Modulation of transmission gain by protons at the photoreceptor output synapse

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ABSTRACT

Synaptic transmission of the light response from photoreceptors to second-order cells of the retina was studied with the whole-cell patch-clamp technique in tiger salamander (Ambystoma tigrinum) retinal slices. Synaptic strength is modulated by extracellular pH in a striking manner: Light-sensitive postsynaptic currents in horizontal and bipolar cells were found to be exponential functions of pH, exhibiting an e-fold increase per 0.23 pH unit over the pH range from 7 to 8. Calcium channel currents in isolated photoreceptors were measured and also exhibited proton sensitivity. External alkalinization from pH 7 to 8 shifted the voltage dependence of channel activation negative by 12 mV. A model of the synaptic transfer function suggested that presynaptic Ca channels could be the primary sites of proton action. Increased Ca influx and transmitter release brought about by alkalinization give rise to larger postsynaptic currents. These results suggest that activity-dependent interstitial pH changes known to occur in the retina, while not alleviating signal clamping at this synapse, may provide an adaptive mechanism controlling gain at the photoreceptor output synapse.

Signal transfer across synapses in the nervous system involves events at both pre- and postsynaptic membranes, many of which are targets of transmission-altering interventions. Presynaptic Ca channels are often the targets of neuromodulators, since the activity of these channels controls Ca-dependent neurotransmitter release. Transmitter release at the photoreceptor output synapse is Ca dependent and graded with membrane potential over a narrow range of presynaptic voltages (1).

In many cell types, extracellular pH modulates Ca channel gating and permeation significantly in the physiological range (2–7). Our present report shows a strong action of extracellular pH on synaptic output from photoreceptors, and this led us to consider the possibility that proton modulation of Ca channels modulates this synapse. By characterizing photoreceptor Ca channel pH sensitivity and adopting these features in a model of synaptic transfer, our work begins to offer an explanation for the strong anoxia and pH dependence reported previously for the horizontal cell membrane potential (8–11). Our result suggests that small light-induced alkalinizations observed in the retinas of several species (12–14) alter transmission gain at this synapse.

MATERIALS AND METHODS

Synaptic Currents in Retinal Slices. Retinal slices (15) were made from dark-adapted eyes of larval tiger salamanders (Ambystoma tigrinum) and cells were whole-cell patch-clamped (Axopatch 1D; Axon Instruments, Foster City, CA) under infrared illumination. Photoreceptors, horizontal cells, and bipolar cells could be identified by their position and morphology in the slice. Some cell identifications were made with 1% Lucifer yellow staining via the patch electrode.

Currents were digitally recorded to hard disk after filtering at 200 Hz. Bath solution contained (in mM): 90 NaCl, 2.5 KCl, 3 CaCl2, 8 glucose, and 10 Hepes. Bath pH was measured (Corning model 140 pH meter) before each experiment and adjusted with NaOH or HCl to ±0.01 pH unit. The pipette solution contained (in mM): 80 KCl, 20 NaCl, 3.5 MgCl2, 1 EGTA, 1.5 Na2ATP, and 10 Hepes (pH 7.2). Solution changes (21–24°C) were complete within 1 min.

Ca Channel Currents in Isolated Photoreceptors. Isolated rod photoreceptors were obtained by mechanical dispersion of the retina. Ba2+ currents were recorded (Axopatch 200) in a bath solution containing (in mM): 65 NaCl, 10 tetaethylammonium bromide, 5 CsCl, 2.5 KCl, 20 BaCl2, 8 glucose, and 10 Hepes. Bath pH was measured before each experiment and adjusted with NaOH or HCl. The pipette solution contained (in mM): 100 CsCl, 3.5 MgCl2, 1 EGTA, 1.5 Na2ATP, and 10 Hepes (pH 7.2). Currents in light-sensitive ion channels were eliminated with bright illumination. CsCl-filled pipettes had tip resistances between 5 and 15 MΩ. Activation curves were derived by dividing the leak-subtracted current–voltage (I–V) relation by the driving force (V − Vrev), in which Vrev is the reversal potential of the current. The activation curves are characterized by the midpoint and slope factor of the least-squares-fitted Boltzmann relation, given by

\[ a = \frac{1}{1 + \exp[(V - V_{mid})/m]}^{-1}, \]

in which V is the membrane voltage, Vmid is the midpoint voltage, and m is the slope factor.

