Persistent Na\(^+\) Current and Ca\(^{2+}\) Current Boost Graded Depolarization of Rat Retinal Amacrine Cells in Culture

AMANE KOIZUMI, SHU-ICHI WATANABE, AND AKIMICHI KANEKO

Department of Physiology, Keio University School of Medicine, Tokyo 160-8582, Japan

Received 17 August 2000; accepted in final form 6 April 2001

Koizumi, Amane, Shu-Ichi Watanabe, and Akimichi Kaneko. Persistent Na\(^+\) current and Ca\(^{2+}\) current boost graded depolarization of rat retinal amacrine cells in culture. J Neurophysiol 86: 1006–1016, 2001. Retinal amacrine cells are depolarized by the excitatory synaptic input from bipolar cells. When a graded depolarization exceeds the threshold level, trains of action potentials are generated. There have been several reports that both spikes and graded depolarization are sensitive to tetrodotoxin (TTX). In the present study, we investigated the contribution of voltage-gated currents to membrane depolarization by using rat GABAergic amacrine cells in culture recorded by the patch-clamp method. Injection of a negative current induced membrane hyperpolarization, the waveform of which can be well fitted by a single exponential function. Injection of positive current depolarized the cell, and the depolarization exceeded the amplitude expected from the passive properties of the membrane. The boosted depolarization sustained after the current was turned off. Either 1 μM TTX or 2 mM Co\(^{2+}\) suppressed the boosted depolarization, and co-application of TTX and Co\(^{2+}\) blocked it completely. Under the voltage clamp, we identified a transient Na\(^+\) current (fast \(I_{\text{Na}}\)), a TTX-sensitive persistent current that reversed the polarity near the equilibrium potential of Na\(^+\) (\(I_{\text{NaP}}\)), and three types of Ca\(^{2+}\) currents (\(I_{\text{Ca}}\); L, N, and the pharmacological agent-resistant type (R type)). These findings suggest that the \(I_{\text{NaP}}\) and \(I_{\text{Ca}}\) of amacrine cells boost depolarization evoked by the excitatory synaptic input, and they may aid the spread of electrical signals among dendritic arbors of amacrine cells.

INTRODUCTION

Retinal amacrine cells are axon-less interneurons that provide a lateral pathway between the radial circuits of the vertebrate retina. Their rich dendrites spread in the second synaptic layer, where the signal is relayed from bipolar cells to ganglion cells. Since GABAergic cells make up the majority of the amacrine cell population (Kolb 1997; Müller and Marc 1990; Yazulla 1986), most amacrine cells are believed to be inhibitory neurons that mediate lateral inhibition (Euler and Masland 2000; Watanabe et al. 2000a). The dendrites of amacrine cells are functioning as both pre- and postsynaptic sites. In many amacrine cells, action potentials are superimposed on light-evoked graded depolarization (mudpuppy, Werblin and Dowling 1969; goldfish, Kaneko 1970; tiger salamander, Barnes and Werblin 1986; Miller and Dacheux 1976; rabbit, Bloomfield 1992; Dacheux and Raviola 1995). The light-evoked graded depolarization of amacrine cells has been thought to be an excitatory postsynaptic potential (EPSP) evoked by the excitatory input from bipolar cells, but a voltage-sensitive mechanism may be also involved. In fact, application of tetrodotoxin (TTX) significantly suppresses the amplitude of the light-evoked graded depolarization in fish (Watanabe et al. 2000b) and rabbit (Bloomfield 1996) amacrine cells. Feigenspan et al. (1998) have suggested that a slowly inactivating Na\(^+\) current exists in dopaminergic amacrine cell, but it is not known how the voltage-sensitive mechanisms contribute to graded depolarization. It is expected that such voltage-sensitive mechanisms play a significant role in signal spread along the dendrites of amacrine cells.

