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Supplemental Data

ATP Consumption

by Mammalian Rod Photoreceptors

in Darkness and in Light

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Supplemental Experimental Procedures

Calculation of ATP Consumption Resulting from Na^+ Influx Through cGMP-gated Channels of Outer Segment

The total flux as current into the rod outer segment is the sum of the current passing through the channels and the current carried by the exchanger [1],

$$i_T = i_{Ch} + i_{Ex} \quad (1)$$

Accordingly, the part of this current carried by Na^+ is the sum of the current carried by Na^+ though the channels plus the current carried by Na^+ through the exchanger,

$$i_{Na(T)} = i_{Na(Ch)} + i_{Na(Ex)} \quad (2)$$

We calculate the Na^+ current carried through the channels first. It is approximately equal to the total current minus the exchange current minus the fraction of the inward current through the channels carried by Ca^{2+},

$$i_{Na(Ch)} = i_T - i_{Ex} - i_{Ca(Ch)} \quad (3)$$

Since the value of the Ca^{2+} current entering the rod at steady state is equal to twice the exchange current [2], we have

$$i_{Na(Ch)} = i_T - 3i_{Ex} \quad (4)$$

Since 4 sodium ions are transported inward by the exchange transporter for each net charge transported inward [3], the value of the Na^+ flux as a current transported inward is four times the value of the exchange current,

$$i_{Na(Ex)} = 4i_{Ex} \quad (5)$$

The total current carried by Na^+ is therefore given by

$$i_{Na(T)} = i_T - 3i_{Ex} + 4i_{Ex} = i_T + i_{Ex} \quad (6)$$

To use this equation for a mouse rod, we must first estimate the value of the exchange current as a fraction of the total current. We did this (M. L. Woodruff and G. L. Fain, unpublished) by fitting the exchange current from the averaged mouse rod response to saturating illumination to an equation of the form

$$i_T = i_{Ch} + i_{Ex}[1 - \exp(-t/\tau)] \quad (7)$$

were i_T is the total photocurrent, i_{Ch} the current due to the cyclic nucleotide-gated channels, i_{Ex} the current due to Na^+/Ca^{2+}-K^+ exchange, and τ the time constant of Ca^{2+} removal from the outer segment. The best fitting values we obtained were as follows: $i_{Ch} = 0.86$, $i_{Ex} = 0.14$, and $\tau = 50.2$ ms. On the assumption that the channels close instantaneously, i_{Ex} can be extrapolated back to t = 0 and can be estimated to represent approximately 14% of i_T. Since however the channels even in the brightest light take several milliseconds to close [4], this is likely to be an overestimate. If the current is extrapolated back to the time when half of the total current has been suppressed [as in 5, 6], the term $i_{Ex}[1 + \exp(-t/\tau)]$ is 0.074, indicating that the exchange current may represent only about 7% of the total current. This value is similar to previous estimates from salamander [5] and guinea-pig rods [1] and is also close to the previous estimate

of 6% of total current for mouse rods of Makino et al. [7]. The total current carried by Na^+ from Eqn. (6) would therefore be equal to $1.07i_T$.

This value is subject to a number of uncertainties. It ignores, for example, the current through the cyclic nucleotide-gated channels carried outward by K^+. We can estimate this current in the dark as a fraction of the Na^+ current from the GHK current equations (see Eqns. (11) below) by taking the resting potential of the rod as -37.3 mV, which is the mean value we have recorded from mouse rods and is similar to values previously recorded in primate rods [8] and amphibians [see for example 9]. We have taken the relative permeability of the channels of K^+ to Na^+ as 0.7 [see 10], the values for the external ion concentrations from the composition of the bathing medium as $[Na]_o$ = 152 mM and K_o = 2.5 mM, and internal ion concentrations from values for other neurons and assumed to be $[Na]_i$ = 30 mM and $[K]_i$ = 100 mM. This gives a ratio of the K^+ to Na^+ current of 0.11, with the K^+ current opposite in sign to the Na^+ current. The K^+ current would therefore cause us to underestimate the value of the Na^+ flux from Eqn. (6) by about 10% in darkness, though the size of this error would decrease in the presence of a steady background light as the rod hyperpolarized away from the Na^+ reversal potential and closer to that for K^+.

