Reciprocal Modulation of Calcium Dynamics at Rod and Cone Photoreceptor Synapses by Nitric Oxide

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1Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106; Departments of 2Anatomy and Neurobiology, 3Ophthalmology and Visual Science, and 5Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia B3H 4H7; and 4Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta T2N 4N1, Canada

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Kourennyi, Dmitri E., Xiao-dong Liu, Jason Hart, Farid Mahmud, William H. Baldrige, and Steven Barnes. Reciprocal modulation of calcium dynamics at rod and cone photoreceptor synapses by nitric oxide. J Neurophysiol 92: 477–483, 2004. First published February 25, 2004; 10.1152/jn.00606.2003. The abundance of nitric oxide (NO) synthesizing enzymes identified in the vertebrate retina highlight the importance of NO as a signaling molecule in this tissue. Here we describe opposing actions of NO on the rod and cone photoreceptor synapse. Depolarization-induced increases of calcium concentration in rods and cones were enhanced and inhibited, respectively, by the NO donor S-nitroso cysteine. NO suppressed calcium current in cones by decreasing the maximum conductance, whereas NO facilitated rod Ca channel activation. NO also activated a nonselective voltage-independent conductance in both rods and cones. Suppression of NO production in the intact retina with Nω-nitro-L-arginine favored cone over rod driven postsynaptic signals, as would be expected if NO enhanced rod and suppressed cone synaptic activity. These findings may imply involvement of NO in regulating the strength of rod and cone pathways in the retina during different states of adaptation.

INTRODUCTION

Nitric oxide (NO) has emerged as an important signaling molecule in the vertebrate retina. Immunocytochemical, immunohistochemical, and histochemical (NADPH-diaphorase) methods have revealed that the isoforms of synthesizing enzymes for NO, NO synthase (NOS), are abundant and found in almost all layers of the retina (Haverkamp et al. 1999; Kelly and Barnes 1997; Shin et al. 2000). NO has also been shown to affect a large and increasing number of different types of ion channels in retinal neurons (for review, see Kelly and Barnes 1997). These include voltage-gated ion channels, ionotropic receptors, and gap junction channels. A significant locus of neuronal NOS (nNOS) in the retina is within and around photoreceptors (Kelly and Barnes 1997; Kurenni et al. 1995; Kurenni et al. 1994; Liepe et al. 1994; Osborne et al. 1993; Weiler and Kewitz 1993), suggesting a role of NO in outer retinal function.

Synaptic transmission from photoreceptors to the second order, horizontal and bipolar, cells is triggered by voltage-dependent calcium entry through L-type Ca channels in rod and cone synaptic terminals (Barnes and Hille 1989; Barnes et al. 1993; Copenhagen and Jahr 1989; Lasater and Witkovsky 1991; Marić and Korenbrot 1988). Calcium entry in turn induces calcium release from intracellular stores via processes that differ between rods and cones (Krizaj et al. 2003). Synaptic cGMP-gated channels, the same type of ion channels that normally are responsible for generating the light-modulated current in photoreceptor outer segments (Fesenko et al. 1985; Haynes et al. 1986; Yau and Baylor 1989), may participate in some aspects of synaptic transmission from cones (Rieke and Schwartz 1994). We showed previously that the NO donor S-nitroso cysteine (SNC) caused a facilitation of Ca channels in salamander rod photoreceptors (Kurenni et al. 1994), an effect due mainly to a shift of the activation curve toward negative potentials.

In this study, we compared the effects of NO on Ca channels in rod and cone photoreceptors using calcium imaging, patch-clamp, and intracellular recording methods. A computer model of the rod and cone responses was constructed to test the potential of the modulatory effects of NO on postsynaptic signals. Our findings show that NO exerts differential modulatory effects on the rod and cone signaling pathways in the vertebrate retina, which suggests that NO could play roles in light-dark adaptation and signal processing at this level of the visual system.

