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Direct Correlation Between the R2 Component of the Early Receptor Potential and the Formation of Metarhodopsin II in the Excised Bovine Retina

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Abstract. The formation of Metarhodopsin II, measured spectroscopically in the excised bovine retina at 37° C, can be described by the sum of two exponentials. The R2 response of a simultaneously recorded early receptor potential can be matched by the difference of two weighted exponentials, whose time constants are identical to those obtained for the spectroscopic signal.

Key words: Flash-spectroscopy – Early receptor potential – Simultaneous recording – Bovine retina.

Since the discovery of the early receptor potential (ERP) in 1964, (Brown and Murakami 1964; Cone 1964) it has often been tried to find a relation between this fast photovoltage signal and spectroscopically observable rhodopsin intermediates. In the excised eye of albino rats, the R2 response of the ERP at 37° C was found to occur in the same time range as an absorbance change observed at 404 nm (Cone 1969) and to have a half-time and temperature dependence closely matched to the formation of Metarhodopsin II (M II), measured at 380 nm in the excised retina of the same animal (Cone and Cobbs III 1969). A detailed analysis of the formation of M II revealed that a possible description of its time course is the sum of two exponentials (see Stewart et al. 1977). The molecular nature of the probably two components is still uncertain.

We report here that in excised bovine retinae, kept under physiological conditions, i.e., 37° C, and superfusion with oxygenated physiological saline, the formation of M II as well as the R2 response is described by the same set of two time constants, a possibility already proposed by Rüppel (1979).

Using a method described elsewhere (Spalink 1979), selected areas of bovine retinae were prepared from dark adapted animals. The samples showed a stable electrophysiological response for up to seven hours when they were kept in a superfusion cell according to Sickel (1965). The cell allowed simultaneous recordings of both absorbance and transretinal voltage changes. At 37° C, ERPs as well as late receptor potentials (a-wave) were measured following a single laser flash (495 nm, 6 ns), which bleached about 8% of the rhodopsin. The formation of M II was traced at 378 nm and 505 nm, which are isosbestic points between the precursor of M II and rhodopsin

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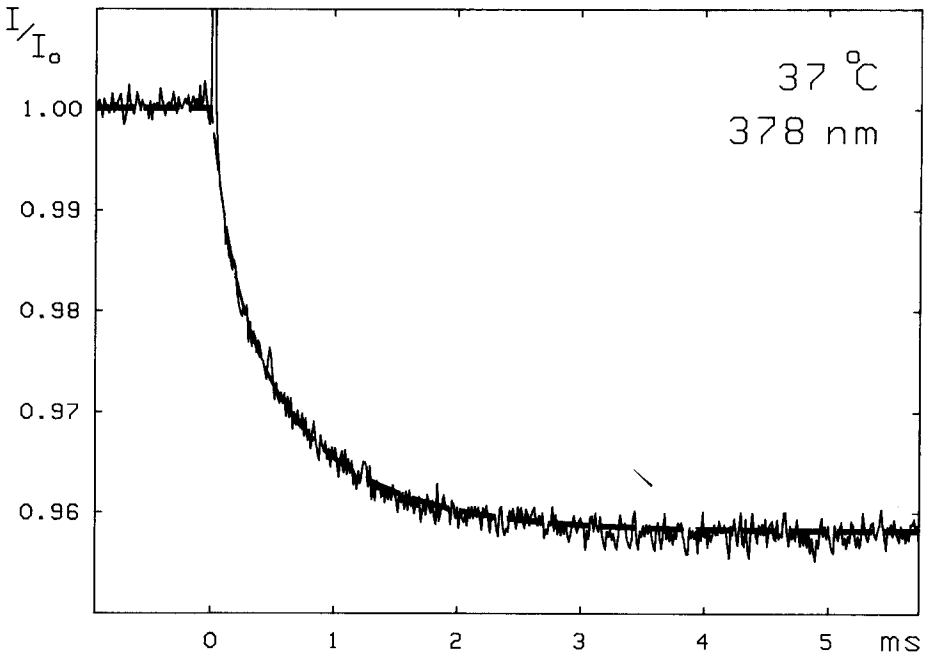

Fig. 1. Formation of M II in an excised bovine retina: transmission change vs. time, measured at 378 nm and 37° C. Single flash experiment, original data (solid line), about 8% rhodopsin bleached, laser flash (495 nm, 6 ns) delivered at t = 0, initial peak due to scattered light of the flash. Dashed line shows computed approximation with a sum of two exponentials (time constants $k_1 = 1250\ s^{-1}$, $k_2 = 5000\ s^{-1}$), weight turned out to be 30/70 (fast/slow). Bandwidth DC – 20 kHz

(Rafferty 1979). At these wavelengths the formation of M II can be measured without interference of other intermediates. Care was taken to avoid transmission changes due to light scattering (Spalink 1979).

