J. Physiol. (1983), **345**, *pp.* 329–351
With 1 plate and 11 text-figures
Printed in Great Britain

IONIC CURRENTS OF SOLITARY HORIZONTAL CELLS ISOLATED FROM GOLDFISH RETINA

BY MASAO TACHIBANA*

From the Department of Neurobiology, Harvard Medical School, Boston, MA 02115, U.S.A.

(*Received 21 December 1982*)

SUMMARY

1. Solitary horizontal cells, dissociated from papain-treated goldfish retinas, produce action potentials and show a non-linear current–voltage relationship. Underlying ion-conductance mechanisms were analysed by a single-micro-electrode voltage-clamp technique. Pharmacological and ion-substitution experiments revealed that ionic currents could be separated into at least four voltage-dependent currents: a Ca current and three types of K currents.

2. The Ca current was activated by membrane depolarization beyond −45 mV, reached a maximal value near 0 mV, and became smaller at more positive potentials. By extrapolation, the reversal potential was estimated to be approximately +50 mV. The Ca current was inactivated by accumulation of intracellular Ca ions but not by membrane depolarization. Co ions (4 mM) blocked this current.

3. The first type of K current showed anomalous (inward-going) rectification near the resting potential ($\simeq -60$ mV). Hyperpolarization from the resting level produced a large, almost steady inward current, while depolarization evoked only a small, steady outward current. The current–voltage relationship revealed a shallow negative resistance region at membrane potentials beyond −50 mV. The current was blocked by Cs (10 mM) or Ba (1 mM) ions.

4. The second type of K current (the transient outward current) was activated by membrane depolarization beyond −25 mV. The peak amplitude increased almost exponentially as the membrane was depolarized. During steady depolarization this current decayed exponentially (time constant $\simeq$ 500 ms at +20 mV). The current was inactivated by conditioning depolarization (> 10 s) beyond −30 mV and blocked by 4-aminopyridine (10 mM).

5. The third type of K current was the maintained outward current which was activated by membrane depolarization beyond −20 mV, increased to a steady level in a few hundred milliseconds, and showed little inactivation. The amplitude increased as the membrane was depolarized. The current was blocked by tetraethylammonium ions (20 mM).

6. A Ca-mediated K current was not detected.

7. Action potentials and the non-linear current–voltage relationship of solitary

* Present address: Department of Information Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan.

horizontal cells can be explained qualitatively by the combination of the four ionic currents.

INTRODUCTION

Horizontal cells are interneurones which probably contribute to lateral interactions in the distal part of vertebrate retinas (Werblin & Dowling, 1969; Naka, 1972, 1977; Toyoda & Kujiraoka, 1982). To understand the precise functions of horizontal cells it is important to know the ion-conductance mechanisms which modify the inputs received, as well as the synaptic transmission mechanisms between horizontal and other cells. However, it is difficult to examine the intrinsic properties of horizontal cells in the intact retina without interference from other retinal neurones: horizontal cells receive inputs not only from photoreceptors but also from interplexiform cells (Ehinger, Falck & Laties, 1969; Dowling, Ehinger & Hedden, 1976), send outputs to photoreceptors (Baylor & Fuortes, 1970; Baylor, Fuortes & O'Bryan, 1971; Pinto & Pak, 1974*a*, *b*; Burkhardt, 1977) or to bipolar cells (Brown, Major & Dowling, 1966; Dowling & Werblin, 1969; Naka, 1976), and are electrically coupled with neighbouring horizontal cells (Naka & Rushton, 1967; Norton, Spekreijse, Wolbarsht & Wagner, 1968; Kaneko, 1971) through gap junctions (Yamada & Ishikawa, 1965; Witkovsky & Dowling, 1969; Stell, 1972; Raviola, 1976).

Several studies have demonstrated that major classes of retinal cells can be isolated from enzyme-treated retinas, and identified morphologically (Drujan & Svaetichin, 1972; Lam, 1972; Anctil, Ali & Couillard, 1973; Bader, MacLeish & Schwartz, 1978; Tachibana, 1981). The solitary cells thus isolated greatly facilitate studies of their physiological and chemical properties by precluding the complex synaptic interactions in the intact retina.

In a previous paper (Tachibana, 1981) the electrical properties of solitary horizontal cells isolated from the goldfish retina were studied by recording membrane potentials. It has been demonstrated that solitary horizontal cells behave as a K electrode over a wide range of potentials and produce Ca-dependent action potentials when their membranes are depolarized by exogenous current pulses. Furthermore, the current–voltage relationship of solitary horizontal cells is quite non-linear, i.e. these cells show both inward- and outward-going rectifications, even after the blockage of their Ca-dependent action potentials.

In the experiments described here a voltage-clamp techique was applied to solitary horizontal cells and at least four ionic currents were identified: a Ca current and three types of K currents. The latter comprised a current flowing through the anomalous (inward-going) rectifier, a transient outward current and a maintained outward current.

METHODS

Animals and dissociation procedure

Experiments were performed on horizontal cells from the retina of the common goldfish, *Carassius auratus* (12–20 cm total length).

The dissociation procedure was slightly modified from that reported previously (Tachibana, 1981). Each fish was kept in darkness for 1 h and then pithed. The eyes were excised under dim light, soaked in 70% ethanol for 30 s, and rinsed three times with a rinse solution (see Table 1). The cornea and lens were removed, and the remaining eye cups were incubated for 20 min at 28 °C

in 14 ml of an oxygenated, gently-swirling enzyme solution containing 5–7 u.papain/ml (Worthington Biochemical Co., no. 3126) and 2·7 mM-DL-cysteine HCl (Sigma). The retina was then detached from the pigment epithelium and cut into small pieces which were incubated for a further 20 min in fresh enzyme solution. At the end of the second incubation the pieces of retina were transferred to a plastic test tube and rinsed three times each with 14 ml of the rinse solution containing 0·1 mg/ml of bovine serum albumin (Sigma, no. A4378). The retina was triturated in 2 ml of this solution with a glass pipette. A 60–80 μl aliquot of the final cell suspension was dispensed into modified culture dishes (Bray, 1970) which contained 2 ml of culture solution (see Table 1). In some cases Medium 199 (GIBCO, no. 320–1153) was added to the culture solution in a ratio of 1 to 20.

The dishes were stored at 10 °C in the presence of constantly circulated, moist filtered air.

Morphology of solitary horizontal cells in culture

Immediately after dissociation, solitary horizontal cells were easily recognized by their large, flat cell bodies and thick dendrites (see Tachibana, 1981). These cells survived in the culture solution supplemented with Medium 199 for over a month; fine processes grew from dendrites, and the cells retained their characteristic morphology (Pl. 1 *A*). In the simple culture solution (without Medium 199) the horizontal cells tended to change in morphology during the period between day 2 and day 4 *in vitro*: the cell bodies became round and the dendrites retracted (Pl. 1 *B*). Fine short processes (< 5 μm length) from the retracted dendrites attached to the surface of the culture dishes.

Resting and action potentials were the same in cells cultured with or without Medium 199. Therefore, the present experiments were performed using the rounded horizontal cells kept in the simple culture solution for 2–4 days, to ensure better space-clamp conditions.

Superfusion system and solutions

For recordings, the culture dish was mounted on the stage of an inverted microscope with phase-contrast optics (Zeiss, IM 35). A stainless-steel ring (O'Lague, Potter & Furshpan, 1978) was put into the dish to increase the rate of superfusate exchange and to improve the heat transfer. Solutions were fed into the dishes through Teflon tubing by a peristaltic pump, and withdrawn by another pump. The perfusion rate was 0·6 ml/min, and it took about 1 min to exchange the solution in the dish. The temperature of the solution in the dish was maintained at 15 °C by a water-cooled holder on the microscope stage.

The composition of solutions is listed in Table 1.

To isolate a specific ionic current(s), the following procedures were combined: change of the holding potential, application of pharmacological agents, and replacement of intracellular cations with the use of an ionophore, nystatin. The pharmacological agents included tetraethylammonium chloride (TEA; Kodak), CsCl (Sigma), 4-aminopyridine (4-AP; Sigma) and $CoCl_2$ (Sigma), which were dissolved freshly before use. Quinine (Sigma, no. Q1625) was dissolved in absolute ethanol and then added to the superfusate. The final concentration of ethanol was 0·1 %, which by itself produced no detectable effect on evoked currents. All of these agents were substituted for an equimolar amount of choline chloride in the standard solution, such that all of the test solutions contained the same amount of Na ions.

Nystatin was used to replace intracellular monovalent cations with Cs ions (Tillotson, 1979). The solitary cells were initially bathed in a Cs-loading solution (see Table 1) containing nystatin (Calbiochem; 60 mg/ml) for 15 min, and then washed with nystatin-free Cs-loading solution for another 30 min. The Cs-loaded horizontal cells were then studied in K-free solutions.

Recording procedures

Since the somata of solitary horizontal cells are characteristically small (around 20 μm in diameter), a single-micro-electrode voltage-clamp technique (Wilson & Goldner, 1975) was applied. The electrical circuit for the voltage clamp was slightly modified from that used by Bader, MacLeish & Schwartz (1979). The micro-electrode was connected to a high input impedance amplifier with an improved capacitance neutralization circuit (Thomas, 1977) and a constant current source (Colburn & Schwartz, 1972). The output of the amplifiier followed a voltage step at the tip of the micro-electrode with a time constant of less than 100 μs when the electrode resistance was 200 MΩ (see section on electrodes). The time constant of solitary horizontal cells near the resting potential was estimated to be about 10 ms. Currents were injected through the micro-electrode for 500 μs at

TABLE 1. Solutions

Solutions (mM)	NaCl	KCl	$MgCl_2$	$MgSO_4$	$NaHCO_3$	NaH_2PO_4	Na pyruvate	Glucose	$CaCl_2$	HEPES	CsCl
Rinse soln.	119·9	2·6	0·5	0·5	1·0	0·5	1·0	16·0	2·5	4·0	—
Enzyme soln.	119·9	2·6	0·5	0·5	1·0	0·5	1·0	16·0	0·5	4·0	—
Culture soln.	112·5	10·0	0·5	0·5	1·0	0·5	1·0	16·0	2·5	4·0	—
Cs-loading soln.	—	—	4·0	—	—	—	—	16·0	—	Tris/2·0	125·0

