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Calcium and Retinal Function

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Abstract

We survey the primary roles of calcium in retinal function, including photoreceptor transduction, transmitter release by different classes of retinal neuron, calcium-mediated regulation of gap-junctional conductance, activation of certain voltage-gated channels for K^+ and $C1^-$, and modulation of postsynaptic potentials in retinal ganglion cells. We discuss three mechanisms for changing $[Ca^{2+}]_i$, which include flux through voltage-gated calcium channels, through ligand-gated channels, and by release from stores. The neuromodulatory pathways affecting each of these routes of entry are considered. The many neuromodulatory mechanisms in which calcium is a player are described and their effects upon retinal function discussed.

Index Entries: Calcium; retina; phototransduction; ganglion cell; calmodulin; glutamate; metabotropic glutamate receptor; GABA.

Introduction

Intracellular calcium and membrane potential are the primary regulators of neuronal excitability. Membrane potential controls the open probability of the various voltage-gated channels in the nerve membrane and sets the driving force for the synaptically driven channels. Calcium, in contrast, has a much broader palette of potential actions. When it is a current carrier at nonspecific cation channels, such as those gated by cyclic nucleotides or neurotransmitters (glutamate, ACh), it influences the rate of ion flux and affects the unitary conductance. It controls the gating of certain voltage-gated K^+ and Cl^- channels. It also modulates the conductance of gap-junctional channels. These 'biophysical' actions, however, are only one facet of calcium's role in neural function because it is itself a diffusible messenger and is coupled to a wide variety of second messenger-mediated pathways. The activities of many intracellular enzymes, for example the kinases, calcium/calmodulin dependent kinase (CaMKII) and some isoforms of protein kinase C (PKC), the phosphatase, calcineurin, and the

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generator of nitric oxide, nitric oxide synthase (NOS), are Ca^{2+}-dependent.

Calcium is highly buffered within neurons through a variety of calcium-binding proteins, e.g., calmodulin, to name just one of the more important. The Ca/calmodulin complex binds to certain membrane proteins and influences their behavior. Ca^{2+} also is sequestered within the neuron in stores created from smooth endoplasmic reticulum and by mitochondria, and is released from stores through the activation of specific receptors. A variety of pumps and transporters interacting with these stores maintain large gradients of $[Ca^{2+}]_i$ within the cell so that certain specialized microdomains, such as the dendritic spine and the synaptic terminal, can achieve $[Ca^{2+}]_i$ levels not experienced generally throughout the neuron and can carry out calcium-dependent functions.

In this brief review we survey a growing literature describing how Ca^{2+} affects retinal cells, synapses, and circuits and thereby influences retinal information processing.

Photoreceptor Transduction

Photoreceptors transduce light capture into an electrical signal through a G-protein-coupled metabotropic pathway *(1).* The specialized outer segment portion of the photoreceptor consists of a stack of flattened disks containing the visual pigment and related molecules of the photoreceptor cascade. The disks are cytoplasmic, and in rods are separated from the plasma membrane of the cell *(2).* The absorbance of a photon by a molecule of visual pigment (either rhodopsin in rod photoreceptors, or one of a variety of visual pigments in cone photoreceptors) leads to activation of multiple molecules of a G-protein, transducin. Each activated transducin, in turn removes inhibitory subunits from multiple molecules of phosphodiesterase (PDE). The activated PDE reduces the cytoplasmic [cGMP], leading to the closure of a variable fraction of cyclic nucleotide gated (CNG) channels located in the plasma membrane of the photoreceptor. Thus, in darkness, a relatively large fraction of CNG channels is open, permitting a steady influx of current, called the 'dark' current, which is carried by Na^+ and Ca^{2+} *(3).* Ca^{2+} carries about 15% of the current flux. In the absence of Ca^{2+}, the conductance of the channel increases greatly because the slow movement of divalent cations through the channel impedes the transmembrane flux of monovalent cations. Light, by initiating a biochemical cascade that reduces [cGMP], evokes a hyperpolarization of the rod or cone cell. The photoreceptor CNG channel has a much greater affinity for cGMP than for cAMP and a Hill plot of [cGMP] against fractional channel activation has an exponent of 2.5–3.0, indicating that three molecules of cGMP bind to the channel *(4,5).*

The steady inward flux of Ca^{2+} has important consequences for photoreceptor function. Under steady-state conditions, i.e., when the light level is constant, Ca^{2+} influx is balanced by Ca^{2+} extrusion affected by a Na/Ca exchanger *(6).* Increased light reduces Ca^{2+} influx without immediately turning off the exchanger, resulting in a drop in $[Ca^{2+}]_i$. This fall impinges on multiple intracellular systems, which are regulated by $[Ca^{2+}]_i$ and which affect the photoreceptor's ability to adapt, i.e., to adjust its operating range to conform to changing light levels. For example, a lowered $[Ca^{2+}]_i$ lead to an increased activity of guanylyl cyclase, the synthesizing enzyme of cGMP. When [cGMP] rises, it partially offsets the actions of PDE, resulting in the opening of some CNG channels and a depolarization of the cell. Photoreceptor outer segments also possess the calcium-binding protein, calmodulin. The Ca/calmodulin complex binds to the CNG channel, decreasing the affinity of its binding site for cGMP. Thus a drop in $[Ca^{2+}]_i$ also results in a dissociation of calmodulin and an increased affinity (lowered $K_{1/2}$) of cGMP for the CNG channel. The effect of the calmodulin-dependent mechanism is in the same direction as the increase in guanylate cyclase, i.e., an increased transmembrane current flow in the presence of light. A third Ca-dependent response is the inactivation of photoactivated rhodopsin, which is a two-step operation.

