Heterogeneous Intrinsic Firing Properties of Vertebrate Retinal Ganglion Cells

TOSHIHIDE TABATA¹ AND MASANOBU KANO²
¹Department of Cellular Neurophysiology, Graduate School of Medical Science, Kanazawa University, Ishikawa 920-8640; and ²Laboratory for Cellular Neurophysiology, Brain Science Institute, Institute of Physical and Chemical Research (RIKEN), Saitama 351-0198, Japan

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INTRODUCTION

Retinal ganglion cells (RGCs) are the output neurons of vertebrate retinae that carry visual information to the brain. RGCs are classified into several functional subtypes that integrate the presynaptic inputs of different types of light responsiveness in different manners. One of the best studied functional classifications is based on the linearity of the spatial summation of presynaptic inputs. Since the original work in the cat (Enroth-Cugell and Robson 1966), vertebrate RGCs clearly showing linear and nonlinear summation have been termed X(-like) and Y(-like) RGCs, respectively. Vertebrate RGCs showing light responsiveness unlike that of X and Y RGCs have been termed W(-like) RGCs (see Stone et al. 1979 for review). Differences in spatial summation and other modes of input integration confer each RGC subtype its characteristic firing pattern, which encodes specific aspects of visual information. For example, there are RGCs that show sustained firing to a step change in illumination and others that show transient firing (sustained and transient RGCs, respectively) (see, e.g., Cleland et al. 1973; Fukuda et al. 1984; Rodieck and Stone 1965; Saito 1983; Stone and Fukuda 1974; Werblin and Dowling 1969). These responses may efficiently encode relatively constant and abruptly changing light stimuli, respectively (Dacey 1994; Merigan and Maunsell 1993). In mammals, most sustained RGCs consist of X-like RGCs and a certain subset of W-like RGCs; most transient RGCs consist of Y-like RGCs and another subset of W-like RGCs (see Stone et al. 1979 for review). In goldfish, RGCs are also classified into sustained and transient subtypes although this classification cuts across the X/Y/W classification (Bilotta and Abramov 1989; Levine and Shefner 1979) (see DISCUSSION).

It has been thought that the sustained and transient light responses of RGCs are shaped depending primarily on the time courses and spatiotemporal interactions of the synaptic inputs. This notion is supported by several studies using in-situ retinal preparations. The basic shapes of both light responses appear to reflect the time courses of synaptic inputs from selective bipolar cells (see Awatramani and Slaughter 2000). Transient light response may be further shaped by transient excitatory synaptic inputs from amacrine cells or by the truncation of sustained excitatory synaptic inputs by amacrine cells (see Nirenberg and Meister 1997).

According to this idea, the intrinsic firing property of an RGC is assumed to play only a minor role in shaping its light response. In some retinal preparations, firing properties do not significantly differ among RGCs and therefore are thought to contribute little to the cell-to-cell distinction of the firing pattern of the light responses. In the turtle, most RGCs show sustained firing to current step stimuli regardless of their light response shapes (Baylor and Fettiplace 1979). In the tiger salamander, most RGCs linearly convert the intensity of the...
synaptic input into firing frequency without major temporal transformation (Diamond and Copenhagen 1995). However, some studies indicate that RGCs may have heterogeneous intrinsic firing properties that could aid the establishment of the light response distinction. In the tiger salamander, the sustained or transient light response of an RGC could be mimicked by light response distinction. In the tiger salamander, the sustained intrinsic transformation (Diamond and Copenhagen 1995). However, some studies indicate that RGCs may have heterogeneous intrinsic firing properties that could aid the establishment of the light response distinction. In the tiger salamander, the sustained or transient light response of an RGC could be mimicked by light response distinction. In the tiger salamander, the sustained intrinsic transformation (Diamond and Copenhagen 1995). However, some studies indicate that RGCs may have heterogeneous intrinsic firing properties that could aid the establishment of the light response distinction. In the tiger salamander, the sustained or transient light response of an RGC could be mimicked by light response distinction. In the tiger salamander, the sustained intrinsic transformation (Diamond and Copenhagen 1995).
venience and the relatively low $R_{\text{series}}$, thereby achieved. However, the “wash-out” of cytoplasmic molecules through a ruptured cell membrane may alter the neuronal firing pattern (Cuevas et al. 1997). To test whether such alteration occurs in goldfish RGCs, we compared the voltage responses recorded consecutively in perforated-patch and ruptured-patch configurations (Fig. 1A). In this experiment, a perforated-patch configuration was first established with amphotericin B in the pipette solution and later was switched to a ruptured-patch configuration (the cell membrane with negative air pressure). In the perforated-patch configuration, electrical access to the intracellular side was obtained without wash-out because amphotericin B formed ionophores in the cell membrane that permeated small ions but not the large cytoplasmic molecules necessary to maintain the normal functions of some ion channels (Horn and Marty 1988). In the cases of RGCs displaying transient firing in the perforated-patch configuration, firing became more sustained after membrane rupture ($n = 5$; Fig. 1A). This alteration cannot be ascribed to cell membrane deterioration caused by amphotericin B entering the cytoplasm because it occurred immediately after membrane rupture (typically within 30 s).

In contrast, if voltage responses were continuously recorded in the perforated-patch configuration, the firing pattern remained unchanged for 10–30 min ($n = 5$; Fig. 1B). To avoid the alterations shown in Fig. 1A, we decided to perform electrophysiological analyses in the perforated-patch configuration.

