16.0 ± 0.5 (12)
++/rdle	8.0 ± 0.2 (30)	11.5 ± 0.4 (12)

Retinas from light or dark adapted ++/++ or ++/rdle mice were obtained and analyzed as described in the text. Each value represents the mean ± SEM of the number of retinas designated in parentheses.

tion distribution showed that there was little overlap between the two populations of mice (Fig. 1). In the group of dark-eared (non-dystrophic), F_2 mice, cyclic GMP levels varied over a wide range between 10 and 55 pmoles/mg protein with 63% of the eyes containing concentrations less than 30 pmoles/mg protein. The measured cyclic GMP concentration distribution in the F_2 population was virtually identical to a distribution profile calculated from measured cyclic GMP levels in populations of rdle/++ and ++/++ mice.

Protein content was the same in all groups of animals (311 µg/retina).

In the dark-adapted retinas of ++/++, cyclic GMP levels were 120% higher than in light-adapted retinas from the same group of animals (Table 1). Dark adaptation also caused a 120% elevation of retinal cyclic GMP levels in rdle/++ mice. However, the concentration in dark-adapted, rdle/++ retinas was only 60% of that in ++/++ animals. Cyclic AMP levels were also elevated in dark-adapted ++/++ and rdle/++ animals, though the elevation in the former was greater than that in the latter, 90 vs. 45% (Table 1).

DISCUSSION

It is clearly apparent from the present results that the cyclic GMP concentration in retinas of animals heterozygous for rdle is reduced by about 40% from the control level, but the level responds to light and dark adaptation as in control animals. Other studies (8) indicate that 98% of the cyclic GMP of a retina is contained in its photoreceptor cells. The fact that heterozygous animals possess numbers of receptors similar to controls is strongly suggested by the virtual identity of protein values for the two retinas since photoreceptors constitute a major part of the retinal mass. Although light microscopic comparisons of eye cups from rdle/++ and ++/++ gave no impression of differences in melanin content in the iris, pigment epithelium or uvea, the le marker gene, which reduces the melanin content of the skin of the ears and tail, also produces some hypopigmentation in ocular tissues in animals homozygous for le, and therefore might conceivably produce less ocular melanin in animals heterozygous for le. Thus, more light would reach the light-adapting retina of heterozygotes and therefore produce a lower cyclic GMP level. But this would not explain the diminished dark-adapted value for cyclic GMP. It therefore seems likely

that it is largely heterozygousity for rd which affects the cyclic GMP system of the photoreceptors.

It has been suggested that mouse retinal dystrophy is a consequence of a deficiency in a cyclic GMP phosphodiesterase (1-3). Were this true, one would expect that, if anything, heterozygotes would have a higher level of cyclic GMP than controls; in fact, the converse seems to be true. On the other hand, if guanylate cyclase activity was limiting in heterozygotes, the dark level of cyclic GMP should be lower than normal, but the response to light unimpaired. This seems more compatible with the present data, and in support of this view are preliminary studies in our laboratory indicating substantial reduction of guanylate cyclase activity in rdle/++ retinas.

Studies on dying photoreceptors in retinas homozygous for rd are valuable but have the inherent limitation that once deterioration is initiated, ramifying effects may tend to obscure the initial event. Thus, a manifestation of an rd effect in a heterozygote may afford a better opportunity both in attempting to understand the genetic lesion and in studying the role of cyclic GMP in photoreceptor function.

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