First, rhodopsin is phosphorylated by rhodopsin kinase, then capped by a small protein, arrestin. Calcium participates in this process by binding to another protein, recoverin, which in its Ca-bound state inhibits rhodopsin kinase. When $[Ca^{2+}]_i$ is clamped experimentally, the operating range of the photoreceptor is severely restricted to about one log unit of steady light. That is, the photoreceptor response is saturated by a 10-fold increase in background illumination above threshold. But when $[Ca^{2+}]_i$ is free to vary, the operating range increases to 3 log units. Additionally, there is evidence for the presence of a Ca/calmodulin-dependent NOS in photoreceptors *(7),* which contributes to the regulation of soluble guanylate cyclase. Whether this pathway operates in the outer segment, as part of the transduction process, is still not fully resolved *(8).* A detailed description of the phototransduction cascade and the multiple roles of calcium in regulating it is found in Pugh et al. *(9)* and Pugh and Lamb *(1).*

Transmitter Release by Photoreceptors

At the opposite end of the cell from the outer segment, each photoreceptor axon expands into a synaptic terminal at which the rod or cone comes into contact with second-order retinal neurons, the horizontal and bipolar cells *(10).* In spite of their unconventional light responses (nonspiking, light-induced hyperpolarizations, graded in amplitude according to the intensity of the light flash), the photoreceptors obey the general rules of synaptic transmission: 1) the transmitter (glutamate) is packaged in vesicles; 2) vesicular release is a Ca-dependent process; and 3) Ca^{2+} entry increases with depolarization (reviewed in ref. *11*). The graded, nonspiking nature of the light-evoked changes in membrane potential, however, does impose special constraints on the Ca channel underlying exocytosis. First the membrane potential of the photoreceptor is relatively depolarized in darkness, –35 to –40 mV, because of the steady cation influx through the CNG channels described earlier. Second, the 'signal' for the second-order retinal neurons is the instantaneous value of the photoreceptor's membrane potential, which remains approximately fixed in steady light, so the Ca current must be noninactivating.

A Dihydropyridine-Sensitive Ca Current Mediates Transmitter Release by Rods and Cones

Apparently the requirements for a photoreceptor calcium current are met by the high voltage-activated subspecies of calcium channel, since this is the one invariably identified whatever the type of photoreceptor or animal species investigated *(12–15).* The most complete pharmacological characterization of the photoreceptor calcium current was carried out by Wilkinson and Barnes *(16)* using the perforated patch method. Exposure to the dihydropyridines, nifedipine or nisoldipine, elicited an incomplete and voltage-dependent block of current, whether carried by Ba^{2+} or by Ca^{2+}. A small fraction of the current was sensitive to the N-type channel blocker, ω-conotoxin GVIA, but unlike the case for N channels, the block was reversible. These workers concluded that the pharmacological profile most closely resembled that of the α1D calcium channel.

In agreement with their conclusion, antibodies to the α1D calcium-channel subunit, immunostain the synaptic terminals of photoreceptors *(17).* In the salamander retina, however, Nachman-Clewner et al. *(18)* localized α1C subunits of L-type Ca channels to photoreceptor terminals, and showed that they clustered in the vicinity of synaptic ribbons. Moreover, a recent study of cloned α1D channels *(19)* found them to be insensitive to G-protein modulation, in particular, G-protein pathways linked to the somatostatin sst2 receptor and the dopamine D2 receptor. In contrast, calcium currents of

rods and cones are modulated by both these G-protein coupled pathways (*see* below; *20,21*). These data imply that not only does the molecular organization of rod and cone calcium channels differ one from the other, but also from that of the α1D calcium-channel subunit studied in other systems. Recent studies of humans afflicted with congenital stationary nightblindness *(22,23)* show that the mutation affects the α_1F subunit of an L-type Ca channel. Evidently, more work is required to establish the molecular identity and possible diversity of photoreceptor calcium channels.

The synaptic gain relation between photoreceptors and horizontal or bipolar cells is approximated by an exponential function over the operating range of the photoreceptors, which extends from –35 to –40 mV in darkness to about –60 mV in the presence of a saturating light. The low synaptic transfer gain at potentials negative to –40 mV is what would be predicted by the diminished open probability of L-type Ca channels. In fact many investigators saw a contradiction here, in that the Ca current sampled by whole-cell patch clamp was unmeasureably small at potentials <–40 mV, suggesting that some other Ca current must underlie synaptic transmission in this voltage range. This apparent result, however, is misleading in that the calcium flux required by photoreceptors is very small.

To make this point clear, consider that the synaptic terminal of a rod is, to a first approximation, a sphere about 3 μm in diameter, having a volume of 10^{-14}L. Fluorescence measurements indicate that, in darkness, the $[Ca^{2+}]_i$ of the terminal is about 100 n*M (24).* Thus, in the transition from saturating light to darkness, the calcium flux to bring $[Ca^{2+}]_i$ from unmeasurably small to 100 n*M* in 0.1 s is $<2 \times 10^{-15}$ coulombs, which is below the measuring capabilities of the whole-cell patch clamp method. Even making the reasonable assumption that the local $[Ca^{2+}]_i$ in the active zone achieves a much higher concentration, the volume of this zone is so small that the numbers are not much altered, and the general conclusion holds.

In fact a quantitative measure of Ca^{2+} influx through the photoreceptor terminal under physiological conditions has not been reported; virtually all the studies of calcium current (I_{Ca}) in photoreceptors have been carried out on pharmacologically treated cells. Typically, Ba^{2+} is substituted for Ca^{2+}, resulting in a rightward shift of the current-voltage relation along the current axis, and interfering currents, e.g., a calcium-dependent chloride current $I_{Cl(Ca)}$ *(25),* that may influence I_{Ca} are blocked. Finally, currents are measured in a potential range (>–30 mV), which the photoreceptor never experiences during normal function.