Solutions (mM)	NaCl	KCl	$MgCl_2$	$CaCl_2$	Glucose	HEPES	Choline Cl	TEA	CsCl	4-AP	$CoCl_2$
Standard soln.	79·0	10·0	1·0	2·5	16·0	2·0	37·25	—	—	—	—
TEA, Cs, 4-AP, Co soln.	79·0	10·0	1·0	—	16·0	2·0	—	20·0	10·0	10·0	4·0
TEA, Cs, 4-AP soln.	79·0	10·0	1·0	2·5	16·0	2·0	2·25	20·0	10·0	10·0	—
Na-free, TEA, Cs, 4-AP soln.	—	10·0	1·0	2·5	16·0	2·0	81·25	20·0	10·0	10·0	—
K-free, Cs soln.	79·0	—	1·0	2·5	16·0	2·0	37·25	—	10·0	—	—
K-free, Cs, Co soln.	79·0	—	1·0	—	16·0	2·0	35·0	—	10·0	—	4·0
TEA, 4-AP, Co soln.	79·0	10·0	1·0	—	16·0	2·0	10·0	20·0	—	10·0	4·0
30 K, TEA, 4-AP, Co soln.	69·0	30·0	1·0	—	16·0	2·0	—	20·0	—	10·0	4·0
Co soln.	79·0	10·0	1·0	—	16·0	2·0	35·0	—	—	—	4·0
Cs, Co soln.	79·0	10·0	1·0	—	16·0	2·0	25·0	—	10·0	—	4·0
4-AP, Cs, Co soln.	79·0	10·0	1·0	—	16·0	2·0	20·0	—	10·0	10·0	4·0
TEA, Cs, Co soln.	79·0	10·0	1·0	—	16·0	2·0	5·0	20·0	10·0	—	4·0

All solutions except the enzyme soln. contained 0·1 mg/ml of bovine serum albumin and Phenol Red (final concentration, 0·001 %). The pH was adjusted to 7·6 with NaOH or HCl. The enzyme soln. contained 0·02 mg/ml of bovine serum albumin and the pH was adjusted to 7·3.

a 1:1 duty cycle. Therefore, at 500 μs after the cessation of a current pulse, the voltage across the micro-electrode decayed almost completely (> 99·9 %), while that across the membrane decreased by about 5 %.

For voltage-clamp experiments, the deviation of the membrane potential from a command voltage, measured at the end of each interval in which current was not injected, was used to determine the amplitude and polarity of the current to be injected during the subsequent time interval, so as to reduce the difference between the membrane and command voltages (for details see Bader *et al.* 1979). In most cases the deviation between the membrane and command voltages was found to be within 10 %.

To minimize the instability or oscillation of the voltage-clamp system during sudden changes of command voltage, the rate of change in command voltage was limited to 5 V/s. Currents observed during the initial 50 ms after potential changes will be ignored in this paper, since the capacitive and ionic currents could not be distinguished during this period.

Both current and voltage outputs of the voltage-clamp amplifier were low-pass filtered (50 Hz, 24 db/oct; ITHACO no. 4302), observed on an oscilloscope and recorded on a pen recorder. At the same time, the data were sampled at 1–10 ms intervals by PDP 11/03 computer and analysed by PDP 11/44 or PDP 11/34.

Electrodes

Micro-electrodes with a short shank were pulled from Ω-dot shaped capillaries (Glass Company of America, 1·5 mm o.d.) or from theta capillaries (R & D Optical Systems, 1·4–1·8 mm o.d., style 1A) by a Brown–Flaming type puller (Sutter Instrument Co., P-77). For recordings, the Ω-dot shaped electrode filled with 4 M-K acetate was used (150–200 MΩ resistance). For ionophoretic injections of Ca or EGTA ions into the horizontal cells under voltage clamp, the theta electrode was used: one barrel was filled with $CaCl_2$ (100 mM) or EGTA (250 mM; pH adjusted to 7·5 with 2 N-KOH), and the second barrel with 4 M-K acetate (150–200 MΩ resistance). Both types of electrodes were coated with wax (Apiezon W wax; Apiezon Products Ltd, London) up to approximately 100 μm from the tip to reduce capacitance through the glass wall.

The indifferent electrode was an Ag/AgCl wire immersed in 2 M-KCl, coupled to the culture dish via an agarose bridge.

Experimental protocol

Experiments were performed by recording from the same solitary horizontal cells following the protocol: (1) recording of resting membrane potentials and action potentials in the standard solution, (2) recording of total ionic currents under voltage clamp in the standard solution, (3) examination of an isolated current in test solutions, (4) blockage of the isolated current, and (5) confirmation of recovery of the isolated current from the blockage. The amplitude of each isolated current recovered to more than 70 % of the original value after a 5–7 min wash. If the cell was lost before the recovery was observed, the data were discarded.

RESULTS

Ionic currents in the standard solution

A total of 146 solitary horizontal cells were studied under voltage-clamp conditions. The complexity of the ion-conductance system will be demonstrated by describing in this section the total currents, and in the rest of the paper the specific components of the currents.

In the standard solution (Table 1) the membrane potential of solitary horizontal cells was clamped to a resting potential and then shifted by a series of command pulses. A hyperpolarizing pulse evoked a large, almost steady inward current (Fig. 1*A*, trace c-1). Membrane depolarization up to −30 mV produced a small outward current (trace c-2) but that beyond −30 mV evoked a current with a complicated wave form (trace c-3): following a capacitive surge, the net current was

first transiently inward (reaching a maximum value at a few hundred milliseconds after the onset of the voltage step), and then outward.

Both of the current–voltage (I–V) relationships, i.e. plots of the amplitudes of membrane current *vs.* membrane potential, each measured at 0·2 s and at 5·3 s after the voltage step onsets, were markedly non-linear (Fig. 1*B*, circles and squares). Anomalous rectification was prominent near the resting potential, i.e. the slope conductance was larger in the hyperpolarizing direction than in the depolarizing one.

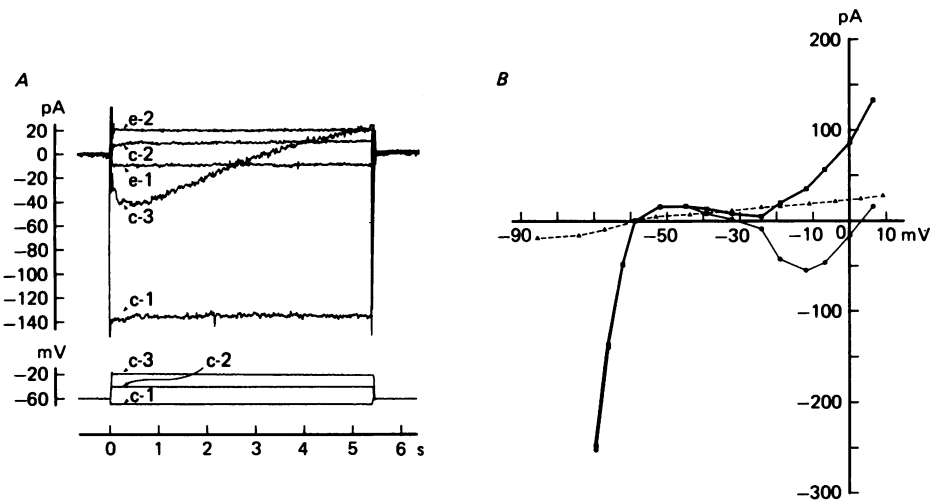


Fig. 1. Total current and leakage current under voltage clamp. *A*, current (upper) and voltage (lower) traces. The membrane potential was held at the resting potential (−59 mV) and then shifted to various levels (c-1, −66 mV; c-2, −39 mV; c-3, −19 mV; e-1, −67 mV; e-2, −11 mV). Traces c-1, c-2 and c-3 (total current) were recorded in the standard solution, while traces e-1 and e-2 (leakage current) were obtained in the solution containing TEA (20 mM), Cs (10 mM), 4-AP (10 mM) and Co (4 mM) ions. Outward and inward currents are shown as upward and downward deflexions, respectively. *B*, I–V relationships replotted from *A*. The amplitude of the currents was measured at 0·2 s (circles) and at 5·3 s (squares) in the standard solution, and at 5·3 s (triangles) in the TEA, Cs, 4-AP, Co solution. The slope resistance in the last solution was 2·7 GΩ.

The slope of the I–V curve at 0·2 s was negative at membrane potentials between −45 and −11 mV and net inward currents were clearly observed up to the positive membrane potentials. Thus, when the membrane potential of unclamped solitary horizontal cells reaches −45 mV, one can expect to record a jump in potential to a positive value, i.e. an action potential. The I–V curve at 5·3 s also showed a negative slope conductance region and a shallow outward-going rectification.

Leakage current

The voltage- and time-dependent currents mentioned above were suppressed almost completely after the standard solution was switched to the solution containing TEA (20 mM), Cs (10 mM), 4-AP (10 mM) and Co (4 mM) ions (the TEA, Cs, 4-AP, Co solution of Table 1). TEA, Cs and 4-AP have been known as K-current blockers and Co as a Ca-current blocker (for review see Stevens, 1980). The remaining current

was time-independent, and linearly proportional to the amount of membrane polarization from the resting level (Fig. 1 *A*, traces e-1 and e-2; Fig. 1 *B*, broken line). The membrane resistance was very high (2·2 ± 0·6 GΩ; $n = 21$) under this condition. These results indicate that the remaining current was a leakage current.

