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TRANSIENT CALCIUM CURRENT OF RETINAL BIPOLAR CELLS OF THE MOUSE

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SUMMARY

1. Isolated bipolar cells were obtained by enzymic (papain) dissociation of the adult mouse retina. The membrane voltage was clamped and the membrane currents were measured by the whole-cell version of the patch-clamp technique. Isolated bipolar cells and horizontal cells of the goldfish retina were also studied for comparison.

2. Hyperpolarization from the holding voltage, V_h, of -46 mV evoked a slowly activating, Cs^+-sensitive, inward current (probably an h-current), and depolarization evoked a TEA- and Cs^+-sensitive outward current (probably a combination of K^+ currents).

3. Depolarization from a more negative V_h (e.g. -96 mV) evoked a transient inward current that had maximal amplitude between -40 and -20 mV. This current was identified as a Ca^{2+} current (I_{Ca}): its amplitude was increased with elevated $[Ca^{2+}]_o$ and was decreased with reduced $[Ca^{2+}]_o$, and it was blocked by 4 mM-Co^{2+}, but not by 5 μM-TTX.

4. Both the perikaryon and the axon terminal generated I_{Ca} with similar properties.

5. The plot of Ca^{2+} conductance (g_{Ca}) against membrane voltage (activation curve) was sigmoidal: in 10 mM $[Ca^{2+}]_o$, g_{Ca} increased for membrane voltages more positive than -65 mV, was half-maximal at about -25 mV, and reached saturation at about $+30$ mV. The plot of inactivation of g_{Ca} against membrane voltage was also sigmoidal: with 1 s conditioning depolarization in 10 mM $[Ca^{2+}]_o$, g_{Ca} decreased for membrane voltages more positive than -80 mV, was half-maximal at about -50 mV, and was fully suppressed for voltages greater than -30 mV.

6. I_{Ca} in the mouse bipolar cells was insensitive to 50 μM-Cd^{2+}, 10 μM-nifedipine and 10 μM-Bay K 8644. In contrast, the calcium currents of bipolar and horizontal cells of the goldfish retina were markedly suppressed by 50 μM-Cd^{2+} and 10 μM-nifedipine, and were augmented several fold by 10 μM-Bay K 8644. The calcium currents of goldfish bipolar and horizontal cells were sustained, and were activated in a more positive range of potentials than the I_{Ca} of mouse bipolar cells.

7. The voltage range at which the I_{Ca} of mouse bipolar cells is activated includes the presumed range of membrane potentials spanned during light-evoked responses; thus, this current may participate in synaptic transmission. The transient character of I_{Ca} may also help to shape transient responses of ganglion cells.

INTRODUCTION

Since the pioneering work by Kuffler (1952), the function of the vertebrate retina has been studied extensively. A vast number of studies have been made on the response properties of the retinal ganglion cells of cat and rabbit. In contrast with the large number of studies of mammalian retinal ganglion cells, however, most studies of retinal photoreceptors and interneurones have been made in lower vertebrates, and studies in mammals are very limited (photoreceptors of monkey, Baylor, Nunn & Schnapf, 1984; mouse horizontal cells, Suzuki & Pinto, 1986; cat retinal neurones, see Kolb & Nelson, 1984). To understand the function of the retina in higher mammals, including man, it is desirable to obtain data from retinal interneurones in mammals, and to determine whether the data from lower vertebrates apply to the mammalian retina.

A compelling reason for studying the mouse retina is that over 500 named mutants are available, at least thirteen of which affect the visual system in subtle ways that do not cause gross degeneration (Balkema, Mangini, Pinto & Vanable, 1984). Comparison of the mutant with the normal animal will allow certain inferences to be made about the function of the affected gene products; for this comparison, a thorough study of the normal animal is a prerequisite.

The retinal bipolar cell is an interneurone that relays the photoreceptor signal to ganglion cells. In our previous voltage-clamp study (Kaneko & Tachibana, 1985) we found that bipolar cells dissociated from the goldfish retina have at least four types of membrane currents, a Ca^{2+} current, an h-current and two types of K^+ currents. In the present study we used a similar protocol to analyse membrane currents of bipolar cells dissociated from the adult mouse retina. We found that most of the ionic currents recorded in mouse bipolar cells are of the same kind as those in the goldfish, but a marked difference was noted in the properties of the calcium current: it is transient in the mouse, but sustained in the goldfish; also, the threshold for its activation is more negative in the mouse than in the goldfish. Preliminary data have been published in abstracts (Kaneko, Tachibana & Pinto, 1988; Pinto, Kaneko & Tachibana, 1988).

METHODS

Preparation

Bipolar cells were isolated from the adult mouse (C57BL/6J and C57BL/6crSlc) retina by a method modified from that initially developed for goldfish horizontal cells (Tachibana, 1981). In brief, mice were killed by dislocation of cervical vertebrae, and both eyes were enucleated. The eyes were opened and hemisected, and the retinae were isolated from the pigment epithelium. Isolated retinae were incubated in Hanks' medium (with a solute composition of: NaCl, 137 mM: KCl, 5·4 mM; glucose, 5·6 mM; HEPES, 2·8 mM; and pH adjusted to 7·4) containing 2·5 U/ml papain (Worthington) and 0·1 mg/ml cysteine for 15 min at 30 °C. After rinsing with Hanks' medium (supplemented with 1·9 mM-$CaCl_2$, 0·6 mM-$MgCl_2$ and 0·1 mg/ml bovine serum albumin), the retina was triturated. Approximately 150–200 μl of cell suspension was dispensed into a plastic culture dish, the bottom of which was replaced with a cover-glass coated with concanavalin A. This dish contained 2 ml of the control medium (solution A, Table 1). The cells were incubated for 1–8 h before use. Initially, incubation and recordings were made at either room temperature (19–22 °C) or 37 °C, but no differences were observed in the waveforms as they appeared on the face of the oscilloscope, and all further incubation and recordings were therefore made at room temperature.