**Tonic and phasic RGCs**

We recorded the voltage responses to depolarizing current step stimuli in the perforated-patch configuration (104 RGCs; $C_m = 15.0 \pm 0.1 \text{ pF}$; $R_{\text{series}} = 57.0 \pm 0.3 \text{ M}\Omega$). The resting potential ($E_{\text{rest}}$) was not adjusted with background current unless otherwise stated. $E_{\text{rest}}$ did not shift by more than a few mV over 30 min (data not illustrated), suggesting that concentrations of major permeant ions were similar in the pipette solution and the cytoplasm.

![FIG. 1. The intrinsic firing property of retinal ganglion cells (RGCs) may be affected by cytoplasmic perfusion. A and B: sample voltage responses to step current stimuli. Dotted lines, initial resting membrane potential ($E_{\text{rest}}$) ($-70 \text{ mV in A}; -74 \text{ mV in B}$). Records were taken from 2 different RGCs. A: responses recorded consecutively in a perforated-patch configuration and in a ruptured-patch configuration (30 s after membrane rupture). Stimulus intensity was fixed at 90 pA. Only in this experiment was the pipette solution supplemented with 2 mM ATP. B: responses recorded at an interval of 10 min in the perforated-patch configuration. Stimulus intensity was fixed at 200 pA.](http://jn.physiology.org/)

We found a striking difference in firing accommodations among the RGCs (Fig. 2). Some of the RGCs displayed sustained spike trains that lasted throughout the stimulus period in a certain range of current stimulus intensity (Fig. 2A, middle two traces), like most RGCs of many other vertebrates (Baylor and Fettiplace 1979; Belgum et al. 1983; Diamond and Copenhagen 1995; Fohlmeister and Miller 1997; Lukasiewicz and Werblin 1988). At stimulus intensities above this range, the spike trains of these RGCs were gradually shortened with stimulus intensity (Fig. 2A, bottom trace). Similar stimulus intensity–dependent spike train shortening has been reported in tiger salamander RGCs (firing truncation) (Lukasiewicz and Werblin 1988). In contrast, in most of the remaining RGCs firing was always accommodated within a few hundreds of milliseconds of stimulus onset. This firing accommodation is qualitatively distinct from firing truncation in that it occurred both at lower and higher stimulus intensities (Fig. 2B); RGCs with firing accommodation never displayed sustained firing in response to current step stimuli, even when stimulus intensity was varied in very small increments (5 pA) from the threshold level (Fig. 3).

We quantitatively compared the firing accommodations of goldfish RGCs, using the duration of the most sustained spike train displayed by each RGC in response to 1-s current step stimuli ($D_{\text{max}}$) (Fig. 3A). To find the most sustained spike train, stimulus intensity was varied in 5-pA increments. The majority (84.6%) of the tested RGCs ($n = 84$) either fell into a group with $D_{\text{max}} < 200 \text{ ms}$ or one with $D_{\text{max}} > 800 \text{ ms}$ (Fig. 3B and...
first two spikes elicited by each current step (Fig. 4A) because this representation can efficiently describe transient responses consisting of only a few spikes (McCormick et al. 1985). When current stimulus intensity was normalized by $C_m$ (current density), cell-to-cell data deviation was small within each RGC group (Fig. 4, B and C). Tonic RGCs fired at very low current densities ($\sim 2$ pA/pF). In contrast, phasic RGCs fired only at higher current densities ($\geq 6$ pA/pF), as indicated by an “in-step” formed at the leftmost part of the F–I plot (Fig. 4C). This may result in truncation of the lower part of the dynamic range (the range of input intensity that could be effectively encoded into firing rate) of the phasic RGCs. We calculated the input resistance ($R_{input}$) at $E_{rest}$ from a steady-state shift in $E_{rest}$ caused by a 10-pA hyperpolarizing current step (Table 1). The mean $R_{input}$ of the phasic RGCs was significantly lower than that of the tonic RGCs. One possibility is that phasic RGCs (but not tonic RGCs) are equipped with an ion current that activates around $E_{rest}$ and effectively counteracts depolarizing stimuli (Figs. 8–11, see DISCUSSION).

We quantitatively compared the shape of the F–I plots of the tonic and phasic RGCs by empirically using sigmoid functions (four-parameter logistic functions) that are well fitted to these plots. The sigmoid function was defined as

$$F(I) = (a - d)[1 + (b/c)^{I}] + d$$

where $F$, $I$, $a$, $b$, $c$, and $d$ are firing frequency, current density, asymptotic minimum, slope parameter, inflection point, and asymptotic maximum, respectively ($d > a$; Fig. 4, B and C, smooth lines). The slope parameter characterizes the “sigmoidality” of the function. When the slope parameter is close to 1, the function lacks its instep. As the slope parameter increases (above 1), the function becomes more sigmoidal with a more acutely curved instep. The slope parameter of the fitted function was much larger for phasic RGCs than for tonic RGCs (Table 1), which confirms the difference in the F–I plot shape between these RGC groups.