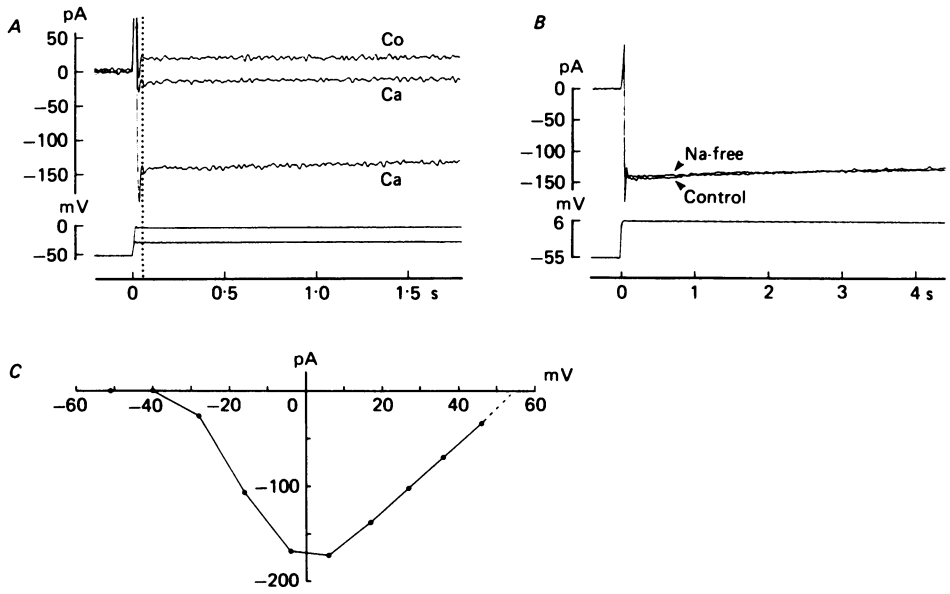


Fig. 2. Ca current. This was isolated from other currents by superfusing a cell with TEA (20 mM), Cs (10 mM) and 4-AP (10 mM). EGTA was continuously injected into the cell ionophoretically through one barrel of the theta electrode under voltage clamp. *A*, Ca current and effect of Co ions. In the TEA, Cs, 4-AP solution membrane depolarization from −52 mV (resting potential) to −28 mV or −4 mV produced sustained inward currents (traces Ca) following capacitive surges and artifacts (< 50 ms: dotted line). The Ca current was blocked by 4 mM-Co ions (the TEA, Cs, 4-AP, Co solution), and a leakage current (trace Co) remained. *B*, effect of external Na ions. Replacement of all external Na ions with choline (the Na-free, TEA, Cs, 4-AP solution) did not affect the evoked current (leakage current not subtracted). The inward current decayed slightly, perhaps because the buffering capacity of the injected EGTA was partially overcome by a large Ca influx. The time scale is slower than that shown in *A*. *C*, *I–V* relationship of the Ca current. The data were replotted from *A* after the correction for leakage currents. The reversal potential was estimated to be +55 mV in this case.

Ca current

Previous studies (Tachibana, 1981; Johnston & Lam, 1981) have shown that solitary horizontal cells produce Ca-dependent action potentials. The expectation then is to observe a substantial Ca current under voltage clamp.

EGTA-injected cells in the TEA, Cs, 4-AP solution. The inward current evoked by membrane depolarization (Fig. 1) was isolated by superfusing the solitary horizontal cell with the solution containing TEA, Cs and 4-AP (Table 1). The Ca-chelating agent EGTA was continuously injected into the cell ionophoretically to maintain the intracellular Ca concentration at a low level. Under these conditions, a steady inward

current was recorded in response to membrane depolarization (Fig. 2*A*, traces Ca; leakage current not subtracted). The steady inward current seemed to be carried mainly by Ca ions for the following reasons: (a) 4 mM-Co ions eliminated this inward current (Fig. 2*A*, trace Co), (b) replacement of the extracellular Na ions by choline did not produce any changes in amplitude and time course of the inward current in any of the three cells examined (Fig. 2*B*), and (c) the injected EGTA probably buffered the concentration of the intracellular Ca ions to a low level, thus minimizing the possibility of contamination by a Ca-mediated K current (Meech, 1974; Meech & Standen, 1975) and/or by a Ca-mediated anionic current (Bader, Bertrand & Schwartz, 1982), if present.

The *I–V* relationship of the Ca current was examined after correction for the leakage current (Fig. 2*C*). It was activated beyond $-45{\cdot}8 \pm 5{\cdot}4$ mV ($n = 9$) and gradually increased in amplitude by depolarizing the membrane potential up to $+0{\cdot}8 \pm 7{\cdot}0$ mV ($n = 9$). Further depolarization decreased the amplitude of the current, probably due to a decrease in the driving force. The current was almost nullified near +50 mV ($+50{\cdot}2 \pm 4{\cdot}9$ mV, $n = 6$). A reversal of Ca current was not observed as in other preparations (see Hagiwara & Byerly, 1981).

Ca-mediated inactivation of the Ca current

Decay of the Ca current. When EGTA was not injected into the cell bathed in the TEA, Cs, 4-AP solution, the inward current evoked by membrane depolarization decayed gradually in all twelve cells examined (Fig. 3*A*, traces Ca; leakage current not subtracted). When leakage currents (Fig. 3*A*, trace Co) were subtracted, the net current flowed inward even during prolonged membrane depolarization (Fig. 3*B*). The decay of the inward current was due to a reduction in the Ca current, but not due to an increase in counteracting outward current. The latter possibility was excluded for the following reasons: (a) the Ca-mediated K current (Meech, 1974; Meech & Standen, 1975) was not detected (these experiments will be described in a later section), (b) the Ca-mediated anionic current (Bader *et al.* 1982) was not observed in experiments in which the extracellular Cl ions (the only anion in the superfusate) were replaced with SO_4 ions (three cells, not illustrated), and (c) the non-specific current has been reported to be activated only at a strong depolarization (> +20 mV) (Kostyuk, Krishtal & Shakhovalov, 1977; Byerly & Hagiwara, 1982) but the inward current decayed even for small membrane depolarizations (Fig. 3*A* and *B*).

It has been reported in molluscan neurones that intracellular accumulation of Ca ions resulting from the Ca influx causes the suppression of subsequent Ca currents (Tillotson, 1979). This hypothesis was examined in the following experiments as an explanation for the decay of the current in solitary horizontal cells.

Double-pulse experiments. The effect of conditioning Ca influx on the Ca current evoked by a test pulse was analysed. A conditioning pulse of various intensities was followed at a fixed interval by a test pulse of constant intensity (Fig. 4*A*). As shown in the preceding section (Figs. 2*C* and 3*B*), the amplitude of the Ca current evoked by a conditioning pulse depended on the magnitude of membrane depolarization (Fig. 4*B*, I_1). Despite the fixed intensity of the test pulse, the current (I_2) *decreased* as the current evoked by the conditioning pulse (I_1) *increased*, and vice versa

(Fig. 4*B*). This relationship is further demonstrated by plotting the total charge transfer (time integral of the current) evoked by the test pulse *vs.* that produced by the conditioning pulse after correction for leakage currents (Fig. 4*C*). The relative charge transfer produced by the test pulse ($I'_2/I'_{2\ max}$) was linearly and inversely related to that produced by the conditioning pulse ($I'_1/I'_{1\ max}$). This result suggests that the size of the Ca influx evoked by test pulses depends on the preceding Ca influx

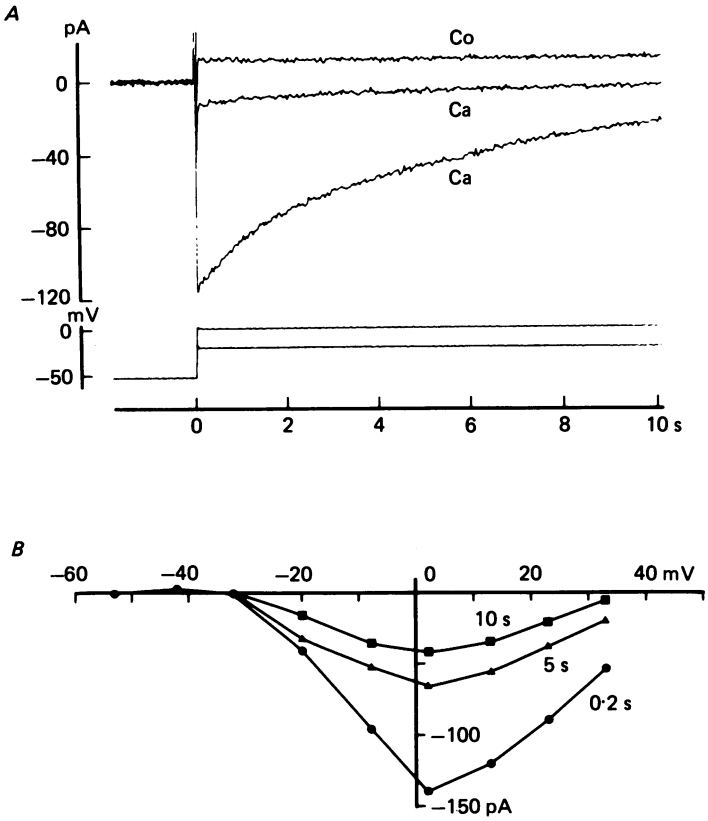


Fig. 3. Decay of the Ca current. A cell not loaded with EGTA was bathed in the TEA, Cs, 4-AP solution. *A*, decaying Ca currents (traces Ca; leakage current not subtracted) and leakage current (trace Co). The Ca current was produced by membrane depolarization from the resting level (−53 mV) to either −20 mV or +2 mV. With the application of 4 mM-Co ions (the TEA, Cs, 4-AP, Co solution) the Ca current was completely blocked and the leakage current remained (depolarization to +2 mV) (trace Co). *B*, *I–V* relationships of the Ca current. Amplitude of each Ca current was measured at 0·2, 5·0 and 10·0 s after the onset of membrane depolarization. The data were replotted from *A* after correction for leakage currents.

and not on the shift in membrane potential produced by conditioning pulses. Similar results were obtained from four other Cs-loaded cells, and four cells which were not loaded with Cs but bathed in the TEA, Cs, 4-AP solution.