The aim of the present study is to determine how the voltage-sensitive mechanisms contribute to the graded depolarization of amacrine cells. We recorded rat GABAergic amacrine cells in culture using a patch-clamp technique and identified two types of TTX-sensitive Na\(^+\) currents (transient and persistent) and three types of Ca\(^{2+}\) currents, L, N, and the pharmacological agent-resistant type (R type). We present evidence that these currents are found generally in GABAergic amacrine cells, where they may contribute to the spread of signals among dendritic arbors of amacrine cells.

METHODS

Culture

The experimental procedure conformed to the Guidelines for the Care and Use of Laboratory Animals, Keio University School of Medicine, and the University Animal Welfare Committee approved our experiments. After decapitating newborn rats (Wistar, P0 and P1), their retinas were isolated and incubated for 25 min in Ca\(^{2+}\)-, Mg\(^{2+}\)-free Hanks’ balanced salt solution with HEPES (10 mM) supplemented with 1 mg/ml trypsin at 37°C. After incubation, they were rinsed with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum and triturated with a fire-polished glass pipette in 10 ml of culture medium. Disso-
associated cells were seeded on poly-L-ornithine-coated glass cover slips at a density of $1.5 \times 10^5$ cells/ml and cultured for 10–14 days in DMEM supplemented with 14 mM NaHCO$_3$, 2 mM glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 5% heat-inactivated fetal bovine serum in a 5% CO$_2$ environment at 37°C. Immediately after dissociation, the cells appeared round, and no dendrites were seen.

The voltage-gated currents developed with days in culture. Until day 5, no voltage-gated inward current was detectable, although morphologically the cell extended multiple dendrites by day 5. On day 7, a transient Na$^+$ current (fast $I_{Na}$) and Ca$^{2+}$ current ($I_{Ca}$) became detectable, but their amplitude was very small. After 10 days in culture, only large cells (soma diameter of >10 μm) survived. Dendrites from their soma extended 100 μm, as seen in the cell shown in Fig. 1D. The fast $I_{Na}$ and $I_{Ca}$ developed to the maximum amplitude and a persistent Na$^+$ current ($I_{Na,p}$) became detectable. This is a good indication that amacrine cells were fully matured by day 10 in culture. We carried out experiments on cells cultured for 10–14 days.

Identification of amacrine cells by immunostaining

Amacrine cells in culture were identified by immunoreaction with the anti-HPC-1/ Syntaxin antibody, a marker for amacrine cells in the retina (Akagawa and Barnstable 1986; Barnstable et al. 1985), and anti-GABA antibody (Gleason et al. 1993; Wexler et al. 1998). Each primary antibody was diluted with 0.01 M phosphate buffer (PBS) containing 5% normal goat serum, 0.05% saponin, and 1% bovine serum albumin. Cells were fixed for 1 h with 4% paraformaldehyde and preincubated for 30 min in a solution containing 5% normal goat serum in PBS. After preincubation, they were reacted in a humidified air chamber for 2 h with mouse anti-HPC-1/Syntaxin antibody (1:5000; Sigma, St. Louis, MO) and for 2 h with rabbit anti-GABA antibody (1:2000; Sigma). The secondary antibodies were diluted to 1:200 in the preceding diluting solution, and the cells were then incubated for 1 h with the secondary antibodies: Texas-Red-labeled anti-mouse IgG (H + L) to anti-HPC-1/Syntaxin antibody and FITC-labeled anti-rabbit IgG (H + L) to anti-GABA antibody (Vector Laboratories, Burlingame, CA). After each process, the cells were washed with PBS.

The reacted preparations were examined with a confocal imaging system (MRC600, Bio-Rad, Hercules, CA) equipped with a krypton-argon laser. FITC was excited with 488 nm laser line and Texas Red with 568 nm line. The barrier filters were BioRad 522DF35 and 585EFLP, respectively. COMOS (Bio-Rad) and Adobe Photoshop (Adobe System, San Jose, CA) softwares were used to process the image data (Satoh et al. 1998).