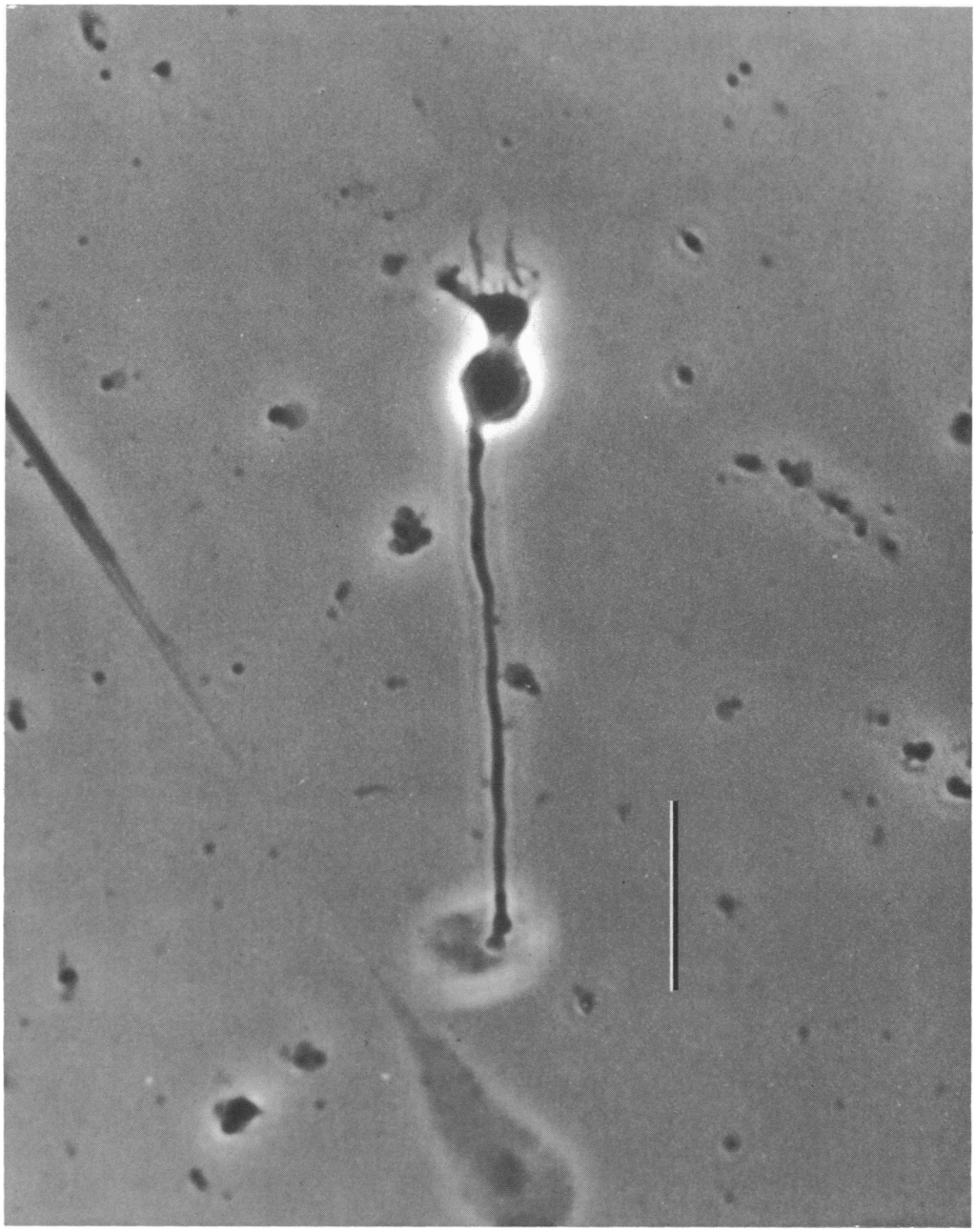

Fig. 1. Isolated bipolar cell of the mouse retina. Note the presence of many dendritic arborizations (top), a long axon and axon terminal (bottom). Scale bar 20 μm.

Dissociation yielded a mixture of isolated retinal cells, but bipolar cells (Fig. 1) were identified easily under the microscope by their characteristic morphology (cf. Plate V of Cajal, 1972). They have a spherical cell body of 5–8 μm in diameter and a stout dendritic stem from which several fine branches emanate. From the opposite pole of the perikaryon a single thin axon originates and extends for 20–50 μm. In some cells the axon terminates in a small swelling.

TABLE 1. Superfusates for mouse retinal cells (mM)

		NaCl	KCl	$CaCl_2$	$MgCl_2$	CsCl	TEA-Cl	$CoCl_2$	HEPES	Glucose
A	Control	135	5	2	1	—	—	—	5	10
B	0 Ca^{2+}	138	5	—	1	—	—	—	5	10
C	10 Ca^{2+}	123	5	10	1	—	—	—	5	10
D	2 Ca^{2+}, TEA, Cs^{+}	95	5	2	1	10	30	—	5	10
E	10 Ca^{2+}, TEA, Cs^{+}	83	5	10	1	10	30	—	5	10
F	TEA, Cs^{+}, Co^{2+}	83	5	—	7	10	30	4	5	10
G	Co^{2+}	131	5	—	1	—	—	4	5	10

pH was adjusted to 7·4 with NaOH. All solutions contained Phenol Red (0·01 mg/ml) and 0·1 mg/ml bovine serum albumin (BSA). Nifedipine and Bay K 8644 were dissolved in ethanol, and this solution was then dissolved in solution D for mouse, and in the superfusate described in the legend of Fig. 10 for goldfish (< 0·1 % final ethanol concentration).

Isolated bipolar and horizontal cells dissociated from the goldfish retina were also used to compare properties of Ca^{2+} currents in the two species of animals. They were obtained and studied as described previously (Kaneko & Tachibana, 1985).

Recording procedures

Membrane currents were recorded by patch pipettes in the whole-cell-clamp configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Pyrex tubing (1·2 mm o.d.) was pulled in two steps on a pipette puller (Narishige Scientific Instruments, PP−83). The outer diameter of the pipette tip was about 3 μm, and the opening was about 1 μm after heat-polishing. Care was taken to minimize stray capacitance of the pipette. The external wall of the pipette was coated, as close to the tip as possible, with an insulating resin (Apiezon wax, Apiezon Products Ltd, London) dissolved in chloroform, and residual capacitance of the pipette was compensated electrically for every pipette. For recording mouse bipolar cells, pipettes were filled with one of two solutions; the first contained (in mM): KCl, 120; $CaCl_2$, 0·5; $MgCl_2$, 1; EGTA, 5; HEPES, 10; Na_2ATP, 3; Na_3GTP, 2; and Na-cGMP, 0·01 (pH 7·2); in the other, KCl was replaced with equimolar CsCl. For recording goldfish isolated cells, pipettes were filled with a solution containing (in mM): CsCl, 120; NaCl, 10; EGTA, 5; and HEPES, 10 (pH 7·2). Resistance of the pipette was about 20 MΩ.