METHODS

Isolated cell preparation

In accordance with the CWRU IACUC, retinas were removed from larval tiger salamanders (Ambystoma tigrinum, Kons Scientific, Germantown, WI). Cells, isolated by trituration following treatment with papain (Sigma, St. Louis, MO), were constantly perfused with a saline solution composed of the following (in mM): 90 NaCl, 2.5 KCl, 3 CaCl2, 10 HEPES, and 10 t-glucose (pH 7.6, NaOH; room temperature). SNC was prepared as described (Kurenni et al. 1994). All experiments were performed at room temperature (21–24°C).

Calcium imaging

Cells were loaded with fluo-3 by incubation in 5–10 µM fluo-3 AM ester and 0.01% Pluronic F-127 (Molecular Probes, Eugene, OR). To stimulate calcium entry, the superfusate was switched from 1 to 8–10 mM K+ solution, which produces mild depolarization (approximately –40 mV) and a small (5–10%) steady-state increase in fluorescence. Solutions with different [K+] were based on the Ringer solution with adjustments in [Na+] to maintain osmolarity. Measurement of fluo-

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rescence was made from the entire inner segment of both rods and cones, because synaptic terminals were not visible. Data are presented as means ± SE and compared using Student’s t-test.

**Patch-clamp recording**

Ca channel currents were recorded in solution composed of the following (in mM): 70 NaCl, 2.5 KCl, 5 CsCl, 3 CaCl₂, 10 TEA·Cl, 20 HEPES, and 10 D-glucose (pH 7.6, NaOH). Barium (5 mM) was also used in some experiments. Ruptured and perforated patch configurations were used. The patch pipette solution contained (in mM) 100 CsCl, 3 MgCl₂, 1 EGTA, 1 ATP·Na₂, and 10 HEPES (pH 7.2, CsOH). For perforated patch-clamp recordings, 150 µg/ml nystatin was added to the pipette solution. Currents were recorded in response to a series of voltage steps between −80 and +40 mV in 5-mV increments. The amplitude of the current at the end of each step was plotted against the corresponding voltage (corrected for the series resistance error) to yield a current-voltage relationship. Leak subtraction of the current voltage relations was performed by subtracting a line fit to the relation between the voltages of −80 and approximately −45 mV. In Fig. 2, both control and test I-Vs had the same leak subtracted to show that the leak conductance increased during SNC application. For analysis of modulation of Ca channel kinetic parameters, activation curves were constructed by dividing the leak-subtracted current-voltage data by a line representing the maximum conductance (linear fit to the data in the +10 to +20 mV range), and were fitted by the Boltzmann function f = 1/(1 + exp(−(V − V_{1/2}))/S)), where V_{1/2} is the half-activation potential and S is the slope factor. Nonselective NO-activated conductance measurements were made in a bath solution containing (in mM) 62 NaCl, 2.5 KCl, 3 CsCl, 0.2 CaCl₂, 10 CsCl, 30 TEA·Cl, 10 HEPES, and 8 D-glucose (pH 7.6, NaOH).

**Intracellular microelectrode recording in intact retina**

Retinal eyecups were prepared by dissecting away the cornea, lens, and iris, and secured in a holder with suction. Continuous superfusion was established at a rate of 1 ml/min with a solution containing (in mM) 95 NaCl, 2.5 KCl, 3 CaCl₂, 1.5 MgCl₂, 30 NaHCO₃, and 3 D-glucose, and bubbled with 95% O₂−5% CO₂ (pH 7.6). Following 1 h of dark-adaptation, 150-200 MΩ microelectrodes filled with 3 M potassium acetate plus 200 mM KCl recorded horizontal cell (HC) light responses. Voltage responses were recorded with a WPI M707 amplifier and digitized with Indec Systems hardware and Basic-Fastlab software. Light flashes were of 500-ms duration with unattenuated intensity of 2 × 10⁻⁷ µW/µm². Recordings were all obtained from individual HCIs that were incubated in control or drug solutions for 1 h prior to recording. A⁵-nitro-l-arginine (NNA) was obtained from Calbiochem (San Diego, CA).

