Commentary

Center-surround Antagonism Mediated by Proton Signaling at the Cone Photoreceptor Synapse

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The identity of the messenger that carries the inhibitory surround receptive field signal from horizontal cells to cone photoreceptors has eluded retinal neurobiologists for nearly three decades. Encoded in horizontal cell membrane potential, the feedback signal presynaptically inhibits neurotransmitter release at the cone terminal. An interesting collection of candidate mechanisms and messengers have come, and some have gone. In this issue of the *Journal of General Physiology*, Hirasawa and Kaneko (2003) take a giant step forward in defining the synaptic messenger. The insight their elegant experiments offer is that protons transmit inhibitory surround information from horizontal cells to cones, and that protons subtly modulate presynaptic Ca channel activity to alter neurotransmitter release dependent on illumination of the surround. That such a ubiquitous signal, e.g., pH, is the messenger in one of the most fundamental steps in visual processing is illuminating in its own right.

Center-surround antagonism is an archetype found in every sensory system design. In visual systems, this form of lateral inhibition was first described in *Limulus* by Hartline (1940). Together with other pioneering work, it was established that this network of laterally interacting elements gave rise to contrast enhancement and edge detection. Ever since the first recordings in vertebrates of a cone photoreceptor response to light (Baylor et al., 1971), it has been known that illumination of the surround counter-acts illumination of the center. In the vertebrate retina, bipolar cells are the recipients of modulated photoreceptor signals and have center-surround antagonistic receptive fields, which they transmit on to the ganglion cells (Werblin and Dowling, 1969). In primate retina, we now know that surround inhibition is also mediated through presynaptic inhibition in cone photoreceptors (Verweij et al., 2003).

A Host of Hypotheses Proposed for the Mechanism of Feedback

Surprisingly, the mechanism(s) by which surround illumination antagonizes the center at the first visual system synapse remain unexplained. Candidates proposed over the years include conventional and unconventional neural messaging systems. Because horizontal cells make intimate synaptic contacts with photoreceptors and have the large receptive fields necessary for the surround signal, these cells have naturally been the focus of attention as the source of the inhibitory feedback (Fig. 1 A). GABA is found in some horizontal cells and GABA receptors have been described both immunocytochemically and physiologically in the cone synaptic terminal. GABAergic mediation of feedback, therefore, has been a prominent hypothesis. However, several studies have tested the persistence of surround inhibition in the presence of GABA receptor antagonists and these have largely ruled out a major role for GABA (for review see Kamermans and Spekreijse, 1999).

A second possible mechanism arose from the unique and characteristic structure of the synaptic contact between cones and horizontal cells (Fig. 1 B). The invaginating synapse, so-called due to the penetration of the cone terminal by the dendrites of horizontal and bipolar cells, gives rise to a protected microenvironment in the synaptic cleft. At these photoreceptor synapses, the invaginating horizontal cell processes require that extracellular current flowing to glutamate-gated channels at the dendrite tips must follow a necessarily resistive path.

Byzov put forward a model of electrical feedback in which the large extracellular flow of current to postsynaptic ion channels in horizontal cell dendrites, which serve as current sinks, produced voltage differences along the extracellular space (Byzov and Shura-Bura, 1986). Extracellular voltage differences, although relatively small in size (estimates place the maximum effect on the order of a few millivolts), affect the voltage-dependent gating of Ca channels similarly as intracellular voltage changes. This ingenious hypothesis postulated that the magnitude of the post-synaptic horizontal cell response itself modulates presynaptic release via an inhibitory, ephaptic feedback loop.

A concern with Byzov's electric feedback model has been that as the central cones become strongly hyperpolarized and reduce their release of glutamate, the feedback is reduced since the glutamate-gated current