Of the 103 large multipolar cells (soma diameter of >10 μm) examined, 99 cells (96.1%) were immunoreactive to HPC-1/Syntaxin, indicating that they were amacrine cells. Most of them (92 cells, 93% of HPC-1/Syntaxin-positive cells) were also immunoreactive to GABA. Examples are shown in Fig. 1. We therefore used cell size and multipolar morphology as useful criteria for identifying GABAergic amacrine cells. Wexler et al. (1998) reported that virtually all large multipolar cells having a soma diameter >12 μm among cultured retinal cells from newborn rats were immunoreactive to HPC-1/Syntaxin, whereas only 5% of large neurons expressed Thy1.1, a ganglion cell marker. The present immunohistological data are all consistent with those of Wexler et al. (1998). A Nomarski photomicrograph of a recorded amacrine cell (Fig. 1D) and its fluorescent (Fig. 1E) image are shown as an example.

Recording procedure

A coverslip to which cultured cells had adhered was placed in a recording chamber, and the chamber was mounted on the stage of an inverted microscope equipped with Nomarski optics (IX-70, Olympus, Japan) and a ×60 objective lens. The chamber was continuously superfused with solutions gravity-fed at a rate of ~1 ml/min at room temperature.

![Fig. 1](image-url). Morphology of rat amacrine cells cultured for 10 days. A–C: immunohistological identification of amacrine cells. A: bright field image of cultured amacrine cells. Large multipolar cells (a and b) and small cells (c and d) are seen. B: cells a and b were immunoreactive to HPC-1/Syntaxin antibody (red). Cells c and d were negative. C: cells a and b were also immunopositive for GABA (green), while cells c and d were negative. Scale bar =10 μm. Thus cells a and b were identified as GABAergic amacrine cells. D and E: an amacrine cell recorded by the patch-clamp technique in the whole cell configuration with a pipette containing 0.1% Lucifer yellow. D: photomicrography taken by the Nomarski optics. The diameter of the dendritic field was ~250 μm. Scale bar = 50 μm. E: the same cell as D observed under the fluorescent microscopy.

J Neurophysiol • VOL. 86 • AUGUST 2001 • www.jn.org
Membrane voltages and currents were recorded by a patch-clamp method in the whole cell configuration (Hamill et al. 1981). The patch pipette was made of Pyrex tubing pulled on a micropipette puller (P-87, Sutter Instrument, Novato, CA). The recording pipette was connected to the input stage of a patch clamp amplifier (CEZ-2400, Nihon Kohden, Japan, and Axopatch 200B, Axon Instruments, Foster City, CA). An Ag-AgCl wire connected to the bath via a ceramic bridge served as an indifferent electrode. The pipette resistance was ~10 MΩ when filled with pipette solution. The input capacitance (~50 pF) and the series resistance (~20 MΩ) were measured by the built-in circuit of the patch-clamp amplifier and electrically compensated as much as possible (series resistance ≤60%). The junction potential was measured under each recording condition and the membrane voltages were corrected for the junction potential. Recorded signals were low-pass filtered (Bessel filter, cutoff frequency 5 kHz) and sampled at 10 kHz with a DigiData 1200 interface and pCLAMP 7 software (Axon Instruments). Data were analyzed with Igor Pro software (WaveMetrics, Lake Oswego, OR).

Solutions

The standard external solution for the current-clamp experiments contained (in mM) 135 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4), and the standard pipette solution contained (in mM) 10 NaCl, 130 K gluconate, 1 CaCl₂, 1.1 EGTA, 10 HEPES, and 10 glucose (pH 7.4), and a pipette solution containing (in mM) 110 NaCl, 2.5 CsCl, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.2). When measuring Na⁺ currents, we used an external solution containing (in mM) 115 NaCl, 2.5 CsCl, 20 TEA-Cl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4) and 1 μM TTX, and a pipette solution containing (in mM) 10 Na gluconate, 130 Cs methanesulfonate, 10 TEA-Cl, 1 CaCl₂, 1.1 EGTA, 10 HEPES, and 2 ATP-Na₂ (pH 7.2) to suppress both Na⁺ and K⁺ currents. To suppress Ca²⁺ currents, external Ca²⁺ of Ca²⁺-containing solution was substituted by equimolar Co²⁺ (“Co²⁺-containing solution”). TTX (Sankyo, Japan), nifedipine (Sigma), diltiazem (Sigma), α-conotoxin GVIA (Alomone, Israel) and α-agatoxin IVA (Alomone) were dissolved into the external solution and applied by pressure from a puffer pipette or by a gravity feeding system.