**Computer simulation of the light response**

The rod photoreceptor model was created using NEURON software with a single-compartment design with leak conductance of 0.01 nS and capacitance of 16 pF. Conductances associated with voltage-gated ion channels were 1) for rods, Ca channels (Kourenny and Barnes 2000; Kurenny et al. 1994), noninactivating potassium Kc channels (Kurenn and Barnes 1994; Kourenny and Barnes 1997), nonselective cation h channels (Malcolm et al. 2003), and delayed rectifier-like K channels (Kamiyama et al. 1996) were included, and 2) for cones, Ca channels (this work), h channels (Barnes and Hille 1989), delayed rectifier-like K channels, and calcium activated K and Cl channels (Kamiyama et al. 1996) were included.

A two-state kinetic scheme was used for Ca channels in rods and cones, and K+ and h channels in rods. In this scheme, the rate constants for activation (α) and deactivation (β) of the channel of type i were described by the equations: 

\[ \alpha_i = \alpha_{0i} \exp\left(\frac{(V - V_{1/2,i})}{S_i}\right) \]

and

\[ \beta_i = \beta_{0i} \exp\left(-\frac{(V - V_{1/2,i})}{S_i}\right) \]

The activation (nᵢ) of the channels was numerically calculated from the equation

\[ n_i = \frac{G_{\text{max},i}}{G_{\text{max}}(V - E_i)} \]

where

\[ G_{\text{max},i} = \frac{G_{\text{max},h}}{2} \]

and

\[ G_{\text{max}} = \frac{G_{\text{max},h} \cdot G_{\text{max},c}}{G_{\text{max},c} + 2 \cdot G_{\text{max},h}} \]

The density of Ca channel kinetic parameters, for Ca channels in rods and cones, was established at a rate of 1 ml/min with a solution containing (in mM): 70 NaCl, 2.5 KCl, 5 CsCl, 3 CaCl₂, 10 TEG, 30 HEPES, 20 HEPES, and 10 D-glucose (pH 7.6, NaOH). Barium (5 mM) was also used in some experiments. Ruptured and perforated patch configurations were used. The patch pipette solution contained (in mM) 100 CsCl, 3 MgCl₂, 1 EGTA, 1 ATP·Na₂, and 10 HEPES (pH 7.2, CsOH). For perforated patch-clamp recordings, 150 µg/ml nystatin was added to the pipette solution. Currents were recorded in response to a series of voltage steps between −80 and +40 mV in 5-mV increments. The amplitude of the current at the end of each step was plotted against the corresponding voltage (corrected for the series resistance error) to yield a current-voltage relationship. Leak subtraction of the current voltage relations was performed by subtracting a line fit to the relation between the voltages of −80 and approximately −45 mV. In Fig. 2, both control and test I-Vs had the same leak subtracted to show that the leak conductance increased during SNC application. For analysis of modulation of Ca channel kinetic parameters, activation curves were constructed by dividing the leak-subtracted current-voltage data by a line representing the maximum conductance (linear fit to the data in the +10 to +20 mV range), and were fitted by the Boltzmann function f = 1/(1 + exp[−(V − V_{1/2}))/S]), where V_{1/2} is the half-activation potential and S is the slope factor. Nonselective NO-activated conductance measurements were made in a bath solution containing (in mM) 62 NaCl, 2.5 KCl, 3 CsCl, 0.2 CaCl₂, 10 CsCl, 30 TEA·Cl, 10 HEPES, and 8 D-glucose (pH 7.6, NaOH).

The delayed rectifier-like Kv channel (Kamiyama et al. 1996) was implemented in rods and cones using standard Hodgkin-Huxley formalism. The Kv current was expressed as

\[ I_{Kv} = G_{\text{max},Kv} (m_{Kv})^{1/2} h_{Kv} (V - E_{Kv}) \]

where

\[ dm_{Kv}/dt = \alpha_{0Kv}(1 - m_{Kv}) - \beta_{0Kv} m_{Kv} \]

and

\[ dh_{Kv}/dt = \alpha_{0Kv}(1 - h_{Kv}) - \beta_{0Kv} h_{Kv} \]