RESULTS

Response to Light of Second-Order Cells Increases with Increasing pH. Tonic release of glutamate by rod and cone photoreceptors in darkness maintains horizontal cells and OFF-bipolar cells in a depolarized state via non-N-methyl-D-aspartate (NMDA-type glutamate receptors and on-bipolar cells in a hyperpolarized state via l-2-amino-4-phosphonobutyrate (APB) receptors (16–18). Fig. 1A summarizes the organization of these cells interacting at the outer plexiform layer, together with their response waveforms. Light hyperpolarizes both rod and cone photoreceptors (Fig. 1A I and A 2), diminishing their release of transmitter, which, for horizontal and OFF-bipolar cells, leads to reduction of inward synaptic current and hyperpolarization (Fig. 1A3). On-bipolar cells become depolarized under these conditions (Fig. 1A4) as inward postsynaptic current is increased. Cells could be identified at the time of recording with Lucifer yellow staining (Fig. 1B).

We voltage-clamped horizontal and bipolar cells in dark-adapted retinal slices and measured postsynaptic currents in these neurons to assess synaptic transfer under conditions of different external pH. Bright light, which strongly hyperpolarizes rods and cones (1), completely suppressed a standing
inward current in horizontal cells (Fig. 2A). After the light step, while rod hyperpolarization persisted, inward current was suppressed by a smaller amount. The magnitude of the synaptic current change was a steep function of bath pH, and responses disappeared entirely at low pH. Superfusion of the slice at pH 7.6 with Cd²⁺ at a concentration which completely blocks photoreceptor Ca channels (23, 24) eliminated all light-suppressible inward current. Changes of synaptic current induced by pH were measured in five horizontal cells and describe a straight line when plotted semilogarithmically (Fig. 2B), indicating an exponential relation between pH and postsynaptic current.

The action of pH is probably not postsynaptic. Voltage-clamp of the horizontal cells eliminated current contributions from voltage- or Ca-gated channels in the cell membrane; thus the observed current changes should arise either from pH sensitivity of the transmitter-gated channels or from increased transmitter release from the presynaptic cell. We recorded little change in the magnitude of glutamate-activated currents in horizontal cells over the pH range from 7.60 to 7.95 (Fig. 3A), the range where we show the largest changes in light-sensitive synaptic current. Reports of work using other preparations confirm that non-NMDA-type glutamate-gated channels (such as those in horizontal and off-bipolar cells) lack pH dependence over the pH range of 7 to 8 (25–27). Also, it seems unlikely that on-bipolar cells, which use a different type of glutamate receptor (APB-sensitive) coupled to cGMP-gated channels (18), would have pH sensitivity similar to that of horizontal cells, as shown in Fig. 3B, if the site of action of protons were solely postsynaptic.

