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Haloperidol Effects on Na Current in Acutely Isolated Rat Retinal Ganglion Cells

Kunio Ito,*† Yoshihiro Nishimura,† Yukitaka Uji*
and Tetsuro Yamamoto†

*Departments of *Ophthalmology and †Physiology, Faculty of Medicine, Mie University, Tsu, Japan*

Abstract: Suppressive effects of haloperidol, one of the neuroleptic compounds that acts on the transient Na current (INa,t) were studied in freshly isolated 5–10-day-old rat retinal ganglion cells using a whole-cell patch clamp technique. High doses of haloperidol reversibly and dose dependently (2–200 μmol) reduced the peak amplitude of INa,t. The half-inhibition dose was 27.1 μmol. The inactivation curve of INa,t was shifted toward a hyperpolarizing direction by about 42% when the amplitude was suppressed with 20 μmol haloperidol. This indicates that haloperidol suppression of INa,t is probably affected through changes in the inactivation of Na channels. **Jpn J Ophthalmol 1997;41:221–225** © 1997 Japanese Ophthalmological Society

Key Words: Haloperidol, Na current, retinal ganglion cells, steady-state inactivation curve, whole-cell patch clamp.

Introduction

A variety of ionic currents contribute to signal transduction in retinal ganglion cells[1] and other neurons. In rats, these include the Na current (INa), the Ca current, K currents (IK, IA), and Ca-activated K current.[2] Although there are two subtypes of INa, transient (INa,t) and persistent (INa,p), rat retinal ganglion cells have only INa,t,[1,2] the major ionic current involved in the rising phase of an action potential.[3]

Recent advances in molecular biology and biophysics revealed the structure and modulation of Na channels by neurotoxins,[4] local anesthetics, or antiarrhythmic agents.[5] However, most of these studies used peripheral nerve fibers and brian neurons. There are few studies of the effects of these chemicals, except for tetrodotoxin,[2] on the Na channels of the retinal cells. Although haloperidol is well known as a dopamine antagonist,[6] it has been reported that haloperidol suppressed INa,t in isolated mammalian central neurons and peripheral nerves at a higher dose than required to antagonize dopamine receptors.[7–9] In isolated retinal ganglion cells, dopamine receptor antagonists, such as haloperidol, spiperone, and SCH23390, have been reported to reduce voltage-dependent Ca current by a mechanism unrelated to dopamine receptors,[10] but there have been no reports on the effects of these agents on INa,t. In the present study, we investigated the effects of haloperidol on INa,t in morphologically identified acutely dissociated rat retinal ganglion cells.

Materials and Methods

Dissociation of Ganglion Cells

Sprague-Dawley rats of both sexes, 5–10 days old, were used for the study. Animals were handled in accordance with the ARVO resolution on the use of animals in vision and ophthalmic research. Two to four days before the recording, 1 μL of 2% DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Molecular Probes, Eugene, OR, USA) dissolved with dimethyl sulfoxide (DMSO) or 5% Fast Blue (FB, Sigma, St. Louis, MO, USA) dissolved in distilled water, was injected bilaterally into the superior colliculus of cryoanesthetized rats using a mi-

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Address correspondence and reprint requests to: Kunio ITO, MD, Department of Ophthalmology, Faculty of Medicine, Mie University, 2-174 Edobashi, Tsu 514, Japan

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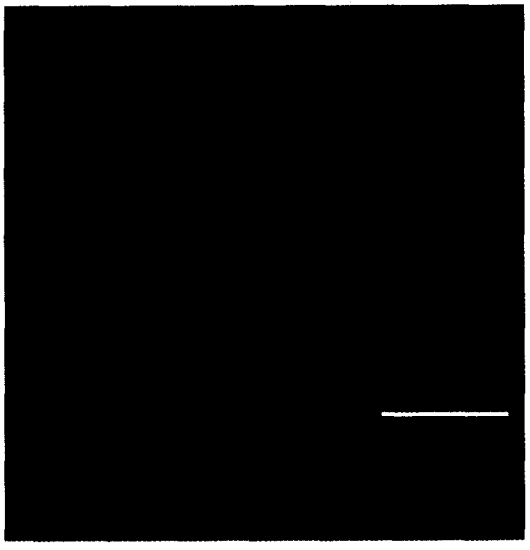

Figure 1. Fluorescent photomicrograph: isolated retinal ganglion cell retrogradely labeled with DiI in 6-day-old rat. Bar = 20 μm.

crosyringe, as reported previously.[2,11,12] The injected fluorescent dyes were transported to the retinal ganglion cells by retrograde axonal flow within 48 hours.