**Input–output dynamics of tonic and phasic RGCs**

We further compared the intrinsic firing properties of tonic and phasic RGCs, using the firing frequency–stimulus intensity (F–I) relation, which is widely employed to characterize the input–output dynamics of functional RGC subtypes (see, e.g., Mobbs et al. 1992; Thibos and Werblin 1978). We represent firing frequency with an inverse of the interspike interval of the

<table>
<thead>
<tr>
<th>Tonic RGCs</th>
<th>Phasic RGCs</th>
<th>Difference</th>
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<tr>
<td>Percentage of total 84 RGCs</td>
<td>28.6 ($n = 24$)</td>
<td>56.0 ($n = 47$)</td>
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<tr>
<td>$E_{rest}$ (mV, mean ± SE)</td>
<td>$-64.9 ± 1.6 (n = 24)$</td>
<td>$-67.4 ± 1.2 (n = 47)$</td>
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<tr>
<td>$R_{input}$ (GΩ, median)</td>
<td>0.56 ($n = 18$)</td>
<td>0.29 ($n = 41$)</td>
</tr>
<tr>
<td>Slope parameter (mean ± SE)</td>
<td>$1.03 ± 0.14 (n = 20)$</td>
<td>$2.45 ± 0.34 (n = 30)$</td>
</tr>
<tr>
<td>$C_m$ (pF, median)</td>
<td>10.6 ($n = 24$)</td>
<td>14.2 ($n = 47$)</td>
</tr>
<tr>
<td>Cross-sectional soma area ($\mu m^2$, median)</td>
<td>218 ($n = 23$)</td>
<td>226 ($n = 44$)</td>
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Slope parameter is a parameter characterizing the shape of the sigmoid function fitted to the firing frequency–stimulus intensity (F–I) relation (see RESULTS for further explanation; cf. Fig. 4). $E_{rest}$, resting membrane potential; $R_{input}$, input resistance; $C_m$, membrane capacitance. * $P$ value obtained by unpaired t-test, $\dagger P$ values obtained by Wilcoxon/Kruskal-Wallis rank sum test.
contrast, the non- tonic, non- phasic RGCs (gray circles) are not concentrated at any point in the plot. The RGCs concentrated on the points in the $C_m$–$D_{max}$ dimension largely overlap those in the soma area–$D_{max}$ dimension (Fig. 5A), suggesting that both measurements describe the same morphological aspect of the RGCs.

To compare in detail the soma size distributions of the tonic and phasic RGCs, we re-plotted their soma areas into histograms (Fig. 5B). The majority of the phasic RGCs had larger values than did the tonic RGCs. Of the tonic RGCs, 60% were concentrated in a range of 100–250 $\mu$m$^2$ whereas 70% of the phasic RGCs were concentrated in a range of 200–500 $\mu$m$^2$ (Fig. 5B). Mean soma area as well as mean $C_m$ were significantly different between the tonic and phasic RGCs (Table 1). In addition, there were a few tonic RGCs that had exceptionally large soma areas (>650 $\mu$m$^2$; Fig. 5B), which corresponds to RGCs with $C_m \cong 34$ pF. These values exceeded those of the largest phasic RGC. It is noteworthy that larger RGCs survived better than did smaller RGCs in vitro (data not illustrated). Thus we might have underestimated the fractional population of smaller RGCs.

Possible ion current underlying firing accommodation

Mathematical models of vertebrate RGCs incorporating previously identified ion currents display sustained firing, but not transient firing, to current step stimuli (see, e.g., Fohlmeister and Miller 1997). This suggests that phasic goldfish RGCs might express an unidentified current which produces firing accommodation. We searched for such an ion current with perforated-patch current-clamp and perforated-patch voltage-clamp techniques.

In central neurons, firing accommodation could be produced by several classes of $K^+$ currents including 1) a low-threshold, noninactivating $K^+$ current with or without coupling to muscarinic acetylcholine receptor (AChR) (we hereafter term this class of currents $I_{K,ln}$); 2) a $Ca^{2+}$-activated $K^+$ current ($I_{K,Ca}$); and 3) a 4-aminopyridine (4AP)-sensitive, slowly inactivating $K^+$ current ($I_{K,4AP}$) (Brown et al. 1990; Del Negro and Chandler 1997; Storm 1990). We used antagonists preferring either of the $K^+$ currents to test for the involvement of these currents in producing firing accommodations in phasic RGCs (Fig. 6).

In the control bath solution, the current-evoked responses of the phasic RGCs did not alter for 10–30 min (Fig. 1B). $Ba^{2+}$, which is known to antagonize $I_{K,ln}$ (Rudy 1988), rapidly abolished firing accommodation at a concentration of 1 mM ($p < 0.05$, Student’s paired $t$-test, $n = 4$). Thus the effect of 1 mM $Ba^{2+}$ could be ascribed to a conductance decrease presumably caused by $I_{K,ln}$ blockade but not to a conductance increase caused by $Ba^{2+}$ influx through $Ca^{2+}$ channels. In contrast, total or partial replacement of $Ca^{2+}$ in a bath solution with $Co^{2+}$ ($n = 3$ for 2.5 mM, $n = 7$ for 2.4 mM), which has

FIG. 4. Firing frequency–stimulus intensity (F–I) relations of tonic and phasic RGCs. A: interval between the first two spikes evoked by a 1-s step current stimulus was measured at various stimulus intensities and was used to calculate firing frequency. F–I plots for 20 tonic (B) and 30 phasic (C) RGCs. Stimulus intensity is normalized by $C_m$ (current density). For clarity, data are binned for each 2-pA/pF band. Large dots and error bars: means ± SE. Small dots in B: raw data points. In the case of tonic RGCs, it was difficult to obtain data at stimulus intensities higher than 30 pA/pF because the tonic RGCs were readily damaged by such a strong stimulus. Smooth lines: sigmoid functions fitted to the data.
and I_K,Ca (Ishida 1991), did not abolish Co_2^+ phases, traces were justi-
mified close-up of the responses in the top panels.