Ca injection. To determine more directly whether accumulation of intracellular Ca ions can suppress the Ca current independently of the membrane potential, Ca ions were ionophoretically injected into horizontal cells bathed in the TEA, Cs, 4-AP

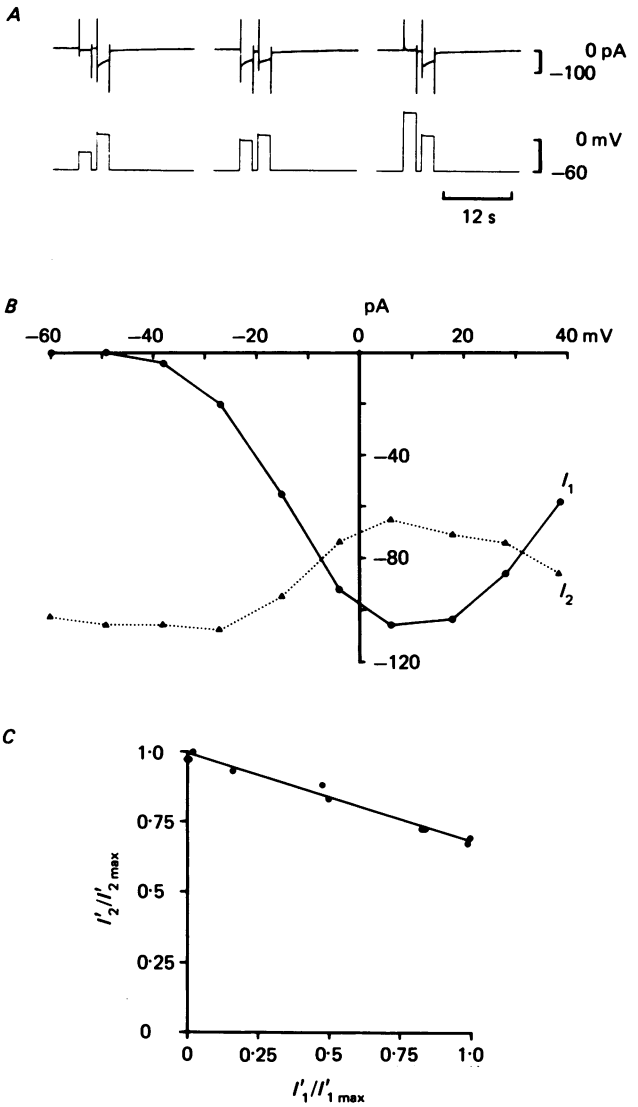


Fig. 4. Effect of conditioning Ca influx on the Ca current. A Cs-loaded cell was used to isolate the Ca current. *A*, the Ca current evoked by a 2·3 s conditioning pulse of various intensities and a 2·3 s test pulse of constant intensity (from −60 to +6 mV). The interval between the onset of the two pulses was fixed at 3·3 s. The cell was superfused with the K-free, Cs solution and the holding potential was −60 mV. Time course of the current decay was similar to that recorded from a cell not loaded with Cs ions but bathed in the TEA, Cs, 4-AP solution (see Fig. 3). Leakage currents were not subtracted. *B*, relationships between the peak amplitude of Ca currents evoked by conditioning pulses (I_1, circles) and that evoked by test pulses (I_2, triangles) as a function of the membrane potential resulting from conditioning pulses. Leakage currents were corrected. *C*, the relationship between the relative amount of time integral of the Ca currents produced by conditioning pulses ($I'_1/I'_{1\,max}$) and that produced by test pulses ($I'_2/I'_{2\,max}$). The time integral of the Ca currents was estimated by measuring the area under each current trace after the correction for leakage currents.

solution while the membrane potential was clamped at the resting level. Under these conditions the evoked Ca current was minimum immediately after the Ca injection, and subsequently recovered to its control amplitude (Fig. 5*A*). When a large amount of Ca ions was injected, the Ca current was strongly suppressed and recovery from suppression was prolonged (Fig. 5*B*). Similar results were obtained from seven cells.

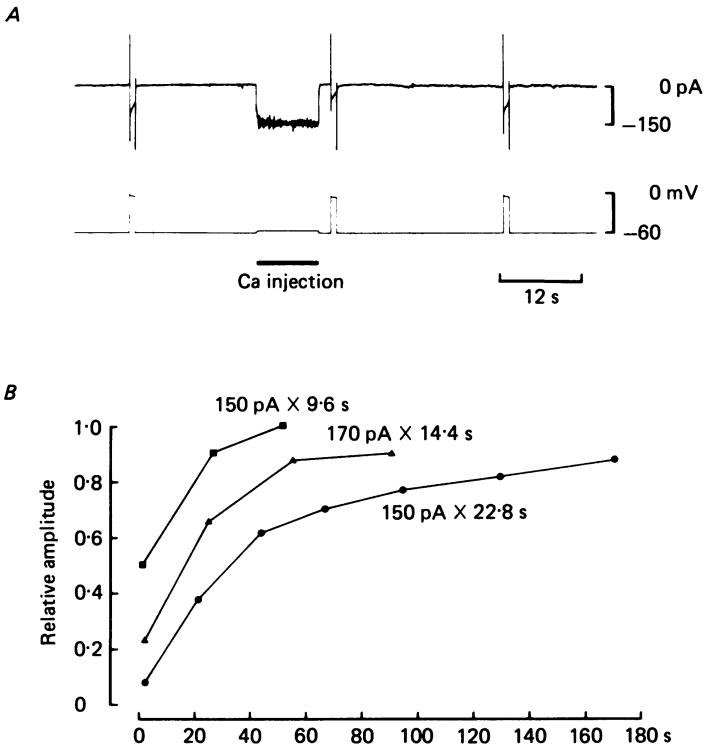


Fig. 5. Effect of intracellular Ca injection on the Ca current. A solitary horizontal cell bathed in the TEA, Cs, 4-AP solution was clamped at the resting potential (−60 mV) through one barrel of the theta electrode and Ca ions were injected into the cell ionophoretically through the other barrel. *A*, the injection of Ca ions (150 pA through the Ca barrel for 9·6 s) and the Ca currents produced by 2 s test pulses. Leakage currents were not subtracted. *B*, relative amplitude of Ca currents (after the correction for leakage currents) produced by test pulses at various time intervals after ionophoretic injection of Ca ions. Amplitude was measured at 0·2 s after the onset of test pulses. Intensities and durations of the applied currents through the Ca barrel are shown for each plot.

Decay of the Ba current. The experiments of Figs. 4 and 5 indicate that the increase in intracellular Ca ions can suppress the Ca current. It may be asked whether the suppression is simply due to a decrease in the driving force caused by the accumulation of divalent cations near the inner surface of the membrane or due to the Ca-mediated inactivation specific for Ca ions (Tillotson, 1979). A way to examine the former possibility would be to measure the reversal potential before and after the decay of the Ca current, but it is technically difficult (see Hagiwara & Byerly, 1981). Therefore,

an alternative method was employed. The experimental design is based on the previous findings that Ba ions can carry a larger amount of current through Ca channels than can Ca ions (Hagiwara, Fukuda & Eaton, 1974) and that cells have a smaller capacity for buffering Ba ions than Ca ions (Connor, Ahmed & Ebert, 1981). Thus, during identical command voltage steps, Ba ions would accumulate near the inner surface of the membrane more quickly than Ca ions, resulting in more rapid decrease in the driving force. It would be expected that the Ba current would decay

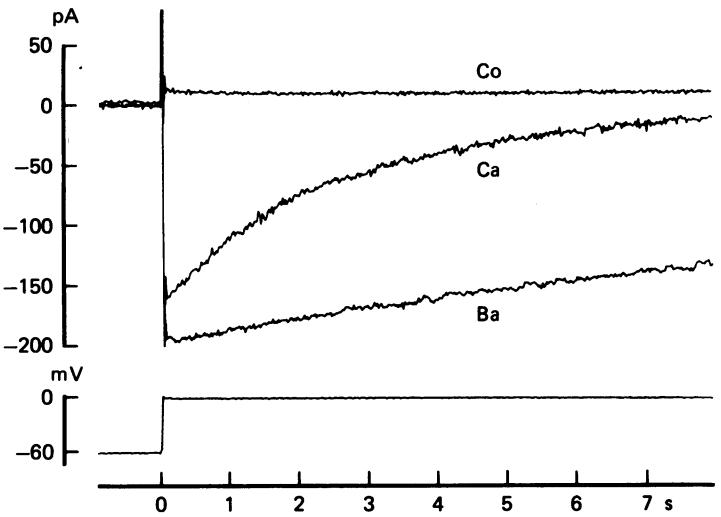


Fig. 6. Decay of the Ba current and the Ca current. Each current trace was recorded from a cell bathed in the TEA, Cs, 4-AP solution which contained either 2·5 mM-Ca ions (trace Ca), 2·5 mM-Ba ions (trace Ba), or 4·0 mM-Co ions (trace Co). The membrane potential was changed from the resting level (−61 mV) to 0 mV. The leakage current (trace Co) was not subtracted from trace Ca or trace Ba.

faster than the Ca current. Contrary to the above assumption, the Ba current decayed more slowly than did the Ca current, although the peak amplitude of the Ba current was larger than that of the Ca current (Fig. 6). All four cells examined showed a similar result. Therefore, it is concluded that the decay of the Ca current in solitary horizontal cells was caused by the Ca-mediated inactivation of the Ca current.

K currents

K current through the anomalous (inward-going) rectifier

The *I–V* relationship of solitary horizontal cells in the standard solution suggested the presence of anomalous rectification (Fig. 1*B*). The property of this rectification shall now be described further.

A current through the anomalous rectifier was separated from the Ca current and other K currents (see the next section) by superfusing the cells with the TEA, 4-AP, Co solution (Table 1). A large inward current preceded by a capacitive surge was evoked by membrane hyperpolarization and a small outward current was produced

by depolarization (Fig. 7*A*). The *I–V* curve bent downwards around the resting membrane potential and became nearly linear at potentials more negative than −70 mV, where the slope resistance was about 30 MΩ (31 ± 14 MΩ, $n = 15$) (Fig. 7*C*). After the leakage currents were corrected, the *I–V* curve showed a shallow negative slope at membrane potentials beyond approximately −50 mV in all the cases examined (fifteen cells). It was difficult to determine the range of the negative resistance region exactly because the outward current was small and because large depolarizations produced much noise.

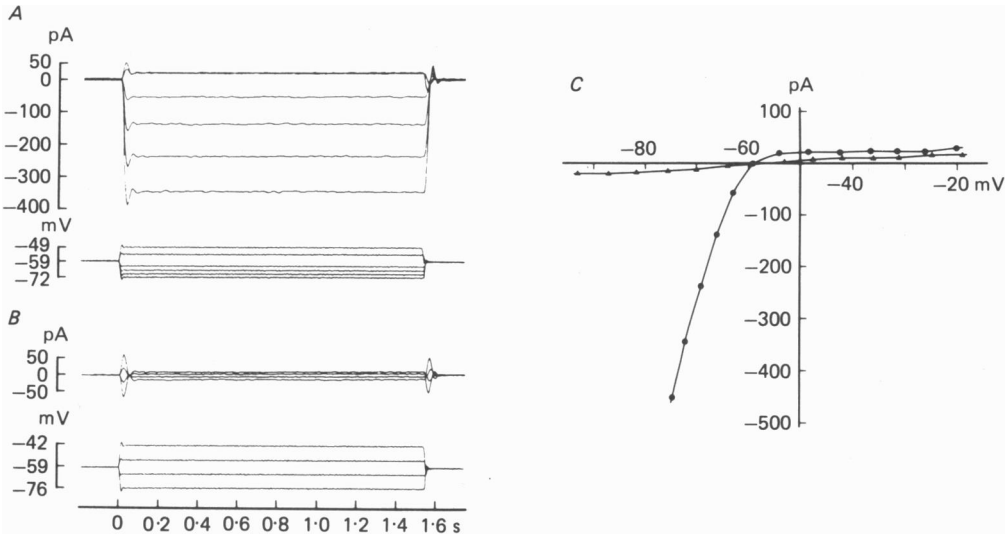


Fig. 7. Current through the anomalous (inward-going) rectifier. *A*, current and voltage traces recorded from a cell bathed in the TEA, 4-AP, Co solution. Currents during the initial 50 ms after potential changes were contaminated with capacitive and other artifacts. Leakage currents were not subtracted. The holding potential was −59 mV. *B*, blocking effect of Cs ions. The same cell was superfused with the solution containing 10 mM-Cs ions (the TEA, Cs, 4-AP, Co solution). Leakage currents remained. *C*, *I–V* relationships before and after the application of Cs ions. The data were replotted from *A* and *B*. Without Cs ions (circles) the slope resistance at more negative potentials than −65 mV was approximately 27 MΩ, and the negative resistance region was observed beyond −50 mV. In the presence of Cs ions (triangles) the *I–V* relationship was approximately linear and the slope resistance was about 1·82 GΩ.