The constant values were

\[ \alpha_{0Kv} = 5 \times 10^{-5}/(1 + \exp[−(V − 100)/42]), \beta_{0Kv} = 9 \times 10^{-5}/(20 − V)/40, \alpha_{0Kx} = 0.15\exp[−V/22], \beta_{0Kx} = 0.4125[1 + \exp(10 − V)/71], \]

and

\[ G_{\text{max},Kv} = 2 \text{ and } E_{Kv} = −80 \text{ mV} \]

The calcium-activated K current (Kamiyama et al. 1996) was expressed with some modifications as

\[ I_{Ca,K} = G_{\text{max},Ca}(m_{Ca})^{1/2} h_{Ca} (V - E_{Ca}) \]

where

\[ dm_{Ca}/dt = \alpha_{0Ca}(1 - m_{Ca}) - \beta_{0Ca} m_{Ca} h_{Ca} \]

and

\[ dh_{Ca}/dt = \alpha_{0Ca}(1 - h_{Ca}) - \beta_{0Ca} h_{Ca} \]

The kinetic constants were

\[ \alpha_{Ca} = 10^{-2} \text{ and } E_{Ca} = −80 \text{ mV} \]

The calcium-activated Cl current (Kamiyama et al. 1996) was expressed with some modifications as

\[ I_{Ca,Cl} = G_{\text{max},CaCl}(m_{CaCl})^{1/2} h_{CaCl} (V - E_{Ca}) \]

where

\[ dm_{CaCl}/dt = \alpha_{0CaCl}(1 - m_{CaCl}) - \beta_{0CaCl} m_{CaCl} h_{CaCl} \]

and

\[ dh_{CaCl}/dt = \alpha_{0CaCl}(1 - h_{CaCl}) - \beta_{0CaCl} h_{CaCl} \]

The kinetic constants were

\[ \alpha_{CaCl} = 10^{-2} \text{ and } E_{CaCl} = −80 \text{ mV} \]

The effects of NO were modeled by changing V_{1/2} for rod I_{Ca} (Kurenn et al. 1994) to −24.3 mV and by reducing cone G_{\text{max},Ca} to 3.79 nS (this work).

**RESULTS**

**NO donor SNC inhibits calcium entry into cones and enhances it in rods**

Our goal was to investigate how SNC affected calcium-dependent fluorescence in rods and cones. We used low concentrations of KCl (8−10 mM) to slightly depolarize the cells to the level expected in the dark-adapted state (near −40 mV) and activate Ca channels. Since Ca channels in photoreceptors do not exhibit pronounced inactivation (Kourenny and Barnes 2000; Kurenny et al. 1994; Wilkinson and Barnes 1996), steady levels of increased fluorescence due to calcium entry were seen. Application of SNC reversibly suppressed the fluorescence signal in cones and enhanced it in rods (Fig. 1). On average, 1 mM SNC suppressed fluorescence by 5.6 ± 0.8% (n = 24) in cones, while 2 mM SNC increased the signal by 2.6 ± 0.3% in rods (n = 21).
Calcium imaging indicated that SNC suppressed Ca channels in cones, a modulation that stands in contrast to the facilitation seen in rods. Changes in calcium-dependent fluorescence in photoreceptors loaded with fluo-3 can be explained almost entirely by the activity of L-type voltage-gated Ca channels (Baldridge et al. 1998). In both rods and cones, calcium-induced calcium release (Krizaj et al. 2003), which could be directly sensitive to NO, as well as modulated extrusion and/or uptake via plasma membrane calcium ATP-ase and sarcoplasmic, endoplasmic reticulum calcium ATP-ase or other systems, could contribute to the modulated fluo-3 signal. Moreover, under some conditions, unclamped photoreceptors can produce regenerative depolarizations, such that NO regulation of channels other than the voltage-gated Ca channels themselves could lead to changes in these responses and hence in Ca\textsuperscript{2+} influx. In salamander rods, NO modulation of $I_{Ks}$ and $I_n$ was not detected (Kurenny et al. 1994). We patch clamped cone photoreceptors to directly measure ionic currents and compare the actions of SNC in photoreceptors.