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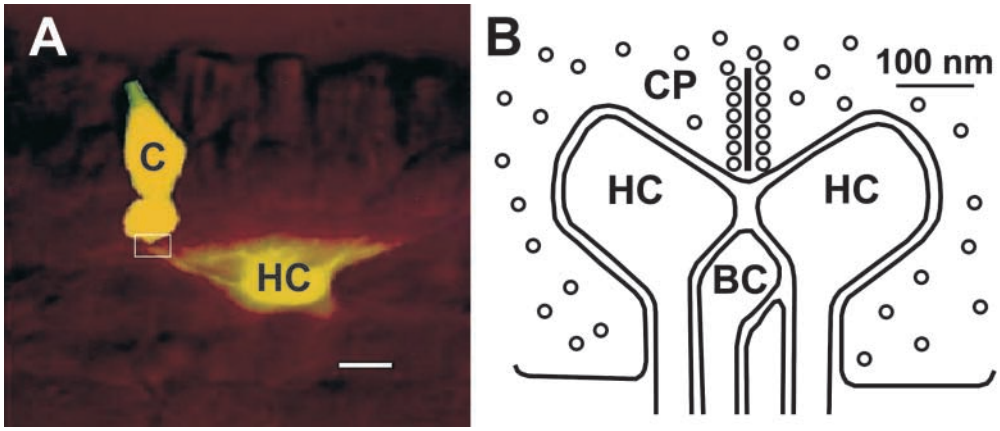

FIGURE 1. Site of the cone-horizontal cell reciprocal synapse—the synaptic invagination. (A) In this diagram of a retinal slice, a cone (C) and a horizontal cell (HC) are depicted as yellow-dyed. The narrow receptive field of the cone is integrated at its synaptic terminal with a broad, inhibitory signal arising in horizontal cells, which receive input from many cones (and are often coupled via gap junctions). The box encompasses the region of synaptic contact between these cells, which is shown greatly enlarged in B. Bar, 10 μm. (B) The invaginating synapse is shown with its four principle elements in this schematic. The synaptic terminal of the cone (CP, cone pedicle) is shown at the site of neurotransmitter release where the ribbon structure directs glutamate-filled vesicles to the presynaptic membrane. Two horizontal cell dendrites (HC) are shown entering the invagination alongside a single dendrite from a bipolar cell (BC) in the typical triad formation. The dendrites of the postsynaptic cells receive signals from the cone, but the horizontal cells feedback onto the cone via a mechanism that now appears to be mediated by protons in this protected synaptic cleft.

sinks no longer exist. Kamermans et al. (2001) adapted Byzov's model by incorporating the finding that hemigap junction channels, composed of connexin 26, are found at the tips of horizontal cell dendrites deep within the invagination (Janssen-Bienhold et al., 2001). This revised model circumvented the perceived problems caused by the gating of glutamate-gated channels, but it remains controversial.

The merits of other possible feedback mediators have also been considered. The lack of conventional presynaptic structures in the horizontal cell dendrites brought forth suggestions of equally unconventional neuromodulators. Nitric oxide has garnered some support as a modulator of presynaptic CNG channels and Ca channels (Kurenny et al., 1994; Savchenko et al., 1997). Signaling through changes in extracellular pH has also been considered but largely refuted since it is known that relatively large and slowly changing pH fluctuations occur in the retina as a function of the state of light and dark adaptation (for review see Barnes, 1998).

Ca Channels in Photoreceptors and their Modulation by Protons

A great deal is known about the pharmacology and biophysics of the L-type Ca channels in photoreceptors (Wilkinson and Barnes, 1996). Functionally, these channels are responsible for the release of the neurotransmitter, glutamate. Identification of the gene that causes night blindness in humans suggests that the photoreceptor Ca channel is composed of $Ca_V1.4$ α1F subunits (Bech-Hansen et al., 1998; Strom et al., 1998). α1F mRNA was localized in photoreceptors by in situ hybridization while antibodies directed against α1F Ca channel subunits labeled photoreceptor synaptic terminals (Strom et al., 1998; Morgans, 2001). Some cones in mammalian retina label with antibodies directed against α1D subunits (Taylor and Morgans, 1998; Morgans, 2000).

External protons affect channel gating by neutralizing fixed negative surface charges distributed over the cell membrane and by interacting with specific protein residues on the channel surface, altering the electric field at the channel's voltage sensor (Hille, 2001). The amount by which external pH shifts channel gating in cones is ~1 mV per 0.1 pH unit (Barnes and Bui, 1991). Acidic conditions reduce Ca channel activity and basic conditions increase it. The consequence for synaptic transmission is that synaptic efficacy is increased as pH is increased (Kleinschmidt, 1991; Barnes et al., 1993; Harsanyi and Mangel, 1993). DeVries (2001) showed that the protons released from presynaptic vesicles together with glutamate provide a fast and potent autoinhibition of cone synaptic release. Illumination induces pH changes on the order of several tenths of a pH unit in the retina, so it might seem that the large environmental pH changes would swamp local fluctuations. But the complex, invaginated structure of the invaginating synapse may protect the synaptic cleft from these systemic changes. The key factor therefore may be the isolation of the local synaptic microenvironment from the retinal milieu afforded by the ensheathing cone terminal membranes.