When the concentration of extracellular K ions was increased from 10 to 30 mM, the resting membrane potential was depolarized by 24 mV (24·3 ± 0·8 mV, $n = 6$), the *I–V* relationship was shifted along the voltage axis, and the slope resistance of the linear region of the *I–V* curve below the resting membrane potential decreased to 62% of the control value (62 ± 8%, $n = 6$) (1·61 times the increase in conductance; not illustrated). On the other hand, when Na or Cl ions in the superfusate were replaced by choline or SO_4 ions, respectively, the wave form and the associated *I–V* relationship did not change significantly (six cells; not illustrated). These results indicate that the current through the anomalous rectifier was carried mainly by K ions and little, if any, by Na or Cl ions.

External Cs ions (10 mM) blocked the anomalous rectification (the TEA, Cs, 4-AP, Co solution). Both the inward and outward currents decreased in amplitude, and the *I–V* relationship became nearly linear (Fig. 7*B* and *C*). The anomalous rectification was also blocked by the application of Ba ions (1 mM) extracellularly (eight cells; not illustrated).

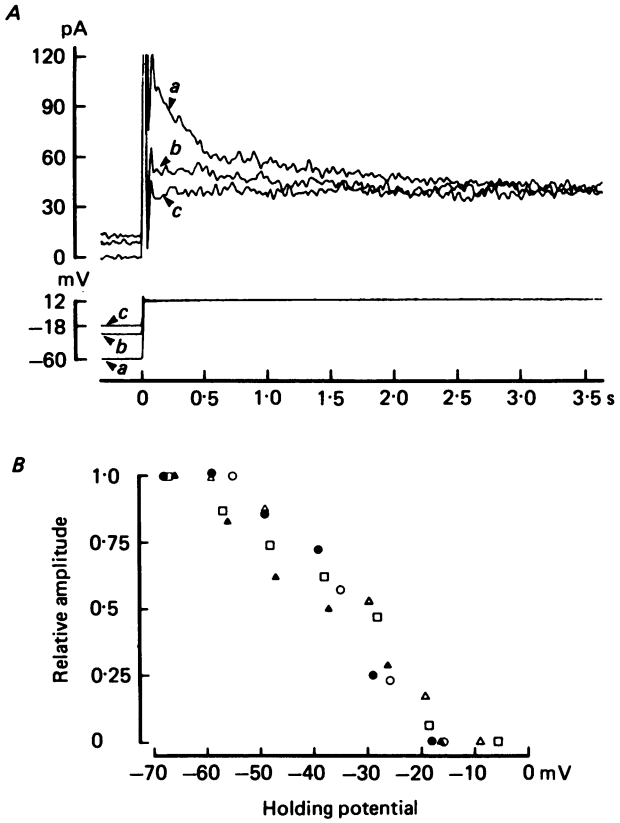


Fig. 8. Effect of holding potentials on the transient and maintained outward currents. *A*, current and voltage recordings from a cell bathed in the Cs, Co solution. Membrane potentials were held at three different levels (*a*, *b* and *c*) by 5 s conditioning pulses, and subsequently shifted to the same level (+12 mV) by test pulses. The transient component was completely eliminated by holding the membrane potential at −18 mV (current trace *c*), while the maintained component was unaffected. The resting membrane potential was −60 mV. *B*, relationship between the amplitude of the transient component and the holding potentials shifted by 5 s conditioning pulses. The amplitude of the transient current was estimated by subtracting the maintained component (trace *c*) from the combined outward current (trace *a* or *b*). Each symbol represents the data obtained from five cells.

Transient and maintained outward currents

Membrane depolarization (> −25 mV) evoked an outward current which decayed gradually to a steady level in a few seconds (Fig. 8*A*, trace *a*) when the K current through the anomalous rectifier and the Ca current were blocked by Cs and Co ions (the Cs, Co solution: Table 1). This outward current could be separated into two

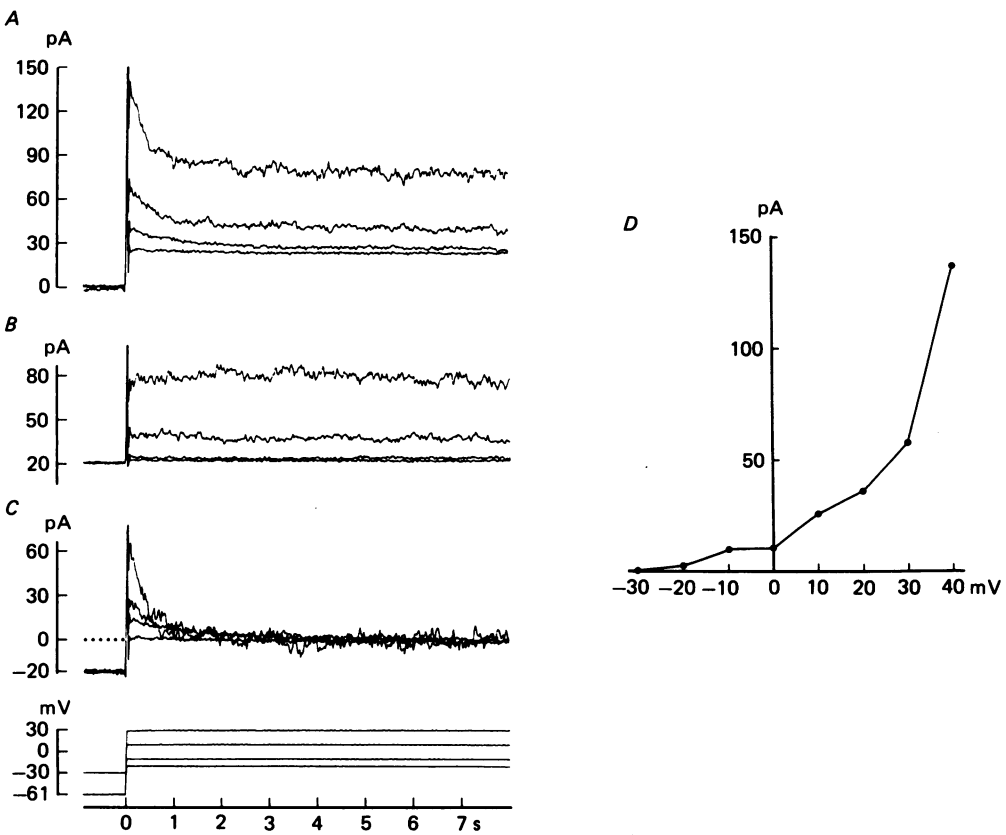


Fig. 9. Transient and maintained outward currents. *A*, combined outward currents (the transient, maintained and leakage currents) recorded from a cell bathed in the Cs, Co solution. The membrane potential was initially held at the resting level (−61 mV). *B*, maintained outward current (leakage current not subtracted). The transient outward current was completely suppressed by holding the membrane potential at −30 mV. A steady current (leakage current) of approximately 20 pA was necessary to hold the potential at −30 mV. *C*, transient outward current isolated by subtracting the current traces in *B* from the corresponding traces in *A*. The difference between the dotted line and current traces before the onset of test pulses corresponded to the leakage current necessary to change the holding potential. Voltage records shown at the bottom can be paired with corresponding current traces in *A*–*C*. *D*, peak amplitude of the transient outward current as a function of membrane potential. Since the falling phase of the current could be described by single exponential curves, the peak amplitude was estimated by extrapolating the exponential curves at time 0 (immediately after the potential change). The data were obtained from *C*.

components: one transient and the other maintained. The two components differed in voltage- and time-dependency, and in sensitivity to blockers.

Transient outward current. As is the A current reported in snail neurones (Connor & Stevens, 1971; Neher, 1971), the transient outward current of solitary horizontal cells was inactivated by conditioning depolarization (Fig. 8). As the holding potential (V_H) was depolarized by conditioning pulses (duration 5 s), peak current amplitudes

to test pulses of a fixed intensity decreased, while the steady level remained intact (Fig. 8*A*, traces *b* and *c*). The transient outward current was separated from the maintained outward current by using the differential effect of V_H, i.e. by subtracting trace *c* from trace *a* (Fig. 8*A*). The transient current was maximum at $V_H < -60$ mV, decreased by conditioning depolarization, and completely inactivated at