Other approaches, however, have linked an L-type Ca current to transmitter release. Schmitz and Witkovsky *(26,27)* utilized a 'reduced' retina, consisting of a layer of photoreceptors *(28)* to study the dependence of glutamate release on light and on calcium. It was found that glutamate release was inhibited by blockers of L-type Ca channels, but not by blockers of N or P-type channels. Light-reduced glutamate release as a monotonic function of intensity. Building on these data, Witkovsky et al. *(29)* showed that, over the full range of rod responsiveness, the function relating rod-membrane potential to fractional glutamate release was well fit by a Boltzmann function for the L-type Ca current studied in rod photoreceptors. In cones, however, an additional calcium current dependent on cyclic nucleotides has been identified *(30,31)* and there is evidence from capacitance measurements that it supports exocytosis. This cGMP-gated calcium current is a target for nitric oxide *(31).* Possibly it may supplement the contribution of L-type Ca current to glutamate releases by cones, particularly at more negative membrane potentials.

Neuromodulation of Photoreceptor Calcium Currents

Before leaving the subject of photoreceptor calcium currents, it is worth pointing out how closely and by how many diverse influences

they are regulated. Their modulation by somatostatin and dopamine was alluded to earlier. Immunocytochemical studies have localized somatostatin sst_{2a} *(20)* and dopamine D2 receptors *(32)* to photoreceptor terminals. Akopian et al. *(20)* showed that somatostatin-14 decreased calcium current in rods, but increased that in cones, actions blocked by pertussis toxin. Stella and Thoreson *(21)* noted that quinpirole, a D2 dopamine agonist, increased the Ca current of rods but decreased that of cones. It is very intriguing that dopamine and somatostatin have similarly divergent actions on rods and cone calcium currents. The activity of these two neuromodulators may well be coordinated, since the dopaminergic neuron also possesses somatostatin sst_{2A} receptors *(33).*

Barnes et al. *(34)* showed that the rod Ca current was sensitive to pH, increasing at more basic pH levels. The effect is mediated by a pH-dependent shift of the activation function along the voltage axis and, using the postsynaptic currents of horizontal and bipolar cells as a metric, the magnitude of the change is substantial: an e-fold increase of postsynaptic current for each 0.23 pH unit between pH 7.0 and 8.0. The implication is that the metabolism/activity of the retina can alter synaptic function through pH. For example, it has been shown *(35)* that illumination causes an alkalinization of up to 0.2 pH units in the extracellular space around the photoreceptors.

Nitric oxide (NO) also increases the Ca current of salamander rods *(36).* A biochemical system for synthesizing NO is indicated by the abundance of the co-factor NADPH in rod and cone ellipsoids *(37),* which is strongly correlated with the enzyme NOS (reviewed in ref. *38*).

Calcium-dependent voltage-gated currents are present in rods and cones. Cones manifest a very strong calcium-dependent I_{Cl}, which produces a large tail current at light offset *(25).* Thoreson et al. *(39)* found that I_{Ca} is directly sensitive to $[Cl^-]$ so that a reduction in $[Cl^-]_i$ causes a drop in I_{Ca}. A determination of the equilibrium potential for chloride (E_{Cl}) in photoreceptors is crucial to understanding the sign and possible significance of this phenomenon. If E_{Cl} is positive to the membrane potential, due to active transport of Cl^-, then a depolarization will cause an influx of Ca^{2+} but an efflux of Cl^- and hence the circuit will behave as a negative feedback modulator of I_{Ca}.

The L-type Ca current is known to be self-inhibiting: a putative mechanism is that increased Ca^{2+} flux activates calmodulin, and the Ca/calmodulin complex binds to the Ca channel, reducing influx. When studied in other systems, however, this phenomenon only became apparent at $[Ca^{2+}]_i$ above 1 μm, a concentration higher than the average cytoplasmic concentration measured in photoreceptor terminals during normal functioning, although it presumably occurs just at the cytoplasmic faces of calcium channels clustered near the active zones where exocytosis occurs. Another factor influencing calcium influx is membrane surface charge. As the concentration of calcium in extracellular space increases, it causes a rightward shift along the voltage axis of the activation function for I_{Ca} *(40).* Thus for a given depolarization there is a reduction in calcium influx. This explains an apparently paradoxical finding *(41)* that lowering $[Ca^{2+}]_o$ increased synaptic transmission from photoreceptors to second-order neurons. Anions also influence membrane surface charge and affect the activation function of voltage-dependent ion channels in rod photoreceptors *(21a).* These authors found that increasing extracellular sulfate and phosphate ion concentrations caused a leftward shift in the activation function of I_{Ca}.

In spite of all these regulatory pathways for the Ca current of photoreceptors, large Ca-dependent, brief spikes and/or sustained depolarizations are sometimes initiated by light. These transients have been studied most in cones of the turtle retina *(42,43).* Thoreson and Burkhardt *(44)* showed that they were inhibited by 0.5 m*M* Co^{2+}, which was too low to affect photoreceptor to horizontal cell transmission, indicating both their dependence on Ca^{2+} and their independence from horizontal cell feedback. A possible role for spikes in signal processing by photorecep-

tors is undetermined, but their presence contributes to a growing body of evidence that the so-called nonspiking neurons of the outer retina, photoreceptors, horizontal cells, and bipolar cells, each possess voltage-gated channels that permit either Na^+ or Ca^{2+} spikes and that may be expressed under special circumstances *(45).*

Calcium Stores in Retinal Neurons

The evidence for the existence of Ca^{2+} stores in a variety of retinal neurons is strong, but on the other hand physiological evidence for their mode(s) of operation is still quite incomplete. Ungar et al. *(46)* described cisternae created from endoplasmic reticulum in the inner segments of photoreceptors in which calcium accumulated. Immunostaining for the ryanodine and inositol trisphosphate (IP_3) receptors typically associated with calcium stores indicated their presence in photoreceptors, bipolar, amacrine, and ganglion cells *(47,48).*

In photoreceptors isolated from salamander retina, exposure to caffeine induces changes in $[Ca^{2+}]_i$ that are antagonized by prior treatment with ryanodine *(49).* These same investigators provided evidence that Ca^{2+} released from stores also participates in release of neurotransmitter. Exposing isolated rods to caffeine transiently raised but then depressed $[Ca^{2+}]_i$ without changing the rod's membrane potential. Caffeine reduced glutamate release and resulted in a hyperpolarization of a second-order retinal neuron. Although the precise mechanism of caffeine's action has to be worked out, it appears to interact with calcium stores and Ca^{2+} transporters.

