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Thermoreception of *Paramecium:* Different Ca^{2+} Channels Were Activated by Heating and Cooling

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Abstract. A *Paramecium* cell responded to heat and cold stimuli, exhibiting increased frequency of directional changes in its swimming behavior. The increase in the frequency of directional changes was maintained during heating, but was transient during cooling. Although variations were large, as expected with this type of electrophysiological recording, results consistently showed a sustained depolarization of deciliated cells in response to heating. Depolarizations were also consistently observed upon cooling. However, these depolarizations were transient and not continuous throughout the cooling period. These depolarizations were lost or became small in Ca^{2+}-free solutions. In a voltage-clamped cell, heating induced a continuous inward current and cooling induced a transient inward current under conditions where K^+ currents were suppressed. The heat-induced inward current was not affected significantly by replacing extracellular Ca^{2+} with equimolar concentrations of Ba^{2+}, Sr^{2+}, Mg^{2+}, or Mn^{2+}, and was lost upon replacing with equimolar concentration of Ni^{2+}. On the other hand, the cold-induced inward current was not affected significantly by Ba^{2+}, or Sr^{2+}, however the decay of the inward current was slowed and was lost or became small upon replacing with equimolar concentrations of Mg^{2+}, Mn^{2+}, or Ni^{2+}. These results indicate that *Paramecium* cells have heat-activated Ca^{2+} channels and cold-activated Ca^{2+} channels and that the cold-activated Ca^{2+} channel is different from the heat-activated Ca^{2+} channel in the ion selectivity and the calcium-dependent inactivation.

Key words: Channels — Ca^{2+} channel — *Paramecium* — Thermoreceptor — Temperature — Ion selectivity

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Introduction

Many organisms have thermosensory receptors, specified as heat and cold receptors. However, the mechanisms of thermosensory-transduction are not well understood. *Paramecium*, a protozoa, can sense temperature change and changes its swimming behavior. Heating above the temperature to which the cells have been adapted, and cooling below this temperature induce an increased frequency of directional changes in the swimming behavior (Nakaoka & Oosawa, 1977; Hennessey & Nelson, 1979). Both heating and cooling stimuli induce depolarization of the membrane potential, and this depolarization triggers the opening of the voltage-dependent Ca^{2+} channel in the ciliary membrane and thus initiates the action potential. The Ca^{2+} influx from the Ca^{2+} channel in the ciliary membrane increases the intraciliary concentration of Ca^{2+} and this Ca^{2+} causes the directional changes in swimming. It has been shown that the depolarization in response to heating is induced by an increase of Ca^{2+} conductance (Hennessey, Saimi & Kung, 1983; Nakaoka, Kurotani & Itoh, 1987; Tominaga & Naitoh, 1994) and the depolarization in response to cooling is also induced by an increase of Ca^{2+} conductance (Kuriu, Nakaoka & Oosawa, 1996). In the present study, we compared the heat-sensitive response and the cold-sensitive response. The results suggest that different Ca^{2+} channels were activated by heating and cooling.

Materials and Methods

Cells

Paramecium tetraurelia nd-6 (the trichocyst nondischarge mutant) was cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. The culture temperature was kept constant at 25°C by incubation in a water bath. *Paramecium* cells in the stationary phase were collected by low-speed centrifugation and suspended in a standard solution. Cells