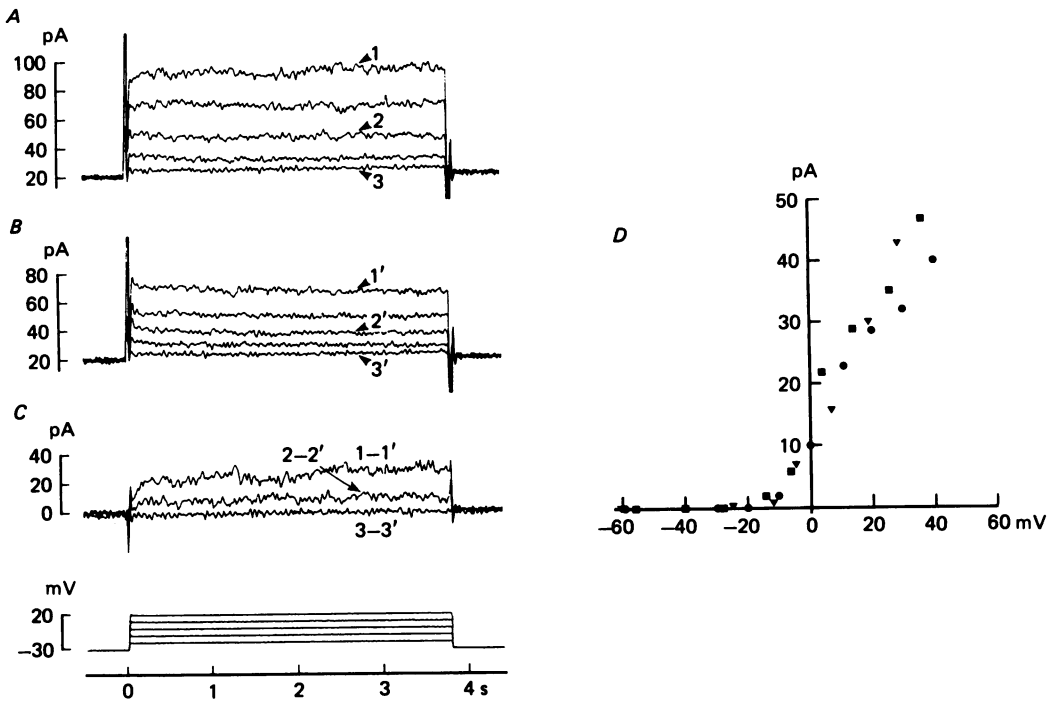


Fig. 10. Effect of TEA on the maintained outward current. *A*, the maintained outward current and the leakage current. The transient outward current was inactivated by holding the membrane potential at −30 mV, the Ca current was blocked by Co ions (4 mM), and the K current through the anomalous rectifier by Cs ions (10 mM). Steady outward current of about 20 pA flowed to hold the potential at −30 mV. *B*, blocking effect of 20 mM-TEA on the maintained outward current. The remaining current mainly consisted of the leakage current (the transient outward current was not completely inactivated in this case). *C*, the maintained outward current isolated by subtracting each current trace shown in *B* from the corresponding trace in *A*. Voltage traces are shown at the bottom. The resting membrane potential was −60 mV. *D*, *I–V* relationship of the maintained outward current (after the correction for leakage currents). The current amplitude was measured at a steady state. The holding potential was either at −30 mV (circles; replotted from *C*) or at resting potentials (triangles and squares). Each symbol represents the data obtained from different cells.

$V_H > -20$ mV (Fig. 8*B*). The inactivation of the transient outward current was also dependent on the duration of the conditioning depolarization. With a conditioning depolarization of more than 10 s, almost complete inactivation occurred at $V_H = -30$ mV (see Fig. 9*B*).

The transient outward current decayed almost completely within a few seconds (Fig. 9*C*). The falling phase of the current could be fitted by single exponential curves,

and the time constant was voltage-dependent, being shorter at more depolarized membrane potentials (about 1·7 s at −10 mV and about 500 ms at +20 mV). The transient outward current was activated beyond approximately −25 mV and the peak amplitude increased nearly exponentially as the membrane was depolarized (Fig. 9*D*). This current was blocked by 10 mM-4-AP but not affected by 20 mM-TEA (seven cells; not illustrated).

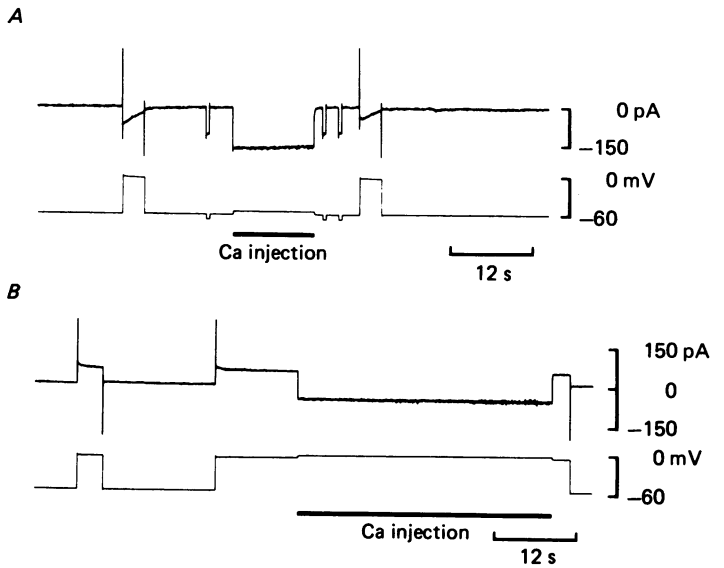


Fig. 11. Effect of Ca injection on the evoked current. A cell was clamped at the resting potential (−58 mV) through one barrel of the theta electrode and Ca ions were injected ionophoretically into the cell through the other barrel. *A*, control experiment. Ca ions were injected into the cell bathed in the standard solution. The net inward current evoked by a 3 s depolarizing pulse decreased immediately after the Ca injection (150 pA through the Ca barrel for 12 s), indicating that intracellular Ca ions were increased by this procedure. Within a minute, the amplitude of the evoked current recovered to the original value. The inward currents produced by hyperpolarizing pulses were almost similar in amplitude, indicating that the membrane conductance near the resting level did not change significantly after the Ca injection. The membrane potential changed by about 5 mV during the Ca injection, probably due to the electrical coupling between the barrels. *B*, Ca injection into the same cell after the Ca current was eliminated by Co (4 mM). Membrane depolarization produced outward current which mainly consisted of the transient and maintained outward currents (leakage currents not subtracted). Ca ions were injected into the cell (100 pA through the Ca barrel for 37 s) while the membrane potential was kept at 0 mV; however, no current was activated.

Maintained outward current. In contrast to the transient outward current, the maintained outward current was sensitive to TEA (20 mM). The maintained outward current was isolated by subtracting the leakage current (Fig. 10*B*) and is shown in Fig. 10*C*. For large depolarizations, the current gradually increased with time, and reached a steady-state value within a few hundred milliseconds without any detectable inactivation. The steady-state *I–V* relationship of the maintained outward current indicates that it was activated at membrane potentials beyond −20 mV

($-21{\cdot}8 \pm 6{\cdot}5$ mV, $n = 8$), and increased in amplitude without evidence of saturation as the membrane was depolarized (Fig. 10*D*).

Absence of the Ca-mediated K current

In the section on Ca current it was shown that the decay of the inward current was not due to the activation of a Ca-mediated K current. In this section, experimental results which support this conclusion will be described.

Effect of quinine. Plant & Standen (1981) have shown that quinine blocks the Ca-mediated K current. However, addition of quinine (0·2–1·0 mM) did not show any significant effect on the amplitude or time course of the decaying inward current recorded from solitary horizontal cells bathed in the TEA, Cs, 4-AP solution (four cells; not illustrated).

Replacement of intracellular monovalent cations by Cs ions. It has been reported that the Ca-mediated K current can be blocked by the presence of Cs ions inside the cell (Tillotson, 1979). Intracellular monovalent cations of the solitary horizontal cells were replaced by Cs ions with the aid of nystatin. After this treatment it was apparent that most of the intracellular K ions were replaced with Cs ions, since the transient and maintained outward currents were not detected. The Cs-loaded horizontal cells produced inward currents (see Fig. 4*A*) which decayed with a similar time course to those in cells which were not loaded with Cs ions (see Fig. 3*A*).

Ca injection. Injection of Ca ions into a cell increased intracellular Ca concentration, which was reflected in a reduction of the net inward current (Fig. 11*A*; see also Fig. 5). This reduction is not due to the development of an outward current, since similar Ca injection did not evoke an outward current in the same cell (Fig. 11*B*) when the Ca current was eliminated by Co ions and the driving force of K ions was increased by holding the membrane potential at 0 mV (the K equilibrium potential under this condition was approximately -60 mV: see Tachibana, 1981). This result indicates that the Ca-mediated K current was not present in solitary horizontal cells. Similar results were obtained from three other cells.

DISCUSSION

In this paper the presence of the Ca current has been clearly demonstrated, confirming what was strongly suggested in a previous paper (Tachibana, 1981). The Ca current of solitary horizontal cells resembles that of *Paramecium* (Brehm & Eckert, 1978) and snail neurones (Tillotson, 1979), in that the Ca conductance is inactivated by accumulation of intracellular Ca ions. It has been reported that Ca channels of other tissues appear to be blocked if the intracellular Ca ion concentration exceeds 1 μM (Hagiwara & Nakajima, 1966; Kostyuk & Krishtal, 1977; Doroshenko & Tsyndrenko, 1978; Takahashi & Yoshii, 1978). It may be estimated that 100 pA of Ca current flowing into a round, 20 μm diameter cell for 100 ms would produce an increase in the intracellular Ca ion concentration of 12 μM. Thus, the Ca current evoked in horizontal cells could very well raise the internal Ca ion concentration high enough to block the Ca current.

The anomalous rectifier found in horizontal cells is similar to the rectifiers found in vertebrate muscles (Hodgkin & Horowicz, 1959) and in egg cells (Hagiwara &

Takahashi, 1974; Miyazaki, Ohmori & Sasaki, 1975; Hagiwara, Miyazaki & Rosenthal, 1976; Hagiwara & Yoshii, 1979) in several aspects: (a) the current is mainly carried by K ions, (b) the conductance depends on the difference between the membrane potential and the K equilibrium potential, (c) the conductance increases in approximate proportion to the square root of the external K concentration, and (d) Cs and Ba ions block the anomalous rectification.

The transient outward current of horizontal cells resembles the A current found in various excitable cells (snail neurones: Connor & Stevens, 1971; Neher, 1971; coelenterate eggs: Hagiwara, Yoshida & Yoshii, 1981; invertebrate photoreceptors: Lisman, Swan & Fain, 1979; cat motoneurones: Barrett & Crill, 1972) in that the current activated by membrane depolarization rapidly decayed to zero and was blocked by 4-AP. The maintained outward current of horizontal cells shows a similarity to the delayed outward current in that it is blocked by TEA, activated with a relatively slow time course, and not detectably inactivated by prolonged depolarization (Hille, 1974; Thompson, 1977). Precise comparison of these properties cannot clearly be made until the kinetics of each current of solitary horizontal cells are examined using a faster voltage-clamp system.

Non-linear I–V relationship and action potentials of horizontal cells

The *I–V* relationship measured in the standard solution was composed of inward- and outward-going rectifying regions and a negative conductance region between them. From the present results, the K current through the anomalous rectifier seems to be responsible for the inward-going rectifying region, the maintained outward current for the outward-going rectifying region, and the Ca current and the K current through the anomalous rectifier for the negative conductance region. The potential jump found in the Co solution under current-clamp conditions (Tachibana, 1981) may be explained by the regenerative property of the anomalous rectifier, since only the anomalous rectifier has this property under these conditions.