For low temperature the formation of M II can be described essentially by one exponential, whose time constant, k_1, is the same at 378 nm and 505 nm. With increasing temperature, the 378 nm signal shows an increasing contribution of an additional component, whose time constant, k_2, is about four times larger than k_1. At 505 nm, no such fast component can be found. For the amplitude of the R2 response a temperature dependence similar to that of the faster component was found by Pak and Ebrey (1966) and Arden and Ikeda (1968). Our analysis of the rise or decay of the R2 response at 37° C shows no strict first order kinetics. The rise seems to have about the same time constant as the fast M II component and the decay might follow the slower one. The typical shape of a R2 response is obtained, when the faster M II component is subtracted from the weighted slower one, with both functions starting at zero time. This operation together with an original ERP is shown in Fig. 2. The ERP was simultaneously recorded with the M II signal of Fig. 1.

Because of the small amplitude of the ERP the measurements at lower temperatures could not yet be evaluated.

Since two time constants for the M II formation are found not only for membrane-bound rhodopsin, but also in various detergent solutions (Stewart et al. 1977), it seems

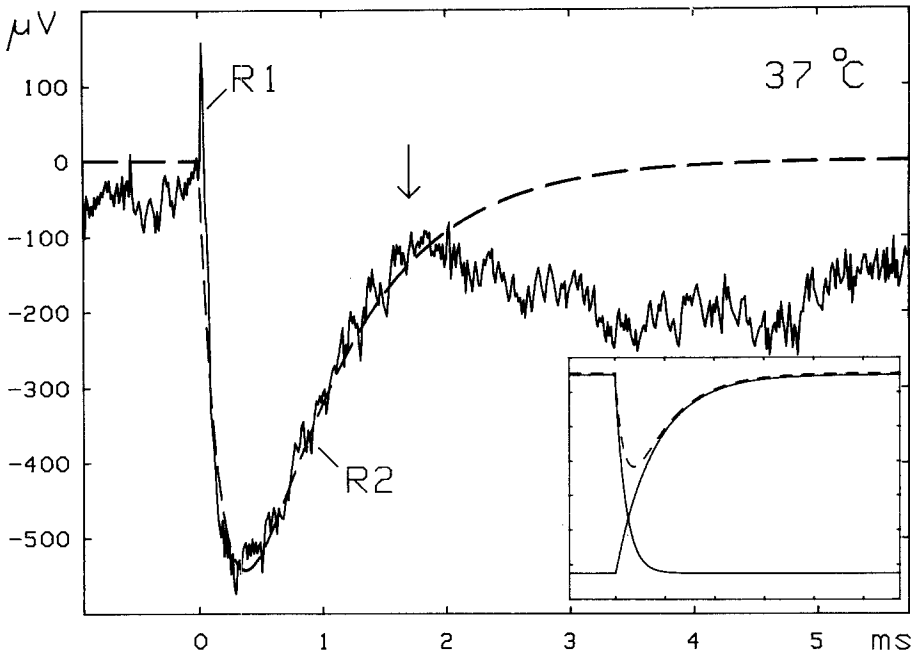

Fig. 2. Transretinal voltage change, recorded from the same retinal area simultaneously with the transmission change of Fig. 1; original data (solid line). Dashed line shows computed approximation with a difference of two exponentials (time constants k_1 and k_2, weighted 1 : 1), for further details see Fig. 1. The arrow indicates the transition between the R2 response and the a-wave, which is weak due to adaptation to the measuring light (378 nm). The insert shows a graph of the two separate exponentials and their computed difference. Bandwidth 0.1 – 20 kHz

reasonable to assume that this effect directly reflects changes in the rhodopsin molecule. It has been suggested (Cone 1969; Hodgkin and O'Bryan 1977), that the ERP is caused by changes in the dipole moment of rhodopsin. Thus it seems to be conceivable to us that the two components may represent two states of the visual pigment, which differ in magnitude and/or orientation of their dipole moment. However, the correspondence may be a mere coincidence. Hodgkin and O'Bryan (1977) showed that the ERP can be described by a capacitive transmembrane current, whose time course depends strongly on the time constant of the plasma membrane.

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