On the recording day, again under deep cryoanesthesia, the eyes of the rats were enucleated and hemisected. The retina was isolated from the vitreous body and the pigment epithelium and sclera were cut into small pieces under a dissecting microscope. Following enzymatic treatment in O_2-bubbled Hanks' solution containing papain (3–5 U/mL, Worthington, Freehold, NJ, USA) and cysteine (0.1 mg/mL, Sigma, St. Louis, MO, USA) for 60 minutes at 31°C, and rinsing with Hanks' solution, retinal cells were dissociated by gentle trituration with a Pasteur pipette.[11,13]

Dissociated cells were plated on Concanavalin A-coated dishes (Sigma, St. Louis, MO, USA) filled with a standard solution (in mM): NaCl 135, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 5, Glucose 10, maintained at 4°C until recording. The dissociated ganglion cells were observed and identified under an epifluorescent microscope (Axioskop, Carl Zeiss, Oberkochen, Germany) with differential interference contrast optics. DiI-labeled ganglion cells were identified before recording with the G filter of the epifluorescent microscope. FB-labeled cells were observed after recording with the V filter to prevent damage to the cells.

Electrophysiological Recording

Borosilicate glass pipettes (1.5 mm OD) had resistances of 5–8 molΩ when filled with the following so-

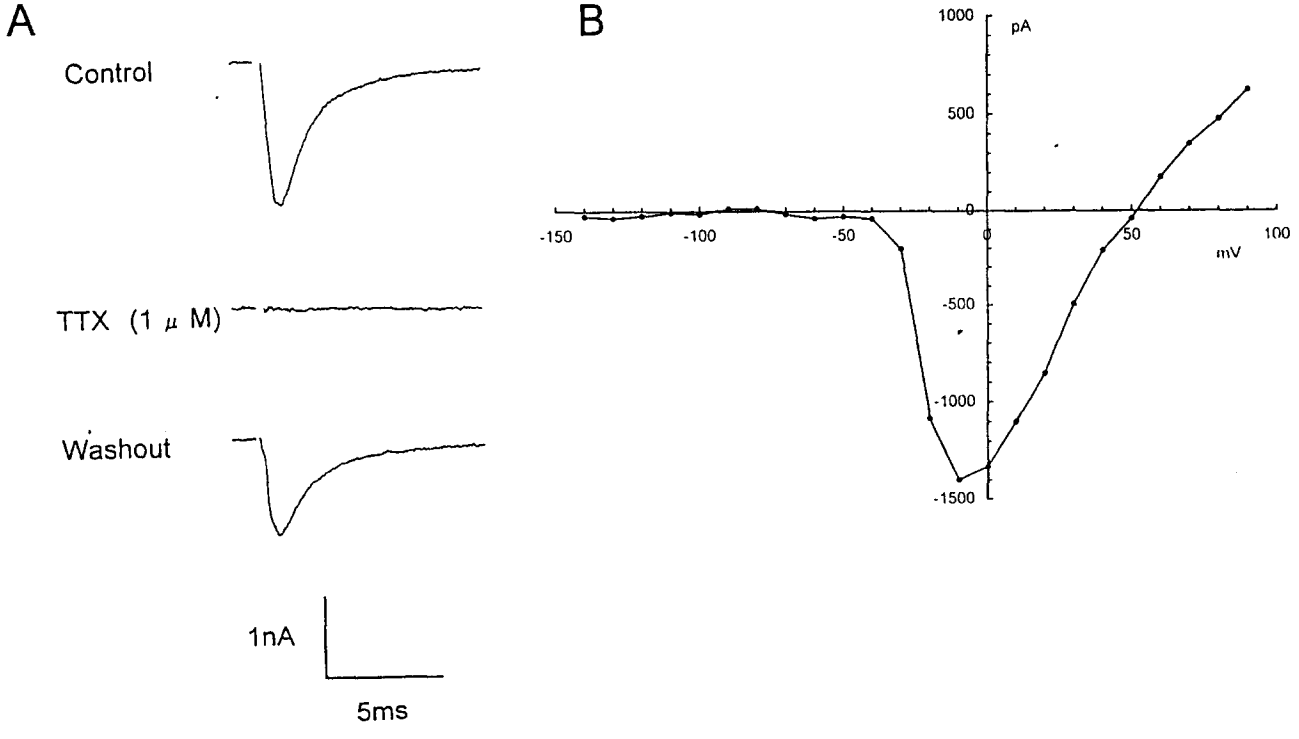

Figure 2. (**A**) Transient inward currents evoked at −20 mV with a Vh of −90 mV before and after application of 1 μmol TTX. The effect of TTX was reversible (washout). (**B**) Current-Voltage relationship of transient inward current (INa,t). Peak amplitude of transient inward currents was plotted against the membrane potential.