For recordings, the culture dish was mounted on the stage of an inverted microscope with phase-contrast optics (Nikon, TMD). A stainless-steel ring (O'Lague, Potter & Furshpan, 1978) was put into the dish to reduce the volume of solutions to approximately 0·15 ml. The cells were continuously superfused (at a rate of 0·6 ml/min) with one of the solutions listed in Table 1. The pipette was connected to a current–voltage converter (List Electronics, Darmstadt, FRG, L/M EPC-7). The indifferent electrode was an Ag–AgCl wire connected to the culture dish *via* an agarose bridge. Recordings of membrane potential were compensated for junction potentials as described previously (Kaneko & Tachibana, 1986). Holding and command voltages were generated by a microcomputer (NEC, Tokyo, PC-9801VX) with a programmable 8-bit D/A converter. The voltage and time resolution of this pulse generator were 1 mV and 0·5 ms, respectively. Data were sampled and digitized by a 12-bit A/D converter (AA11-K) attached to a VAX 11/750 computer (Digital Equipment Corp., Maynard, MA, USA) after passing through an 8-pole Bessel filter (NF Circuit Design Block Co. Ltd, Yokohama, Model FV-625A). The cut-off frequency of the filter was set to one-half of the sampling frequency, a value that did not introduce significant aliasing for the present experiments, which did not employ repetitive stimuli or measurement of spectra. Sampling rate was set at an adequate value between 0·2 and 20 ms, depending on the type of analysis.

Application of drugs

Several pharmacological agents were used for isolating a specific ionic current(s): tetrodotoxin (TTX) for blocking Na^+ current, tetraethylammonium chloride (TEA) and CsCl for blocking K^+ current, and $CoCl_2$ and $CdCl_2$ for blocking Ca^{2+} current. Dihydropyridines (nifedipine and Bay K 8644) were also used to modify Ca^{2+} currents. Divalent cations and other pharmacological agents were dissolved in the superfusate and applied from a pipette (20 μm diameter tip) by using pressure (290 Pa, 5–20 s in duration). The pipette was positioned approximately 50 μm away from the recorded cell (Ishida, Kaneko & Tachibana, 1984; Tachibana, 1985), and delivered test solutions to the entire cell surface. To apply the test solution locally, we used a pipette with a small opening (the same type as the patch pipette placed 1–2 μm away from the membrane). Complete recovery was observed in most cases after a brief application of the agents. Pressure application of the superfusate alone did not produce any change in membrane current. $CoCl_2$, $NiCl_2$, $CdCl_2$, and CsCl were purchased from Katayama Chemical Industries Co. (Osaka), TEA from Tokyo Chemical Industry Co. (Tokyo), TTX from Sankyo Co. (Tokyo) and nifedipine and Bay K 8644 from Sigma Chemical Co. (St Louis, MO, USA).

A total of 428 cells were studied. Results presented in this report were obtained from at least three cells.

RESULTS

Membrane currents recorded under voltage-clamp conditions

The current–voltage relation of isolated bipolar cells was non-linear when examined in the control solution (Fig. 2). The resting voltage (at which no net

current was recorded) was about −45 mV. When the membrane was depolarized by a 2 s test pulse from a holding voltage (V_h) of −46 mV to a voltage more positive than −30 mV, a sustained outward current was generated (Fig. 2*A*), and its amplitude increased with increasing depolarization. The outward current showed a large

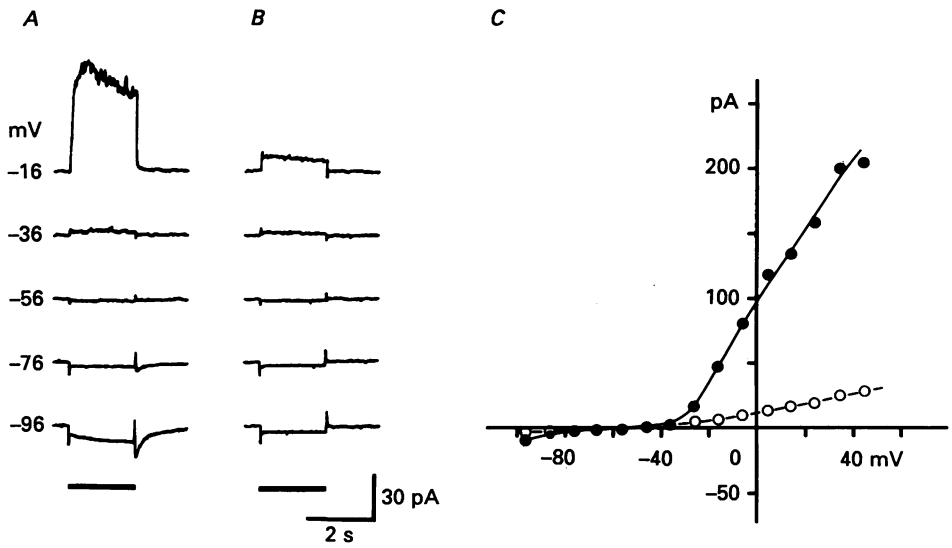

Fig. 2. Membrane current induced by steps to various membrane voltages (values in *A*) while cell was bathed in control solution (*A*, Table 1 solution A), and a mixture of blockers (*B*, Table 1 solution F). *C*, plot of membrane current (immediately before the cessation of the voltage pulse) against voltage for control solution (●) and a mixture of blockers (○). Holding voltage, −46 mV; pipette solution contained 120 mM-KCl.

fluctuation, and a mixture of 10 mM-Cs^+, 30 mM-TEA^+ and 4 mM-Co^{2+} blocked the current (Fig. 2*B*). Thus, the outward current was probably a combination of K^+ currents. When the membrane was hyperpolarized below −80 mV, a slowly activating inward current was seen (Fig. 2*A*). This current was presumably an h-current, because it had similar activation voltage and time course to the h-current recorded in isolated bipolar cells dissociated from the goldfish retina (Kaneko & Tachibana, 1985), and was blocked by external Cs^+ (not illustrated).

Since a sustained Ca^{2+} current was evoked by a 2 s depolarization (from $V_h \simeq$ −40 mV) to around −10 mV in goldfish bipolar cells (Kaneko & Tachibana, 1985), we expected to see a similar Ca^{2+} current in the present preparation. However, no such current was seen, even after the opposing K^+ currents were suppressed by application of Cs^+ and/or TEA to the outside of the cell or by replacement of K^+ of the pipette solution with Cs^+. However, when test pulses (from V_h = −46 mV) more negative than −70 mV were terminated, a transient inward current was evoked (bottom two traces of Fig. 2*A*). We thought this inward tail current might have been either a Na^+ or Ca^{2+} current with fast inactivation kinetics. Perhaps the current had been inactivated while the cell was held at −46 mV and was reactivated during the prolonged (> 2 s) hyperpolarizing test pulse.

To test this possibility, we next recorded (with higher time resolution) the membrane currents that were evoked by depolarizing the membrane from a more negative V_h (Fig. 3*A*). When the cell was depolarized to −36 mV from the V_h of −86 mV, a transient inward current was generated. In this experiment, K^+ currents were partially blocked by dialysing the cytoplasm with high-Cs^+ pipette solution.