Our value of Na^+ influx also ignores the inward current carried by Mg^{2+}. The fraction of the current carried by Mg^{2+} is unknown for a mouse rod but has been estimated as about one-third the value of the Ca^{2+} current in toad rods [5]. This Mg^{2+} current may therefore represent about 2-3% of the total current and would lead us to overestimate the part of the current carried by Na^+ by approximately this percentage. Finally, in Fig. 1A we have estimated the ratio of the exchanger current to the ionic current only for the rod in darkness, and this ratio would change in

background light as a result of changes in Na^+ and Ca^{2+} driving forces and the voltage dependence of Na^+/Ca^{2+}-K^+ transport. These effects are likely, however, to be small.

We have estimated the value of the normal resting dark current of a mouse rod as 25 pA. Taking $i_{Na(T)}$ as approximately $1.1i_T$, 25 pA is equivalent to the influx into the rod of about $(1.1)(2.5 \times 10^{-11}$ coul $s^{-1})(1.6022 \times 10^{-19}$ coul per $Na^+)^{-1}$ or 1.72×10^8 Na^+ ions per second. Since 3 Na^+ are transported outward for every ATP utilized [11], the rod in darkness must consume approximately 5.7×10^7 ATP per second in darkness to support the influx of Na^+ ions. This can be converted into moles per liter from the dimensions of a mouse rod outer segment, which we took from the results of electron tomography of rapidly frozen isolated outer segments of Nickell et al. [12]. They report an outer segment length of 23.8 μm and a diameter of 1.32 μm. Their measurements show that the cytoplasm occupies only 31% of the space inside the outer segment, with the rest taken up by the disk membranes and intradiskal space. They therefore estimate the volume of the cytoplasm as about 1×10^{-14} liters. The influx of Na^+ amounts then to about 30 mM Na^+ per second, an enormous load of Na^+ influx into the cell, and the rate of ATP turnover is equivalent to about 10 mM ATP per second.

Calculation of Light Dependence of ATP Consumption by Guanylyl Cyclase

The maximal rate of the cyclase occurs when the outer segment Ca^{2+} is minimal [see for example 13] and is approximately 1 nmole cGMP per retina per min at 30 ^oC [14] and therefore approximately 2 nmoles cGMP per retina per min at the body temperature of a mouse (38-39 ^oC), or 2×10^{13} cGMP s^{-1}. Since there are 6.5×10^6 rods per mouse retina [15], this is 3×10^6 cGMP synthesized per rod per second. The minimum rate of synthesis is 0.1 of this value [16], or 3 x

10^5 cGMP s^{-1}. Since 2 ATPs are required to regenerate the GTP from which cGMP is synthesized by guanylyl cyclase, the expenditure of ATP for cGMP synthesis would range from about 6 x 10^5 ATP s^{-1} in darkness to 6 x 10^6 ATP s^{-1} in bright light.

We have estimated the dependence of the cyclase on light intensity in the following way. We assumed that the free-Ca^{2+} concentration in the rod outer segment is proportional to the outer segment current, as seems very likely from experiments on both salamander [17] and mouse [13]. The free-Ca^{2+} concentration was then estimated from the equation

$$[Ca]_{free} = ([Ca]_{\max} - [Ca]_{\min})(1 - i_{step}) + [Ca]_{\min} \quad (8)$$

where $[Ca]_{free}$ is the free-Ca^{2+} concentration in the outer segment, $[Ca]_{max}$ and $[Ca]_{min}$ are the maximum and minimum values of the free-Ca^{2+} concentration, and i_{step} is the mean normalized photocurrent response of a mouse rod to steps of light from Woodruff et al. [18]. The values of $[Ca]_{max}$ and $[Ca]_{min}$ were taken from Woodruff et al. [13] as 240 nM in darkness and 46 nM in saturating light.