lution (in mmol): CsCl 110, NaCl 10, $CaCl_2$ 0.5, EGTA 5, and HEPES 10, adjusted to pH 7.2 with CsOH. Sylgard was used to minimizing stray capacitance. The tip of the pipette was heat-polished to allow gigaseal formation more easily. The recording bath was superfused continuously at 1.0 mL/minute and grounded by an Ag-AgCl indifferent electrode. To isolate INa,t, the perfusate included (in mmol): NaCl 101, CaCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 5, glucose 10, TEA-Cl 30, $CoCl_2$ 4, and 0.01% bovine serum albumin (Sigma, St. Louis, MO, USA) maintained at pH 7.4 with CsOH.[11] After rupturing the membrane patch under the pipette tip, ionic currents were recorded in the whole-cell configuration[14] at a holding potential (Vh) of −90 mV, using a patch-clamp amplifier (AXOPATCH 200A, Axon Instruments, Foster City, CA, USA) with a low-pass filter of 5 kHz and an on-line simultaneous recording and stimulating system (pCLAMP, Axon Instruments). Leak currents were subtracted from the recorded currents using scaled currents obtained by averaging four pulses of opposite polarity. Recording was done at room temperature; data were analyzed by the Mann-Whitney test.

Drug Application

Haloperidol (Sigma, St. Louis, MO, USA: final concentration, 2–200 μmol) was initially dissolved with DMSO. Before studying the effects of haloperidol, we verified that DMSO (final concentration in perfusate ≤ 1%) had no effect on INa,t. Recording and perfusing systems were shielded from light to prevent the degeneration of the drugs.

Results

Because most of the dissociated ganglion cells did not have complete neurites due to the trituration procedure, we confirmed the identification of the retinal ganglion cells by retrograde transport of Dil or FB. The recorded cells were round or ovoid with a smooth surface. The present data were derived from nine Dil-labeled (Figure 1) and 47 FB-labeled cells.

Transient inward currents were examined in 56 cells by a 50-millisecond step command (−140 to +90 mV; Vh, −90 mV). One of these was identified as INa,t: It was observed when Ca and K currents were suppressed by Co^{2+}, Cs^+, and TEA in the perfusate and Cs^+ in the pipette, and was reversibly blocked

