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cGMP modulates spike responses of retinal ganglion cells *via* a cGMP-gated current

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Abstract

Certain ganglion cells in the mammalian retina are known to express a cGMP-gated cation channel. We found that a cGMP-gated current modulates spike responses of the ganglion cells in mammalian retinal slice preparation. In such cells under current clamp, bath application of the membrane-permeant cGMP analog (8-bromo-cGMP, 8-*p*-chlorophenylthio-cGMP) or a nitric oxide donor (sodium nitroprusside, S-nitroso-N-acetyl-penicillamine) depolarized the membrane potential by 5–15 mV, and reduced the amount of current needed to evoke action potentials. Similar effects were observed when the membrane potential was simply depolarized by steady current. The responses to cGMP are unaffected by inhibitors of cGMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase. The response to cGMP persisted in Ca^{2+}-free bath solution with Ca^{2+} buffers in the pipette. Under voltage clamp, cGMP analogs did not affect the response kinetics of voltage-gated currents. We conclude that cGMP modulates ganglion cell spiking simply by depolarizing the membrane potential *via* the inward current through the cGMP-gated channel. Modulation of this channel *via* the long-range NO-synthase amacrine cell may contribute to control of contrast gain by peripheral mechanisms.

Keywords: Retina, Ganglion cell, Guinea pig, Patch clamp, cGMP-gated channel

Introduction

Certain ganglion cells in mammalian retina express a cGMP-gated cation channel (Ahmad et al., 1994; Kawai & Sterling, 1999). This channel is modulated when nitric oxide (NO) released from amacrine cells (Sandell, 1985; Vincent & Kimura, 1992; Haberecht et al., 1998) stimulates the ganglion cell's soluble guanylyl cyclase (Ahmad & Barnstable 1993; Gotzes et al., 1998; Kawai & Sterling, 1999). The effect of current through this channel had not been studied and was complicated to address because cGMP can have several effects. For example, cGMP can modulate voltage-gated currents directly (Levitan & Levitan, 1988) and *via* cGMP-dependent protein kinase (PKG) (Paupardin-Tritsch et al., 1986; Sumii & Sperelakis, 1995; Lohmann et al., 1997; Wei et al., 1998). Here we investigated the effect of current through the cGMP-gated channel on ganglion cell responses, isolating this effect from that of the PKG cascade and from the effect of Ca^{2+} entering *via* the cGMP-gated channel on voltage-gated currents.

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Methods

Preparation and recording

Slices from adult guinea pig retina were cut at 200 μm (Kawai & Sterling, 1999) and viewed on a Zeiss upright microscope with differential interference contrast optics (40× water-immersion objective). Ganglion cells were identified in the slice by their position and size. Membrane voltages and currents were recorded in the whole-cell configuration using a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Foster City, CA) linked to a computer. The current- and voltage-clamp procedures were controlled by the pCLAMP software (Axon Instruments). Data were low-pass filtered (4-pole Bessel type) with a cut-off frequency of 5 kHz and then digitized at 10 kHz by an analog-to-digital interface. All experiments were performed at room temperature (23–25°C).

Solutions and drugs

In most experiments, the tissue was perfused with Ames medium (which is buffered with 22.6 mM bicarbonate), bubbled with 95% O_2/5% CO_2. In some experiments, Ringer's solution was used instead of Ames' solution to eliminate Ca^{2+} in the bath. The Ringer's solution contained (in mM) 135 NaCl; 5 KCl; 10 HEPES;

and 15 glucose. The solution was adjusted with NaOH to pH 7.4. $CdCl_2$ (200 μM), picrotoxin (100 μM), and strychnine (1 μM) were also added to block synaptic transmission. The recording patch pipette contained (in mM): 140 KCl; 1 $CaCl_2$; 5 EGTA or BAPTA; 10 HEPES; and 2 Mg-ATP. The solution was adjusted with KOH to pH 7.4. Pipette resistance was about 7 MΩ.

Test substances were applied either through the bath [8-bromo-cGMP, 8-*p*-chlorophenylthio-cGMP, 8-*p*-chlorophenylthio-cAMP, sodium nitroprusside (SNP), or S-nitroso-N-acetyl-penicillamine (SNAP)], *via* the patch pipette (KT5823, or KN-62). KT5823, KN-62, and S-nitroso-N-acetyl-penicillamine were purchased from Calbiochem (San Diego, CA). Other chemicals were from Sigma (St. Louis, MO).

Results

Two spike patterns to current injection

Recordings in control solution with synaptic input blocked ($CdCl_2$, 200 μM + picrotoxin, 100 μM + strychnine, 1 μM) showed two types of firing. In some cells, small current injections (30 and 100 pA) evoked sustained firing, and larger currents (140 pA) gave initial spiking that was ultimately blocked—presumably by inactivation of Na^+ channels at sustained depolarizations above −40 mV (Figs. 1A–1C). In other cells, even small current injections evoked phasic spiking that soon inactivated (Figs. 1D–1F). About three-quarters of the cells (52/70) that we recorded in adult retina showed the tonic spiking; and the rest (18/70) were phasic. This resembles previous observations on cells in the ganglion cell layer of adult rat (Wang et al., 1997).