Fig. 2. Postsynaptic current in horizontal cells is an exponential function of external pH. (A) Synaptic currents evoked by 500-ms white light flashes (58 μW·cm⁻², timing indicated by the bar above each trace) in a horizontal cell voltage-clamped at -60 mV in a tiger salamander retinal slice. Salmander horizontal cells sample rod and cone input (21, 22). During bright illumination 60 pA of inward current was suppressed in pH 7.60 solution. While rods remained hyperpolarized for about 7 s, 43 pA was suppressed. Changing to bath pH 7.83 increased inward current in the dark, such that when the bright light was presented, 188 pA was suppressed. At pH 7.31 an inward current of 26 pA was suppressed by light. Superfusion with pH 6.94 eliminated all light-suppressible inward current, as did 100 μM Cd²⁺ applied at pH 7.60. All changes were reversible. (B) Semilogarithmic plot summarizing synaptic regulation by pH from five horizontal cells. Current is normalized at pH 7.6. □, Synaptic current suppression during light application when there is rod and cone input. ◯, Synaptic current suppression measured during persistant rod hyperpolarization after light application. The least-squares-fitted solid line is given by I = exp(4.36pH - 33).
Protons Alter Gating of Ca Channels. We focus in this report on presynaptic Ca channels as possible targets of pH-induced changes in synaptic transfer to explain these results. Fig. 4 shows pH-induced changes in Ca channel properties in rods similar to those for cones (7) and other cell types (2–6). Rod Ca channel currents were measured in solutions of different pH (Fig. 4 A and B). After the I–V relations had been converted to activation curves and fitted with the Boltzmann function (Fig. 4C), the shift of each activation curve midpoint was plotted relative to that measured at pH 7.40 (Fig. 4D). The activation curve midpoint, which undergoes a positive lateral shift in low pH and a negative shift in high pH, exhibits overall a 12-mV shift between pH 7 and 8. Ba$^{2+}$ at 20 mM, used to enhance currents in Ca channels, does not interfere with the actions of protons as judged from the similar shifts of Ca channel and Ca-activated channel activation curves recorded in 3 mM Ca (7). Protons induce gating and permeation changes in Ca channels by binding on or near the channel protein and affecting the electric field seen by the channel voltage sensor, reducing local external Ca concentration or blocking the channel (2–6).

A Model of Synaptic Transfer Supports Ca Channel Involvement in pH Action. We used the rod-to-horizontal cell synaptic transfer function measured by Attwell et al. (1) to test whether the simple pH-induced gating shifts of Ca channels would account for the observed exponential dependence of postsynaptic current on pH. To simulate how pH-induced changes of Ca channels could account for our transmission results, the synaptic transfer function, determined at pH 7.6 (1), was shifted laterally along the presynaptic voltage axis as shown in Fig. 5. The magnitude of each shift was prescribed by the observed pH dependence of Ca channel activation (from the data of Fig. 4D).

The synapse operates on a different curve at each pH. For example, at pH 7.6, the horizontal cell response to bright light is represented in Fig. 5 as follows: In darkness, with the photoreceptors sitting near −40 mV (Vdark on the horizontal axis), the horizontal cell produces the inward current shown on the vertical axis (about 60 pA of inward current, depicted in the upward direction in the figure). Bright light hyperpolarizes the photoreceptor strongly to Vlight or beyond, and the horizontal cell response on the vertical axis declines to near zero. The net expected change of postsynaptic current is described by the vertical line drawn at the right side of the figure and labeled 7.60.

The expected changes in postsynaptic current due to bright illumination at each pH are illustrated as vertical lines at the right side of the figure. These expected responses, expressed relative to the response at pH 7.6, are summarized in the Inset to Fig. 5. They fall close to the line describing pH dependence of postsynaptic current obtained in Fig. 2, suggesting that pH-induced shifts of Ca channel activation could account for transmission modulation. Note that the gain of the synaptic transfer function is altered, not the overall range over which transmission occurs.

The foregoing model assumes that presynaptic Ca channel activity forms the basis for the synaptic transfer function. Thus the known pH-induced shifts of the Ca channel activation curve were implemented as shifts of the exponential transfer function. To test further this approach, we examined the relation between the synaptic transfer function and the Ca channel activation curve. A close approximation of the exponential synaptic transfer function over the voltage range from −40 to −50 mV (1) was obtained by raising the Ca channel I–V relation to the third power and scaling (Fig. 6).


**DISCUSSION**

**Effect of pH and the Site of Proton Action.** We recorded the pH sensitivity of postsynaptic currents in second-order cells and the pH sensitivity of presynaptic Ca currents in photoreceptors and related the two with a simple model of synaptic transfer. Our results show that pH does little to alleviate a major problem faced by the photoreceptor output synapse, namely, signal clipping resulting from the narrow 5-mV transmission range (1). The results do indicate that pH exerts control over the gain of synaptic transfer function, which is reflected in the instantaneous slope of the exponential curves in Fig. 5. As the pH becomes higher, smaller fluctuations around a given postsynaptic membrane potential result in larger responses in the second-order cell. Our quantitative analysis suggests that this gain control can be explained predominantly by the pH sensitivity of presynaptic Ca channels, although pH should be expected to modify many cell processes, as discussed below. In accord with known mechanisms of synaptic transmission in numerous other preparations, Cd$^{2+}$ block of transmission at the photoreceptor output synapse suggests that Ca channels link membrane depolarization with transmission.