In solitary ON bipolar cells from carp retina, caffeine-sensitive Ca^{2+} stores were identified in the cell soma, but not the synaptic terminal of the cell *(50).* On the other hand, using the same preparation and a double-pulse protocol, Kobayashi et al. *(51)* found a potentiation of $[Ca^{2+}]_i$ that was not attributable to increased calcium current, and so was presumed to reflect Ca^{2+} release from stores. These stores were insensitive to ryanodine and only slightly affected by caffeine, but the possibility that release was mediated by IP_3 was not tested. Parenthetically, there is as yet no identification of the neuroactive substances which generate IP_3 in retinal neurons.

Shen and Slaughter *(52)* provide evidence for the presence of both ryanodine- and IP_3 receptors in salamander retinal ganglion cells. Their data on the suppressive effects of Ca^{2+} store activation on trans-membrane Ca currents suggest that IP_3R activation leads to Ca release and that the resulting increase in $[Ca^{2+}]_i$ may cause activation of ryanodine receptors.

Glutamate-Dependent Current of 'ON' Bipolar Cells

Bipolar neurons, like photoreceptors, are nonspiking, responding to light with graded potentials. Bipolar cells, differ from photoreceptors, however in being divided into two major subgroups. One, the ON bipolars, responds to light with depolarization, the other, the OFF bipolars, is hyperpolarized by light. OFF bipolars respond to glutamate through AMPA or Kainate-type iGluRs *(53),* whereas the ON bipolar utilizes a metabotropic GluR, mGluR6 *(54).* The pathway coupling mGluR6 to a change in membrane current involves a G-protein, G_o *(55),* that, through a cascade not yet fully understood, reduces a cGMP-dependent membrane cation current *(56,57).* This pathway bears an obvious similarity to the phototransduction cascade, another measure of which is the regulation of the cation current in ON bipolar cells by calcium *(58,59).* Dialysis of ON bipolar cells with BAPTA eliminates a loss of sensitivity to glutamate, reflected by a re-opening of cation channels in the continued presence of glutamate. The mechanism of this calcium-dependent adaptation appears to implicate calmodulin.

Transmitter Release by Bipolar Cells

Immunocytochemical studies *(60,61)* indicate that both types of bipolar utilize glutamate as a transmitter. Both T- and L-type Ca channels have been identified in bipolar cells *(62–64).* The latter authors showed that only L-type channels were found in the terminal region. With regard to the relation between Ca current and glutamate release, however, all the studies have been carried out on a depolarizing bipolar cell found in the goldfish retina, which is favored for these investigations because it has a large, bulbous synaptic terminal, about 10 μm in diameter. Using isolated bipolar cells of this type, and with a glutamate-sensitive neuron brought close to the terminal to serve as an indicator of release, Tachibana et al. *(65)* elegantly demonstrated that the liberation of glutamate was blocked by a dihydropyridine, consistent with the demonstration of a dihydropyridine-sensitive calcium current, but it remains to be shown that the result of Tachibana et al. applies to all other bipolar cells.

Heidelberger et al. *(66)* probed the dependence of exocytosis on $[Ca^{2+}]$. For large depolarizations a fourth power relationship was found, requiring $[Ca^{2+}]_i$ to exceed 100 μ*M* for very large and rapid release. Lagnado et al. *(67)* suggest, however, that basal (submicromolar) levels of $[Ca^{2+}]_i$ support a low level of exocytosis, corresponding to what the ON bipolar cell might release in darkness. High rates of exocytosis depend on the rate at which vesicles can be brought to the release sites. Gomis et al. *(68)* studied this phenomenon with paired pulse stimulation and capacitance measures. Vesicles became available for exocytosis in a two-stage process of which the fast phase was inhibited by EGTA. Their model calls for release of vesicles at different rates, dependent on Ca^{2+}-sensing proteins with different affinities.

Some bipolars are found to possess T-type Ca currents *(62,63),* which have more transient kinetics and are activated at more hyperpolarized potentials than the L-type channels. It has not been shown that T-type channels contribute directly to exocytosis, but they might influence that process indirectly. Recently it was found that bipolar cells exhibits prominent Ca^{2+} transients or full spikes, which may arise spontaneously in darkness *(69)* or be light-driven *(70,71).* The Ca channels underlying spikes have not been characterized, but in any event such transients, by locally raising $[Ca^{2+}]_i$, will certainly influence transmitter release and might be additionally important in activating one or more of the Ca-dependent neuromodulatory pathways. In that regard, a Ca-dependent isomer of PKC has been found to facilitate transmitter release by bipolar cells *(72)* and also to decrease GABAergic input to bipolar neurons *(73).* Heidelberger and Matthews *(74)* identified a GABA-dependent inhibition of Ca^{2+} influx into goldfish bipolar cells that was GTP-dependent. The mechanism was to shift the activation function of Ca^{2+} to the right along the voltage axis, i.e., a given depolarization induced a smaller Ca current. Lukasiewicz and Werblin *(75)* suggested that the $GABA_c$ receptor-elicited chloride current could act as a shunt in salamander bipolar cells, decreasing Ca current at the terminal.