Effects of the cGMP-gated current on spike responses

Under voltage clamp, we established that certain cells in the guinea pig retina express a cGMP-gated current. When membrane-permeant analogs of cGMP (1 mM 8-bromo-cGMP or 250 μM 8-*p*-chlorophenylthio-cGMP = pCPT-cGMP) were bath applied to a cell voltage clamped at −50 mV, there was a sustained inward current of approximately 200 pA (10/24 cells; data not shown). This matches previous observations in rat retina (Ahmad et al., 1994; Kawai & Sterling, 1999).

Under current clamp, cGMP analogs increased the spike rate. Fig. 2A shows a tonic cell that was depolarized by 15 mV in response to 250 μM pCPT-cGMP. This raised the spike rate to 45 spikes/s (Fig. 2B). Nearly half of the tonic cells (9/21) responded similarly. Bath application of 1 mM 8-bromo-cGMP (3/6 cells), 1 mM sodium nitroprusside (SNP; 4/7 cells), and S-nitroso-N-acetyl-penicillamine (SNAP; 2/5 cells) produced similar effects. However, 250 μM pCPT-c**A**MP was ineffective (0/6 cells). Fig. 2C shows a phasic cell that was depolarized by ~10 mV in response

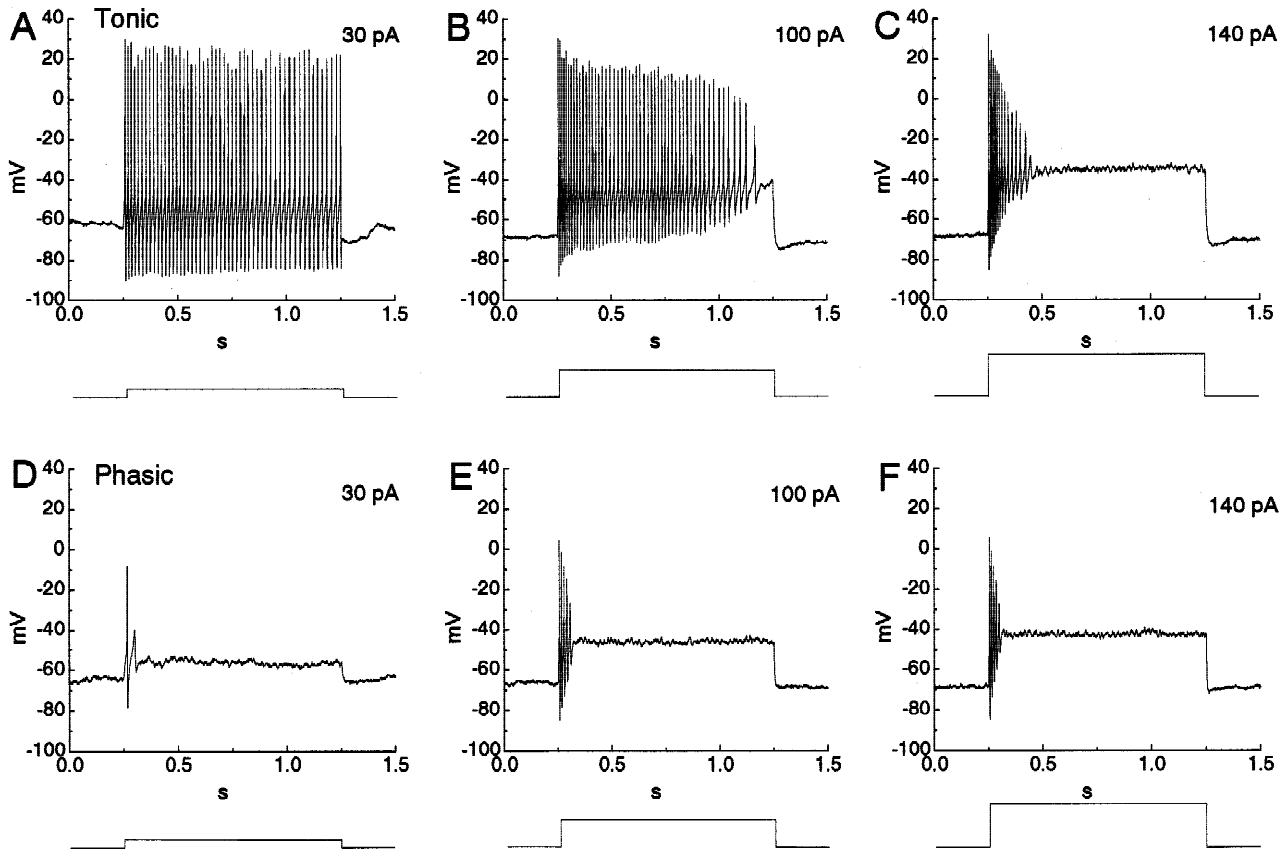

Fig. 1. Action potentials induced by the current injection in the control solution. (A–C) Spike responses of one cell to current injection, respectively, of 30 pA, 100 pA, and 140 pA for 1 s between 0.25 s and 1.25 s. Note that the cell exhibits a tonic firing pattern. (D–F) Different cell shows phasic firing. Spike responses to current injection, respectively, of 30 pA, 100 pA, and 140 pA for 1 s between 0.25 s and 1.25 s.