Carbachol (an agonist for muscarinic and nicotinic AchR) (50–
4-aminopyridine (4AP; 1 mM) (F), or DIDS (1 mM) (F) abolished firing
accommodation. Ca_2^+ rings are not modi-
ied.

been reported to suppress depolarization-induced increase in
cyttoplasmic free calcium levels ([Ca_2^+]) (Ishida et al. 1991) and I_{K,CA} (Ishida 1991), did not abolish firing accommodation
(Fig. 6B). Also, apamin (1 μM) (Blatz and Magleby 1986) (Fig. 6C) and iberiotoxin (IbTx; 50 nM) (Galvez et al. 1990)
(Fig. 6D), specific blockers against major small- and large-
conductance Ca_2^+ -activated K_+ channels (SK and BK chan-
nels), respectively, did not abolish firing accommodation. To
confirm the potency of the batches of apamin and IbTx used in
the present study, we used cultured rat cerebellar Purkinje
neurons that express both SK and BK channels (unpublished
data). 4AP (40 μM—1 mM, n = 6, Fig. 6E) did not abolish firing
accommodation. These results suggest that, among the
three classes of K_+ currents, I_{K,Ino} is the most important for
producing firing accommodation in RGCs. Moreover, a current
underlying firing accommodation might not couple to musca-
rinic AChR because neither muscarine (30 μM, n = 5) or
carbachol (an agonist for muscarinic and nicotinic AChR) (50–
500 μM, n = 4) affected firing accommodation (data not
illustrated).

In addition, we tested for the involvement of an outwardly
toologically activating Cl^- current (I_{Cl}) identified in goldfish RGCs (Tabata
and Ishida 1999) because I_{Cl} shares nonactivating kinetics with I_{K,ln}. At a concentration of 1 mM, DIDS, a potent blocker
against I_{Cl} (Tabata and Ishida 1999), did not abolish firing
accommodation (n = 4) (Fig. 6F). Thus I_{Cl} might be less
important for producing firing accommodation.

**Contribution of Ba_2^+-sensitive current to intrinsic firing
property heterogeneity**

To examine the extent of the contribution of Ba_2^+-sensitive
current to the differences in firing property between tonic and
phasic RGCs, we measured the F–I relation of phasic RGCs in
the presence of 1 mM Ba_2^+ (Fig. 7) (n = 5). Under this
condition, the instep characteristic of the F–I plot for untreated
phasic RGCs (Fig. 4C) completely disappeared. The sigmoid
function originally generated for the F–I plot of untreated tonic
RGCs (Fig. 4B) was well fitted to that of Ba_2^+-treated phasic
RGCs (Fig. 7); the function was scaled along the y-axis but its
parameters were not modified to preserve the shape of the
original function). Thus Ba_2^+ treatment makes the input–
output dynamics of phasic RGCs indistinguishable from those
of tonic RGCs. This result suggests that the Ba_2^+-sensitive
current expressed in phasic RGCs may largely explain the
differences in firing properties between tonic and phasic RGCs.

**Activation kinetics of Ba_2^+-sensitive current**

We characterized the Ba_2^+-sensitive current under voltage-
clamp conditions in the perforated-patch configuration. The
results shown in Figs. 8–11 were obtained from large RGCs
with C_m of 15.3 ± 1.1 pF (n = 31; R_series = 33.3 ± 5.4 MΩ),
most of which were expected to be phasic, based on the C_m
distribution (Fig. 5A).

In the control bath solution containing Co_2^+ (2.4 mM), 4AP

![FIG. 6. Effects of various ion channel blockers on firing accommodation. A–F: sample voltage responses to 1-s step current stimuli before and during extracellular application of indicated drugs. In A, response after drug wash-out (Recovery) is also shown. Dotted lines: E_{rest} in control bath solution (−58 to −79 mV). Stimulus intensity was fixed at a certain level (40–210 pA) throughout each set of experiments. Records were taken from 6 different RGCs. A: Ba_2^+ (1 mM) abolishes firing accommodation reversibly. Inset: close-up of the responses in the top panels. To compare the depolarizing phases, traces were justified at the peak of the second spike (*). B–D: neither Co_2^+ (2.5 mM) (B), apamin (1 μM) (C), iberiotoxin (IbTx; 50 nM (D), 4-aminopyridine (4AP; 1 mM) (E), or DIDS (1 mM) (F) abolished firing accommodation. Ca_2^+ in the control bath solution was totally replaced with Co_2^+ to minimize change in membrane surface charge.

![FIG. 7. F–I relation of Ba_2^+-treated phasic RGCs. Plot of F–I relation measured in 5 phasic RGCs in the presence of 1 mM extracellular Ba_2^+. Firing frequency was measured as in Fig. 4. For clarity, data are binned for each 2-pA/pF band. Dots and error bars: mean ± SE. Smooth line: sigmoid function originally fitted to the F–I plot for 20 tonic RGCs (Fig. 4B). The function is scaled along the y-axis at a factor of 1.2 but the values character-
izing the parameters of the original function’s shape are not modified.
(1 mM), and TTX (1 µM), the total whole-cell current activated during a depolarizing test potential step was seen as an outward current (Fig. 8A, control). At the concentration at which it abolished firing accommodation (1 mM), extracellular Ba\(^{2+}\) selectively blocked two components of the total current that are demonstrated as differences between currents recorded before and during Ba\(^{2+}\) application (Fig. 8A, difference). One component was a "fast" current that was activated within a few msec and was inactivated within 200 ms (Fig. 8A, arrow). The other component was a "slow" current that became obvious following the decay of the fast current and was sustained throughout a 2-s test potential step without inactivation. The slow current also was distinguished from the fast current in its lower activation threshold. As shown by its I-V plot (Fig. 8B), the slow current was activated at potentials of \(-70\) mV and above. In contrast, the fast current was not activated even at a test potential as positive as \(-50\) mV (Fig. 8A, difference). These differences between inactivation and activation kinetics indicate that these two currents are carried by different ion channels. In the following analyses, we focus on the slow