Verification of space clamp and estimation of membrane parameters

It seems hard to voltage-clamp the long and narrow dendrites of cultured amacrine cells uniformly (Fig. 1, D and E), but we have evidence that at least the soma was clamped satisfactorily (Fig. 2). Here, two recording pipettes were placed on the soma of the same cell and made in the whole cell configuration. A command voltage was given by the first pipette connected to an amplifier that was set in the voltage-clamp mode, and the membrane voltage was recorded by the second pipette connected to another amplifier set in the current-clamp mode. The amplitude of the recorded signal (Fig. 2B) was almost identical (>-95%) to that of the command signal (Fig. 2A). The rising and falling phases of the recorded signals were delayed by ~5 ms due to the remaining stray capacitance. Membrane parameters were determined from the voltage change evoked by injecting a −20 pA negative current from a patch pipette (Fig. 2C, a). The current-induced graded voltage change was fitted by a single exponential function used to fit the trace a. The reversal potential was calculated as −0.8 ± 0.66 mV (n = 17), and the conductance was calculated as 0.8 ± 0.02 pS (n = 17). The I-V relationship was linear between −100 and −10 mV. The conductance was calculated as 0.8 ± 0.02 pS (n = 17), and the reversal potential was −66 ± 1 mV.
a single exponential function (Fig. 2C, c). The good agreement with a single exponential function indicates that in the hyperpolarizing voltage range, no voltage-gated mechanism was activated. In fact, the waveform of the hyperpolarizing voltage was not affected by the application of 1 μM TTX and 2 mM Co²⁺ (Fig. 2C, b). Similar observations were made on six cells. The membrane capacitance, input resistance and time constant were 40 ± 1 pF, 917 ± 16 MΩ and 38 ± 1 ms (means ± SE, n = 6). In analyzing the depolarizing waveform induced by a brief current pulse, a mirror image of the waveform induced by a negative current pulse of the same current amplitude and duration was used as a control “passive waveform,” because no “active” components were evoked by hyperpolarization.

The leakage current was measured by giving voltage commands in the Co²⁺-containing solution (see Solutions). The solutions contained Co²⁺ to block Ca²⁺ current, TTX to block Na⁺ current, TEA⁺ and Cs⁺ to block K⁺ current. The current-voltage (I-V) relationship is shown in Fig. 2D. It was linear between −100 and −10 mV. The leak conductance was estimated as 0.8 ± 0.02 nS (n = 17) and the reversal potential was −66 ± 1 mV.

RESULTS

Depolarization induced by positive current injection

To mimic the synaptic currents generated in amacrine cells, extrinsic step currents were injected from a patch pipette into the cultured amacrine cell recorded under the current-clamp condition (resting potential −65 ± 1 mV, n = 54). As shown in Fig. 3A, injection of a positive current of 2.5 pA to an amacrine cell with a resting potential of −64 mV induced a depolarization (red line) that exceeded the passive waveform (yellow line) in amplitude. The enhanced depolarization was seen on the rising phase, and it persisted after the current was terminated. The deviation from the passive waveform disappeared when 1 μM TTX and 2 mM Co²⁺ were added to the medium (blue line), indicating that Na⁺ and Ca²⁺ currents are contributing to the amplitude enhancement. Figure 3B illustrates the relation between the absolute peak potential and the amount of injected current. Enhanced depolarization was noted by positive current injection of more than +5 pA (membrane voltage more positive than −55 mV; Fig. 3B, filled red circle). The enhanced depolarization was absent in the presence of TTX and Co²⁺ (Fig. 3B, open blue square). To evaluate the effect of TTX on the enhanced depolarization, we applied TTX (1 μM) alone (Fig. 3C). TTX dramatically reduced the enhanced depolarization (Fig. 3C). The application of Co²⁺ (2 mM) alone also blocked the enhanced depolarization significantly (data not shown). These observations suggested that TTX- and Co²⁺-sensitive components in concert contributed to the enhanced depolarization. Figure 3D illustrates the relation between the absolute peak potential and the amount of injected current in nine cells. The enhanced depolarization appeared at membrane voltages more positive than −55 mV. Simultaneous application of TTX and Co²⁺ blocked the response enhancement completely, and the relation became linear (Fig. 3D).