The cyclase activity could then be calculated at each light intensity from the free-Ca^{2+} concentration and the equation

$$A = \frac{(A_{\max} - A_{\min})}{1 + ([Ca]_{free} / K_m)^n} + A_{\min} \quad (9)$$

as given in Olshevskaya et al. [16], where A is the activity of the enzyme, A_{max} and A_{min} the maximum and minimum activities (3 x 10^6 cGMP s^{-1} and 3 x 10^5 cGMP s^{-1}), $[Ca]_{free}$ the free-Ca^{2+} concentration taken from Eqn. (8), and K_m and n constants equal to 70 nM and 2.2 [16]. The enzyme activities were then multiplied by 2 in order to calculate the ATP expenditure required to synthesize cGMP.

Calculation of ATP Required for Extrusion of Na^+ Entering Inner Segment Through i_h

We estimated the Na^+ flux entering the rod through i_h in the following way. We first calculated i_h as a function of voltage from Eqn. (1) of Demontis et al. [19], which provides an empirical description of voltage-gated currents from guinea-pig rods. The component of their equation due to i_h is as follows:

$$i_h = \frac{G_h(V - E_h)}{1 + \exp(\frac{V - V_{1/2h}}{S_h})} \tag{10}$$

where G_h is the maximum conductance (1 nS), $V_{1/2h}$ the voltage for half activation (-75 mV), and S_h a constant equal to 8.2 mV. The numbers in parentheses are the mean values of the parameters averaged from 30 cells [19]. E_h, the reversal potential for the current, was kept fixed at -31 mV as in Demontis et al.[19].

Once we calculated the total current due to i_h, we next used the ion permeability ratio of the conductance to calculate separately the Na^+ and K^+ current through the channels as a function of voltage from the GHK current equations,

$$I_{h(Na)} = P_{Na} V_m \frac{F^2}{RT} \frac{[Na^+]_o - [Na^+]_i e^{FVm/RT}}{1 - e^{FVm/RT}} \quad and \quad I_{h(K)} = P_K V_m \frac{F^2}{RT} \frac{[K^+]_o - [K^+]_i e^{FVm/RT}}{1 - e^{FVm/RT}} \tag{11}$$

We have taken $[Na^+]_o$ = 140 mM and $[K^+]_o$ = 3.6 mM from the composition of the external solution in Demontis et al. [19] and assumed that $[Na^+]_i$ = 30 mM and $[K^+]_i$ = 100 mM. This leaves the permeabilities as the only unknowns. Since however the ratio of permeabilities P_{Na}/P_K was determined by Demontis et al. [19] as approximately 0.3, in agreement with previous

values from salamander rods [20, 21], we can use this ratio together with the value of i_h from Eqn. (10), which is equal to the sum of $I_{h(Na)}$ and $I_{h(K)}$, to calculate the values of the sodium and potassium currents individually and to estimate the influx of Na^+ into the inner segment through the i_h channels as a function of voltage. This was then combined with data in Fig. 2B of our paper to calculate the value of the influx as a function of steady illumination, which was divided by 3 to give the rate of ATP consumption required by the Na^+/K^+ ATPase for removing the Na^+ entering through the i_h channels.