**NO suppresses calcium channel activity in cones**

In our previous study (Kurenny et al. 1994), we showed that 2 mM SNC facilitated Ca channels in rods by shifting the activation curve to negative potentials by about 4 mV with an insignificant increase in the maximum conductance. Here we show that SNC inhibits currents through Ca channels in cones via a different mechanism. Figure 2 shows an example of a calcium current recording in a cone made with the perforated patch-clamp technique. Current amplitude was suppressed at all potentials where Ca channels are typically activated in cones, but at potentials negative to this range, leak...
conductance was increased. The Ca channel activation curve was slightly shifted (2 mV) to positive potentials in this cone.

SNC suppressed Ca channel currents in cones by decreasing the maximum conductance. Compared with the control conditions, maximum Ca channel ensemble conductance was reduced to 78 ± 3% (n = 5, P < 0.01) in 0.1–0.2 mM SNC and to 77 ± 6% (n = 17, P < 0.01) in 0.4–1 mM SNC. When the Boltzmann equation was fit to leak subtracted data, no statistically significant shift in I/V0.5 of cone Ca channels was observed: 1.8 ± 2.4 mV (n = 5, P > 0.4) in 0.1–0.2 mM SNC and 0.9 ± 1.1 mV (n = 17, P > 0.4) for 0.4–1 mM SNC. As was the case in rods, there was no significant change in the slope factor: 1.12 ± 0.05 (n = 5, P > 0.05) and 1.03 ± 0.04 (n = 17, P > 0.5) of the control value for 0.1–0.2 mM and 0.4–1 mM SNC, respectively.

Similar to the results in rods (Kurennyy et al. 1994), we found that SNC increased the slope of the current-voltage relationship at negative potentials (below −50 mV), where Ca channels are deactivated (Fig. 2). We studied properties of this conductance while other currents were blocked by cadmium, cesium, and TEA. Under these conditions, conductance was significantly increased from 1.89 ± 0.22 nS (n = 32) in control to 3.68 ± 0.24 nS (n = 16) in 2 mM SNC (P < 0.001). The SNC-induced conductance was voltage independent and its reversal potential was 0.8 ± 1.6 mV (n = 16). The membrane conductance recorded in SNC solutions that had been allowed 1–2 days to de-gas NO from the solution, 2.50 ± 0.44 nS (n = 4), did not differ significantly from the control (P > 0.3), indicating that NO must be present in the solution to activate this conductance.

We excluded the possibility that this conductance was due to activation of sodium dependent glutamate transporter (Eliasof and Werblin 1993) by substituting lithium for sodium (n = 4) and by using the transporter blocker α-threo-β-hydroxyaspartate (βHA; n = 4). In both cases, SNC activated a conductance that did not differ statistically from the conductance activated by SNC in control solution (P > 0.25). We also tested the hypothesis that the SNC-induced conductance was due to activation of cGMP-gated channels. As found previously with rods, diltiazem (20 μM), a blocker of cGMP-gated channels, reduced the SNC-induced conductance in cones. On average, the conductance decreased from 4.03 ± 0.47 (n = 4) to 2.27 ± 0.46 nS (n = 4). In another series of experiments we applied 8-bromo-cGMP, a membrane permeable analog of cGMP, to activate cGMP-gated channels. In the presence of 8-bromo-cGMP (1 mM), membrane conductance was increased to 3.92 ± 1.60 nS (n = 4), and diltiazem also blocked this conductance.

Blocking NO production favors cone over rod input to HCs

HCS in tiger salamander retina receive input from both rods (peak sensitivity, ~500 nm) and red cones (peak sensitivity, ~600 nm) (Yang and Wu 1996). We recorded the responses of HCs to 500–600 nm flashes of light of different wavelengths in eyecup preparations in control and after incubation in NNA (0.5 mM), a selective inhibitor of nNOS. Whereas we had used the NO donor, SNC, in our imaging and electrophysiological studies of isolated rods and cones, now in the intact retina system we felt it best to use an inhibitor of endogenous NO production. Since the rods and cones in the previous studies were isolated from the retinal network and most of the endogenous NO production sites, it seemed that adding NO to the isolated cells would offer the best modulatory signal. In the dark-adapted, intact retinal eyecup, where it can be argued that endogenous NO levels would be at their peak, we felt that the most prominent modulatory signal would be the elimination of NO. This approach also minimized contributions from the by-products of the NO donors, which are easily tested for in isolated systems using degassed solutions, but present significant obstacles in difficult eyecup recording conditions.