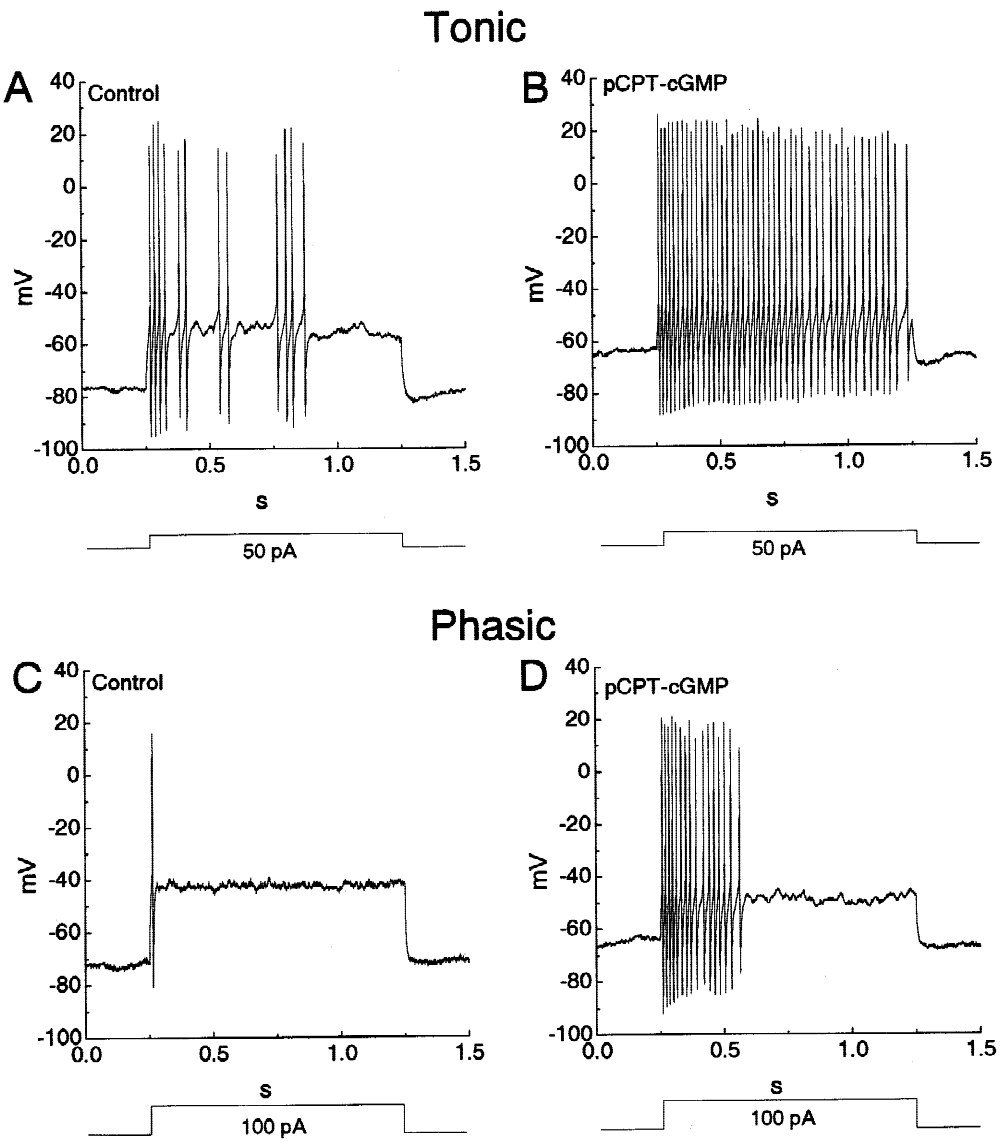

Fig. 2. Bath application of pCPT-cGMP depolarized the resting potentials and increased the spike rates. (A,B) Spike responses of a tonic cell to current injection of 50 pA in the control solution (A) and the solution containing 250 μM pCPT-cGMP (B). Current was injected for 1 s between 0.25 s and 1.25 s. (C,D) Spike responses of a phasic cell to current injection of 100 pA in the control solution (C) and the solution containing 250 μM pCPT-cGMP (D).

to 250 μM pCPT-cGMP. This increased the spike rate to 16 spikes/s (Fig. 2D; 3/7 cells).

cGMP also altered the relationship between injected current and spike rate. Application of pCPT-cGMP (250 μM) to tonic cells markedly lowered the amount of current needed to evoke spikes (Fig. 3A). Furthermore, current injections between 10 and 40 pA increased spike rate almost linearly, the effect peaking at 70 pA (50 spikes/s). Larger current injections (80–140 pA) decreased spike rate. The effect of pCPT-cGMP on reducing the current needed for spikes was evident during the first 100 ms, and shifted the dynamic range of spike rate (Fig. 3B). The currents needed to evoke spikes under the control and pCPT-cGMP conditions were 40 pA and 10 pA, respectively. Spike rates under both conditions increased almost monotonically between 10 pA and 140 pA. A similar result was obtained in nine cells.