Action potentials observed in horizontal cells might be explained qualitatively by combination of four isolated currents: the regenerative property of the Ca current seems to initiate the action potential (the K current through the anomalous rectifier may contribute to the initiation of action potentials as mentioned above); the activation of the transient outward current (which is inactivated within several hundred milliseconds) antagonizes the inward Ca current during the upstroke of the action potential, resulting in the delay of action potential peak by a few hundred milliseconds after the onset of current stimulus; the activation of the maintained outward current and the inactivation of the Ca current induce the gradual decay of the plateau potential; and the regenerative properties of the Ca current and the K current through the anomalous rectifier may bring back the membrane potential to the resting level.

Function of the Ca current

At present, our understanding of how ionic currents contribute to the photoresponse of horizontal cells in the retina is still far from complete. Horizontal cells are depolarized in the dark to between approximately -10 and -30 mV by neurotransmitter(s) released from photoreceptors (Trifonov, 1968; Byzov & Trifonov,

1968; Kaneko & Shimazaki, 1975). Under these conditions, the Ca current would flow into the cell, increase the intracellular Ca ion concentration and in turn inactivate the Ca conductance. The final concentration of the Ca ions would depend on the Ca regulating system of the cell. Free intracellular Ca ions may be related to some function(s), such as regulation of the coupling ratio between the electrically coupled horizontal cells, or release of transmitter substances. It has recently been reported that the release of γ-aminobutyric acid (GABA) from frog horizontal cells was independent of the extracellular concentration of Ca ions (Schwartz, 1982). However, at present it is not clear whether the substance released from horizontal cells is only GABA. In fact, in the fish retina, only one of four subclasses of horizontal cells appears to use GABA as a transmitter (Lam & Steinman, 1971; Marc, Stell, Bok & Lam, 1978; Lam, Su, Swain, Marc, Brandon & Wu, 1979). Therefore, the other subclasses might release other transmitter(s) in a Ca-dependent manner. It is also plausible that the GABA-releasing horizontal cell may have an additional mechanism which releases other transmitter(s) in a Ca-dependent manner.

Horizontal cells *in vivo* show graded responses to light (MacNichol & Svaetichin, 1958; Tomita, 1963), while solitary horizontal cells *in vitro* produce action potentials. However, using a special method of uniform polarization of electrically coupled horizontal cells, Trifonov and his colleagues have demonstrated that horizontal cells *in vivo* have a non-linear $I-V$ relationship with a negative conductance region after the synaptic transmission between photoreceptors and horizontal cells is suppressed by bright light (Trifonov, Byzov & Chailahian, 1974; Byzov, Trifonov, Chailahian & Golubtzov, 1977). Therefore, it seems reasonable to suppose that the active properties shown in solitary horizontal cells are present in normal horizontal cells, but suppressed in the presence of transmitters by some mechanism(s): for example, transmitters released from photoreceptors and/or interplexiform cells may modulate the ionic conductance(s) and suppress the active properties; intracellular and extracellular ionic composition may be changed under the influence of transmitters, resulting in the partial inactivation of the ionic conductance(s); a feed-back system from horizontal cells to photoreceptors may prevent potential jumps. It is interesting that the photoreceptors contain several kinds of voltage- and Ca-dependent conductances (Fain & Lisman, 1981; Bader *et al.* 1982), although the cells show a graded response to light illumination. These and the present results indicate that the response of neurones may consist of complicated conductance mechanisms even when the cells show a slow, graded response.

I thank Torsten Wiesel for his continued encouragement and support of this work, Peter MacLeish for his valuable advice, discussion and encouragement, Akimichi Kaneko and Andrew Ishida for their comments on the manuscript, John Kanki and Jim Voyvodic for their technical assistance, Cathy Cross and Hiroshi Maebashi for their assistance with photography. This work was supported by an N.I.H. grant EY 00606 to T. N. Wiesel and in part by Grant in Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture to M.T.

REFERENCES

ANCTIL, M., ALI, M. A. & COUILLARD, P. (1973). Isolated retinal cells of some lower vertebrates. *Rev. Can. Biol.* **32**, 107–119.

BADER, C. R., BERTRAND, D. & SHWARTZ, E. A. (1982). Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. *J. Physiol.* **331**, 253–284.

BADER, C. R., MACLEISH, P. R. & SCHWARTZ, E. A. (1978). Responses to light of solitary rod photoreceptors isolated from tiger salamander retina. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3507–3511.

BADER, C. R., MACLEISH, P. R. & SCHWARTZ, E. A. (1979). A voltage-clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol.* **296**, 1–26.

BARRETT, J. N. & CRILL, W. E. (1972). Voltage clamp analysis of conductances underlying cat motoneuron action potentials. *Fedn Proc.* **31**, 305.

BAYLOR, D. A. & FUORTES, M. G. F. (1970). Electrical responses of single cones in the retina of the turtle. *J. Physiol.* **207**, 77–92.

BAYLOR, D. A., FUORTES, M. G. F. & O'BRYAN, P. (1971). Receptive fields of cones in the retina of the turtle. *J. Physiol.* **214**, 265–294.

BRAY, D. (1970). Surface movements during the growth of single explanted neurons. *Proc. natn. Acad. Sci. U.S.A.* **65**, 905–910.

BREHM, P. & ECKERT, R. (1978). Calcium entry leads to inactivation of calcium channel in *Paramecium*. *Science, N.Y.* **202**, 1203–1206.

BROWN, J. E., MAJOR, D. & DOWLING, J. E. (1966). Synapses of horizontal cells in rabbit and cat retinas. *Science, N.Y.* **153**, 1639–1641.

BURKHARDT, D. A. (1977). Responses and receptive-field organization of cones in perch retinas. *J. Neurophysiol.* **40**, 53–62.

BYERLY, L. & HAGIWARA, S. (1982). Calcium currents in internally perfused nerve cell bodies of *Limnea stagnalis*. *J. Physiol.* **322**, 503–528.

BYZOV, A. L. & TRIFONOV, Y. A. (1968). The response to electric stimulation of horizontal cells in the carp retina. *Vision Res.* **8**, 817–822.

BYZOV, A. L., TRIFONOV, Y. A., CHAILAHIAN, L. M. & GOLUBTZOV, K. W. (1977). Amplification of graded potentials in horizontal cells of the retina. *Vision Res.* **17**, 265–273.

COLBURN, T. R. & SCHWARTZ, E. A. (1972). Linear voltage control of current passed through a micropipette with variable resistance. *Med. biol. Engng* **10**, 504–509.

CONNOR, J. A., AHMED, Z. & EBERT, G. (1981). Diffusion of Ca^{2+}, Ba^{2+}, H^+ and arsenazo III in neural cytoplasm. *Neurosci. Abstr.* **7**, 15.

CONNOR, J. A. & STEVENS, C. F. (1971). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *J. Physiol.* **213**, 21–30.

DOROSHENKO, P. A. & TSYNDRENKO, A. Y. (1978). Action of intracellular calcium on the inward calcium current. (In Russian.) *Neirofiziologiya* **10**, 203–205. (Translated in *Neurophysiology* (1978) **10**, 143–145.)

DOWLING, J. E., EHINGER, B. & HEDDEN, W. L. (1976). The interplexiform cell: a new type of retinal neuron. *Invest. Ophthal.* **15**, 916–926.

DOWLING, J. E. & WERBLIN, F. S. (1969). Organization of retina of the mudpuppy, *Necturus maculosus*. I. Synaptic structure. *J. Neurophysiol.* **32**, 315–338.

DRUJAN, B. D. & SVAETICHIN, G. (1972). Characterization of different classes of isolated retinal cells. *Vision Res.* **12**, 1777–1784.

EHINGER, B., FALCK, B. & LATIES, A. M. (1969). Adrenergic neurons in teleost retina. *Z. Zellforsch. mikrosk. Anat.* **97**, 285–297.

FAIN, G. L. & LISMAN, J. E. (1981). Membrane conductances of photoreceptors. *Prog. Biophys. molec. Biol.* **37**, 91–148.

HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *A. Rev. Neurosci.* **4**, 69–125.

HAGIWARA, S., FUKUDA, J. & EATON, D. C. (1974). Membrane currents carried by Ca, Sr, and Ba in barnacle muscle fiber during voltage clamp. *J. gen. Physiol.* **63**, 564–578.

HAGIWARA, S., MIYAZAKI, S. & ROSENTHAL, N. P. (1976). Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. gen. Physiol.* **67**, 621–638.

HAGIWARA, S. & NAKAJIMA, S. (1966). Differences in Na and Ca spikes as examined by application of tetrodotoxin, procaine, and manganese ions. *J. gen. Physiol.* **49**, 793–806.

HAGIWARA, S. & TAKAHASHI, K. (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. Membrane Biol.* **18**, 61–80.

HAGIWARA, S., YOSHIDA, S. & YOSHII, M. (1981). Transient and delayed potassium currents in the egg cell membrane of the coelenterate, *Renilla koellikeri*. *J. Physiol.* **318**, 123–141.

HAGIWARA, S. & YOSHII, M. (1979). Effects of internal potassium and sodium on the anomalous rectification of the starfish egg as examined by internal perfusion. *J. Physiol.* **292**, 251–265.

HILLE, B. (1974). Ionic selectivity of Na and K channels of nerve membranes. In *Membranes: A Series of Advances*, vol. 3, ed. EISENMANN, G., pp. 256–323. New York: Dekker.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol.* **148**, 127–160.

JOHNSTON, D. & LAM, D. M. K. (1981). Regenerative and passive membrane properties of isolated horizontal cells from a teleost retina. *Nature, Lond.* **292**, 451–454.

KANEKO, A. (1971). Electrical connexions between horizontal cells in the dogfish retina. *J. Physiol.* **213**, 95–105.

KANEKO, A. & SHIMAZAKI, H. (1975). Effects of external ions on the synaptic transmission from photoreceptors to horizontal cells in the carp retina. *J. Physiol.* **252**, 509–522.

KOSTYUK, P. G. & KRISHTAL, O. A. (1977). Effects of calcium and calcium-chelating agents on the inward and outward current in the membrane of mollusc neurones. *J. Physiol.* **270**, 569–580.