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**FIG. 8.** Activation kinetics of barium-sensitive current (I\(_{\text{Ba-s}}\)). A: in this and in Figs. 9–11, I\(_{\text{Ba-s}}\) is extracted as the difference between whole-cell currents recorded under voltage clamp before (Control) and during (Ba\(^{2+}\)) extracellular application of 1 mM Ba\(^{2+}\). The control and test bath solutions contained 2.4 mM Co\(^{2+}\), 1 µM TTX, and 1 mM 4AP to reduce voltage-gated Ca\(^{2+}\) and Na\(^{+}\) currents and voltage-gated K\(^{+}\) currents, which might be less important for firing accommodation (cf. Fig. 6). Note that I\(_{\text{Ba-s}}\) (slow current) is preceded by a fast current (arrow) when the holding potential is set as negative as \(-90\) mV (see RESULTS for further explanation). Bottom schematics: time-courses of command potentials. For simplicity, only 4 traces are shown in “Difference.” B: steady-state I-V plot of I\(_{\text{Ba-s}}\). Steady-state current amplitude was measured as mean current amplitude during 0.9–1.0 s of a test potential step and normalized by \(C_m\) (current density). Dots and error bars: means ± SE. Data were taken from 6 RGCs. C: I\(_{\text{Ba-s}}\) elicited at indicated test potentials. Note that the fast current in A is absent when the holding potential is set at \(-65\) mV. Gray lines: single exponential functions fitted to the I\(_{\text{Ba-s}}\). Bottom schematics: time courses of command potentials. D: time constant of exponential functions fitted to I\(_{\text{Ba-s}}\) as in C plotted against test potential. Dots and error bars: means ± SE. Data were taken from 4 RGCs.

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**FIG. 9.** Carrier ion species and deactivation kinetics of I\(_{\text{Ba-s}}\). A–C: tail currents of I\(_{\text{Ba-s}}\) on repolarization from 0 mV (400 ms) to test potentials of \(-40\) to \(-110\) mV (increment, 10 mV; schematics in A). I\(_{\text{Ba-s}}\) was extracted by the subtraction method as in Fig. 8A. A: typical examples of tail currents recorded from one RGC (dotted lines). For simplicity, only the tail currents at 3 test potentials are shown. Smooth lines: single exponential functions fitted to the data. B: instantaneous I-V relation of tail currents measured at 3.5 ms of repolarization. Current amplitude is normalized by the value at a test potential of \(-80\) mV. Dots and error bars: means ± SE. Data were taken from 4 RGCs. The \(E_{\text{rev}}\) estimated by linear regression to the data (line) is \(-92\) mV and close to the potassium equilibrium potential (\(E_K\)) (\(-98\) mV). C: time constant of the fitted function plotted against test potential. Large dots and error bars: means ± SE. Small dot: raw data point. Smooth line: sigmoid function fitted by eye. Data were taken from 4 RGCs.
around the normal Erest (approximately -65 mV) (Table 1) and this value is close to the K
permeability of Na⁺ over K⁺ (PNa/PK) would be as small as 0.005 (Goldman-Hodgkin-Katz equation with ENa of 68 mV). Therefore, I_{Ba-s} is thought to be selectively carried by K⁺. The tail currents were well fitted by single exponential functions (Fig. 9A). The time constant of the fitted functions decreased with more hyperpolarized test potentials (Fig. 9C), suggesting that the deactivation of I_{Ba-s} is accelerated by hyperpolarization.

**Pharmacology of I_{Ba-s}**

We examined whether I_{Ba-s} is identical to any of the voltage-gated K⁺ currents with slow kinetics previously reported in vertebrate RGCs. Goldfish RGCs possess a tetraethylammonium (TEA)-resistant, voltage-gated K⁺ current with very slow inactivation kinetics (see Tabata and Ishida 1996, 1999). In goldfish RGCs preincubated with extracellular TEA (30 mM), additional application of 1 mM Ba²⁺ did not block a time-dependent current (Fig. 10A). Thus I_{Ba-s} is sensitive to TEA and is distinguished from the TEA-resistant, voltage-gated K⁺ current.

Vertebrate RGCs possess I_{K,Ca} (see, e.g., Lipton and Tauck 1987; Lukasiewicz and Werblin 1988; Rothe et al. 1999; Wang...
The actual contribution of $g_{Ba-s}$ to the total conductance depends on the voltage-gated Ca$^{2+}$ entry through voltage-gated Ca$^{2+}$ channels. However, SK channel did not appear to mediate $I_{Ba-s}$. We could extract $I_{Ba-s}$ in RGCs preincubated with the full-blocking concentration of apamin (1 $\mu$M) (Blatz and Magleby 1986) (Fig. 10B). Also, BK channel, the other major $I_{K,Ca}$-responsible channel, did not mediate $I_{Ba-s}$ because $I_{Ba-s}$ could be extracted in RGCs preincubated with the full-blocking concentration of IbTx (50 nM) (Galvez et al. 1990) (Fig. 10C). In addition, the steady-state densities of the apamin- and IbTx-sensitive components of the total current activated at a potential of 30 mV (mean across 0.9–1.0 s of the test potential step; 2.5 mM Ca$^{2+}$, Co$^{2+}$ was not included in the bath to allow Ca$^{2+}$ influx through the voltage-gated Ca$^{2+}$ channels) (Fig. 10, B and C) were 5.32 ± 4.29 pA/pF ($n=5$, data not illustrated).