Figure 3A shows voltage responses to 500-, 550-, and 600-nm light, normalized to the response at 550 nm to allow comparisons between the responses in control and in NNA from different cells. NNA reduced the relative response amplitude at 500 nm and increased it at 600 nm. A reduction of...
the depolarizing rollback during the light step also occurred in the presence of NNA. The effect of NNA on the spectral response curve (Fig. 3B) was to sharpen the peak, essentially increasing the red (cone-driven) while reducing the green (rod-driven) inputs. In a sample of 10 horizontal cells recorded under control conditions, the 600 to 500 nm ratio was $1.58 \pm 0.09$, whereas in 4 cells treated with NNA, the ratio was increased significantly by $\sim 20\%$ to $1.90 \pm 0.2$ ($P < 0.05$). Since the red/green strengthening occurs when NO signaling is blocked, the inverse would be that NO should tend to enhance green input and reduce the red input to the HC, consistent with the effects of NO on Ca channels in isolated rods and cones.

**Synaptic model**

We constructed models of rod and cone photoreceptors using NEURON software to predict how changes in Ca channels would affect the light response in these cells and in postsynaptic HCs. When fed with a simulated photocurrent signal (waveforms taken from Attwell et al. 1982 for cones and from Baylor et al. 1984 for rods), this model recreated a photovoltage that corresponded well to the recorded light responses. The HC response was simulated using the transfer function:

$$V_{HC} = 39 \exp \left( \frac{|V_R| - 40}{2.1} \right) - 1$$

for the rod input (Attwell et al. 1987) and

$$V_{HC} = 33.4 \exp \left( \frac{|V_C| + 39.5}{8.1} \right) - 1$$

for the cone input (our fit to Fig. 3E in Rieke 2001), where $V_{HC}$, $V_R$, and $V_C$ are voltages in the HC, the rod, and the cone, respectively.

We simulated the effects of SNC in rods by shifting $V_{1/2}$ for Ca channels by $-4.3$ mV and in cones by reducing $G_{max,Ca}$ to 77% of the control value. Other voltage-gated channels are not affected by SNC (Kurenny et al. 1994). Figure 4 shows that the predicted amplitude of the HC response is increased for the rod input and decreased for the cone input. The postsynaptic rod response more than doubled ($\sim 220\%$ of control) and there was
a modest (≈9%) reduction in the postsynaptic cone response, amounting to a rod/cone ratio change much greater than the ≈20% change observed during NOS inhibition in the intact retinal eyecup.

The nonspecific conductance increase during SNC application to rods and cones could not be included in the photoreceptor voltage simulations or in the synaptic transfer functions since the added conductance strongly depolarized the photoreceptors. Thus the model indicates that a conductance increase of this magnitude may not normally occur in photoreceptors and that conditions associated with patch clamp recording may tend to amplify such membrane conductance responses to the NO donor.

DISCUSSION

We show that the NO donor SNC has important actions on the Ca channels of cone photoreceptors, that these actions are dramatically different from those in rods, and that together, these changes in calcium signaling alter the balance of rod and cone synaptic strength measured postsynaptically in retinal neurons. In a previous investigation of the actions of NO on rod photoreceptors, we showed that SNC facilitated the activation of L-type Ca channels by shifting the channel open probability (activation) curve to more negative potentials (Kurennyi et al. 1994). Not only is the functional effect of opposite polarity in cones, in that Ca channel activity is suppressed, but the modulatory mechanism appears to be different as well. While the negative-shift in channel activation in rods implied changes in channel gating properties, the decrease of the maximum calcium conductance in cones suggests a drop in the number of available channels or a reduction of the conductance of individual channels.