Calculation of ATP Consumption from Ca^{2+} Pumping in Inner Segment

To calculate the size of the Ca^{2+} current and the ATP required to remove entering Ca^{2+} as a function of steady light intensity, we began with the voltage-clamp measurements of Morgans et al. [22] from mouse rods. They give the value of the Ca current from the chord conductance equation as

$$I_{Ca} = G_{Ca}(V - E_r) \tag{12}$$

where I_{Ca} is the calcium current, G_{Ca} the calcium conductance, V the voltage, and E_r the reversal potential of the calcium current (approximately +1 mV). They showed that the voltage dependence of G_{Ca} could be adequately described by a Boltzmann equation of the form

$$G_{Ca} = \frac{G_{\max}}{1 + \exp[\frac{-(V - V_{0.5})}{k_{act}}]} \tag{13}$$

with mean values of G_{max}, $V_{0.5}$, and k_{act} equal to 1.2 nS, -36.5 mV, and 6.1 mV. This gives a value for the inward Ca current at a dark resting membrane potential of -37.3 mV equal to about 21.5 pA. This value may underestimate the Ca^{2+} influx, since the low value of the reversal

potential of about -1 mV in the experiments of Morgans et al. [22] would suggest that the channels also had a significant permeability to the Cs^+ included in the internal solution of their patch pipettes, an ion known to be able to permeate Ca^{2+} channels [see for example 23]. An outward Cs^+ current would result in a lower net current and an underestimate of the influx of Ca^{2+} as well as an underestimate of the light-dark difference in ATP utilization. Since however neither the relative permeability of the channel to Cs^+ nor the free-Ca^{2+} concentration of the synaptic terminal is known, we made no attempt to correct for the Ca^{2+} efflux due to Cs^+ permeation.

The Ca^{2+} currents recorded by Morgans et al. [22] were in response to a relatively rapid voltage ramp (100 mV s^{-1}), but steady changes in membrane voltage are likely to produce some inactivation of the Ca^{2+} current entering the rod synaptic terminal. Measurements in salamander [24] indicate that a combination of Ca^{2+}-dependent inactivation and depletion of Ca^{2+} in the synaptic cleft is likely to reduce the Ca^{2+} current by about 50% at voltages near the rod resting membrane potential (47% at -40 mV). The inactivation increases with positive voltage and the amplitude of the Ca^{2+} current and therefore decreases as the rod hyperpolarizes during illumination. In mammals, the rod channel is apparently a member of the α_F calcium channel subtype [22, 25-27], which shows little Ca^{2+}-dependent inactivation but a pronounced voltage-dependent inactivation with slow kinetics [26, 28]. The inactivation of expressed α_F channels also increases with increasing depolarization and would be expected to decline during hyperpolarization. Quantitative measurements of the rate and voltage-dependence of mammalian rod calcium currents have not yet been made under physiological conditions. We therefore followed Rabl and Thoreson [24] and take the steady-state current entering the rod in darkness as

approximately half that measured by Morgans et al. [22], or about 11 pA. This gives an inward flux of Ca^{2+} in darkness of (1.1 x 10^{-11} pA)(1.6022 x 10^{-19} coul per electronic charge), divided by 2 (for the valence of the Ca^{2+} ion), equal to 3.4 x 10^{7} Ca^{2+} s^{-1}. Since most of the Ca^{2+} entering the inner segment is apparently extruded by a Ca^{2+} ATPase [29-31], which expends one ATP per Ca^{2+} ion pumped [32], the ATP required to maintain the inward Ca^{2+} current in darkness is also about 3.4 x 10^{7} ATP s^{-1}.

During steady illumination, the rod hyperpolarizes. We again used Eqns. (12) and (13) to calculate the current as a function of voltage. It makes relatively little practical difference in these calculations what assumption we make about the voltage dependence of inactivation, since as the rod hyperpolarizes, the Ca current declines rather precipitously, and the effect of inactivation becomes rather small on the total influx of Ca^{2+}. It is nevertheless likely that inactivation decreases with hyperpolarization, and we therefore made the arbitrary assumption that the extent of inactivation is proportional to the amplitude of the Ca^{2+} current. A relation of this sort would be more likely to describe inactivation in a salamander rod, where it is apparently Ca^{2+} dependent, than in a mammalian rod, where it appears to be primarily voltage dependent. In either case, however, the extent of inactivation would decline with hyperpolarization, and the difference between a dependence on current and a dependence on voltage is likely to be small.

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