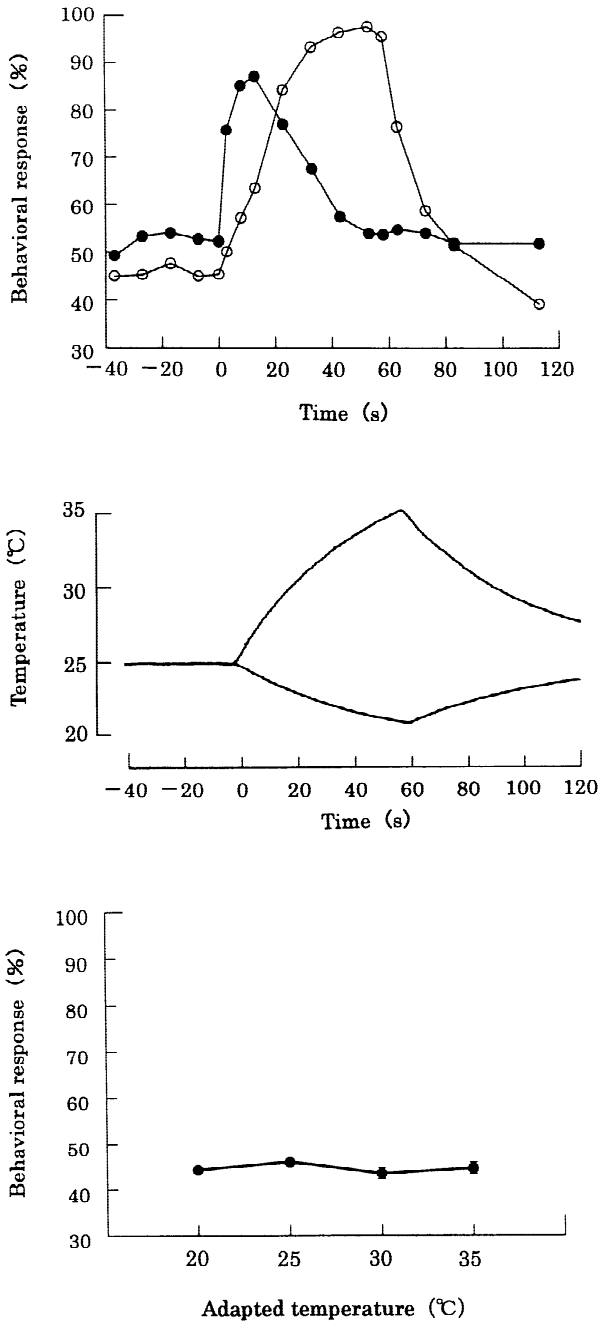

Fig. 1. Behavioral response to temperature changes. Cells were placed in the vessel at 25°C, then subjected to the initial rate of heating, +0.27°C/sec (open circles) or cooling, −0.15°C/sec (filled circles) at 0 sec on the abscissa. The behavioral responses are expressed by % of cells showing directional changes in swimming (top, *see* Materials and Methods). Similar data were obtained from different experiments (n = 3, in heating; n = 3, in cooling). The middle traces give the temperature changes in the vessel. Behavioral response was not changed by adapted temperature when the temperature was constant (bottom). The cells were incubated in the standard solution for 3 hr at the adapted temperature before commencement of the experiments (bottom); this means that cells can adapt to new temperature in 3 hr.

were pre-incubated in this solution at 25°C for 1 hr or more prior to examination, except for the behavioral experiment in Fig. 1, bottom.

Solutions

The standard solution contained 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 4 mM KCl and 1 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid)-Tris[Tris(hydroxymethyl)aminomethane] (pH 7.2). When the membrane potential under the current clamp was recorded, the cells were in the standard solution or a Ca^{2+}-free solution containing 0.5 mM $MgCl_2$, 4 mM KCl and 1 mM HEPES-Tris (pH 7.2). The solution used to bathe the cells during voltage clamp (Ca^{2+}/TEA^+ solution) contained (in mM): 1 $CaCl_2$, 10 TEA-Cl (tetraethylammonium chloride) and 1 HEPES-Tris (pH 7.2). In some experiments, 1 mM $CaCl_2$ was replaced with equimolar concentrations of $MgCl_2$, $BaCl_2$, $SrCl_2$, $MnCl_2$, or $NiCl_2$.

Behavioral Assays

Cells suspended in the standard solution were placed in a glass vessel whose temperature was controlled by water flowing beneath it (Nakaoka & Oosawa, 1977). The temperature was changed by switching a valve connected to outlets of two different temperature-regulating units. The temperature of the cell suspension was measured with a thermocouple probe placed in the vessel. The swimming behavior of cells was monitored with a CCD camera mounted just above the vessel and was recorded by a videotape recorder. Swimming tracks of 1-sec duration were obtained from the video record by using a real time video image quality improvement system (DVS-1000, Hamamatsu Photonics, Japan). Among the swimming tracks of 90–160 cells, those that changed swimming direction were counted and this fraction was taken as the frequency of directional change.

