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Carriers of the Mouse rd Gene Have Reduced Levels of the Beta Subunit of the Retinal Cyclic GMP Phosphodiesterase

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Polyclonal antipeptide antisera have been utilized to quantitate the amount of retinal rod outer segment cGMP phosphodiesterase α and β catalytic subunits present in retinas from C57BL/6J mice which are normal or carriers for the rd gene defect. Results suggest that the quantity of PDE-β subunit is reduced in carrier mice while PDE-α and PDE-γ are not affected. In 21-day-old mice, the PDE-β was reduced by about one-half while adult carrier mice had even more reduced levels of PDE-β. Since PDEα was not reduced, this suggests that synthesis of PDEα and PDEβ may not be coordinately controlled.

In the rod outer segment visual signal transduction occurs through the photoisomerization of rhodopsin (1). This then causes a cascade of events which includes the activation of transducin, and finally, the activation of the cyclic GMP phosphodiesterase (PDE) through the removal of the gamma inhibitory subunits (1). The PDE is a holoenzyme complex consisting of alpha and beta catalytic subunits and two gamma inhibitory subunits (2,3). Sequences for cDNA and genes have been reported for these proteins (4,5,6). The functional domain mapping has been reported for the inhibitory subunits and, partially, for the catalytic subunits (7,8). The alpha and beta subunits have high sequence identity within the C-terminal domain which is thought to contain the catalytic region (9). Partial proteolysis of the alpha subunit confirms this (7). The N-terminal region binds the gamma inhibitory subunit (8).

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Abbreviations: cGMP - guanosine 3',5'-cyclic monophosphate; PDE - phosphodiesterase; ROS - rod outer segment; PMSF - phenyl methylsulfonylfluoride.

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In the rod outer segment (ROS) of rd mice, abnormal cGMP accumulation is associated with a recessive genetic mutation which causes early selective degeneration of photoreceptor rod outer segments which is followed by the degeneration of cones (10,11,12). No photoreceptor layer remains by 21 days post-natally. The genetic defect has been identified as resulting from a mutation in codon 347 of the beta catalytic subunit of the cyclic GMP phosphodiesterase. The mutation results in premature termination of the synthesis of the beta subunit (13,14,15).

Although individual catalytic subunits of the phosphodiesterase have not been isolated in active form, PDE activity may require both the alpha and beta subunits. Early reports noted the deficiency in PDE activity and in immunoreactivity of the PDE catalytic proteins in the homozygous affected rd mouse (10-12,16,17).

Carriers of various retinal degenerative defects have been reported to have lesions which, although not as severe as the homozygous individuals, are still not that which is observed in normal individuals (18). A defect in PDE Km, in cyclic GMP levels, and in cyclic GMP binding has been reported to occur in the rd mice which are heterozygous for the rd gene (19-22). In this paper, we report the results of measurements of amounts of PDE protein present in 21 day old and adult mice which are heterozygous for the rd gene defect.

MATERIALS AND METHODS

C57 BL/6J mice which were normal at the rd and le loci (+/+ +/+) and C57 BL/6J mice which were carriers (rd/+ le/+) or homozygous affected (rd/rd le/le) were from an in-house colony originally purchased from Jackson Laboratory. Animals were pooled male and female, 21-day-old or adult (older than 1 year). Retinas were dissected under room light and homogenized in 10 mM Tris (pH 7.5), 1 mM EDTA, 1% Triton-X-100, 1 μg/ml leupeptin and 1 mM PMSF for SDS-PAGE. For PDE assays, 10 retinas of each type were homogenized in 1 ml of 10 mM Tris (pH 7.5), 1 mM EDTA, 1 μg/ml leupeptin.

PDE assays were as previously described (8,23). The reaction mixture contained 50 mM Tris-HCl (pH 7.2), 4 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 1 mg/ml histone, and cGMP with 50,000 cpm ^{3}H-cGMP (ICN; specific activity 6 Ci/mmole).

Peptides were synthesized, cleaved and purified as previously described (24,25,26,8). Peptide antisera, listed in Table I, were produced by cross-linking peptides to KLH (27) and injecting with or without adjuvant as previously reported (8).

SDS-PAGE (28) and Western blots (29) were as described (8). PDE α/β subunits were resolved on 15% acrylamide/0.08% bisacrylamide (2) while PDEγ was resolved on 10% acrylamide/ 0.3% bisacrylamide (31). Protein concentrations were determined by the method of Bradford (30) using BSA as a standard. Individual proteins were standardized using scanning gel densitometry of autoradiograms.

Table I. Antisera Directory

Antisera	Peptide Residues	Identity
α-NT	1-15	PDE-α
α-β	21-35, 36-50	PDE-β
α-Pγ	73-87	PDE-γ
α-TαC	1-15	T-α

Peptide antisera were produced by injecting rabbits subcutaneously with synthetic peptides linked to KLH. Bovine sequences for PDEα, PDE-β, and PDEγ were as published (4,6,9). The Tα sequence was also bovine (32). PDE = cyclic GMP phosphodiesterase; Tα = alpha subunit of transducin.