Moreover, the total currents (including $I_{Ba-s}$) activated at test potentials from −110 to 30 mV were not reduced by muscarine (100 $\mu$M, $n=5$, data not illustrated).

### Contribution of $I_{Ba-s}$ to total membrane conductance

To understand how $I_{Ba-s}$ produces firing accommodation in phasic RGCs, we evaluated the relative contribution of $I_{Ba-s}$ to total membrane conductance (Fig. 11). First, we measured the steady-state $I$-$V$ relations of control current and $I_{Ba-s}$, as in Fig. 8. Next, we used these $I$-$V$ slopes to calculate the conductance of the control component ($g_{control}$) and that of the Ba$^{2+}$-sensitive component ($g_{Ba-s}$) (Fig. 11A). Finally, we plotted the relative contribution ($g_{Ba-s}/g_{control}$) as a function of test potential (Fig. 11B). In this experiment, we suppressed Ca$^{2+}$ currents with extracellular Co$^{2+}$ because repetitive depolarization might cause the cytoplasmic deposit of Ca$^{2+}$ and the gradual facilitation of $I_{K,Ca}$.

At test potentials between −90 and −30 mV, $g_{Ba-s}$ constitutes nearly half of $g_{control}$ (Fig. 11B). At more positive potentials, in contrast, $g_{Ba-s}$ constitutes only ~10% of $g_{control}$ (Fig. 11B). The actual contribution of $g_{Ba-s}$ to the total conductance in this range of $E_m$ might be smaller than this value because $I_{Ca}$ and $I_{K,Ca}$, which typically activate at potentials above ~45 mV (Bindokas and Ishida 1996; Tabata et al. 1996), were not included in $g_{control}$. These results suggest that $I_{Ba-s}$ may substantially affect subthreshold changes in $E_m$ but not the waveforms of individual spikes (see DISCUSSION).

### DISCUSSION

#### Heterogeneity of intrinsic firing properties in goldfish RGCs

In the perforated-patch, whole-cell configuration, many goldfish RGCs displayed phasic current-evoked responses (Figs. 2 and 3). This result contrasts with previous observations that most RGCs in other vertebrates display only tonic current-evoked responses (Baylor and Fettiplace 1979; Belgem et al. 1983; Diamond and Copenhagen 1995; Fohmleinter and Miller 1997; Lukasiewicz and Werblin 1988), except for some RGCs in the tiger salamander (Mobbs et al. 1992) and the immature rat (Barres et al. 1988). This discrepancy may be caused by species variation and/or differences in recording configuration. Some of the previous observations were made in ruptured-patch configurations. Under these recording conditions, cytoplasmic disturbance might alter the intrinsic firing properties, including firing accommodation (Fig. 1).

The majority of the goldfish RGCs examined in the perforated-patch configuration were classified into tonic and phasic subtypes based on maximal firing duration (Fig. 3). The tonic RGCs made little adaptation in firing frequency throughout current stimuli (Fig. 2). Tiger salamander RGCs with similar intrinsic firing properties show light responses whose temporal patterns directly reflect the time-course of the synaptic inputs (Diamond and Copenhagen 1995). Thus tonic goldfish RGCs may exhibit various temporal patterns of light response in vivo, depending on the synaptic inputs. Some studies using isolated retinae (Cohen 1998; Mobbs et al. 1992) showed that, in the tiger salamander and the cat, most RGCs receive depolarizing synaptic inputs that slowly decay during excitatory light stimuli. If this is also true of goldfish, tonic RGCs will exhibit sustained light responses. However, if some of the tonic RGCs are predominantly governed by fast decaying excitatory synaptic drives generated with the aid of amacrine cells (see Nirenberg and Meister 1997), they will exhibit transient light responses. In contrast, in phasic goldfish RGCs, firing accommodation may emphasize the transience of the light response, reducing an RGC’s excitability during prolonged excitatory synaptic inputs.

Do the differences in the intrinsic firing properties of tonic and phasic RGCs actually contribute to shaping the distinct light responses seen in vivo? Although there is no direct evidence of this, there are some observations that support this possibility. First, tonic and phasic goldfish RGCs had input–output dynamics similar to those of sustained and transient vertebrate RGCs, respectively. As shown by the instep of the F–I plots (Fig. 4), phasic goldfish RGCs had a higher threshold than did the tonic RGCs. A similar difference is seen in situ between the transient and sustained RGCs of the tiger salamander (Mobbs et al. 1992) and the mudpuppy (Thibos and Werblin 1978). Second, tonic and phasic goldfish RGCs had soma size distributions similar to those of sustained and transient goldfish RGCs, respectively. Goldfish RGCs are classified into on and off subtypes, which display relatively sustained responses to stepped illumination of a particular wavelength (typically red), and the on-off subtype, which displays a relatively transient response (see, e.g., Spekreijse et al. 1972). An intracellular recording/staining study (Vallerga and Djamgoz 1991) and an axonal conduction velocity measurement (Northmore and Oh 1998) suggested that on-off goldfish RGCs correspond to a morphological subtype with the largest somata (Cook et al. 1992; Hitchcock and Easter 1986). A few tonic RGCs with exceptionally large somata (Fig. 5) may correspond to off RGCs. These RGCs might also overlap Y-like RGCs to a large extent because off RGCs often are identified as Y-like RGCs (Bilotta and Abramov 1989). Moreover, the velocity measurement (Northmore and Oh 1998) suggests that the on subtype contains more small RGCs than does the on-off subtype. With respect to relative soma size (Fig. 5; Table 1), tonic and phasic RGCs might partly correspond to the on and the on-off RGCs, respectively. In addition, most on and on-off RGCs are identified as W- and Y-like...
RGCs (Bilotta and Abramov 1989). Assuming that the morphological correlation of X/Y/W RGCs in the cat (cat X, Y, and W RGCs have medium-sized, large, and small somata, respectively; see Stone et al. 1979 for review) is applicable to goldfish, the main components of tonic and phasic RGCs might be W- and Y-like RGCs, respectively.