The axon terminals of depolarizing bipolar cells in goldfish retina have calcium-dependent K^+ channels of the BK variety *(76),* i.e., are of relatively large conductance and are blocked by TEA and charybdotoxin *(77).* Experimental manipulation of $[Ca^{2+}]_i$ indicated a value of 8 μ*M* for a 0.5 open probability of the Ca-dependent K^+ channel. Assuming that the calcium and Ca-dependent K^+ channels are very closely associated in the terminal membrane and given other studies (e.g., *78*) showing that, in response to large depolarizing steps, $[Ca^{2+}]_i$ rises to tens of μ*M*, it is probable that the Ca-dependent K^+ channels play a role in the normal functioning of the cell. One can predict that in response to large depolarizations induced by bright-light stimuli, the effect of a Ca-dependent K^+ current will be to reduce transmitter release, i.e., it functions as a negative feedback pathway. It is worth noting also

that the Ca-dependent K^+ current is a target for neuromodulation *(79).* A full treatment of the transmitter-release process in bipolar cells is found in von Gersdorff and Matthews *(79a).*

Transmitter Release by Third-Order Retinal Neurons

Transmitter release by amacrine neurons has been studied relatively little. Perhaps the most important reason for this neglect is the large variety of amacrine cells: some subtypes of amacrine cell fire spikes whereas others respond only with graded, slow potentials (reviewed in ref. *80*). Gleason et al. *(81,82)* and Borges et al. *(83)* utilized amacrine neurons isolated from embryonic chick retina and grown in culture. The cells they studied had spiking Na^+ currents and L-type Ca currents. Depolarization permitted Ca^{2+} entry and exocytosis, the latter process occurring in two phases. The more rapid one presumably reflected release of readily available vesicles, whereas the slower phase was found to be prolonged when the activity of a Ca^{2+}/Na^+ exchanger was inhibited.

Transmitter release by retinal ganglion cells has been little studied. Taschenberger and Grantyn *(84)* stimulated cultured retinal ganglion cells with current and recorded evoked currents in neighboring neurons. That these currents were EPSCs was indicated by their sensitivity to an AMPAR blocker (in some cases). This study showed that transmitter release was dependent on ω-conotoxin sensitive calcium current, i.e., presumed N-type calcium channels. Other workers *(85)* have provided pharmacological evidence for the presence of N-type Ca currents in ganglion cells, although the same ganglion cells also possess L-type Ca channels.

Calcium and Electrical Coupling

Horizontal cells and some amacrine cells are known to be joined by large and/or numerous gap junctions *(86,87),* which permit signal transmission within the layer of horizontal cell with space constants extending to hundreds of micrometers. Smaller gap junctions join photoreceptors *(88)* and these can be between like cells (e.g., neighboring rods) or unlike cells (rod-cone junctions) *(24).* Similarly, some bipolar cells also may be joined by gap junctions, *(89)* and this coupling has been noted between bipolar neurons of the same or different subclass *(90).*

The gap-junctional structure mediating electrical coupling is universal and well-understood (reviewed in ref. *91*). A group of six proteins called connexins are joined in a barrel like arrangement to create a connexon, the hemi-gap junction. Connexons in the membranes of neighboring cells join to create a tube-like structure, which incorporates a central cytoplasmic channel that bridges the coupled cells. In relation to the central theme of this review, the important topics are the effects of $[Ca^{2+}]_o$ on hemi-gap junctional conductance and the neuromodulatory effects of $[Ca^{2+}]_i$ on gap-junctional properties.

DeVries and Schwartz *(92)* created hemi-gap junctions by killing one of a pair of coupled horizontal cells in culture. The conductance of the exposed hemi-gap junctions showed a sigmoidal dependence on $[Ca^{2+}]_o$ with a half maximum near 220 μ*M* and only a 4% maximal conductance at physiological $[Ca^{2+}]_o$. The conditions that permit the maintenance of hemi-gap junctions in intact retinas are unknown. In a recent study *(93),* connexin 26 was localized to horizontal cell terminals in cone photoreceptor bases of the goldfish retina. The absence of a gap-junctional structure supports the idea that the connexins form hemi-gap junctions. The authors propose that these hemi-gap junctions provide a current path, which is part of a negative feedback pathway affecting photoreceptor Ca currents and influencing photoreceptor transmitter release. It was estimated that at physiological $[Ca^{2+}]_o$ the hemi gap junctions had only 4% maximum conductance (cf. *92*), but that even this low fractional conductance and a small (<400) number of hemi-channels were

sufficient to account for the 4.6 mV hyperpolarization in cones observed when the hemichannels were blocked pharmacologically.

With regard to intracellular modulation of gap-junctional permeability, the influence of dopamine *(94,95)* and nitric oxide (NO) *(96)* is well-known. Lu and McMahon *(97)* reported that in cultured horizontal cells, activation of the NO system by Na nitroprusside was not associated with a change in $[Ca^{2+}]_i$. This does not mean, however, that an increase in $[Ca^{2+}]_i$ would be without effect on an NO-mediated neuromodulatory response, since NOS is known to be stimulated by Ca^{2+} *(38).* Moreover, McMahon and Mattson *(98)* report that when horizontal cells are cultured in a lowered Ca^{2+} medium, gap-junctional conductance is reduced, an effect which took a few hours to become manifest and whose basis is unknown.

There is, as well, a complex interaction between glutamate, $[Ca^{2+}]_i$ and pH in horizontal cells, an interaction that bears on gap-junctional conductance, because acidification reduces gap-junctional permeability. Takahashi and Copenhagen *(99)* and Takahashi et al. *(100)* have documented that the Ca current of horizontal cells is affected by pH; increasing alkalinization increases the Ca current, as was described earlier for the Ca current of photoreceptors *(34).* L-glutamate acidifies the horizontal cell *(101),* resulting in a reduction in the Ca current. GABA also contributes to the maintenance of pH, because the Cl^- channel opened by GABA also fluxes $HCO3^-$, which in turn affects carbonic anhydrase and proton production *(102).* Thus both the main excitatory and inhibitory amino acid transmitters influence Ca current and coupling indirectly through a modulation of intracellular and extracellular pH *(99).*