pCPT-cGMP also lowered the thresholds of a phasic cell and increased the dynamic range. For example, the current threshold during 1-s current injection was 100 pA under the control and 20 pA under pCPT-cGMP (Fig. 3C). Spike rate under the control condition increased monotonically between 100 pA and 150 pA; whereas under the pCPT-cGMP condition it increased between 20 pA and 40 pA, decreasing between 90 pA and 140 pA. The maximum spike rates were 3 spikes/s (at 140 pA) under the control and 22 spikes/s (at 40 pA) under pCPT-cGMP conditions (Fig. 3C). The current thresholds during the first 100 ms under the control and pCPT-cGMP conditions were 100 pA and 30 pA, respectively (Fig. 3D). Spike rates under both conditions increased almost monotonically. The maximum spike rates under the control and pCPT-cGMP conditions were 30 spikes/s (at 140 pA) and 89 spikes/s (at 120 pA), respectively. A similar result was obtained in three cells.

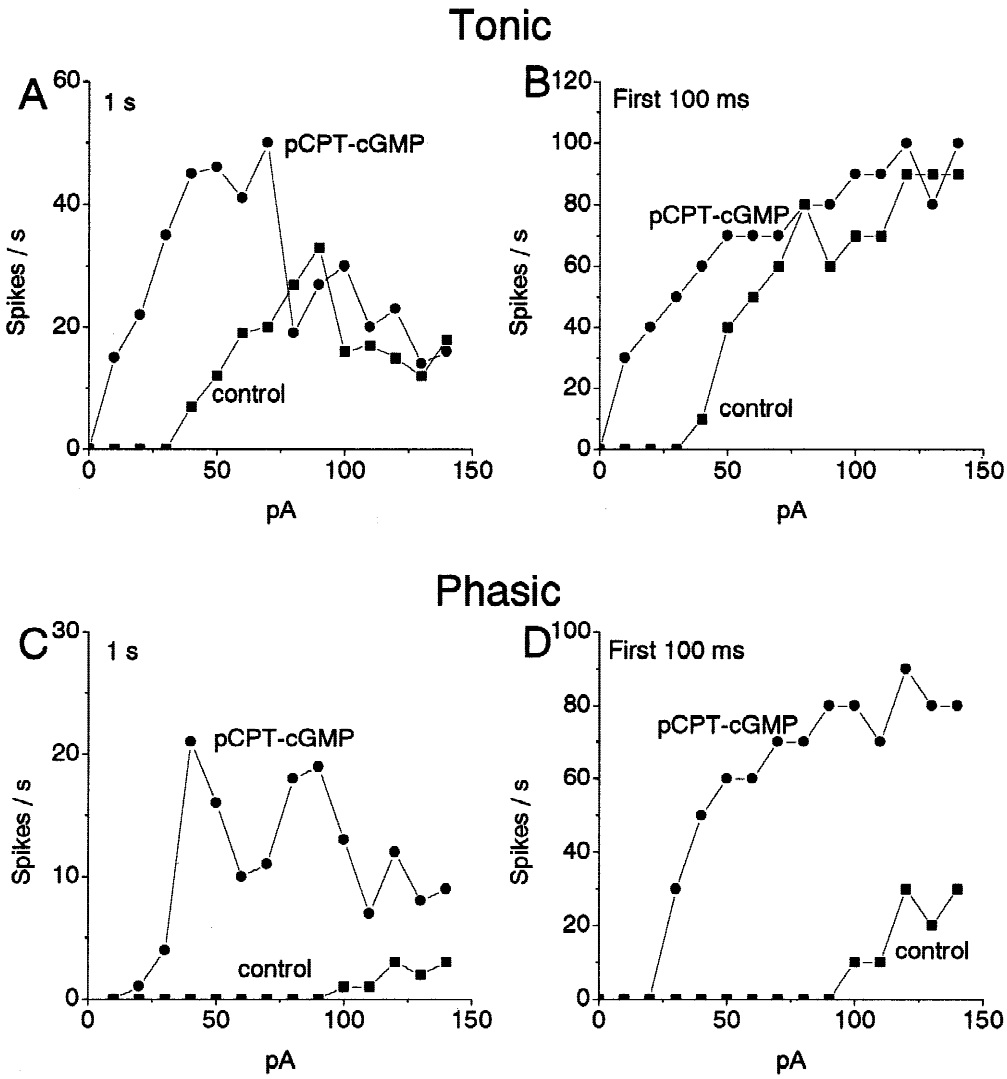

Fig. 3. Effects of pCPT-cGMP on the spike rates. (A,B) Stimulus–response relationships recorded from a tonic cell in the control solution (filled square) and the solution containing 250 μM pCPT-cGMP (filled circle). (C,D) Stimulus–response relationships recorded from a phasic cell in the control solution (filled square) and the solution containing 250 μM pCPT-cGMP (filled circle).