Intracellular Recording

Membrane potentials and membrane currents of *Paramecium* were recorded using the method described previously (Naitoh & Eckert, 1972; Nakaoka & Iwatsuki, 1992; Kuriu et al., 1996) and retained on a chart recorder with the paper moving at a speed of 2 cm/min. The capillary microelectrodes used for the current clamp contained 1 M KCl with a tip resistance of about 50 MΩ. Voltage clamp electrodes contained 1 M CsCl with a tip resistance of about 50 MΩ. For these measurements, the cells were deciliated by incubation in the standard solution containing 5% ethanol for 0.5–1 min and then transferred to the various experimental solutions (Ogura & Machemer, 1984). The deciliated cells were placed in a glass vessel mounted on an inverted microscope. The temperature was varied by switching the water flow beneath the vessel, and was monitored with a thermocouple probe placed near the specimens in the vessel (Nakaoka et al., 1987; Kuriu et al., 1996).

Results

Behavioral Response

When the temperature of the cell vessel started to rise from 25°C (culture temperature) to 35°C, *Paramecium tetraurelia* gradually increased the frequency of directional changes in swimming (Fig. 1, top). This increase was maintained during heating. As soon as the temperature started to drop to 25°C, the frequency started to decrease and returned to the original rate. On the other hand, when the temperature of the cell vessel started to drop from 25° to 20°C, *Paramecium tetraurelia* rapidly increased the frequency of directional changes in swim-

ming, attained a peak value after 13 sec, and decreased gradually thereafter even when cooling continued (Fig. 1, top). After the cells were adapted for 3 hr at the temperature between 20° and 35°C, the frequency of directional changes was measured at each constant adapted temperature. The frequency of directional changes was constant at the adapted temperature between 20° and 35°C (Fig. 1, bottom).

Electrophysiological Recording

A *Paramecium* cell responded to heating from 25° to 30°C with a membrane depolarization (Fig. 2, left, top). This depolarization was maintained during heating, and when the temperature started to drop to 25°C, the membrane potential started to return to the original potential. In the Ca^{2+}-free solution this depolarization was small (Fig. 2, left, middle). The amplitudes of the depolarization (mean ± SE) were 9.4 ± 1.0 mV ($n = 25$) and 4.1 ± 0.7 mV ($n = 16$) in the standard solution and in the Ca^{2+}-free solution, respectively. These depolarizations are significantly different from 0.0 mV (*t*-test, $P < 0.05$, in the standard solution and $P < 0.10$, in the Ca^{2+}-free solution). Cooling from 25° to 20°C also caused a membrane depolarization (Fig. 2, right, top), but this depolarization was transient and had a peak value at about 5 sec from the start of cooling in the standard solution. In the Ca^{2+}-free solution, no such depolarization was observed (Fig. 2, right, middle). The amplitudes of the depolarization (mean ± SE) were 9.7 ± 2.1 mV ($n = 5$) and 0.04 ± 0.3 mV ($n = 8$) in the standard solution and in the Ca^{2+}-free solution, respectively.

Heating of the cell while voltage clamped at −25 mV (near the resting potential in the standard solution) elicited a continuous inward current during heating in Ca^{2+}/TEA^+ solution (Fig. 3, left, top). Cooling also elicited an inward current in Ca^{2+}/TEA^+ solution, but this was transient (Fig. 3, right, top). A heat-induced inward current was also observed in a solution with 1 mM $CaCl_2$ replaced by 1 mM $MgCl_2$ (Mg^{2+}/TEA^+ solution) (Fig. 3, left, middle). The average amplitude of the heat-induced inward current was −54.4 ± 5.7 pA ($n = 7$) in Ca^{2+}/TEA^+ solution and −50.9 ± 6.3 pA ($n = 3$) in Mg^{2+}/TEA^+ solution at −25 mV. On the other hand, a cold-induced inward current was observed in Ca^{2+}/TEA^+ solution, but it was lost in Mg^{2+}/TEA^+ solution (Fig. 3, right, middle, $n = 2$). In these experiments, the K^+-currents were suppressed by the use of a voltage clamp electrode containing 1 M CsCl, and by including 10 mM TEA-Cl in the bath solution (Hinrichsen & Saimi, 1984).