Calcium Waves in Retinal Neurons and Glia

Waves of neural activity involving substantial increases in intracellular Ca^{2+} occur in both retinal neurons (amacrine and ganglion cells) and in retinal glia (astrocytes and Muller cells). The neuronal waves have been studied in developing mammalian retinas and are considered to play an important role in the formation of central connections *(103,104).* The Ca^{2+} rise in neurons of developing retinas accompanies spiking and is abolished by TTX *(105).* The question is whether the wave of increased Ca^{2+} has a functional role or is merely a concomitant of spiking activity. Certainly elevated Ca^{2+} would favor transmitter release, and there is evidence that acetylcholine, acting through ionotropic nicotinic receptors on retinal ganglion cells, plays a role in propagating the waves *(106).* Another possibility, discussed by Catsicas and Mobbs *(107)* is that elevated $[Ca^{2+}]_i$ triggers gene transcription. It is well-established that this can be mediated by a biochemical cascade involving calmodulin, nuclear CaMKIV, and CREB or by a direct elevation of nuclear $[Ca^{2+}]$ leading to CREB-initiated transcription *(108).*

Glial waves involving elevated $[Ca^{2+}]_i$ can be initiated in mature mammalian retinas by mechanical, electrical, or neurochemical stimulation *(109).* In a subsequent study these authors *(110)* found that glial waves either excited or inhibited neurons in the path of the wave through a mechanism involving release of glutamate from glial cells. The glutamate then either directly activates ganglion cells through iGluRs and/or causes release of GABA or glycine from amacrine cells. Glial cells appear to exhibit Ca-dependent calcium release from stores, since prior treatment with thapsigargin decreased the Ca^{2+} signal. Newman *(111)* reports that mechanical stimulation releases ATP and that a pulse of exogenous ATP evokes a Ca wave.

Regulation of Intracellular Calcium in Retinal Ganglion Cells

Ganglion cells are the final output neurons of the retina and each ganglion cell axon con-

tributes a temporal pattern of spikes to the central receiving areas for vision. Ganglion cell spikes may arise spontaneously or be light induced. In the latter case, the most general division of firing patterns is transient vs sustained. The factors that determine which pattern a particular ganglion cell adopts are: the temporal mix of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs); the location on the neuron (dendritic tree, perikaryon) of the synaptic inputs; and the intrinsic voltage-dependent channels, which influence spike firing. Both synaptic and intrinsic channels are subject to neuromodulatory influences, of which a subsets are Ca-dependent.

The important role of intracellular Ca^{2+}, and Ca^{2+}-activated neuroregulatory processes in modulating ligand- and voltage-gated channels in third-order retinal neurons (amacrine and ganglion cells) has received considerable attention in recent years (summarized in ref. *112*). Still, it is fair to say that the impact of such neuromodulation on signal processing by amacrine and ganglion cells is little understood. A full consideration of signal processing is beyond the scope of this review (*113,114* for reviews); we confine ourselves to a consideration of the sources of calcium and of Ca-dependent neuromodulatory effects on cellular responses, such as ganglion-cell spiking.

The sources of Ca^{2+} for retinal ganglion cells are the same three discussed earlier in relation to photoreceptors and bipolar cells, but the repertoire of responses available to ganglion cells is greater than for distal retinal neurons, because they receive a greater variety of synaptic inputs and they produce action potentials. The sources are: 1) Ca^{2+} influx through voltage-gated Ca channels. Since the open probability is increased by depolarization, Ca^{2+} entry by this route is increased when the ganglion cell receives excitatory drive through synaptic channels *(115),* and when the cell spikes. 2) Permeation of Ca^{2+} through one or more divalent ion permeable postsynaptic receptor channels. 3) Release of Ca^{2+} from intracellular stores *(116–118).* Retinal ganglion cells possess Ca^{2+}-permeable, ligand-gated channels activated by glutamate *(119,120),* ATP *(121),* and acetylcholine *(122).*

In relation to monitoring Ca^{2+} fluxes in retinal neurons, a recent, very relevant technological advance is two-photon excitation of calcium-sensitive dyes *(123).* The method offers sufficiently high spatial resolution to resolve local Ca^{2+} changes, e.g., in dendritic processes and synaptic terminals. Denk and Detwiler took advantage of a secondary peak in the excitation spectrum of the dye, calcium green, to use a 930 nm wavelength to monitor Ca^{2+} dynamics, thus avoiding visible light excitation of rods and cones. On the other hand, visible light was used to evoke responses from retinal neurons, which were sampled electrophysiologically and temporally related to changes in $[Ca^{2+}]_i$. Individual upward steps in the fluorescence signal were found to be coincident with action potentials, indicating that in these ganglion cells, voltage-gated Ca channels are responsible for at least a part of the light-induced influx of Ca^{2+}.

In relation to calcium influx through ligand-gated channels, most attention has been paid to glutamate, which is the principal excitatory neurotransmitter of vertebrate retinas *(11,124). In situ* hybridization studies and immunocytochemical measures have established that all the different receptor subunits for AMPA and kainate receptors, i.e., GluR 1–4, GluR 5–7, and KA1,2 and the NMDA receptor subunits NMDAR1 and NMDAR2 *(125)* are found in ganglion cells. The precise subunit compositions of native AMPA, KA, and NMDA receptors, however, are still unknown. This information is crucial to understanding the behavior of the receptors in relation to Ca^{2+}-permeability, desensitization, and kinetic properties *(126).*

Whether KA receptors are generally found on ganglion cells and how they might contribute to their light-evoked responses are still largely unexplored topics. In this regard Lukasiewicz et al. *(127)* reported that the non-NMDA component of salamander ganglion-cell EPSCs is mediated by AMPA-preferring receptors, whereas KA-preferring receptors do

not contribute significantly to the EPSC. On the other hand, it is well-established that both AMPA and NMDA receptor contribute to the light-evoked excitatory postsynaptic responses of ganglion cells *(128–130).* NMDA receptors have a substantial permeability to Ca^{2+}, but whether a given AMPAR fluxes Ca^{2+} is a function of its subunit composition and further post-translational editing.