KOSTYUK, P. G. & KRISHTAL, O. A., with an appendix by SHAKHOVALOV, YU A. (1977). Separation of sodium and calcium currents in the somatic membrane of mollusc neurones. *J. Physiol.* **270**, 545–568.

LAM, D. M. K. (1972). Biosynthesis of acetylcholine in turtle photoreceptors. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1987–1991.

LAM, D. M. K. & STEINMAN, L. (1971). The uptake of [γ-^{3}H]aminobutyric acid in the goldfish retina. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2777–2781.

LAM, D. M. K., SU, Y. Y. T., SWAIN, L., MARC, R. E., BRANDON, C. & WU, J.-Y. (1979). Immunocytochemical localisation of L-glutamic acid decarboxylase in the goldfish retina. *Nature, Lond.* **278**, 565–567.

LISMAN, J. E., SWAN, M. D. & FAIN, G. L. (1979). Limulus photoreceptors have a transient outward current not dependent on Ca^{2+} entry. *Biophys. J.* **25**, 268a.

MACNICHOL, E. F. JR & SVAETICHIN, G. (1958). Electric responses from the isolated retina of fishes. *Am. J. Ophthal.* **46**, 26–46.

MARC, R. E., STELL, W. K., BOK, D. C. & LAM, D. M. K. (1978). GABA-ergic pathways in the goldfish retina. *J. comp. Neurol.* **182**, 221–246.

MEECH, R. W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol.* **237**, 259–277.

MEECH, R. W. & STANDEN, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. *J. Physiol.* **249**, 211–239.

MIYAZAKI, S., OHMORI, H. & SASAKI, S. (1975). Potassium rectifications of the starfish oocyte membrane and their changes during oocyte maturation. *J. Physiol.* **246**, 55–78.

NAKA, K.-I. (1972). The horizontal cells. *Vision Res.* **12**, 573–588.

NAKA, K.-I. (1976). Neuronal circuitry in the catfish retina. *Invest. Ophthal.* **15**, 926–935.

NAKA, K.-I. (1977). Functional organization of catfish retina. *J. Neurophysiol.* **40**, 26–43.

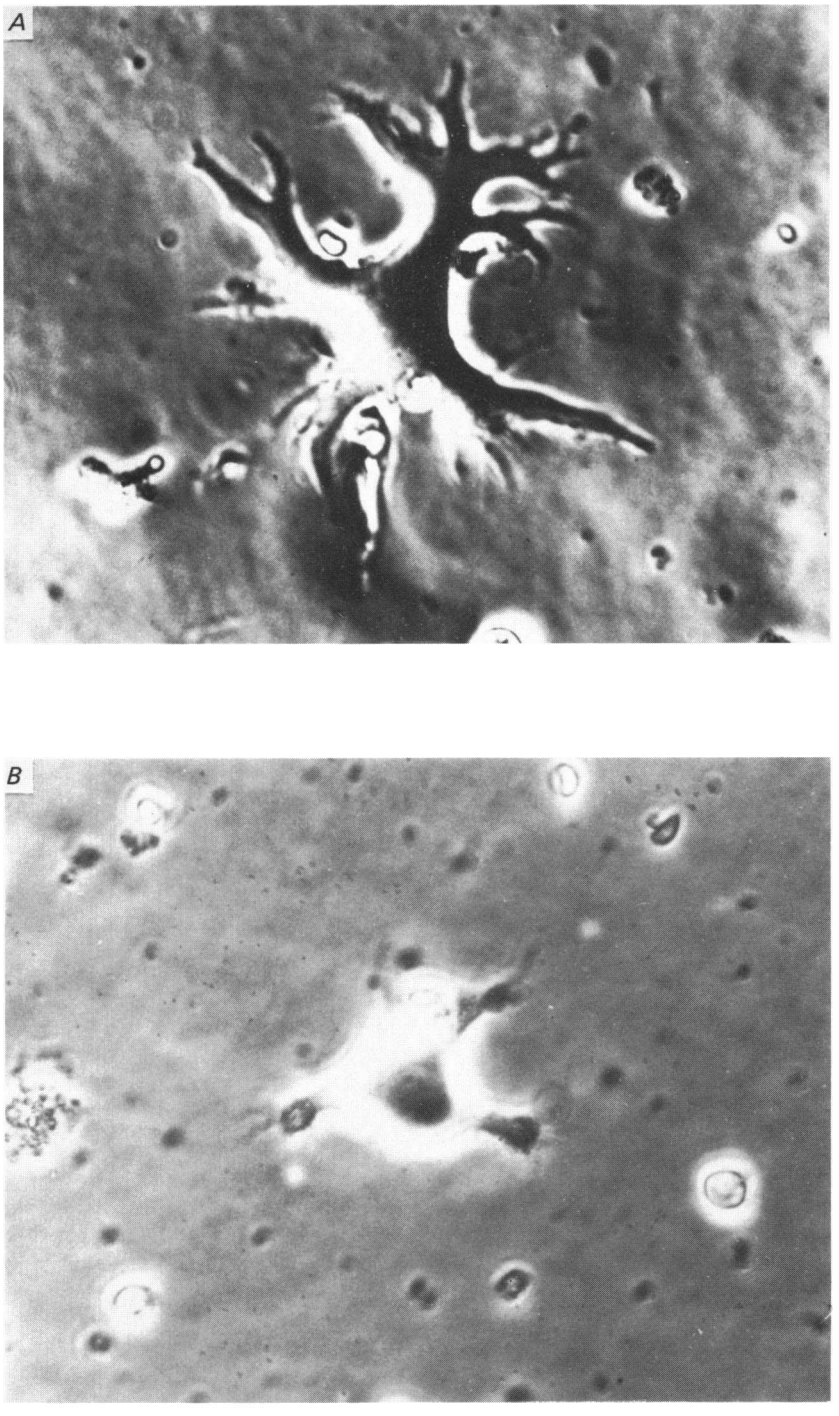
NAKA, K.-I. & RUSHTON, W. A. H. (1967). The generation and spread of S-potentials in fish (Cyprinidae). *J. Physiol.* **192**, 437–461.

NEHER, E. (1971). Two fast transient current components during voltage clamp on snail neurons. *J. gen. Physiol.* **58**, 36–53.

NORTON, A. L., SPEKREIJSE, H., WOLBARSHT, M. L. & WAGNER, H. G. (1968). Receptive field organization of the S-potential. *Science, N.Y.* **160**, 1021–1022.

O'LAGUE, P. H., POTTER, D. D. & FURSHPAN, E. J. (1978). Studies on rat sympathetic neurons developing in cell culture. I. Growth characteristics and electrophysiological properties. *Devl Biol.* **67**, 384–403.

PINTO, L. H. & PAK, W. L. (1974*a*). Light-induced changes in photoreceptor membrane resistance and potential in gecko retinas. I. Preparations treated to reduce lateral interactions. *J. gen. Physiol.* **64**, 26–48.



(*Facing p.* 351)

PINTO, L. H. & PAK, W. L. (1974*b*). Light-induced changes in photoreceptor membrane resistance and potential in gecko retinas. II. Preparations with active lateral interactions. *J. gen. Physiol.* **64**, 49–69.

PLANT, T. D. & STANDEN, N. B. (1981). Calcium current inactivation in identified neurones of *Helix aspersa*. *J. Physiol.* **321**, 273–285.

RAVIOLA, E. (1976). Intracellular junctions in the outer plexiform layer of the retina. *Invest. Ophthal.* **15**, 881–895.

SCHWARTZ, E. A. (1982). Calcium-independent release of GABA from isolated horizontal cells of the toad retina. *J. Physiol.* **323**, 211–227.

STELL, W. K. (1972). The morphological organization of the vertebrate retina. In *Handbook of Sensory Physiology*, vol. VII/2, *Physiology of Photoreceptor Organs*, ed. FUORTES, M. G. F., pp. 111–213. Berlin, Heidelberg & New York: Springer.

STEVENS, C. F. (1980). Ionic channels in neuromembranes: methods for studying their properties. In *Molluscan Nerve Cells: From Biophysics to Behavior*, ed. KOESTER, J. & BYRNE, J. H., pp. 11–31. Cold Spring Harbor Laboratory.

TACHIBANA, M. (1981). Membrane properties of solitary horizontal cells isolated from goldfish retina. *J. Physiol.* **321**, 141–161.

TAKAHASHI, K. & YOSHII, M. (1978). Effects of internal free calcium upon the sodium and calcium channels in the tunicate egg analysed by the internal perfusion technique. *J. Physiol.* **279**, 519–549.

THOMAS, M. V. (1977). Input capacitance feedback improves neutralization. *Electronics* **50**, 112–113.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol.* **265**, 465–488.

TILLOTSON, D. (1979). Inactivation of Ca conductance dependent on entry of Ca ions in molluscan neurons. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1497–1500.

TOMITA, T. (1963). Electrical activity in the vertebrate retina. *J. opt. Soc. Am.* **53**, 49–57.

TOYODA, J.-I. & KUJIRAOKA, T. (1982). Analyses of bipolar cell responses elicited by polarization of horizontal cells. *J. gen. Physiol.* **79**, 131–145.

TRIFONOV, Y. A. (1968). Study of synaptic transmission between the photoreceptor and the horizontal cell using electrical stimulation of the retina. *Biofizika* **13**, 809–817.

TRIFONOV, Y. A., BYZOV, A. L. & CHAILAHIAN, L. M. (1974). Electrical properties of subsynaptic and nonsynaptic membrane of horizontal cells in fish retina. *Vision Res.* **14**, 229–241.

WERBLIN, F. S. & DOWLING, J. E. (1969). Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**, 339–355.

WILSON, W. A. & GOLDNER, M. M. (1975). Voltage clamping with a single microelectrode. *J. Neurobiol.* **6**, 411–422.

WITKOVSKY, P. & DOWLING, J. E. (1969). Synaptic relationships in the plexiform layers of carp retina. *Z. Zellforsch. mikrosk. Anat.* **100**, 60–82.

YAMADA, E. & ISHIKAWA, T. (1965). The fine structure of the horizontal cells in some vertebrate retinae. *Cold Spring Harb. Symp. quant. Biol.* **30**, 383–392.

EXPLANATION OF PLATE

Phase-contrast micrographs of solitary horizontal cells isolated from the same retina and maintained for 3 days *in vitro*. *A*, a cell kept in the culture solution supplemented with Medium 199 (5%, v/v). *B*, a cell maintained in the simple culture solution without Medium 199. Cells similar to the cell in *B* were used for voltage-clamp experiments.