Deviation from the passive waveform was more pronounced when more current was injected (Fig. 3E, +30 pA, red line). Plateau depolarization lasted long after the termination of the positive current injection and action potentials of various amplitudes were superimposed on the sustained depolarization. During the sustained depolarization, injection of a brief hyperpolarizing current terminated the plateau depolarization and the membrane voltage returned close to the resting level (data not shown). Suprathreshold sustained depolarization triggered by the brief positive current was sensitive to both TTX (Fig. 3E) and Co²⁺ (Fig. 3F). TTX suppressed the action potentials as well as the sustained depolarization (Fig. 3E, green line). Action potentials were blocked by TTX in all recorded cells (n = 17), and the sustained depolarization was significantly suppressed (16 of the 17 cells). The sustained depolarization was also suppressed partially by 2 mM Co²⁺ (Fig. 3F, purple line, n = 3). Co-application of TTX and Co²⁺ suppressed the sustained depolarization nearly completely (Fig. 3E, blue line, n = 11), and the waveform was fitted by the passive waveform (Fig. 3F, yellow line). These findings strongly suggest that both the TTX-sensitive current and Ca²⁺ current (I_{Ca}) contribute to the sustained depolarization.

Similar voltage responses to current injection were observed in 47 of the 54 cells examined, and the duration of the sustained depolarization was 170 ± 10 ms (ranging between 150 ms and 2 s). The remaining seven cells did not show the enhanced and sustained depolarization.

Persistent inward currents

TTX-SENSITIVE PERSISTENT INWARD CURRENT. To estimate the effect of TTX on the current of amacrine cells, they were voltage clamped by the whole cell patch-clamp technique in the presence and absence of TTX (Fig. 4A). TTX (1 μM) application not only blocked transient inward current but also increased the sustained outward current (Fig. 4A, left). Figure 4A, right, illustrates the relation between the membrane potential and the persistent current amplitude, measured at 200 ms after the onset of the command pulse, in the presence and the absence of TTX (n = 6). TTX application increased the amplitude of net outward current obviously between −27 and +23 mV. These observations strongly suggest the presence of a TTX-sensitive persistent inward current.

To evaluate the TTX-sensitive current more quantitatively, the TTX-sensitive current was isolated by computer subtraction of a current recorded in the presence of TTX (1 μM) from the current recorded without TTX (Fig. 4B). K⁺ currents were blocked by TEA⁻ (20 mM in pipette solution) and Cs⁺ (2.5 mM in extracellular solution), and Ca²⁺ currents were blocked by Co²⁺ (4 mM in extracellular solution).

Depolarization of a cell to −43 mV from the holding voltage of −93 mV induced a sustained inward current of small amplitude (Fig. 4B, left and right, 2nd traces). Depolarization to −33 mV evoked a transient inward current of large amplitude and the persistent inward current (Fig. 4B, left and right, 3rd traces). The TTX-sensitive transient inward current is likely to be the conventional Na⁺ current (fast I_{Na}). In 5 of the 12 cells examined, the persistent component appeared at more negative voltage than the fast I_{Na} by 10 mV. In the remaining seven cells, both types of currents started to flow at the same voltage, but in no cell was the fast I_{Na} activated at more negative voltage than the persistent component. The I-V relationships of the persistent component (filled circle) and the fast I_{Na} (open square) is shown in Fig. 4C (n = 12). The currents were normalized for comparison. The peak current amplitude of persistent current was −16 ± 3 pA (n = 12), whereas that of the fast I_{Na} was −329 ± 48 pA (n = 12). The persistent component flowed long (200 ms) after the fast I_{Na} was inactiv-
vated. Thus there were clear differences between the persistent current and the fast $I_{\text{Na}}$; the activation voltage and kinetics.