There are other possible sites of action for protons at this level in the retina that could contribute to our observations. As discussed in Results above, we believe the postsynaptic glutamate receptors do not undergo pH sensitivity of transmission. A report describing glutamate uptake in retinal glia (37) suggests that if uptake were reduced under alkaline conditions, more transmitter might be present at the synapse and this could produce larger postsynaptic currents. Bath pH changes could alter coupling between horizontal cells (30–32), but the clamped cell was dialyzed with 10 mM Hepes at pH 7.2, and this might be expected to maintain gap junction permeability at a low level (33). Cells filled rapidly and thoroughly with Lucifer yellow (M., 457) (Fig. 1B), suggesting that Hepes (M., 238) might also be extensively distributed. Although this does not rule out electrical coupling, no filled cell showed evidence of dye coupling with neighbors. Broad-field illumination, which polarizes cells.
Neurobiology: Barnes et al.

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equally, eliminates lateral current flow in coupled networks such as the photoreceptors, but it could produce current changes, similar qualitatively to those observed here, if one cell in the network were voltage-clamped. As the membrane potential of uncoupled neighboring cells became closer to or farther away from its holding potential, lateral current flow would change. For example, if one horizontal cell were clamped at -60 mV and the membrane potential of coupled neighboring cells were -30 mV, current would flow laterally through gap junctions into the clamped cell. During the light response, the neighbors' membrane potential would hyperpolarize and diminish the potential difference between the cells, reducing inward current flow. At the same time, if gap junction permeability were reduced by acidification, less current would flow into the clamped cell and the lateral current change during a light response would be smaller. However, unlike the case in fish and some other species (16), horizontal cell bodies in salamander do not form a tightly coupled syncytium (21, 31), a fact reflected in our recordings as high input resistances (ranging from 0.8 to 2.2 GΩ, the same range we find in isolated cells), so lateral current flow, if affected by our external pH changes, should be minimal.

Features of the photoreceptor response itself (dark current, Na-Ca-K exchange, aspartate-isolated receptor potential of the electroretinogram) are little changed over the range of pH having effects on transmission with the time course described here (34–36). Proton modulation of IK, the voltage-gated K current at the inner segment that sets the photoreceptor dark potential by counterbalancing the inward flow of current through GMP-gated channels in the outer segment, has been described (23), but it would have an effect on photoreceptor membrane potential opposite to that required to account for our results. A decreased concentration of protons shifts K channel activation in the negative direction (23), and this would lead to greater K current activation and hyperpolarization of the photoreceptor. If this effect were significant, it would presumably result in less transmitter release in darkness and horizontal cells would respond with less inward postsynaptic current, not increased inward current as observed.

Relevance of This Result to Retinal Function. The pH sensitivity of transmission demonstrated in the present work is a strictly biophysical observation made under unphysiological conditions. Similar pH sensitivity of horizontal cell light responses has been recorded by others in fish and salamander retinas (8–11). These observations could be relevant to adaptation in the retina when considered together with the pH changes (0.02–0.2 pH unit) known to occur as a result of reduced metabolic activity in the outer segments during their response to light (12–14). Even the smallest change observed in amphibia (0.02 pH unit) could, according to our analysis, increase postsynaptic current by 9%. This suggests that a novel form of negative feedback may operate on photoreceptor output. As photoreceptors hyperpolarize in response to light and reduce transmitter release, delayed light-induced alkalization of extracellular pH would act to increase transmitter release. Slow pH changes, decreasing in darkness and increasing in the light, could take part in adapting synaptic gain to the ambient level of illumination.