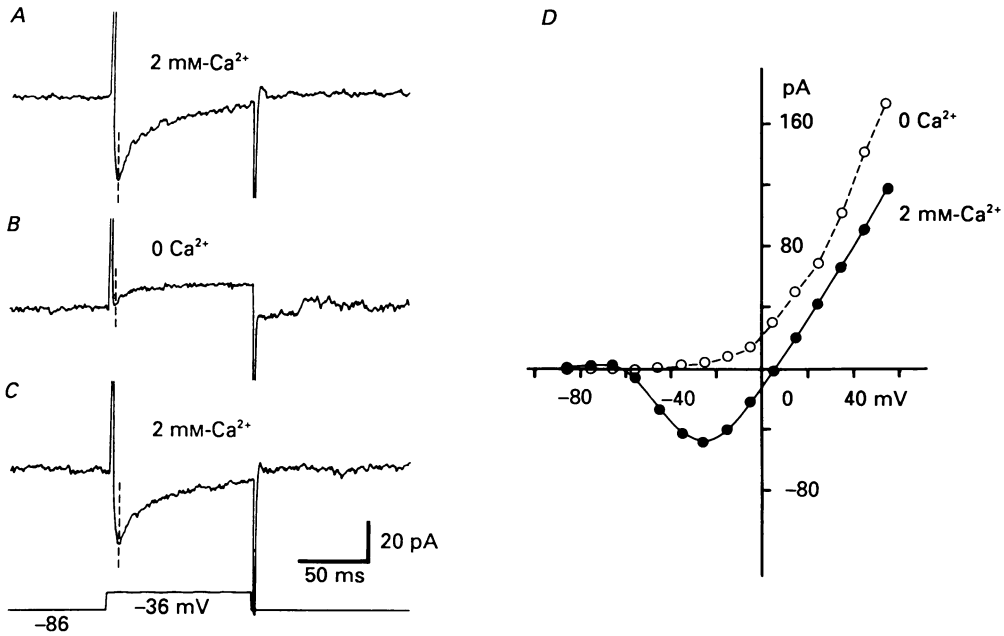

Fig. 3. Amplitude of I_{Ca} decreased with lowered $[Ca^{2+}]_o$. Membrane currents evoked by a voltage pulse to −36 mV (−86 mV holding voltage) in the presence of 2 mM $[Ca^{2+}]_o$ (*A* and *C*, Table 1 solution A) and zero added Ca^{2+} (*B*, Table 1 solution B). *D*, membrane current, measured at 5 ms after the onset of voltage pulses (interrupted vertical lines in *A*–*C*), is plotted against membrane voltage during the pulse. Pipette solution contained 120 mM-CsCl.

The inward current reached its peak within 12 ms of depolarization, and decayed to about one-fifth of the peak value during the 100 ms depolarizing command.

The leakage current that remained after the superfusion with a mixture of TEA^+, Cs^+ and Co^{2+} was directly proportional to the membrane voltage in both depolarizing and hyperpolarizing directions, and was time-independent. The leakage resistance was 17·5±7·4 GΩ (mean±S.D., $n = 18$).

It might be argued that the membrane was not uniformly voltage clamped, since the cell has a thin axon (about 1 μm in diameter and 50 μm long). The calculated resistance of the axoplasm was less than 300 KΩ. The high input resistance, taken together with this low axoplasmic resistance, would have resulted in the membrane being nearly isopotential under the conditions used. Support for this notion comes from the results of experiments in which the reversal potential was measured for currents induced by local application of GABA to either the axon terminal or the soma (unpublished observation; the recording pipette was placed at the soma). In these experiments, the reversal potential was 2·5±3·7 mV (mean±S.D., $n = 15$) when GABA was applied to the axon terminal, and was 2·3±3·6 mV ($n = 21$) when GABA was applied to the soma.

Identification of the transient inward current

To identify the species of ion(s) carrying the transient inward current we performed several experiments. First, pressure application of 5 μM-TTX did not produce any detectable change in either the amplitude or the time course of the inward current. The same concentration of TTX immediately and completely

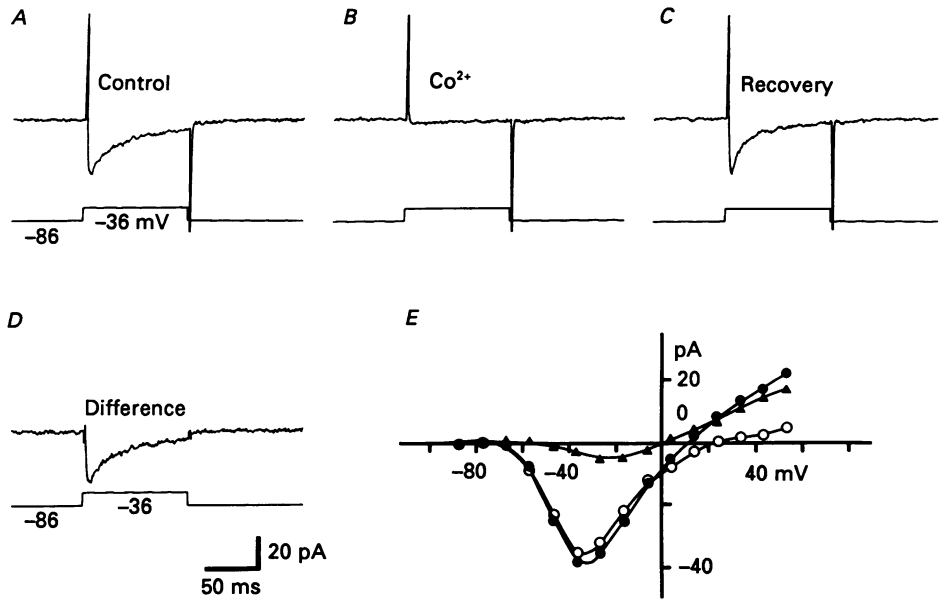

Fig. 4. Cobalt-sensitive current. *A–C*, Membrane currents evoked by a voltage pulse to −36 mV (−86 mV holding voltage) in the presence of control solution (*A* and *C*) and solution to which 4 mM-Co^{2+} had been added (*B*, Table 1 solution G). *D*, the Co^{2+}-sensitive current obtained by subtracting the above two records. *E*, current–voltage relations of a mouse bipolar cell measured in the presence of control solution (●), a solution containing 4 mM-Co^{2+} (▲) and the difference (○). Currents were measured at the peak during the pulse. Pipette solution contained 120 mM-CsCl.

blocked action potentials recorded extracellularly from presumed isolated ganglion cells (identified by large soma size, with four to five long dendrites extending radially from the perikaryon) found in the same culture dish. Action potentials recovered within a few minutes after removal of TTX.

Secondly, the transient inward current became larger when the concentration of Ca^{2+} in the superfusate, $[Ca^{2+}]_o$, was increased. With 10 mM $[Ca^{2+}]_o$, the peak amplitude of the inward current was nearly twice as large as that recorded with 2 mM $[Ca^{2+}]_o$ (not illustrated). Removal of Ca^{2+} from the superfusate eliminated the transient inward current (Fig. 3*B*).