Effects of steady current injection on spike responses

To investigate the mechanism underlying spike modulation by cGMP, we analyzed the effects of resting potential on the spike rate, because cGMP analogs depolarized the resting potentials of ganglion cells (Fig. 2). Fig. 4A shows the spike response to current injection recorded from the same cell as in Figs. 2A and 2B. In the control solution, by injecting a steady current to the cell, the resting membrane potential was depolarized to the same voltage (approximately −65 mV) as that in Fig. 2B. During a 1-s step current injection of 50 pA, we observed 42 spikes (Fig. 4A), similar to the number of spikes (44 spikes) at 50 pA under the pCPT-cGMP condition (Fig. 2B), however much larger than the number (12 spikes) under the control condition (Fig. 2A). Fig. 4B shows effects of the resting potential on the mean spike rate in the control solution. As the resting potential was depolarized by injecting steady current, the spike rate was increased. This suggests that the steady membrane depolarization increases the response to the step depolarization.

The stimulus–response relationship under the steady current injection was quite similar to that under application of pCPT-cGMP. The steady current injection markedly lowered the mean current threshold and shifted the dynamic range of spike rate during both 1 s (Fig. 4C) and the first 100 ms (Fig. 4D). The mean current threshold under the steady current injection was 10 pA (filled triangle in Figs. 4C and 4D), which was identical with that (10 pA) under application of pCPT-cGMP (filled circle in Figs. 4C and 4D). In Fig. 4C, the mean maximum spike rates were similar under the steady current injection and under the pCPT-cGMP condition. In addition, the maximum rates were also quite similar during the first 100-ms injection under the steady current injection and under the pCPT-cGMP condition (Fig. 4D). These results suggest that pCPT-cGMP modulates spike rate by depolarizing the resting potential *via* the cGMP-gated inward current.

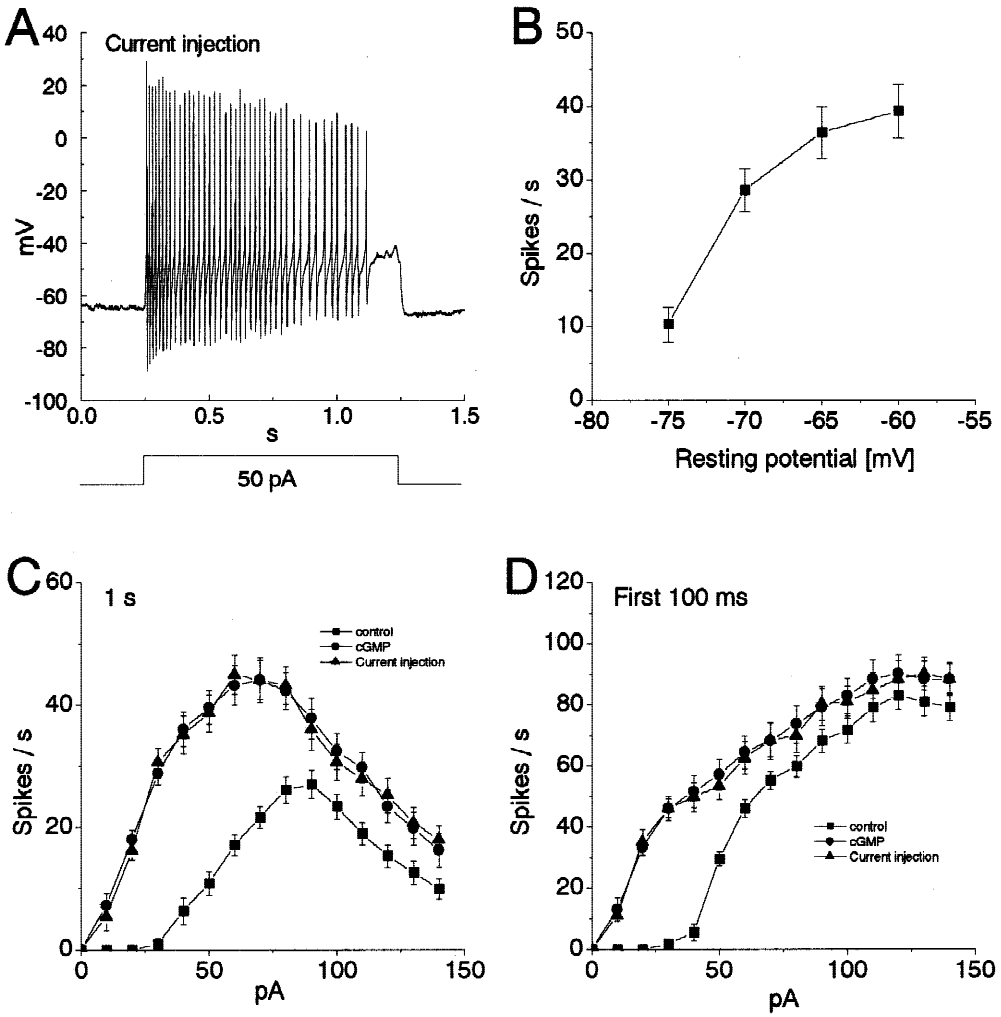

Fig. 4. Effects of steady current injection on the spike rates in tonic cells. (A) Spike responses, recorded from the same cell as in Figs. 2(A,B), to the step current injection of 50 pA in the control solution. The resting potentials were depolarized by 14 mV by injecting the steady current of +25 pA. The step current was injected for 1 s between 0.25 s and 1.25 s. (B) Effect of the resting potential on the mean spike rate in the control solution. The resting potential of each cell was adjusted to −75 mV, −70 mV, −65 mV, and −60 mV by injecting the steady current of various intensities. The step current of 50 pA was injected for 1 s. Each symbol represents mean of four cells. (C,D) Mean stimulus–response relationships in the control solution (filled square, filled triangle) and the solution containing 250 μM pCPT-cGMP (filled circle). Filled triangle and square show the spike rate with and without the steady current injection. Each symbol represents mean of five cells.