Effects of Divalent Cations

The ability of other divalent cations to cause the heat- or cold-induced inward current was tested. The heat-induced inward current was lost when the extracellular Ca^{2+} in Ca^{2+}/TEA^+ solution was replaced with an equimolar concentration of Ni^{2+} ($n = 3$), but it was not affected significantly by replacing Ca^{2+} with equimolar concentrations of Ba^{2+}, Sr^{2+} or Mn^{2+} (Fig. 4, left). The average amplitude of the heat-induced inward current in Ba^{2+}/TEA^+ solution, Sr^{2+}/TEA^+ solution, Mn^{2+}/TEA^+ solution was −55.0 ± 2.7 pA ($n = 4$), −59.5 ± 8.5 pA ($n = 3$), −54.7 ± 4.7 pA ($n = 3$), respectively, at −25 mV. On the other hand, the cold-induced inward current was diminished or lost when the extracellular Ca^{2+} in Ca^{2+}/TEA^+ solution was replaced with equimolar concentrations of Mn^{2+} or Ni^{2+} (Mn^{2+}: $n = 3$, Ni^{2+}: $n = 2$), but

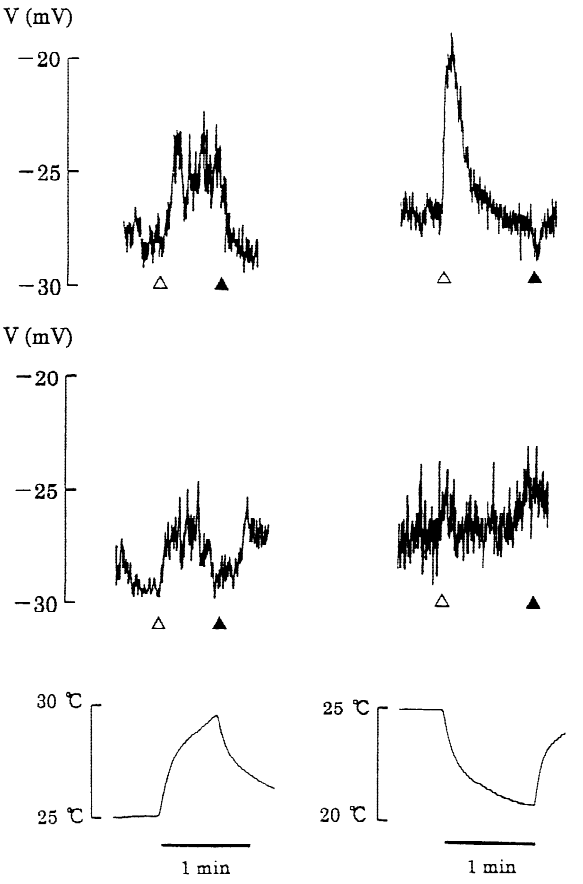

Fig. 2. Membrane potential responses to heat stimuli (left) and cold stimuli (right). Deciliated cells were used for all measurements of the electrophysiology. Changes in membrane potential of *Paramecium* cells elicited by heating and cooling in the standard solution (top). Changes in membrane potential of *Paramecium* cells elicited by heating and cooling in the Ca^{2+}-free solution (middle). The depolarization caused by heating in Ca^{2+}-free solution became small, but was not lost. On the other hand, the depolarization caused by cooling in Ca^{2+}-free solution was lost. Mg^{2+} caused this small depolarization in heating. Because Mg^{2+} can permeate through the heat-activated Ca^{2+} channel, but cannot permeate through the cold-activated Ca^{2+} channel (*see* Fig. 3). Open arrowheads indicate the start of heating or cooling. Filled arrowheads indicate the end of heating or cooling. The data were obtained from different cells. The bottom traces are records of temperature.