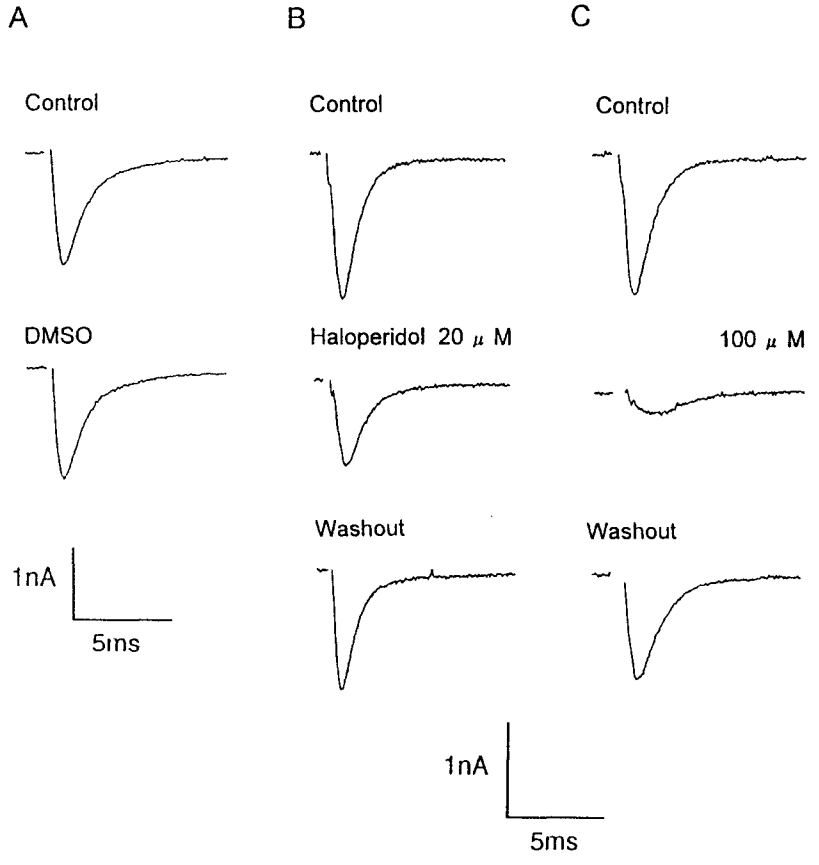

Figure 3. (**A**) INa,t recorded by step command of −20 mV from a Vh of −90 mV before and after application of 1% DMSO. (**B**) INa,t induced by depolarizing pulse of −20 mV from a Vh of −90 mV. Peak current was suppressed by 42% after application of 20 μmol haloperidol. INa,t was recovered to 84% control value after washout of haloperidol. (**C**) INa,t recorded by step command of −20 mV from a Vh of −90 mV in the other cell. Haloperidol (100 μmol) reversibly suppressed it by 91%. Right scales apply to (**B**) and (**C**).

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by the 1 μmol tetrodotoxin (TTX)[15] (Figure 2A). The evoked current showed a voltage and time dependence similar to the INa,t reported in rat retinal ganglion cells by Lipton and Tauck[2] (Figure 2B). The averaged peak amplitude of the current was −1.7 ± 0.7 nA (mean ± SD).

Although INa,t was not influenced by DMSO, alone, added to the perfusate (Figure 3A), 20 μmol haloperidol dissolved in DMSO reduced the peak amplitude that was evoked by a step command from −90 mV to −20 mV (42%) (Figure 3B). The INa,t returned to the control level after washout of the haloperidol. The depression of INa,t by 20 μmol haloperidol averaged 41.7 ± 5.4% (n = 7). The INa,t activated at −20 mV was reversibly depressed by 89% with 100 μmol haloperidol (Figure 3C).

We studied the effects of haloperidol in INa,t at various doses: 2 (n = 3), 10 (n = 7), 50 (n = 6), 100 (n = 4), and 200 μmol (n = 1). Figure 4 plots the ratio of the peak amplitude of INa,t in haloperidol compared to the control at various doses. The dose response curve fit well with Hill's equation:

$$I/Imax = 1 - x^n/(x^n + \chi_{50}^n)$$

in which x is the dose of haloperidol, n is the Hill coefficient (n = 1.3), and χ_{50} is the half-inhibition dose (χ_{50} = 27.1 μmol).

Figure 5 shows the steady-state inactivation curves of INa,t obtained in control and haloperidol-containing solutions. Both curves fit well with Boltzmann's equation:

$$I/Imax = 1/\{1 + \exp(x - \chi_{50}^n)/k\},$$

where χ_{50} is the potential at which the current is half-inactivated and k is a factor describing the steepness of the inactivation curves. In the control solution, χ_{50} was −40.4 mV and k was 4.5. The inactivation curve of INa,t was similar to that reported by Lipton and Tauck.[2] Haloperidol 20 μmol shifted the curve to the left, a hyperpolarizing direction, by 15.5 mV. In five cells, the half-inactivation potential shifted significantly ($P < 0.01$) from −42.5 ± 4.9 mV to −56.0 ± 5.6 mV in response to the haloperidol.

