Biochemical and electrophysiological abnormalities in the photoreceptors of mice heterozygous for the rd gene

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Several causes have been proposed for the rod dysplasia that occurs in mice homozygous for the photoreceptor degeneration gene designated as rd, but none has been established. Rod breakdown is preceded, at about 8 days of age, by a large increase in the concentration of cyclic guanosine monophosphate (cyclic GMP), resulting from a reduction in the activity of the rod specific cyclic GMP phosphodiesterase (PDE; Farber & Lolley, 1976). Abnormal cross-reactivity with a specific anti-PDE monoclonal antibody (Lee et al., 1985) has provided evidence for a structural change in the enzyme and this is supported by the fact that it cannot be activated by histone (Lolley et al., 1987), but doubt remains as to the primary genetic defect, since rhodopsin phosphorylation and antibody interaction are also abnormal (Shuster & Farber, 1986; Takemoto et al., 1985) and inaccessibility of the C-terminal of rhodopsin has been detected as early as 7 days of age (Takemoto et al., 1985).

Mice heterozygous for the rd gene (+/rd) have normal retinal structure and a full complement of rhodopsin (Doshi et al., 1985). However, the retinal concentration of cyclic GMP is reduced by 40% (Ferrandelli & Cohen, 1976; Doshi et al., 1985), and the amplitude/intensity relationship of the photoresponse is changed (Arden & Low, 1980; Low, 1987). There is no observable change in the activity of guanylate cyclase (GC) but the Km of the PDE, as measured in a homogenate of bleached retina, is increased (Doshi et al., 1985).

About 90% of the cyclic GMP present in normal photoreceptors is bound, principally to non-catalytic binding sites on the PDE (Yamazaki et al., 1980; Pugh & Cobbs, 1986), although other sites exist (Fesenko & Krapivinsky, 1986; Shinozawa et al., 1987). Since the overall concentration of cyclic GMP is reduced by 40% in +/rd retinas, there must be a reduction in cyclic GMP binding, potentially to the PDE. In contrast, rhodopsin phosphorylation, and by implication, the C-terminal is reported as normal in +/rd retinas (Shuster & Farber, 1986).

Rod GC is progressively inhibited by increasing concentrations of calcium up to a level of 10−5 M (Pepe et al., 1986). Thus if a retina is superfused with a medium containing the calcium chelator, EGTA, the concentration of intracellular

Abbreviations used: PDE, rod specific cyclic GMP phosphodiesterase; GC, guanylate cyclase; PHI, photoreceptor response waveform.

Fig. 1. Effect of 3.0 mM-EGTA on PHI amplitude and retinal cyclic GMP in C57 mice, heterozygous for the rd gene

Glutamate-isolated, trans-retinal responses to light flashes (4 ms duration) were obtained on a Medelec Mainframe system. Dark-adapted mouse retinas were incubated in normal Earle’s medium until the response stabilized. The medium was then changed to calcium-free Earle’s medium, containing 3.0 mM-EGTA and photoreponses elicited every 30 s over the following 30 min period. Cyclic GMP was measured by radioimmunoassay as described by Doshi et al. (1985). Each point represents the mean of at least four separate estimations ± S.E.M. (a) Retinal cyclic GMP; (b) PHI amplitude; □, retinas from +/+ mice; +, retinas from +/rd mice.

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cyclic GMP in the photoreceptors transiently increases (Fig. 1a). The rise is due to GC activation and the subsequent decline to PDE activity. We have found that the rise in cyclic GMP after EGTA is significantly delayed in intact +/rd retinas (Fig. 1a). The kinetics of GC and its response to calcium withdrawal, as assessed in a retinal homogenate in vitro, are normal (Doshi et al., 1985). Therefore, it is possible that an increase in endogenous calcium is causing the delay. An increase in the $K_m$ of PDE will lead to an increase in free cyclic GMP and this, in turn, would be expected to lead to an increase in the intracellular concentration of calcium (Pugh & Cobbs, 1986).