Protons Mediate the Surround Signal from Horizontal Cells

The article by Hirasawa and Kaneko (2003, in this issue) goes a long way to establishing that protons are the major, if not the only, messenger needed for feed-

back inhibition. Their data confirm that a surround-illumination-induced Ca channel activation shift (~3 mV in the negative direction) in cones is sensitive to the activity of horizontal cells (Verweij et al., 1996). By increasing the buffering power of the retinal environment, they can swamp the local changes of pH that must be occurring in the synaptic cleft. They speculate that the Ca channel shift is due to pH changes in the synaptic invagination caused by proton exchange/transport in horizontal cells. Although there may yet be room for GABA and other systems to be operative at this synaptic site, protons offer the most parsimonious explanation of the new data.

Protons affect many different channels, enzymes, and receptors. We know that another voltage-gated channel important in photoreceptor activity, the hyperpolarization-activated cation channel responsible for I_h, shows about the same sensitivity to external pH as the Ca channel (Malcolm et al., 2003). The Ca-activated Cl channel in cones also shows strong pH sensitivity, apparently due to the underlying pH-sensitivity of the Ca channels (Barnes and Bui, 1991). Yet, owing to the leveraged role that calcium ions play in synaptic transmitter release, the sensitivity of voltage-gated Ca channels to pH keeps these most critical ion channels at the center of interest.

What Remains to be Elucidated?

It will now be essential to establish that horizontal cell dendritic membranes transfer protons in response to membrane potential and in the right direction. Recent work examining proton fluxes across isolated horizontal cell membranes indicate that hyperpolarization is associated with an increase in pH (Molina et al., 2000), the opposite polarity that would be expected for the proton-mediated feedback described by Hirasawa and Kaneko (2003, in this issue). This issue is discussed in the present work and may reflect a problem of pH buffering, which may necessitate measurements in a bicarbonate-based system.

Can the extracellular, intrainvagination pH change be measured? The authors attempted this with pH-sensitive microelectrodes, but had no success. The volume of interstitial fluid where the pH signaling takes places is extremely small (Fig. 1 B). It will be interesting if optical methods, perhaps performed with multiphoton microscopy, can achieve the recording within these tight confines. For now, the pH change in such a small and protected space can only be inferred.

Conclusion

Hirasawa and Kaneko (2003, in this issue) defend a controversial mechanistic hypothesis for the formation of center-surround receptive field organization at the first synapse in the visual system. The elegant experiments strongly support the notion that protons carry the feedback signal from horizontal cells to cones. Proton-mediated feedback may not only generate center-surround antagonistic receptive fields, but chromatic antagonism in the retina as well (Stratis and Baldridge, 2002). The mechanism may have applicability at synapses throughout the nervous system. This work defines a new era of research on synaptic messengers and synaptic modulation.

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REFERENCES

Barnes, S., V. Merchant, and F. Mahmud. 1993. Modulation of transmission gain by protons at the photoreceptor output synapse. *Proc. Natl. Acad. Sci. USA.* 90:10081–10085.

Barnes, S. 1998. Modulation of vertebrate retinal function by pH. *In* pH and Brain Function. K. Kaila and B.R. Ransom, editors. John Wiley & Sons Inc., New York. 491–505.

Barnes, S., and Q. Bui. 1991. Calcium-activated chloride current modulation via pH-induced changes of Ca channel properties in cone photoreceptors. *J. Neurosci.* 11:4015–4023.

Baylor, D.A., M.G.F. Fuortes, and P.M. O'Bryan. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol.* 214:265–294.

Bech-Hansen, N.T., M.J. Naylor, T.A. Maybaum, W.G. Pearce, B. Koop, G.A. Fishman, M. Mets, M.A. Musarella, K.M. Boycott. 1998. Loss-of-function mutations in a calcium-channel alpha1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. *Nat. Genet.* 19:264–267.

Byzov, A.L., and T.M. Shura-Bura. 1986. Electric feedback mechanism in the processing of signals in the outer plexiform layer of the retina. *Vision Res.* 26:33–44.