Such an opposing effect of NO on rod and cone Ca channels could contribute to the mechanism that alters the balance of rod and cone input to the second-order retinal neurons. A change in relative rod-cone strength is known to occur when the level of ambient illumination changes and may be under the control of a circadian clock (Mangel et al. 1994; Wang and Mangel 1996; Witkovsky et al. 1988, 1989; Yang and Wu 1996). Rod signals are enhanced and cone signals suppressed in second-order neurons following dark-adaptation or during the subjective night. We show that the selective nNOS inhibitor NNA changes horizontal cell responses to rod- and cone-dominating wavelengths, decreasing rod and increasing cone signals recorded postsynaptically. Our modeling of rod, cone, and horizontal cell responses predicts that changes of the appropriate Ca channel parameters ($V_{1/2}$ for rods and $G_{\text{max}}$ for cones) alters the synaptic transfer functions and results in an increase, in the case of rods, or a decrease, in the case of cones, of the HC response produced by a fixed change of the presynaptic photoreceptor voltage.

It is interesting to consider that the most proximal source of NO production in the retina that could modulate photoreceptor Ca channels is the photoreceptors themselves. Photoreceptors contain nNOS, the activity of which is positively correlated with intracellular levels of calcium (Bredt and Snyder 1994; Goldstein et al. 1996; Hu and el-Fakahany 1996; Mayer et al. 1992). In darkness, calcium is elevated in photoreceptors, and this could result in elevated NOS activity and NO production. In rods, this would in turn facilitate Ca channels and allow even more calcium to enter the cell, producing a positive feedback system in rods that could increase the sensitivity of the rod pathway by increasing the output of rods onto second-order neurons. For cones, whose Ca channels are suppressed by NO, the production of NO in darkness would have the opposite effect, yielding a negative feedback loop that would stabilize calcium levels.

Dopamine was previously shown to modulate the balance of rod and cone input into second order retinal neurons, enhancing cone input and decreasing rod input (Witkovsky et al. 1988, 1989). Like NO, dopamine modulates Ca channels differently in rods (facilitation) than in cones (suppression) (Stella and Thoreson 2000), but this result was viewed as paradoxical since the enhancement of rod Ca channel activity should increase the input of these cells to second-order neurons, not decrease it. It has been suggested that calcium-activated chloride current ($I_{\text{CaCl}}$) in rods, altered via modulation of Ca channels by dopamine, provides an explanation for this apparent paradox (Thoreson et al. 2002). Our data show NO-induced Ca channel facilitation and increased rod neurotransmission, suggesting that additional steps may contribute to the NO- and dopamine-mediated Ca channel synaptic strength modulations.

It has been shown that cGMP-gated channels might play a role in synaptic transmission from cones (Rieke and Schwartz 1994; Savchenko et al. 1997). Activation by NO of cGMP-gated channels introduces another important mechanism for a modulatory influence of NO in the outer retina. Activation of a nonspecific conductance by NO might act in unison with the facilitation of voltage-gated Ca channels in rods, but counteract the inhibitory effect of NO on cone Ca channels. These apparently contrasting mechanisms may again highlight the different signal processing strategies demanded for the synapse by rod and cone mediated visual transduction: high amplification, and sensitivity for the rod output system and stability and lower sensitivity for that of the cones. The increase in leak conductance in rods and cones, measured with patch-clamp recording techniques, was not evident in calcium imaging experiments, and it is not accounted for in the experiments performed in eyecup. Modeling of the synaptic transfer of rod and cone membrane potential changes during treatment with NO-donors also could not accommodate the relatively large conductance increases seen during patch clamp recording. Such conductance increases may be specific to conditions encountered during patch clamp recording.

Taken together, the results of these investigations of calcium signal modulation in photoreceptors and of synaptic transmission to horizontal cells, demonstrate opposing actions of NO on rod and cone synapses that could be relevant to the roles of these photoreceptor systems in the duplex retina. NO modulation of Ca channels may reflect an important mechanism aligning rod/cone bias in the dark and light adapted states of the retina and may contribute to signal processing strategies that are unique for the two types of photoreceptor synapses.

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REFERENCES