A common method for studying Ca^{2+} permeability of iGluRs is to compare their current-voltage relation in a high Na^+/low Ca^{2+} and a high Ca^{2+}/low Na^+ medium. High Ca^{2+} permeability is associated with a positive shift in the reversal potential in the high Ca^{2+} medium. Gilbertson et al. *(116)* provided this data for bipolar cells of salamander retina showing that their non-NMDA iGluRs had a small but perceptible permeability to Ca^{2+}. Tachibana *(131)* had earlier obtained similar data for horizontal cells of a teleost retina, before the specific blockers were available for AMPARs vs. NMDARs. Ganglion cells of the rat retina were found to show either a low or a high degree of Ca^{2+} flux through AMPARs *(120).* Correlative molecular analysis of the population of ganglion cells show that low Ca^{2+} permeability may be associated with the 'flop' version of the GluR2 subunit. A single cell-PCR analysis of the AMPARs of individual ganglion cells, however, remains to be carried out.

The role of glutamate in relation to the regulation of $[Ca^{2+}]_i$ in retinal ganglion cells extends beyond simple permeation of Ca^{2+} through the ligand-gated channel. Ganglion cells possess different subunits of metabotropic glutamate receptors, $mGluR_{1,2,5,7,8}$ *(132–134)*, which include those linked to IP_3-, and ryanodine-sensitive internal Ca^{2+} stores *(48,52,135,136).* An exemplary analysis was carried out by Shen and Slaughter *(52)* on ganglion cells of the salamander retina. These neurons possess both L- and N-type voltage-gated calcium channels. Permeation of calcium through kainate-activated ionotropic glutamate receptors inhibited the N-type Ca current, an action that was reduced by inhibitors of calmodulin and of phosphatases. Activation of metabotropic glutamate receptors, primarily of the group III sub-type, caused Ca^{2+} release from stores, resulting in an inhibition of L-type Ca current. In the *Xenopus* retina, Akopian and Witkovsky *(48)* reported a similar inhibition of L-type Ca current in retinal ganglion cells through a pathway, involving group I metabotropic glutamate receptors. In isolated mouse retinal ganglion cells, both inhibition and enhancement of high voltage-activated Ca currents was reported to occur through G-protein coupled mGluRs, but which did not involve Ca^{2+} release from intracellular stores *(137).* Modulation of voltage-gated Ca channels in ganglion cells by other retinal neurotransmitters and neuromodulators such as GABA *(85,138,139),* dopamine *(140),* and NO *(141)* have been reported in different retinal preparations. Thus, the principal retinal neurotransmitters, through activation of both ionotropic and metabotropic receptors, exert a major modulatory influence on the level of intracellular $[Ca^{2+}]_i$ in retinal ganglion cells.

Calcium-Induced Modulation of Neurotransmitter Receptors

Increases in $[Ca^{2+}]_i$ during synaptic transmission are associated with important physiological processes, such as synaptic plasticity, neuronal differentiation, and neuromodulation *(142,143).* Both voltage- and ligand-gated channels are a target for modulation by intracellular Ca^{2+} in different retinal neurons (reviewed in ref. *112*). Here our focus is the neuromodulatory actions of $[Ca^{2+}]_i$ on neurotransmitter receptor function. As an example, the affinity of the metabotropic $GABA_B$ receptors in salamander ganglion cells is reduced by Ca^{2+} released from internal stores *(144).* In the same preparation calmodulin modulated the activity of baclofen-sensitive $GABA_B$ receptors both upregulation through kinase activation and downregulation through phosphatase activation, whereas *cis*-aminocrotonic acid- (CACA)-sensitive $GABA_B$ receptors were upregulated

by NO stimulation of cGMP *(139).* Other studies have noted the presence of calcium calmodulin-dependent protein kinase II (CaMKII) in inner retinal cells *(145)* and its regulation by glutamate acting through ionotropic receptors *(146).* Ca^{2+}-dependent intracellular regulatory mechanisms also produce differential effects on ionotropic GABA receptors. For instance, activation of PKC reduces $GABA_C$ receptor-activated current in rat retinal bipolar cells *(73).* In turtle retinal ganglion cells, Ca^{2+} released from intracellular stores suppresses $GABA_A$-induced currents through the intermediation of one or more Ca^{2+}-dependent enzymes *(147).* Interestingly, in these cells, a comparable decrease in GABA-evoked current was not observed when Ca^{2+} influx was activated during a sustained application of GABA, like the one observed in frog sensory neurons *(148),* indicating that the modulatory action of Ca^{2+} depended on the source of Ca^{2+}. A similar dependence of Ca^{2+}-induced regulatory processes on the source of $[Ca^{2+}]_i$ elevation was reported earlier in rat central neurons *(149,150),* and more recently in salamander retinal ON bipolar cells *(59).* These data clearly support a spatial compartmentalization of Ca^{2+} signaling, by which each Ca-dependent intracellular pathway is coupled to a specific fraction of the intracellular Ca^{2+} pool *(151).*

Suppression of $GABA_A$ currents in turtle, and $GABA_B$ currents in salamander retinal ganglion cells was mediated by calcium released from IP_3- and ryanodine-sensitive stores, and involved a Ca/CaM-dependent cascade. Thus, an underlying probable scenario is that metabotropic glutamate receptors linked to IP_3, stimulate a release of internal calcium *(48,52),* which subsequently inhibits the action of $GABA_A$ or $GABA_B$ receptors in retinal ganglion cells. The crosstalk between glutamate-dependent excitatory signals and GABA-mediated inhibitory signals must influence the balance of excitation and inhibition being processed by the ganglion cell.

As mentioned earlier, the excitatory component of the postsynaptic response is a mixture of AMPA and NMDA receptor-mediated currents. Akopian and Witkovsky *(130)* found that an increase in $[Ca^{2+}]_i$, reduced primarily the NMDA component of the light-evoked EPSCs in salamander retinal On-Off ganglion cells. Both Ca^{2+} influx through voltage-gated calcium channels and IP_3-mediated calcium release from stores were effective in reducing the NMDA-dependent component of the EPSC. Extrapolating to intact retina in which light-evoked changes in voltage occur, their data indicate that synaptic depolarizations are accompanied by an increased influx of Ca^{2+} through voltage-gated Ca channels and a release of Ca^{2+} from stores. The same depolarization will open Ca^{2+}-permeable NMDA channels *(129)* by reducing the voltage-dependent Mg block of these receptors. Elevation of $[Ca^{2+}]_i$ by these various means and the resultant inhibition of synaptic response serves as a negative feedback mechanism reducing the Ca^{2+} accumulation in postsynaptic cell caused by repetitive synaptic activity. Given the large contribution of NMDA receptors to ganglion cell EPSCs and the relatively slow kinetics of the NMDA-dependent response *(129),* its reduction by Ca^{2+} also will influence the kinetics of the ganglion cell's spiking pattern.