Taken together, the results of the present current-clamp study demonstrate that goldfish RGCs have heterogeneous intrinsic firing properties that may be consonant with the temporal patterns of light responses.

Identification and possible function of IBa-s

Under voltage clamp conditions, we extracted a Ba\(^{2+}\)-sensitive, voltage-dependent K\(^+\) current in isolated goldfish RGCs as a difference between currents recorded before and after external Ba\(^{2+}\) application (IBa-s) in isolated goldfish RGCs. IBa-s had a low activation threshold negative to \(-70\) mV, was activated and deactivated slowly with time constants of 10–100 ms, and was not inactivated during depolarization for as long as 2 s (Figs. 8 and 9).

There are at least two general possibilities concerning the molecular nature of IBa-s. One is that IBa-s is a new current mediated by a K\(^+\) channel(s) highly sensitive to Ba\(^{2+}\). Another possibility is that IBa-s reflects a time-dependent reduction of a voltage-gated K\(^+\) current(s) caused by Ba\(^{2+}\)-induced K\(^+\) channel modulation such as facilitation of inactivation. At present, a biological toxin that selectively blocks a specific channel(s) responsible for IBa-s has not been found. Positive identification of the molecular nature of IBa-s has yet to be made by single-channel recordings and molecular analyses of the putative channel protein. However, several lines of evidence obtained in this and previous studies support the former possibility. IBa-s was resistant to 4AP and Co\(^{2+}\) (Fig. 8) but was completely blocked by TEA (Fig. 10). Therefore, if the latter possibility were the case, IBa-s might be derived from a 4AP/Co\(^{2+}\)-resistant, TEA-sensitive, voltage-gated K\(^+\) current(s), which mainly consists of a delayed rectifier K\(^+\) current(s) (IK,V) in vertebrate RGCs (see Ishida 1995 for review). Contrary to this expectation, IBa-s has a much more negative activation threshold than those IK,V’s (above \(-55\) mV) (see, e.g., Lipton and Tauck 1987; Lukasiewicz and Werblin 1988; see Ishida 1995 for review). Moreover, IK,V is generally known as a primary ionic mechanism that forms the repolarizing phase of a spike (Storm 1990). Thus Ba\(^{2+}\) would hamper the repolarizing phase of a spike, assuming the second possibility, whereas Ba\(^{2+}\) did not reduce or slow the repolarizing phase in goldfish RGCs (n = 5) (Fig. 6A, inset). In addition, closely related retinal cells (photoreceptors) possess a Ba\(^{2+}\)-sensitive K\(^+\) current that may be mediated by a specific channel (see the following paragraphs). We compare in detail the basic properties of IBa-s with those of various K\(^+\) currents with slow kinetics.

Vertebrate RGCs possess IK,Ca as well as IK,V (see Ishida 1995 for review). IK,V constitutes the major part of depolarization-activated K\(^+\) currents in vertebrate RGCs (see, e.g., Lukasiewicz and Werblin 1988; Sucher and Lipton 1992). Therefore, the kinetics of the Ba\(^{2+}\)-resistant current measured in the present study (Fig. 8A) may reflect those of IK,V. IBa-s appears to differ from IK,V because IBa-s showed much more slow activation than did the Ba\(^{2+}\)-resistant current. IBa-s shares voltage sensitivity with IK,Ca mediated by BK channel (see Sah 1996 for review). However, IBa-s differs from BK channel-mediated current in its resistance to IbTx (Fig. 10). In addition, IBa-s differs from IK,Ca mediated by amput-sensitive SK channels (see Sah 1996 for review) in its resistance to amput (Fig. 10). Recent studies (Hirschberg et al. 1998; Kohler et al. 1996) show that mammalian central neurons express an amput-sensitive SK channel (SK1). Moreover, mammalian neurons possess an amput-insensitive IK,Ca that is probably mediated by another channel. However, IBa-s may also differ from these two amput-insensitive currents because the activation of IBa-s is voltage-dependent (Fig. 8), unlike these currents (Hirschberg et al. 1998; Sah 1996). In the present study, we measured IBa-s using a bath solution containing 2.4 mM Co\(^{2+}\) and 0.1 mM Ca\(^{2+}\) (Figs. 8, 9, and 10A). Under this condition, the [Ca\(^{2+}\)] of goldfish RGCs is fixed to the resting level, even when E\(_m\) is depolarized (\(-120\) mV) (Ishida et al. 1991). Thus the voltage-dependence of IBa-s is not an artifact produced by an increase in [Ca\(^{2+}\)] caused by Ca\(^{2+}\)-influx through voltage-gated Ca\(^{2+}\) channels or by Ca\(^{2+}\)-induced Ca\(^{2+}\) release from the intracellular store associated with such Ca\(^{2+}\) influx. Taken together, the data show that IBa-s may differ from major IK,Ca’s, although we do not exclude the possibility that IBa-s belongs to a previously unidentified class of IK,Ca.