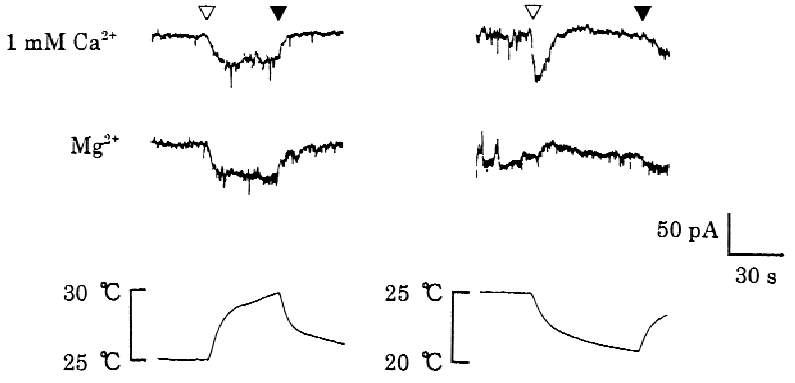

Fig. 3. Current responses to temperature change. Heat-induced membrane currents in Ca^{2+}/TEA^{+} solution and in Mg^{2+}/TEA^{+} solution (left) and cold-induced membrane currents in Ca^{2+}/TEA^{+} solution and in Mg^{2+}/TEA^{+} solution (right). The holding potential was −25 mV. Open arrowheads indicate the start of heating or cooling. Filled arrowheads indicate the end of heating or cooling. The data were obtained from different cells. The bottom traces are records of temperature.

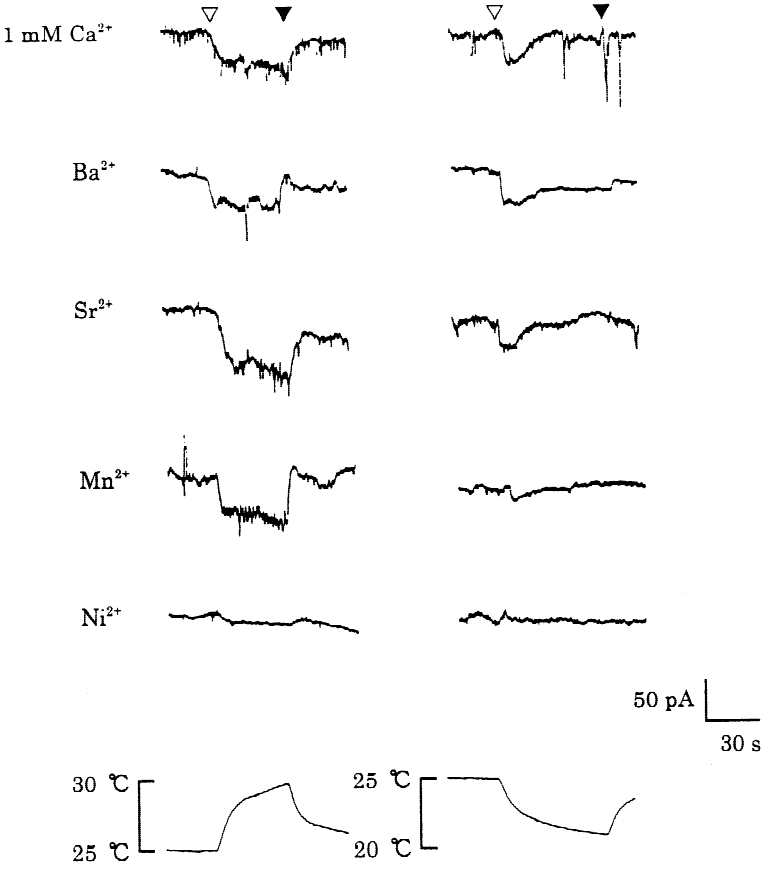

Fig. 4. Divalent cation selectivity of inward currents. Currents elicited by heating (left) or cooling (right) in Ca^{2+}/TEA^{+} solution, and upon replacing 1 mM $CaCl_2$ with equimolar concentrations of $BaCl_2$, $SrCl_2$, $MnCl_2$, or $NiCl_2$. The holding potential was −25 mV. Representative recordings are shown from different cells in each solution. It was difficult to collect several data from a single cell because cells were often damaged in the second application of stimulus. Open arrowheads indicate the start of heating or cooling. Filled arrowheads indicate the end of heating or cooling. The bottom traces are records of temperature.