Effects of cGMP on voltage-gated currents

cGMP is known to modulate voltage-gated currents in other preparations directly (Levitan & Levitan, 1988) or through cGMP-dependent protein kinases (PKG; Paupardin-Tritsch et al., 1986; Sumii & Sperelakis, 1995; Lohmann et al., 1997; Wei et al., 1998). This may raise the possibility that pCPT-cGMP changed spike responses of ganglion cells by modulating their voltage-gated currents rather than by depolarizing their membrane potentials. To examine this possibility, we first recorded the voltage-gated currents in the presence and absence of pCPT-cGMP under the voltage clamp.

In the control solution, depolarizing step pulses from the holding potential of −100 mV induced time- and voltage-dependent currents (Fig. 5A). At step voltages between −90 mV and +40 mV, current responses consisted of a transient inward current and a sustained outward current. pCPT-cGMP (250 μM) did not change significantly the kinetics of either the inward or outward current (Fig. 5B). Fig. 5C show I–V relationships of the transient inward current and the sustained outward current. pCPT-cGMP did not change markedly either I–V curve. A similar result was obtained in five cells. This suggests that cGMP did not significantly change the voltage-gated currents in ganglion cells.

To test whether PKG affects the spike responses evoked by cGMP, we applied a specific inhibitor in the recording pipette (10 μM KT5823; Fig. 6; Kase, 1988; Wu et al., 1998). Bath application of 250 μM pCPT-cGMP still depolarized the resting potential by approximately 10 mV, and increased markedly the spike rates (Fig. 6). A similar result was obtained in four cells. Apparently cGMP's modulation of spike responses does not require PKG.