Present evidence suggests that the photoreceptor membrane current is principally determined by the concentration of free cyclic GMP, and that photoic responses are due to a reduction in its concentration through binding (Fesenko & Krapivinsky, 1986) and hydrolysis (Pugh & Cobbs, 1986). Assuming equilibrium between free and bound cyclic GMP, it should be possible to correlate the total retinal content (95% of which is present in photoreceptor cells; Orr et al., 1976) with the photoreceptor response waveform (PIL). However, there is no correlation in the +/rd retinas exposed to EGTA (Fig. 1). For, whereas the peak in PIL corresponds with the normal retina and occurs within 2.0 min, the cyclic GMP decay is delayed to 4.0 min. Therefore, factors other than cyclic GMP appear to be influencing the +/rd response. The plasma membrane of the normal photoreceptor also contains pores responsive to calcium and magnesium (which may or may not be those affected by cyclic GMP), and there are complex interactions between the valent cations and cyclic GMP (Pugh & Cobbs, 1986; Stern et al., 1987). It is possible that the effects, partially expressed in +/rd retinas, is perturbing the interaction sufficiently for separate processes to be discerned. The model may, therefore, be informative as regards the processes involved in normal phototransduction and adaptation in rod photoreceptor cells.

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Effect of dexamethasone on pyruvate carboxylation and decarboxylation in isolated hepatocytes from starved rats

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Treatment of animals with dexamethasone is known to affect pyruvate carboxylation and decarboxylation in subsequently isolated mitochondria and these effects have been implicated in the enhanced rate of gluconeogenesis following steroid administration (Adam & Haynes, 1969; Watk & Haynes, 1977; Martin et al., 1984; Allan & Titheradge, 1984). The aim of this work was to investigate whether mitochondrial pyruvate carboxylation and decarboxylation are enhanced by steroid treatment in the intact cell and to correlate these changes with alterations in gluconeogenesis.

Male Sprague-Dawley rats, starved for 24 h, were injected with dexamethasone acetate (15 $\mu$mol/l00 g) or 0.9% (w/v) NaCl, 90 min before the preparation of the hepatocytes. Dexamethasone sodium phosphate (1 $\mu$m) was included in all media in contact with the steroid-treated liver throughout the experiment. The experimental protocol was essentially that used by Agius & Alberti (1985). The cells were incubated in Krebs-Ringer buffer containing 20 mm-

Abbreviation used: PEPC, phosphoenolpyruvate carboxy-

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Hepes, pH 7.4, under an atmosphere of 100% $O_2$ to minimize loss of Na$^{14}$CO$_2$ from the incubation medium. 1 mm-$^{14}$Cpyruvate (labelled in the 1 or 3 position) was included as the substrate. After 15 min, the cells were deproteinized and the $^{14}$CO$_2$ collected by trapping in hyamine. The flux through pyruvate dehydrogenase was calculated by subtraction of the $^{14}$CO$_2$ released from the citric acid cycle (estimated from $^{14}$CO$_2$ released from $[3-^{14}$C]pyruvate) plus that released by malic enzyme and phosphoenolpyruvate carboxykinase (PEPC), from the total $^{14}$CO$_2$ output from $[1-^{14}$C]pyruvate (Claus & Pilis, 1977; Agius & Alberti, 1985). The activity of malic enzyme and PEPC was calculated from the rate of glucose synthesis from $[1-^{14}$C]pyruvate after allowing for recycling of phosphoenolpyruvate into pyruvate plus lactate via pyruvate kinase. The latter was measured by the incorporation of Na$^{14}$CO$_2$ into glucose, pyruvate and lactate (Rognstad & Katz, 1977). Pyruvate carboxylase flux was determined by the subtraction of the pyruvate dehydrogenase flux from total pyruvate metabolized.

The results of dexamethasone treatment are shown in Table 1. The effect of the steroid is compared with that of vasopressin, a peptide known to increase pyruvate carboxylation and pyruvate dehydrogenase activity in isolated mitochondria from fed animals (Hems et al., 1978; Allan et al., 1983; Oviasu & Whittton, 1984) and $^{14}$CO$_2$ production from $[1-^{14}$C]pyruvate in perfused liver (Sies et al., 1983). In agree-