Discussion

This study revealed the inhibitory action of haloperidol on INa,t in rat retinal ganglion cells. A concentration of 27 μmol produced half inhibition in this experiment. This is one-third of the concentration found in isolated rat brain neurons[7] and about 20 times that in rat peripheral nerve fibers.[8,9]

Neuroleptic compounds such as haloperidol and chlorpromazine are believed to block dopamine (D1, D2) receptors.[6] The IC_{50} (concentrations producing a 50% blockade of drug binding) of haloperidol and chlorpromazine, in antagonizing D1 and D2 receptors were 1.2 nmol and 29 nmol, respectively. These are nearly equal to the serum concentration of free haloperidol used in the treatment of schizophrenia patients,[6] but they are much lower than the IC_{50} of haloperidol that produced INa,t inhibition in our experiment.

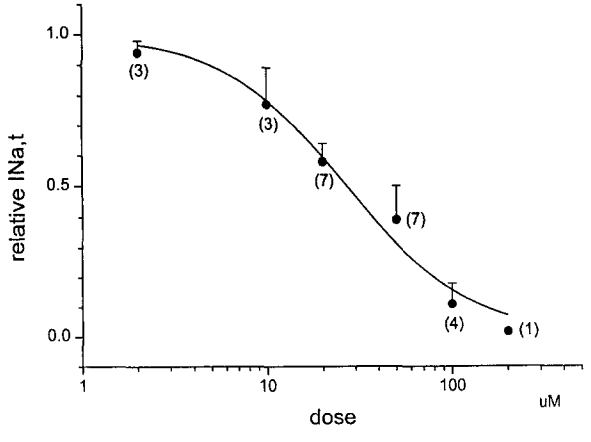

Figure 4. Dose response curve: haloperidol depression of peak current of INa,t. Suppressive effects were evaluated by relative INa,t to the amplitude of the control current. Points and bars indicate the mean values and standard deviations, respectively. Number of cells examined is shown in parenthesis. Plotted points fit well with Hill's equation.

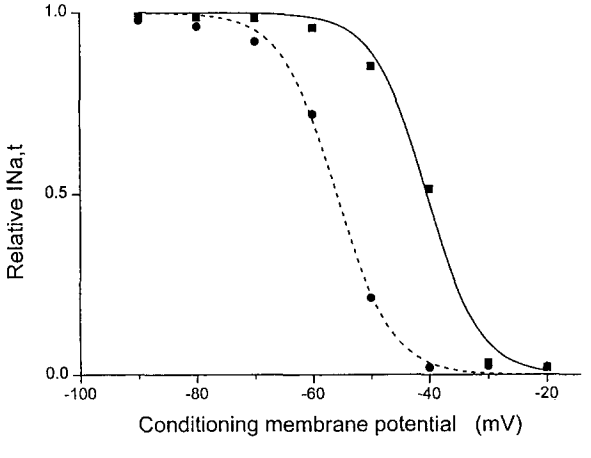

Figure 5. Inactivation curve of INa,t before (■) and after (●) application of 20 μmol haloperidol. Abscissa represents conditioning prepulse of 450 milliseconds duration; Ordinate is peak amplitude of INa,t induced by the test pulse up to −20 mV normalized to the maximal value of INa,t. In both cases the plotted points fit well with Boltzmann's equation.

Dopamine receptors are localized in the ganglion cell layer of the adult rat retina.[16] Endogenous dopamine is, however, first observed histochemically in 10-day-old rats[17] but dopamine receptors are not yet completely established on the retinal ganglion cells of 5–10-day-old rats.[10] Therefore, this study may refute the theory that suppression of the Na current is mediated by dopamine receptors in rat retinal ganglion cells. Guenther et al[10] also reported that haloperidol reduced Ca currents without dopamine receptors mediating the response. Their dose-dependence data were similar to ours.

Reduction of the amplitude of INa,t by haloperidol shifted the inactivation curve in the hyperpolarizing direction. This shift has been reported both in brain neurons[7] and peripheral nerve fibers,[8] leading us to conclude that haloperidol, like local anesthetics, affects the process of inactivation of INa,t.

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