Thirdly, the transient inward current was blocked by Co^{2+}. As shown in Fig. 4, application of 4 mM-Co^{2+} reversibly suppressed the inward current. All of these observations strongly suggest that the transient inward current activated by

depolarization is carried by Ca^{2+}. Thus, the difference between the current records obtained under the control condition and those obtained during the application of Co^{2+} gives the Co^{2+}-sensitive component of the current, which is essentially Ca^{2+} current (I_{Ca}).

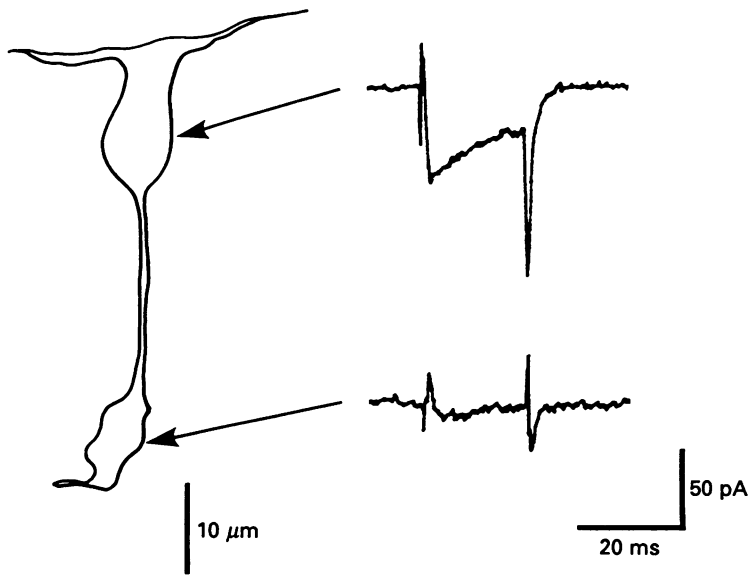

Fig. 5. Spatial distribution of Co^{2+}-sensitive current. Solution containing Co^{2+} (Table 1 solution F) was applied to the soma (upper arrow) and axon terminal (lower arrow) while the cells were superfused with solution E, Table 1; the Co^{2+}-sensitive current is shown for each location (right) for a 20 ms pulse to −36 mV from a holding voltage of −96 mV. Pipette solution contained 120 mM-CsCl.

I_{Ca}, isolated by Co^{2+} application, was detectable for membrane potentials more positive than −60 mV, was maximal at about −30 mV, and became undetectable at around +40 mV. This latter decrease in amplitude with strong depolarization was probably due to a reduction of the driving force, and was not due to a reduction of calcium conductance (see Fig. 6).

Spatial distribution of I_{Ca}

Bipolar cells have an elongated, polarized structure with distal dendrites and a proximal axon. Within the axon terminals lie synaptic ribbons. Therefore, the spatial distribution of I_{Ca} is important for considering the possible physiological role of I_{Ca} (e.g. transmitter release). In the present experiments we measured the total calcium current with a patch pipette attached to the perikaryon. However, the conductance that gave rise to this total current might have been distributed in the perikaryon, axon or axon terminal. We estimated the contribution that each of these regions made to the total current by applying a blocking agent (4 mM-Co^{2+} from a pipette with *ca* 1 μm diameter tip) focally to each of these parts of the cell. Focal application of Co^{2+} to the perikaryon resulted in a greater reduction of the total current than did focal application to the axon terminal (Fig. 5). In a few cases we recorded I_{Ca} from

bipolar cells that had lost their axon terminal during dissociation. The time course of I_{Ca} from these cells was similar to that recorded from cells that had intact axon terminals. These observations show that the majority of I_{Ca} flows through the membrane of the perikaryon and argue against the possibility that the transient nature of I_{Ca} was merely an artifact of an inhomogeneous space clamp of the cell.

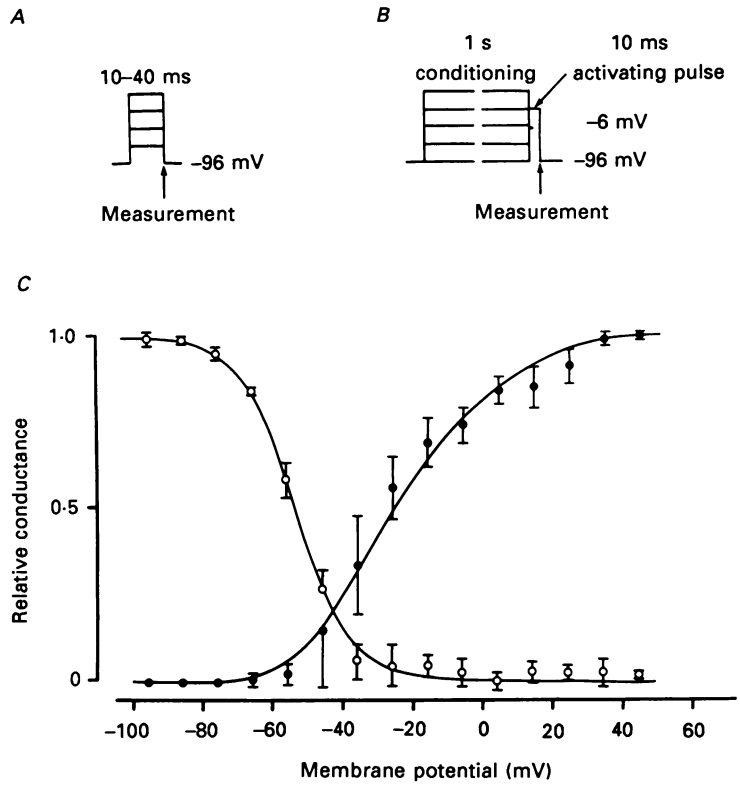

Fig. 6. Activation and inactivation curves. Filled circles represent relative conductance, measured using tail currents (time course of pulse, *A*), plotted against test pulse voltage. For each value of test pulse voltage, the duration was varied from 10 to 40 ms, and the duration that evoked the largest current was chosen for measurement. The points show the mean (±S.D.) for four cells. Open circles represent relative conductance, measured using the tail current to a −6 mV activating pulse, that followed a long conditioning pulse of each of many voltages (time course of pulse, *B*), plotted against voltage of the conditioning pulse. The points show the mean (±S.D.) for four cells. Holding voltage, −96 mV. Superfused with solution containing 10 mM $[Ca^{2+}]_o$ (Table 1 solution E); pipette solution contained 120 mM-CsCl.

Voltage dependence of activation and inactivation of I_{Ca}

As demonstrated in preceding section, I_{Ca} of the mouse bipolar cells showed a strong voltage dependence. To study this property quantitatively, we used the method of tail current analysis, in which membrane current is measured while membrane voltage is held constant (−96 mV for the present experiment) immediately after the offset of an activating pulse. This method offers the advantages

of (1) maintaining a large, constant driving force upon Ca^{2+}, which results in a large tail current and (2) not requiring knowledge of the reversal potential for Ca^{2+}. This method was valid for I_{Ca} of the mouse bipolar cells, since the time course of inactivation was slower than that of activation (see pp. 624–625).