RESULTS AND DISCUSSION

Results of Western blots of retinal homogenates from adult or 21-day-old normal or heterozygous mice indicate that the amount of beta subunit protein is reduced in the heterozygous animals (Figure 1 and Table II). The amount of reactivity with αNT (Gel A, Figure 1) was

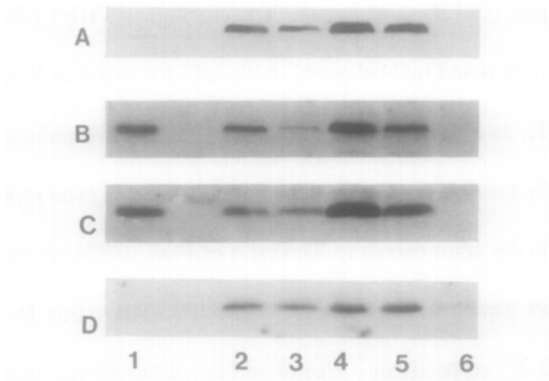

Figure 1. Western Blots of PDE Samples

PDE-α, PDE-β and T-α were resolved on 15% SDS-PAGE (2) while PDE-γ was resolved on 10% SDS-PAGE (31). Retinal homogenates were loaded at 200 μg protein/lane. Following Western blots (29) antisera (see Table I) were added at 1:100. Visualization is with ^{125}I-protein A and autoradiography as previously described (8). Gel-A - Western blot with α-NT antisera to PDE-α; Gel-B - with antisera, α-β, to PDE-beta; Gel-C - with antisera, α-P-γ, to PDE-γ;Gel-D - with antisera, α-TαC, to transducin alpha. Lane 1 = 5 μg bovine purified PDE $\alpha/\beta/\gamma_2$; Lane 2 = adult normal retinal homogenate; lane 3 = adult carrier retinal homogenate; Lane 4 = 21-day-old normal retinal homogenate; Lane 5 = 21-day-old carrier retinal homogenate; Lane 6 = 21-day-old rd/le affected retinal homogenate. Results of densitometric scans are in Table II.

Table II. Densitometric Scans of Western Blots

Samples	Antisera	Peak Area	Ratios		
			α-β/α-NT	α-Pγ/α-β+ α-NT	α-TαC/α-NT
Adult-Normal	α-NT	1780129	0.23	0.34	0.58
	α-β	403501			
	α-Pγ	740287			
	α-TαC	1033715			
Adult-Carrier	α-NT	1080787	0.09	0.41	0.85
	α-β	94493			
	α-Pγ	487249			
	α-TαC	928695			
21-day-old Normal	α-NT	3529121	0.50	0.99	0.41
	α-β	1781970			
	α-Pγ	5276020			
	α-TαC	1463266			
21-day-old Carrier	α-NT	2493522	0.27	0.85	0.68
	α-β	663913			
	α-Pγ	2689710			
	α-TαC	1689649			

Samples were prepared as described in Materials and Methods. Antisera are as listed in Table I and Figure 1. Peak areas are results of densitometric scans from a Gilford multimedia densitometer using a Shimadzu integrator.

approximately equal for adult normal (lane 2) or adult carrier (lane 3) or for 21-day-old normal (lane 4) or carrier (lane 5) samples. However, the amount of reactivity with α-β was reduced in adult or 21-day-old carrier samples (Gel B, same lanes). The amounts of PDEγ (Gel C) or Tα (Gel D) were identical. No reaction was noted in 21-day-old, rd/le affected samples (lane 6, all gels). Lane 1 is a sample of bovine PDE, as a control. The data suggest a selective reduction in the beta protein in heterozygous mice. We have observed that the amount of beta is even more reduced, to about one eighth that of normal, in the adult carriers. The ratio of PDE β/PDEα was 0.23 and 0.09 for adult normal and adult carrier samples, respectively (Table II). The ratios for 21-day-old normal and carriers was 0.50 and 0.27, respectively. Use of additional polyclonal antisera gave similar results (data not shown). We are uncertain as to what effect this may have on carrier mice since they have been reported to have normal morphology and do show a normal light-induced reduction in cyclic GMP levels (18-22). Perhaps the ratio of PDE to transducin and rhodopsin is not as critical as was previously thought. It is of interest that even though the amount of beta subunit is reduced the amount of alpha subunit is not significantly altered. This suggests that there is not a coordinated

Table III. Characteristics of Histone-Activated PDE

Samples	Km	Specific Activity (nmole/min/μg protein)
Normal Adult	35 μM	0.86
Carrier Adult	84 μM	0.48

PDE assay is described in Materials and Methods. Km is for cGMP, plus histone. Specific activity is in nmole cGMP hydrolyzed per minute per μg total retinal protein.

control of the synthesis of the two catalytic subunits in the rod cell. It remains to be determined where this exists and if it is active when not bound to the beta subunit.

The Km values and specific activities of the adult normal and adult carrier samples are listed in Table III. As previously reported (18), carrier samples exhibit a higher Km for cGMP in the presence of histone. At 50 μM cGMP, the specific activity of adult normal PDE was 0.86 nmole/min/μg retinal protein while that of the carrier was 0.48 nmole/min/μg retinal protein. The approximate half value of the carrier would suggest that the PDE alpha of the carrier is not active. However, this protein must be purified to establish whether it has PDE enzymatic activity.

We do not observe the presence of the truncated form of the beta subunit at up to 200 μg of protein. Our results using an in vitro translated truncated version of both PDE alpha and beta do indicate that these proteins would still bind the gamma inhibitory subunit (our unpublished results). However, it is uncertain as to whether this occurs in vivo or whether the excess alpha subunits of the carriers would also bind to gamma proteins.

Reduction in levels of mRNA caused by nonsense mutations have been reported to occur in several other systems including the Drosophila ninaE mutation (33). The mechanism by which this reduction occurs is not known. In this paper, we report that mice which are heterozygous for the rd gene defect synthesize less of the beta subunit protein while the alpha subunit protein is synthesized at normal levels.

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