TTX-SENSITIVE PERSISTENT INWARD CURRENT WAS CARRIED BY $\text{Na}^+$.

The TTX-sensitive persistent inward current reversed its polarity near the equilibrium potential of $\text{Na}^+$ ($E_{\text{Na}}$). The reversal potential of the persistent current ($+24 \pm 2 \text{ mV}$, $n = 8$) measured with a pipette solution containing 50 mM $\text{Na}^+$ was close to $E_{\text{Na}}$ ($+25 \text{ mV}$). When the pipette solution containing 20 mM $\text{Na}^+$ ($E_{\text{Na}} = +48 \text{ mV}$) was used, the reversal potential shifted to $+42 \pm 2 \text{ mV}$ ($n = 7$, Fig. 5). These results clearly indicate that $\text{Na}^+$ carried the persistent current ($I_{\text{NaP}}$).

$\text{Ca}^{2+}$ CURRENTS. As shown in Fig. 3F, $\text{Co}^{2+}$-sensitive components also contributed to the enhanced and the sustained depolarization. To isolate $\text{Ca}^{2+}$ currents, $\text{K}^+$ currents were

duced.
PERSISTENT CURRENTS IN CULTURED RAT AMACRINE CELLS

The present study demonstrates that amacrine cells from the newborn rat retina, cultured for 10–14 days in vitro, possess INaP and several types of ICa as well as fast ICaNa and ICa significantly contribute to the enhanced and the sustained depolarization of amacrine cells. They are activated by membrane depolarization to voltages more positive than −55 mV and, once activated, are maintained as long as depolarization lasted. Because of the preceding properties, depolarization produced by injection of an extrinsic current is boosted both in length and amplitude, and generation of the action potentials is facilitated. Under the current-clamp conditions, INaP and ICa work in concert. Therefore application of a blocker selective to either one of the two current components results in suppressing not only its target but also another current.

The enhanced depolarization was observed at membrane voltages more positive than −55 mV, but neither INaP nor ICa was not clearly detected at −55 mV. Under our voltage-clamp recording conditions, the noise level was at a few picoamperes. Because the input resistance of amacrine cells was ~1 GΩ, inward current of 1 pA depolarizes the cell by 1 mV. In this sense, measurement of the membrane voltage under the current clamp is a more sensitive method to detect a small amount of membrane current. Thus it is highly likely that INaP or ICa of a few picoamperes are activated at about −55 mV, which induces enhanced depolarization in a cell under current clamp.

Significance of the enhanced and the sustained depolarization in signal propagation in the inner plexiform layers

In amacrine cells having dendritic arbors spanning hundreds of micrometers, propagation of excitatory synaptic signals along the dendrite is important for processing input signals and forming the receptive field of neurons in the inner retina. Cook et al. (1998) showed that long-distance lateral inhibition (spreading 250 μm) in the large-field amacrine cells of the mudpuppy is mediated by a TTX-sensitive mechanism and that a local transient inhibition of ganglion cells does not require a voltage-sensitive mechanism. Cook and McReynolds (1998) suggested that lateral inhibition in the inner retina is mediated by a sustained spiking activity in GABAergic amacrine cells. Taylor (1999) also showed that TTX attenuates surround inhibition in rabbit retinal ganglion cells. They proposed that TTX suppressed the propagating action potentials, and the spread of signals within a large-field amacrine cell was limited.

In amacrine cells, it has been shown that TTX and Ni 2- (2,6-dimethylphenyl carbamoyl methyl) triethylammonium (QX314) significantly reduce the amplitude of both light-evoked graded potentials and action potentials (Bloomfield...
Currents recorded in the present study perhaps originated mainly in the soma and nearby dendritic stems since it is unlikely that dendrites were uniformly voltage clamped judging from their thin and long structure. By extrapolating the present observation, it is tempting to speculate that $I_{NaP}$ and $I_{Ca}$ of similar characteristics are also present in the dendritic membrane. If they really exist in the dendrites, they should contribute to the spread of subthreshold depolarization over a wide dendritic field of an amacrine cell. In conclusion, $I_{NaP}$ and $I_{Ca}$ play an important role in the lateral spread of signals in the inner plexiform layer.