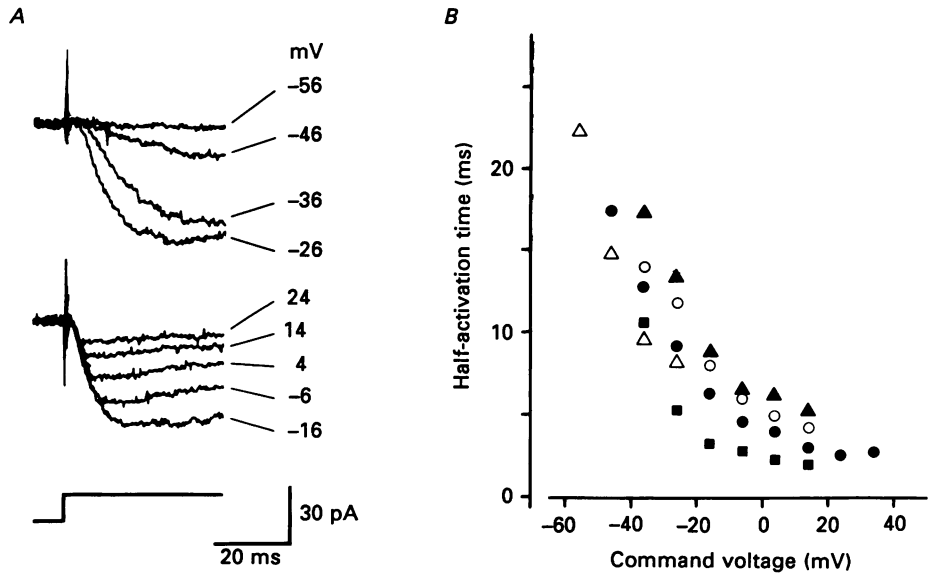

Fig. 7. Time course of the onset of membrane current evoked by a voltage step from a holding voltage of −96 mV to each of many test voltages (*A*). Note the more rapid time course for more depolarized voltages. *B*, plot of half-activation time (time to reach half-maximal current) *vs.* voltage during the step. Each symbol gives data from a different cell. Superfused with solution E, Table 1; pipette solution contained 120 mM-CsCl.

Activation

I_{Ca} was detected in the range of membrane voltages between −60 and +40 mV, but decreased in amplitude with increasing depolarization between −30 and +40 mV (see Fig. 4). This decrease in amplitude might have resulted either from a decrease in calcium conductance (g_{Ca}) or decrease in driving force. To distinguish between these possibilities we used tail current analysis to estimate relative g_{Ca}. The tail currents we measured were the difference between the current recorded with superfusate lacking Co^{2+} and with superfusate containing 4 mM-Co^{2+}. Since activation was slow for negative membrane voltages but fast for positive voltages, we selected, for each voltage, the duration of test pulse (between 10 and 40 ms) which generated the largest tail current (for method see Fig. 6*A*).

Figure 6*C* (●) illustrates the relation between normalized g_{Ca} (measured in 10 mM $[Ca^{2+}]_o$ to augment I_{Ca}) and the membrane potential. g_{Ca} was activated by depolarization of the membrane (from $V_h = -96$ mV) to potentials more positive than −65 mV. The relation between conductance and membrane voltage was sigmoidal, and reached saturation at around +30 mV. g_{Ca} was half-maximal at about −25 mV.

Inactivation

Inactivation of I_{Ca} was also voltage dependent. Figure 6*C* (○) illustrates the results of a series of experiments in which tail currents (activated by depolarization to −6 mV from V_h of −96 mV) were measured after 1 s conditioning pulses of various

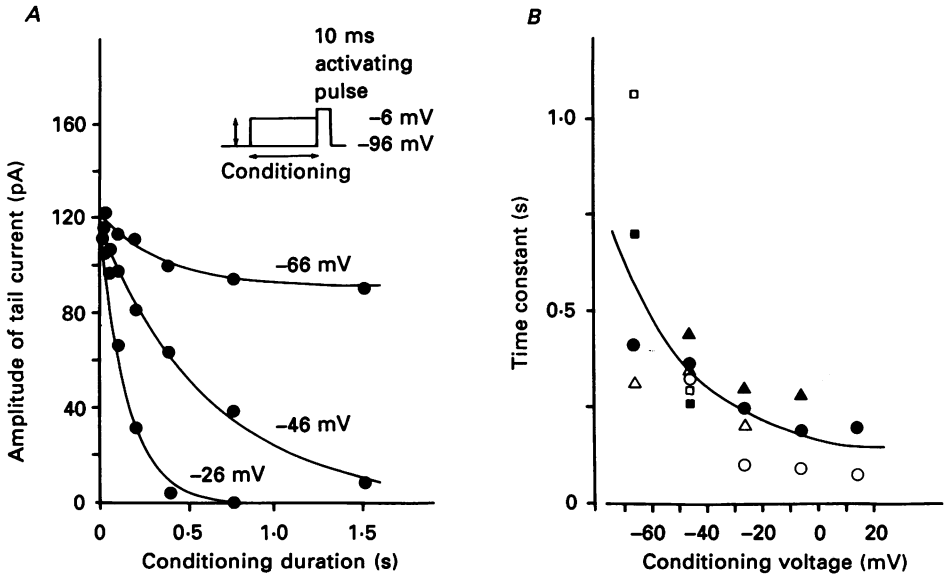

Fig. 8. Dependence of inactivation upon duration and amplitude of depolarization. *A*, amplitude of the tail current to a −6 mV activating pulse that followed conditioning pulses of each of many durations (and each of three voltages). Note that for the more depolarized conditioning pulses (1) the amplitude decreased more quickly with duration of the conditioning pulse, and (2) the amplitude decreased to a lower fraction of its original value. The lines are single-exponential decays fitted by the method of least squares to the untransformed data. *B*, time constant of the least-squares lines fitted to the data in (*A*) plotted against conditioning pulse voltage. Each symbol gives data from a different cell. Holding voltage, −96 mV. Superfused with solution E, Table 1; pipette solution contained 120 mM-CsCl.

voltages (for method see Fig. 6*B*). No reduction of g_{Ca} was seen for conditioning pulses more negative than −80 mV, but g_{Ca} was suppressed nearly entirely by conditioning pulses more positive than −30 mV of 1 s duration. Between these two extremes the relation between conductance and membrane voltage was sigmoidal. The membrane voltage at which half-maximum inactivation occurred was about −50 mV.