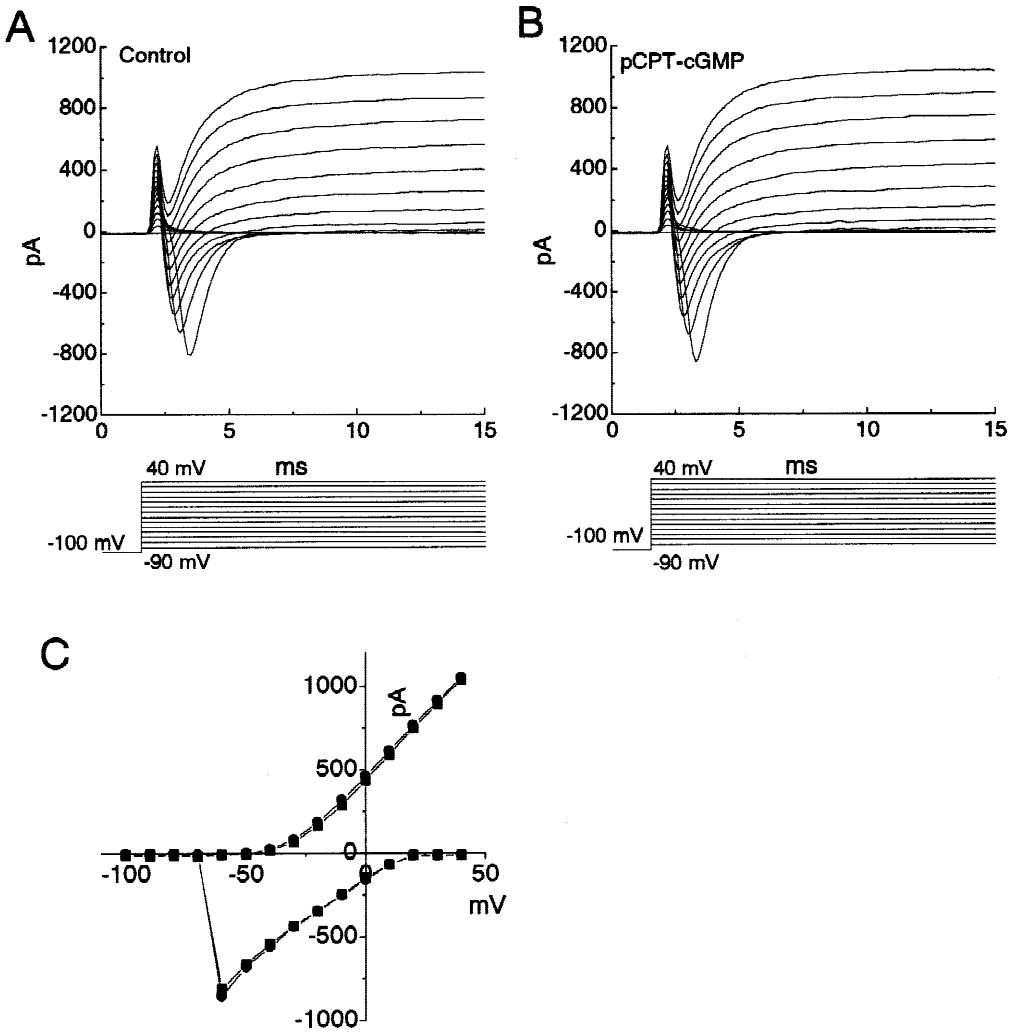

Fig. 5. Effects of pCPT-cGMP on the voltage-gated currents. (A,B) Membrane currents induced by depolarization from the holding voltage (−100 mV) in the control solution (A) and the solution containing 250 μM pCPT-cGMP (B). Command voltages were increased in 10-mV steps from −90 mV to +40 mV. To inhibit the sustained inward current through the cGMP-gated channel, 3 mM Cd^{2+} was added to the bath solution. (C) Peak amplitudes of inward and outward currents were measured from (A,B) and plotted against the command voltages. Filled square and circle show the amplitude in the control and pCPT-cGMP conditions, respectively.

Effects of Ca^{2+} influx through the cGMP-gated channel on spike responses

An accumulation of intracellular Ca^{2+} is also known to modulate voltage-gated currents in other preparations through Ca^{2+}- and calmodulin-dependent protein kinase (CaM kinase; Browning et al., 1985; Arreola et al., 1998). To examine the possibility that Ca^{2+} influx through the cGMP-gated channel may change spike responses, we first examined effects of cGMP on spike responses in the Ca^{2+}-free Ringer's solution using the recording pipette containing 5 mM EGTA. Even in the Ca^{2+}-free solution, bath application of 250 μM pCPT-cGMP depolarized the resting potential by approximately 10 mV, and increased markedly the spike rates (Fig. 7; $n = 4$). A similar result was obtained by using the pipette containing 5 mM BAPTA ($n = 3$). This suggests that the Ca^{2+} influx is not significantly involved in the modulation by cGMP of the spike responses.

To test whether CaM kinase affects the spike responses evoked by cGMP, we applied a specific inhibitor in the recording pipette (10 μM KN-62; Hidaka & Yokokura, 1996), Bath application of 250 μM pCPT-cGMP depolarized the resting potential by 5–10 mV, and increased markedly the spike rates ($n = 3$; data not shown). Apparently CaM kinase is not essential for cGMP's modulation of the spike responses.

Discussion

In the present study, we studied the effects of cGMP, SNAP, and SNP on spike responses of the ganglion cells in the guinea pig retina. We found that these agents lowered the current threshold by depolarizing the membrane potential through the cGMP-gated inward current.

Spiking patterns in the control solution

In the control solution, most cells (74%) in the ganglion cell layer showed tonic firing, the rest (26%) showed phasic. This is similar

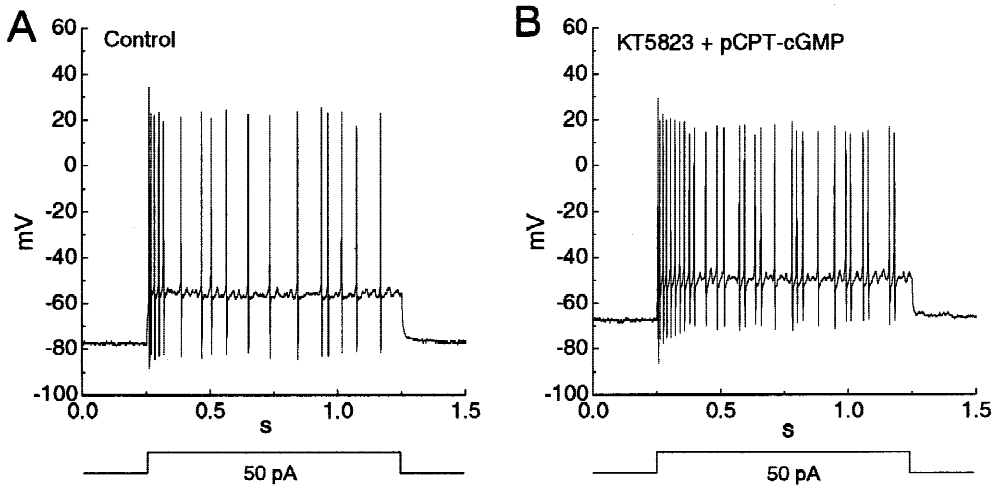

Fig. 6. Effects of pCPT-cGMP on spike responses when the recording pipette included KT5823, a selective PKG inhibitor. (A) Spike response to step current injection of 50 pA in the control solution. The step current was injected to a cell for 1 s between 0.25 s and 1.25 s. The recording pipette contained 10 μM KT5823. Voltage response was recorded 5 min after rupture of the patch membrane. (B) Spike response recorded from the same cell as in (A) in the solution containing 250 μM pCPT-cGMP using the pipette containing 10 μM KT5823. The superfusate was changed 6 min after rupture of the patch membrane. The same step current as in (A) was injected to the cell.

to recordings in adult rat retina (Wang et al., 1997). Since our main purpose was to examine cGMP effects on spike response, we did not analyze the mechanisms for tonic/phasic spiking.