Contribution of $I_{NaP}$ and $I_{Ca}$ to the enhanced and the sustained depolarization

In the present study, we showed that $I_{NaP}$ and $I_{Ca}$ boost a small and brief depolarization produced in cultured amacrine cells. The boosting action of the TTX-sensitive sustained current has been demonstrated in several preparations. Llínás and Sugimori (1980) have demonstrated an example in the cerebellar Purkinje cell. A positive extrinsic current injected into a cerebellar Purkinje cell evoked a plateau depolarization that outlasted the duration of current injection by several hundred milliseconds. The plateau potential was not blocked by Cd$^{2+}$, but it was abolished by removing the extracellular Na$^+$ or adding TTX. They proposed that the action potential was generated by the Hodgkin-Huxley-type fast Na$^+$ conductance that inactivates rapidly and that the plateau response was generated by the persistent Na$^+$ conductance. In fact, Raman and Bean (1999) showed that a TTX-sensitive persistent current contributed to spontaneous activities of action potentials in mouse cerebellar Purkinje cells. Stuart and Sakmann (1995) have shown that the TTX-sensitive current generated in the soma and the axon amplifies a subthreshold EPSP of neocortical pyramidal neurons. The fact that the amplification mechanism was sensitive to TTX and had a slow time course suggests that the underlying mechanism is $I_{NaP}$. The enhanced and the sustained depolarization we recorded in amacrine cells in the present study was very similar to that described by Llínás and Sugimori (1980) in Purkinje cells at a supra-threshold level. Stuart and Sakmann (1995) have shown that the TTX-sensitive persistent current generated in the soma and nearby dendritic stems of the rabbit and in some goldfish amacrine cells. It is reasonable to speculate that the suppression of $I_{NaP}$ narrows lateral inhibition mediated by GABAergic amacrine cells. In the rat, amplification should be operating at least in GABAergic amacrine cells because all GABAergic amacrine cells in our culture possessed $I_{NaP}$ and $I_{Ca}$.
the rat spinal cord in a slice preparation. As the plateau potential was highly sensitive to dihydropyridine (DHP), they concluded that the plateau potential of deep DHNs is supported mainly by Ca\(^{2+}\) influx through the L-type Ca\(^{2+}\) channels. The contribution of \(I_{\text{NaP}}\) to the plateau potential of DHNs was not examined because their solution always contained TTX to block presynaptic action potentials.

We showed that \(I_{\text{NaP}}\) and \(I_{\text{Ca}}\) contribute to the enhanced and sustained depolarization in cultured rat amacrine cells and that both of them are activated near the resting membrane potential. These two components in concert contribute to the boosting mechanism of the graded depolarization.

**Identification of \(I_{\text{NaP}}\) in amacrine cells**

Feigenspan et al. (1998) reported a TTX-sensitive slowly inactivating \(I_{\text{Na}}\) as contributing to the interspike slow depolarization in acutely dissociated mouse dopaminergic amacrine cells. This slowly inactivating \(I_{\text{Na}}\) may be identical to the \(I_{\text{NaP}}\) we recorded in the present study. \(I_{\text{NaP}}\) has been identified in neurons of the mammalian neocortex, thalamus, entorhinal cortex, hippocampus, and cerebellum (Crill 1996; French et al. 1990).

In the present experiments, we demonstrated that \(I_{\text{NaP}}\) was activated at more negative voltages than the fast \(I_{\text{Na}}\). However, we cannot tell whether \(I_{\text{NaP}}\) and fast \(I_{\text{Na}}\) flow through the same
channel or not. Although there are still ambiguous interpretations of \( \text{Na}^+ \) channel itself, difference in the kinetics and the activation voltage suggests that \( I_{\text{NaP}} \) effectively plays a different role from fast \( I_{\text{Na}} \).