DeVries, S.H. 2001. Exocytosed protons feedback to suppress the Ca^{2+} current in mammalian cone photoreceptors. *Neuron.* 32: 1107–1117.

Harsanyi, K., and S.C. Mangel. 1993. Modulation of cone to horizontal cell transmission by calcium and pH in the fish retina. *Vis. Neurosci.* 10:81–91.

Hartline, H.K. 1940. The nerve messages ogf the fibres of the visual pathway. *J. Opt. Soc. Am.* 30:239–247.

Hille, B. 2001. Ion Channels of Excitable Membranes. 3rd ed. Sinauer Associates, Inc., Sunderland, MA. 646–660.

Hirasawa, H., and A. Kaneko. 2003. pH changes in the invaginating synaptic cleft mediate feedback from horizontal cells to cone photoreceptors by modulating Ca channels. *J. Gen. Physiol.* 122: 657–671.

Janssen-Bienhold, U., K. Schultz, A. Gelhous, P. Schmidt, J. Ammermuller, and R. Weiler. 2001. Identification and localization of connexin26 within the photoreceptor-horizontal cell synaptic complex. *Vis. Neurosci.* 18:169–178.

Kamermans, M., and H. Spekreijse. 1999. The feedback pathway from horizontal cells to cones. A mini review with a look ahead. *Vision Res.* 39:2449–2468.

Kamermans, M., I. Fahrenfort, K. Schultz, U. Janssen-Bienhold, T. Sjoerdsma, and R. Weiler. 2001. Hemichannel-mediated inhibition in the outer retina. *Science.* 292:1178–1180.

Kleinschmidt, J. 1991. Signal transmission at the photoreceptor synapse. Role of calcium ions and protons. *Ann. NY Acad. Sci.* 635:468–470.

Kurenny, D.E., L.L. Moroz, R.W. Turner, K.A. Sharkey, and S.

Barnes. 1994. Modulation of ion channels in rod photoreceptors by nitric oxide. *Neuron*. 13:315–324.

Malcolm, A.T., D.E. Kurenny, and S. Barnes. 2003. Protons and calcium alter gating of the hyperpolarization-activated cation channel current (I_h) in rod photoreceptors. *Biochim. Biophys. Acta*. 1609:183–192.

Molina, A.J., P.J. Smith, and R.P. Malchow. 2000. Hydrogen ion fluxes from isolated retinal horizontal cells: modulation by glutamate. *Biol. Bull.* 199:168–170.

Morgans, C.W. 2000. Calcium channel heterogeneity among cone photoreceptors in the tree shrew retina. *Eur. J. Neurosci*. 11:2989–2993.

Morgans, C.W. 2001. Localization of the alpha (1F) Calcium channel subunit in the rat retina. *Invest. Ophthalmol. Vis. Sci*. 42:2414–2418.

Savchenko, A., S. Barnes, and R.H. Kramer. 1997. Cyclic nucleotide-gated channels in synaptic terminals mediate feedback modulation by nitric oxide. *Nature*. 390:694–698.

Stratis, A.K., and W.H. Baldridge. 2002. Effects of aminosulfonates on the feedback responses of goldfish horizontal cells. *ARVO Meeting Abstracts*. 43:3776.

Strom, T.M., G. Nyakatura, E. Apfelstedt-Sylla, H. Hellebrand, B. Lorenz, B.H. Weber, K. Wutz, N. Gutwillinger, K. Ruther, B. Drescher, et al. 1998. An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nat. Genet*. 19:260–263.

Taylor, W.R., and C.W. Morgans. 1998. Localization and properties of voltage-gated calcium channels in cone photoreceptors of Tupaia belangeri. *Vis. Neurosci*. 15:541–552.

Verweij, J., E.P. Hornstein, and J.L. Schnapf. 2003. Surround antagonism in macaque cone photoreceptors. *J. Neurosci*. In press.

Verweij, J., M. Kamermans, and H. Spekrejse. 1996. Horizontal cells feed back to cones by shifting the cones calcium-current activation range. *Vision Res*. 36:3943–3953.

Werblin, F.S., and J.E. Dowling. 1969. Organization of the retina of the mudpuppy, Necturus maculosus. II. Intracellular recording. *J. Neurophysiol*. 32:339–355.

Wilkinson, M.F., and S. Barnes. 1996. The dihydropyridine-sensitive calcium channel subtype in cone photoreceptors. *J. Gen. Physiol*. 107:621–630.