We selectively recorded from the cells of the large soma size (approximately 15 μm diameter) in the ganglion cell layer, so most cells recorded in the present experiments are likely to be ganglion cells. However, we cannot completely rule out the possibility that some cells recorded here may be displaced amacrine cells, which are found in the ganglion cell layer (Wang et al., 1997). The phasic cells could be displaced amacrine cells.

Mechanisms underlying the modulation by cGMP of spike activity

Under voltage clamp, bath application of pCPT-cGMP did not change significantly the response kinetics or voltage dependency of voltage-gated currents, when the recording pipette contained KT5823. This suggests that PKG is not responsible for the modulation of spike responses in the guinea pig retinal neurons. By contrast, in other systems cGMP modulates voltage-gated I_{Na}, I_{Ca}, and I_K directly or *via* PKG. Thus cGMP increases I_{Na} in some

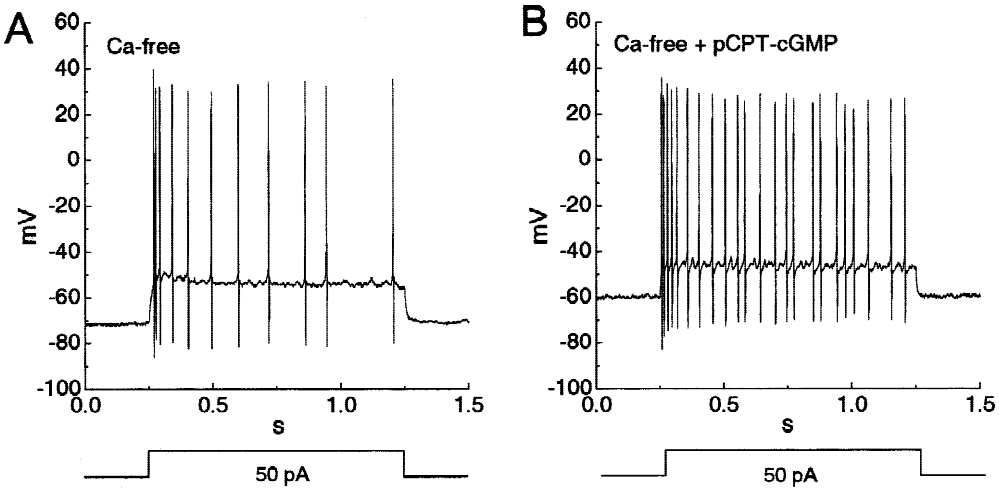

Fig. 7. Effect of pCPT-cGMP on spike responses in Ca^{2+}-free Ringer's solution. (A) Tonic cell injected with 50 pA, recording pipette contained 5 mM EGTA. (B) Same cell with 250 μM pCPT-cGMP added to bath.

molluscan neurons (Connor & Hockberger, 1984) and I_{Ca} in others (Paupardin-Tritsch et al., 1986); whereas the Aplysia R15 neuron pCPT-cGMP directly reduces I_{Ca} (Levitan & Levitan, 1988) and in catfish retinal horizontal cells, cGMP upregulates an inward rectifier K^+ current via PKG (Dixon & Copenhagen, 1997).

cGMP can also act *via* cAMP-dependent phosphodiesterase (PDE; Beavo et al., 1971*a*,*b*; Wexler et al., 1998). Thus, bath application of cGMP analogs might modulate the spiking by changing the cAMP concentration. However, in the present study, bath application of 250 μM pCPT-cAMP did not change the membrane potentials or spike rates.

We also examined the effects of intracellular Ca^{2+} accumulation on the spike rate. Even in Ca^{2+}-free Ringers with the recording pipette containing 5 mM EGTA or BAPTA, bath application of 250 μM pCPT-cGMP markedly increased the spike rates. In addition, when the recording pipette contained 10 μM KN-62 (specific inhibitor of CaM kinase), application of pCPT-cGMP also increased the spike rates. These results suggest that effects of cGMP on the spike responses are independent of Ca^{2+} and CaM kinase.

The cGMP-gated current in ganglion cells does not desensitize over many minutes irrespective of whether the channel was activated by cGMP analogs or SNP (Ahmad et al., 1994; Kawai & Sterling, 1999). The effect of this cGMP-gated current is similar to that of an injected steady current (Fig. 4C). Apparently, cGMP increases the spike activity in ganglion cells mainly by modulating the resting potential *via* the cGMP-gated inward current.

Physiological function of the cGMP-gated current in ganglion cells

The retina contains numerous sources of NO, any or all of which might modulate the ganglion cell's cGMP-gated inward current. However, due to its proximity, perhaps the most likely candidate is the axonal arboration of the amacrine cell whose long axons, rich in NO synthase, form a dense plexus in the middle stratum of the inner plexiform layer. NO released in this stratum diffusing radially for at most 10 μm would affect all ganglion cell dendrites. Such amacrine cells with long axons probably spike and might serve as one mechanism by which long-range, lateral connections regulate the ganglion cell's contrast gain (Demb et al., 1999).

Acknowledgments

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