In other cell types, there are several classes of low-threshold, noninactivating K\(^+\) currents (IK,ln) (including S current in Aplysia sensory neurons (IS) (Siegelbaum et al. 1982), standing outward current in rat cerebellar granule cells [IK(SSO)] (Watkins and Mathie 1996), muscarinic-sensitive current in vertebrate neurons (IM) (Brown and Adams 1980), and IK,ln’s that kinetically resemble IM but lack muscarinic sensitivity. IBa-s differs from IK,SSO either in pharmacological properties or kinetics. IS is resistant to 10 mM Ba\(^{2+}\) (Shuster and Siegelbaum 1987). IK,SSO is rapidly activated and deactivated with time constants of sub-millisecond (Watkins and Mathie 1996). IBa-s more resembles IM, sharing susceptibilities to 10–3 M of Ba\(^{2+}\) and 10–2 M of TEA, resistance to 10–3 M of 4AP, and slow activation and deactivation kinetics (time constants, 10–100 ms) (Adams et al. 1982a,b). However, IBa-s was not down-regulated by muscarinic agonists (data not illustrated), unlike IM (Brown and Adams 1980), although vertebrate RGCs express muscarinic AChRs (see Fischer et al. 1998). IBa-s also differs from IM in its lower activation threshold (Adams et al. 1982a). With respect to activation threshold, IBa-s resembles a muscarinic-insensitive IK,ln found in smooth muscle cells (Evans et al. 1996). Recently, a muscarinic-insensitive IK,ln was found in closely related retinal cells (IK,S in salamander rod photoreceptors) (Beech and Barnes 1989; Wollmuth 1994). Therefore, one possibility is that IBa-s forms a new class of IK,ln with IK,S. Further identification of IBa-s would be established by molecular comparisons between IBa-s channels and previously cloned IK,ln channels such as ether à go-go (Hoshi et al. 1998; Warmke et al. 1991) and aK5.1 (Zhao et al. 1994).

At the concentration at which it selectively blocked IBa-s (1 mM), external Ba\(^{2+}\) abolished firing accommodation in phasic RGCs (Fig. 6). Thus IBa-s is an ionic mechanism sufficient to explain the firing accommodation. In an analogy of IK, a K\(^+\) current kinetically resembling IBa-s (see the previous paragraph) (Brown et al. 1990; McCormick 1990), a possible action of IBa-s is depicted as follows. When a
goldfish RGC rests at a potential of approximately −65 mV (Table 1), the membrane conductance largely consists of leak K⁺ and Na⁺ currents (Tabata and Ishida 1996, 1999) and I_{Ba-s} (Figs. 8 and 11). At the onset of a depolarizing current step stimulus, E_m is readily depolarized because an opposing electrical force against the depolarizing stimulus, caused by the resting membrane conductance, is relatively small. E_m rapidly reaches the spike threshold (approximately −45 mV) (Ishida 1991). Following the repolarizing phase of each spike, the current stimulus again depolarizes E_m toward the spike threshold. The activation level of I_{Ba-s} may be slightly increased by depolarization during each spike (Fig. 8). This additional activation can be temporarily summated because of I_{Ba-s}'s slowly deactivating property (Fig. 9). This summation causes a gradual increase in I_{Ba-s}'-mediated K⁺ conductance through repetitive firing. Therefore, at the late period of a current step stimulus, the electrical force opposing the depolarizing stimulus should be greatly increased. With this increased opposing force, E_m becomes more resistant to depolarize and stays at subthreshold levels for a longer time. This prolonged subthreshold depolarization causes failure of spike firing by hampering the disactivation of, and by facilitating the inactivation of, voltage-gated Na⁺ channels (McCormick 1990). The involvement of I_{Ba-s} in the prolongation of subthreshold depolarization is indicated by the accelerated subthreshold depolarization seen after Ba²⁺ application (n = 5) (Fig. 6A, inset; note a Ba²⁺-induced change in the time-course after the asterisked spikes).

Voltage-gated K⁺ currents other than I_{Ba-s} and major I_{K,CA}'s appeared to be less important for firing accommodation than did I_{Ba-s} (Fig. 6). Total conductance recorded in the absence of external Ba²⁺ shows a drastic increase at potentials more than approximately −50 mV (Fig. 11), indicating that the majority of Ba²⁺-resistant K⁺ currents have relatively high activation thresholds as compared with I_{Ba-s}. In addition, I_{K,V}, the primary component of voltage-gated K⁺ currents in vertebrate RGCs (see, e.g., Lukasiewicz and Werblin 1988; Sucher and Lipton 1992) is deactivated within a few msec of the cessation of depolarization. These kinetic properties may prevent these voltage-gated K⁺ currents from cooperating with I_{Ba-s} to form an electrical force opposing depolarizing current stimuli at subthreshold potentials. In some neurons, I_{K,CA}'s (particularly those mediated by SK channels) are gradually activated by Ca²⁺, which enters during repetitive firing, and modulate the firing pattern in a time-dependent manner (Brown et al. 1990; McCormick 1990). However, potent blockers of I_{K,CA}'s did not abolish firing accommodation in goldfish RGCs (Fig. 6). The mean densities of the apamin- and IbTx-sensitive currents (at test potentials of 30 mV, 5.32 ± 4.29 and 7.46 ± 2.87 pA/pF, respectively; see results) were less than half that of I_{Ba-s} (15.73 ± 4.25 pA/pF) (Fig. 8). Thus the functional contribution of I_{K,CA}'s may be relatively smaller than that of I_{Ba-s} in goldfish RGCs.

The F-I relation of tonic RGCs was indistinguishable from that of phasic RGCs whose I_{Ba-s} was blocked with Ba²⁺ (Fig. 7). This suggests that the functional contribution of I_{Ba-s} is negligible in tonic RGCs. Therefore, I_{Ba-s} may be the primary factor producing the heterogeneity in intrinsic firing property between tonic and phasic RGCs.

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