Time course of activation and inactivation of I_{Ca}

Activation

The time course of I_{Ca} activation was more rapid for more depolarized membrane voltages (Fig. 7) and a delay occurred between the onset of depolarization and the

rising phase of inward current (Fig. 7*A*). We tried to fit the current record with an exponential function (raised to each of several powers), but were unsuccessful because of interference from capacitative artifacts. However, the time required for half-maximal activation could be measured reliably and was found to be shorter for more depolarized membrane voltages (Fig. 7*B*).

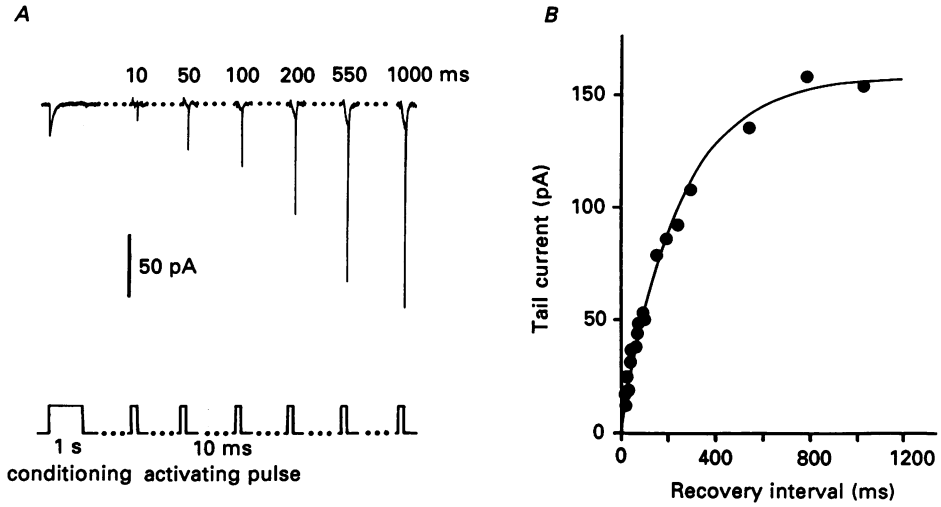

Fig. 9. Recovery from inactivation. At each of several times after offset of a 1 s inactivating pulse (−6 mV), a 10 ms (−6 mV) activating pulse (*A*, lower) was applied. The resulting membrane currents (*A*, upper) increased in amplitude with increasing time after the offset of the inactivating pulse (recovery interval). *B*, the peak amplitude of the tail current is plotted against recovery interval. Holding voltage, −96 mV. The line is a single-exponential function that had a time constant of 244 ms for this cell. Superfused with solution E, Table 1; pipette solution contained 120 mM-CaCl.

Inactivation

The time course of I_{Ca} inactivation was also more rapid for more depolarized membrane voltages (Fig. 8). The data illustrated in Fig. 8 were obtained from a series of experiments in which an activating pulse (to −6 mV, 10 ms duration) was applied at the end of each of several conditioning pulses of various durations and magnitudes. Tail currents were measured immediately after offset of the activating pulses while membrane voltage was held at −96 mV. The tail currents we measured were the difference between the current recorded with superfusate lacking Co^{2+} and with superfusate containing 4 mM-Co^{2+}. The amplitude of the tail current was plotted against the duration of the conditioning pulse for each of several conditioning voltages (Fig. 8*A*). For each value of conditioning voltage the time course of inactivation could be fitted with a single-exponential function (Fig. 8*A*). The time constant of this exponential function was shorter for more depolarized membrane voltages (Fig. 8*B*). This finding suggests that the process of inactivation of I_{Ca} could be simulated by a model in which the translocation of a single charged particle closes each gate (cf. p. 56 in Hille, 1984).

Recovery from inactivation

Maintaining the membrane potential in a hyperpolarized state permitted I_{Ca} to recover from inactivation with an exponential time course. In the experiment of Fig. 9, I_{Ca} was initially inactivated almost completely by maintaining the cell at

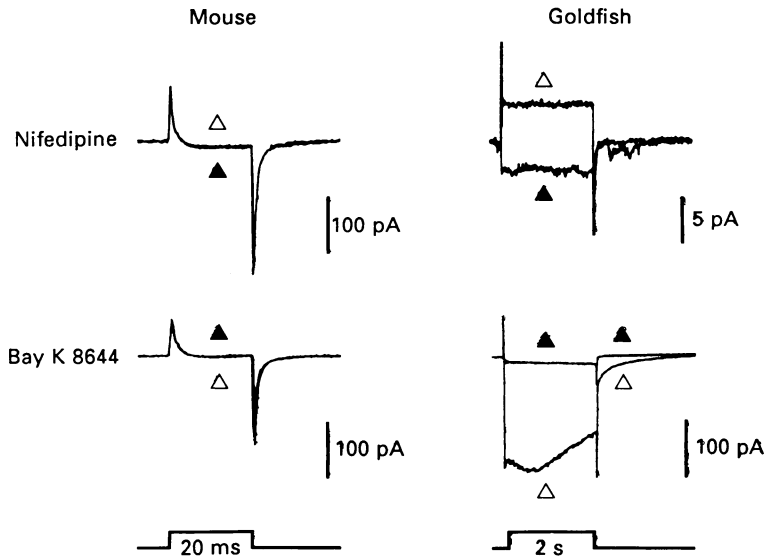

Fig. 10. Comparison of membrane currents of mouse bipolar cells to a brief depolarizing pulse to −6 mV (left) and goldfish bipolar cells to a prolonged depolarizing pulse to −6 mV (right). The transient I_{Ca} of the mouse bipolar cell can be best evaluated by examining the tail current at offset of the pulse, which includes a small capacitative artifact, whereas the long-lasting calcium current of the goldfish bipolar cell can be evaluated during the pulse. Neither nifedipine (10 μM) nor Bay K 8644 (10 μM) affected the mouse bipolar cells (superimposed control and test traces), but decreased and increased, respectively, the calcium currents of goldfish bipolar cells (open triangle indicates record in test solution; record marked by filled triangle is control). During recording mouse bipolar cells were superfused with solution D, Table 1 (pipette solution contained 120 mM-CsCl), and goldfish bipolar cells with solution containing 79 mM-NaCl, 10 mM-KCl, 2·5 mM-$CaCl_2$, 1 mM-$MgCl_2$, 10 mM-CsCl, 25 mM-TEA-Cl, 2 mM-HEPES, 16 mM-glucose and 0·1 mg/ml bovine serum albumin (pH 7·4). Pipette solution contained 120 mM-CsCl. Holding voltage was −96 mV for mouse and −46 mV for goldfish.

−6 mV for 1 s. Activating pulses were applied at various times after the end of the conditioning pulse, and tail currents were measured after the activating pulses. I_{Ca} showed 50% recovery in about 150 ms and 100% recovery in about 1 s. The time course of recovery was fitted by a single-exponential function with a time constant of 360 ± 104 ms (mean ± S.D., $n = 10$, $V_h = -96$ mV).