it was not affected significantly by replacing Ca^{2+} with equimolar concentrations of Ba^{2+} or Sr^{2+} (Fig. 4, right). The peak amplitude of the cold-induced inward current in Ca^{2+}/TEA^{+} solution, Ba^{2+}/TEA^{+} solution, Sr^{2+}/TEA^{+} solution, was −47.6 ± 8.5 pA (n = 4), −51.2 ± 6.3 pA (n = 3), −73.3 ± 14.5 pA (n = 3), respectively, at −25 mV. However, in Ca^{2+}/TEA^{+} solution, the inward current decayed within about 15–30 sec after the start of cold stimuli, while the decay was slower when the extracellular Ca^{2+} was replaced by either Ba^{2+} or Sr^{2+} (Fig. 4, right).

Discussion

Heat- and Cold-Sensitive Behavioral Response and Membrane Depolarization

Heating above the temperature to which the cells have been cultured induced increased frequency of directional changes in swimming behavior. This frequency increased gradually with rising temperature — 98% of the cells showing this response at the peak — and was maintained during heating. As soon as the temperature started

to drop to 25°C, the frequency started to decrease and returned to the initial rate. On the other hand, cooling below the culture temperature induced transient increases in the frequency of directional changes in swimming behavior. The cells showed a peak in frequency of directional changes at about 13 sec after the temperature changed, after which the frequency decreased gradually — even when cooling continued — and returned to the initial rate. These results indicate that heat-sensitive behavioral response was different from cold-sensitive behavioral response.

The membrane potential response of the cell to heating was a continuous depolarization, while the response to cooling was a transient depolarization. The depolarization in response to heating and cooling became small and was lost, respectively, upon removing extracellular Ca^{2+}. It suggested that this depolarization was mainly caused by extracellular Ca^{2+} and that the heat-induced depolarization was partly caused by extracellular Mg^{2+}. Both heating and cooling of the voltage-clamped cell induced Ca^{2+} current under conditions where K^+ current was suppressed (Fig. 3). These results indicate that *Paramecium* cells have both heat-activated Ca^{2+} channels and cold-activated Ca^{2+} channels.

Inactivation of Cold-Induced Ca^{2+} Current

The Ca^{2+} current was induced by heating of the voltage-clamped cell and maintained during heating stimuli. The Ca^{2+} current was also induced by cooling of the voltage-clamped cell but this current subsequently decayed within about 15–30 sec and this decay was slowed when the extracellular Ca^{2+} was replaced by either Ba^{2+} or Sr^{2+} (Fig. 4; Kuriu et al., 1996). This inactivation explains the transient nature of the cold-induced depolarization. In *Paramecium*, Ca^{2+} influx leads to inactivation of depolarization- or hyperpolarization-activated Ca^{2+} channels (Brehm & Eckert, 1978; Preston, Saimi & Kung, 1992). The decay of the cold-induced inward current may also result from Ca^{2+}-dependent inactivation of cold-sensitive Ca^{2+} channels. The inactivation of cold-induced Ca^{2+} current may cause the cold-induced increase of the frequency of directional changes to become transient. However the heat-induced Ca^{2+} inward current did not show inactivation (Fig. 3).

Ion Selectivity of Heat- and Cold-Activated Ca^{2+} Channels

The heat-induced inward current was not affected in amplitude significantly by replacing Ca^{2+} with equimolar concentrations of Mg^{2+} or Mn^{2+}, but the cold-induced inward current was lost or diminished by replacing Ca^{2+} with equimolar concentrations of Mg^{2+} or Mn^{2+}, respectively. These results indicate that the heat-activated Ca^{2+} channel and the cold-activated Ca^{2+} channel have different ion selectivity. On the other hand, the cold-induced inward current was not affected in amplitude significantly by replacing Ca^{2+} with equimolar concentrations of Ba^{2+} or Sr^{2+}, however the decay of the inward current was slowed. In conclusion, *Paramecium* has heat-activated Ca^{2+} channels and cold-activated Ca^{2+} channels, and the cold-activated Ca^{2+} channel is different from the heat-activated Ca^{2+} channel in its ion selectivity and calcium-dependent inactivation.

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