It might be argued that \( I_{\text{NaP}} \) is an immature type of \( I_{\text{Na}} \). As stated in METHODS, we also followed the developmental changes of cultured amacrine cells, and we used cells cultured >10 days in which \( I_{\text{Na}} \) had fully developed. At least in fish retina, \( I_{\text{NaP}} \) has been recorded in mature neurons. Hidaka and Ishida (1998) reported \( I_{\text{NaP}} \) as distinct from the fast \( I_{\text{Na}} \) in the mature ganglion cells acutely isolated from the goldfish. Watanabe et al. (2000b) also detected \( I_{\text{NaP}} \) in amacrine cells of the mature goldfish slice preparation.

Another argument on the identity of the prolonged inward \( \text{Na}^+ \) current is whether it is the current carried by a \( \text{Na}^+-\text{Ca}^{2+} \) exchanger. The \( \text{Na}^+-\text{Ca}^{2+} \) exchanger was shown to exist in amacrine cells cultured from chick embryos (Gleason et al. 1995). It is activated by the intracellular \( \text{Ca}^{2+} \) that flowed into the cell through the voltage-activated \( \text{Ca}^{2+} \) channel. In our preparation, however, this possibility is unlikely since the sustained inward current was recorded in the presence of \( \text{Ca}^{2+} \) channel blockers and was TTX sensitive.

Identification of \( I_{\text{Ca}} \) in amacrine cells

\( I_{\text{Ca}} \) was also detected in our cultured amacrine cells. Pharmacological experiments revealed that \( I_{\text{Ca}} \) consisted of several subtypes. Gleason et al. (1994) reported that the L-type current is the major component of \( I_{\text{Ca}} \) in chick amacrine cells in culture. However, they reported that nifedipine reduced \( \text{Cd}^{2+} \)-suppressible \( \text{Ca}^{2+} \) currents by an average of 59\%, and the remaining current was not sensitive to other antagonists. In our own preparation, the \( \text{Cd}^{2+} \)-suppressible \( I_{\text{Ca}} \) was not completely blocked by nifedipine and \( \omega \)-conotoxin GVIA. It is known that the R-type \( I_{\text{Ca}} \) shows resistance to any pharmacological agent (Randall and Tsien 1995; Tottene et al. 1996). Thus the DHP-, \( \omega \)-conotoxin GVIA- and \( \omega \)-agatoxin IVA-resistant \( \text{Ca}^{2+} \) current in the present study is likely to be the R-type \( I_{\text{Ca}} \). \( I_{\text{Ca}} \) of amacrine cells had an unusually low activation voltage of approximately −50 mV, which is close to the resting potential and more negative than the activation voltage of the fast \( I_{\text{Na}} \) [see also, rat all amacrine cells (Boos et al. 1993) and cultured chick amacrine cells (Gleason et al. 1994)]. The low activation voltage suggests a strong possibility that \( I_{\text{Ca}} \) is also contributing to the enhancement of graded depolarization at subthreshold level.

We are grateful to H. Satoh, N. Mukainaka, and Y. Yamada for technical assistance. This work was supported in part by a grant from the Keio Health Counseling Center Foundation, by the Keio University Grant-in-Aid for Encouragement of Young Medical Scientists (A. Koizumi), by Research Grants for Life Sciences and Medicine from the Keio University Medical Fund and Keio Gijuku Academic Development Funds (S.-I. Watanabe), by a grant-in-aid for scientific research from the Japanese Ministry of Education, Science and Culture (06454715), and by a grant from Research for the Future Program of Japan Society for the Promotion of Science under the Project “Cell Signaling” (JSPS-RFTP97L00301) (A. Kaneko).

Present address of S.-I. Watanabe: Dept. of Physiology, Saitama Medical School, 38 Morohongo, Moroyama, Saitama 350-0495, Japan.

REFERENCES


RANDALL A AND TSIEH NW. Pharmacological dissection of multiple types of...