Summary and Conclusions

The data summarized in this report clearly indicate the indispensable nature and multiple roles of calcium in the functioning of every retinal cell class. Intracellular calcium is essential for phototransduction, for signal processing by ON bipolar cells and for neurotransmitter release. It interacts in various ways with signal transfer through gap junctions and at chemical synapses to influence the magnitude and kinetics of the postsynaptic response. Most all of the investigations we discussed concern relatively rapid events occurring on a time scale of seconds or less, but there are clear indications that calcium also is linked to slower processes, such as the turning on of specific genes. One suspects that in the day/night transition experienced

daily by the retina there are other important changes in calcium-dependent processes, and this remains an interesting possibility for future studies to explore.

Although the details vary from neuron to neuron, our survey clearly points out that intracellular [Ca^{2+}] is controlled through a large number of separate and quasi-independent mechanisms. Three basic pathways exist for increasing $[Ca^{2+}]_i$: calcium flux through voltage-gated channels, through ligand-gated channels, and by release from intracellular stores. Each of these mechanisms is subject to a variety of controls, which come both from within the cell and through the extracellular medium. Inside the cell, calcium transporters and exchangers either move Ca^{2+} against a gradient into compartments (endoplasmic reticulum and mitochondria) or extrude Ca^{2+} from the cell. In addition, a large number of Ca^{2+} buffers bind calcium. Some of these buffers appear just to hold calcium, whereas others, e.g., calmodulin, play an active role in neuronal physiology by binding to membrane proteins and influencing their activity. The cytoplasm contains a number of important Ca-dependent enzymes, including CaM kinase, calcineurin, PKC and NOS, and each month brings new reports of their neuromodulatory activities that set the functional state of retinal neurons. Outside the cell, transmembrane calcium flux is influenced strongly by pH, which is itself dependent on cellular metabolism, and by membrane surface charge. In addition, a host of regulatory molecules impinge on each neuron, interact with it through specific membrane receptors, and influence $[Ca^{2+}]_i$. We note receptors for amino acid transmitters, monoamines, catecholamines, peptides, and purinergic receptors.

In a mechanistic sense the properties of calcium permit tremendous flexibility of control in space and in time. The spatial dimension is provided by the compartmentalization of Ca^{2+} in the cytoplasm and the local control of $[Ca^{2+}]_i$ through the buffers and transporters/exchangers just mentioned. Thus, although the average $[Ca^{2+}]_i$ in the cytoplasm might be 100 n*M*, a local region such as the subsynaptic space may achieve a concentration 1000 × higher *(66).* In the temporal dimension, Ca^{2+} is acted on, and acts through multiple feedback and feedforward pathways, each having its own timescale. For example, somatostatin modulates photoreceptor I_{Ca} through a relatively rapid, membrane-delimited pathway, which can be turned on or off within a few ms *(20)* whereas, inhibition of L-type I_{Ca} in ganglion cells through a metabotropic glutamate receptor takes many seconds to turn on fully and also dissipates relatively slowly *(48).*

The calcium-dependent mechanisms work on multiple time scales. Acting simply as a divalent cation, calcium slows the rate of cation flux through channels and activates Ca-dependent K^+ and Cl^- channels. In the retina, the cyclic nucleotide-gated channels of photoreceptors and ON-bipolar cells, and the NMDA-gated channels of amacrine and ganglion cells are examples of channels that flux Ca^{2+}. Within the cell, calcium acts directly to gate transmitter release, probably by combining with a calcium sensor molecule such as synaptotagmin, a process that occurs on a submillisecond time scale. When bound to calmodulin, the Ca/calmodulin complex influences a number of membrane-bound molecules, including the cyclic-nucleotide gated channel, the N-type Ca channel, and possibly some neurotransmitter receptors as well and this modulatory influence is experienced within 1 s. Activation of Ca-dependent enzymes takes longer and persists longer, leading to neuromodulatory influences acting for seconds or minutes. Finally, the turning on and off of genes and the resultant production of new proteins takes many minutes to occur and may persist for hours.

Another measure of flexibility and local control is provided by the presence of multiple mechanisms having different sensitivities to Ca^{2+}. A possible example is low baseline rates of exocytosis vs the much higher rates initiated by strong depolarizations being dependent on Ca^{2+}-sensing proteins with different affinities for Ca^{2+}. It is worth noting that this is still a

hypothesis, pending identification and characterization of the proteins. In other systems, however, the balance between the phenomena of long-term potentiation vs long-term depression depends on the local $[Ca^{2+}]_i$. Similarly, the balance between activation of calcium-dependent kinases vs calcium-dependent phosphatases is a function of $[Ca^{2+}]_i$.

The presence of multiple Ca-dependent mechanisms within a single neuron poses a difficult problem for physiological analysis: to what degree do the various mechanisms function independently? Full independence would imply a tremendous degree of compartmentalization, with subpopulations of surface receptors coupled to very local pools of calcium and calcium-dependent proteins. It is more probable that the Ca-dependent mechanisms often interact. Whatever may be the final resolution of this question, the underlying theme of this essay is that the functional state of every retinal neuron is under continuous revision, a revision mediated by a large number of surface-membrane proteins coupled to intracellular machinery. Evidently, in the vertebrate retina, as in all parts of the nervous system, the local availability, mobility, charge, and binding properties of calcium are appropriate to its integration into a large number of surface membrane and intracellular neuromodulatory pathways.

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