Effects of dihydropyridines on I_{Ca} of bipolar cells: a comparison of I_{Ca} in mouse and goldfish bipolar cells

Various dihydropyridine compounds are known to modify various Ca^{2+} channels in a way that depends on the channel type (Nilius, Hess, Lansman & Tsien, 1985).

I_{Ca} of mouse bipolar cells (examined either by measuring the current during a voltage pulse or the tail current) was unaffected either by nifedipine (5–50 μM) or Bay K 8644 (10 μM, Fig. 10). In contrast to the results with mouse bipolar cells, the calcium current in goldfish bipolar cells was immediately blocked by 10 μM-nifedipine (trace marked with open triangle; note also the sustained nature of the calcium current). Recovery of the calcium current was nearly complete after 1 min wash-out. Furthermore, the calcium current in goldfish bipolar cells was augmented by nearly 10−fold by application of 10 μM-Bay K 8644 (Fig. 10). Similar effects were also observed with the calcium current of goldfish horizontal cells. We also observed a difference in the sensitivity to 50 μM-Cd^{2+}; I_{Ca} in mouse bipolar cells was little affected, while the calcium current in goldfish horizontal cells was blocked nearly completely (not illustrated).

In addition to these differences in sensitivity to various agents, a difference was obvious in the time course of the calcium current for mouse and goldfish bipolar cells. I_{Ca} in mouse bipolar cells was transient and showed a rapid inactivation, while the calcium current in goldfish bipolar cells was sustained (Fig. 10; cf. Fig. 3 of Kaneko & Tachibana, 1985).

DISCUSSION

The present study has demonstrated that mouse bipolar cells have a Ca^{2+} current. The current is blocked by Co^{2+}, but is resistant to TTX. The threshold for activation is about −65 mV, and the current is maximal at about −30 mV. The time course of this I_{Ca} is transient, even when possibly opposing K^+ currents are blocked by drugs and minimized by reducing driving force with holding potential near E_K. The I_{Ca} of mouse bipolar cells differs from the calcium current of goldfish bipolar cells in a number of ways. First, for goldfish bipolar cells the threshold for activation (−30 mV) and the voltage at which maximal current is induced (+10 mV) are more depolarized than for mouse bipolar cells. Secondly, the calcium current is sustained for goldfish bipolar cells (Fig. 10, control records; cf. Kaneko & Tachibana, 1985, their Fig. 10), but is transient for mouse bipolar cells. Finally, the calcium current in the two species has different sensitivity to divalent cations and to dihydropyridine; I_{Ca} in the mouse was insensitive to 50 μM-Cd^{2+}, 10 μM-nifedipine, or 10 μM-Bay K 8644. In contrast, the calcium current of bipolar cells of the goldfish was suppressed by both 50 μM-Cd^{2+} and 10 μM-nifedipine, and was augmented by 10 μM-Bay K 8644.

Recently, it has been demonstrated that there is a variety of potential-dependent Ca^{2+} channels in neurones and cardiac cells; a long-lasting (L) type, a transient (T) type and a 'neither' (N) type (Nowycky, Fox & Tsien, 1985; Hagiwara, Irisawa & Kameyama, 1988). L-type Ca^{2+} channels contribute a long-lasting current in response to strong depolarization, while T-type channels contribute a transient current activated by weak depolarization. Of the three types of Ca^{2+} channels, only the L−type is sensitive to dihydropyridine. Thus, according to the criteria of time course and effect of blocking agents, I_{Ca} of mouse bipolar cells is T-type, while the calcium current of goldfish bipolar cells is L-type.

It is well known that mammalian retinal ganglion cells can be classified into on-type and off-type, depending on their responses to light. A similar classification has

been established for bipolar cells of lower vertebrates, and such a classification may also apply to mammalian bipolar cells. In the lower vertebrates, bipolar cells of the two response types have different morphology; on-type bipolar cells have a long axon, the terminal of which ends in the proximal half (sublamina *b*) of the inner plexiform layer, while the axon terminal of off-bipolar cells ends in the distal half (sublamina *a*) of the inner plexiform layer (Famiglietti, Kaneko & Tachibana, 1977). It would have been interesting to ask whether the specific subtype of I_{Ca} that we studied occurred in either on- or off-type bipolar cells. However, we did not find any hint that two subpopulations of bipolar cells occurred among the population of over 400 cells that we studied.

Inactivation of I_{Ca} in mouse bipolar cells is likely to be voltage dependent because the time constant of inactivation was shorter for more depolarized values of membrane voltage at which I_{Ca} was smaller (cf., for example, -46 mV with $+14$ mV, Figs 4*E* and 8*B*), and because inactivation was seen with membrane voltages which were less negative than the activation threshold (between -80 and -65 mV; see Fig. 6). However, we cannot exclude the possibility of some Ca^{2+}-dependent inactivation, because the Ca-EGTA buffer we used in our pipette solution would not have prevented local elevation of free $[Ca^{2+}]_i$ near the calcium channel during Ca^{2+} entry (cf. Tsien, Hess, McCleskey & Rosenberg, 1987).

The finding that I_{Ca} is generated both at the perikaryon and at the axon terminal indicates that this current may participate in synaptic transmission, and may help to shape the transient responses of ganglion cells. In a superfusate that contains 10 mM $[Ca^{2+}]_o$, I_{Ca} can be activated at membrane potentials between -65 and -10 mV, a range that probably includes the voltages that occur in bipolar cells during the response to light. Since I_{Ca} will cause the rise in free $[Ca^{2+}]_i$ that is needed for synaptic transmitter release, it seems reasonable that the release from mouse bipolar cells might have a transient component. It has long been a puzzle how the transient on- or off-responses of amacrine or ganglion cells are formed. The present finding may suggest a basis for understanding how these transient responses are generated.

Several of the ionic currents of mouse bipolar cells are similar to those found in bipolar cells of the goldfish (Kaneko & Tachibana, 1985). Hyperpolarization induced a slowly activated, Cs^+-sensitive inward current (probably an h-current), and depolarization evoked a TEA- and Cs^+-sensitive K^+ current. We did not attempt to classify the subtypes of K^+ currents rigorously. However, the large current fluctuation that accompanied the outward current induced by strong depolarization (see top record, Fig. 2*A*) is characteristic of a Ca^{2+}-dependent K^+ current (see Marty, 1981). Thus, the K^+ currents recorded from mouse bipolar cells may be a combination of a voltage-activated (delayed rectifier) K^+ current and a Ca^{2+}-dependent K^+ current.

The present study demonstrates that retinal neurones can be dissociated from the adult mouse and maintained for physiological experiments. We have also found that it is not essential to keep these cells at body temperature for maintaining the various ionic conductances of the plasma membrane. Thus, it seems possible to apply this technique to other mammalian retinal neurones.

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