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IONIC MECHANISM OF THERMORECEPTION IN *PARAMECIUM*

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SUMMARY

The localization of thermoreceptors in *Paramecium*, and the ionic basis of thermoreception, was investigated in posterior and anterior fragments of cells. Transverse section of the animals was used to obtain these fragments, which sealed up and swam actively. In the anterior fragment, an increase in the frequency of directional changes in swimming and depolarization of the membrane was produced by cooling below the temperature of the culture. In the posterior fragment, these effects were produced by warming above culture temperature. Reversal potentials of these effects were found by injection of constant current to change membrane potential. In the anterior fragment, the reversal potential of the response to cooling was more negative than the resting potential and was potassium-dependent (52 mV/ $\log[K^+]_o$). In the posterior fragment, the reversal potential of the warming response was above resting potential and was primarily calcium-dependent (28 mV/ $\log[Ca^{2+}]_o$). It is concluded that cooling results in changes in the frequency of directional changes in swimming of *Paramecium* by causing a transient change in the membrane conductance for potassium, whereas warming produces its effects by a transient change in calcium conductance.

INTRODUCTION

Thermoreception is found in many living organisms (Hensel, 1974; Jennings, 1906; Maeda & Imae, 1979), but its molecular mechanism remains unknown. The protozoan, *Paramecium*, responds to warming above the temperature to which it has been adapted, and to cooling below this temperature, by producing transient changes in the frequency of directional changes in swimming (Nakaoka & Oosawa, 1977). It has been shown that both types of temperature change induce a slow transient depolarization of the membrane potential which triggers spike-like depolarization and in turn causes the directional changes in swimming (Hennessey, Saimi & Kung, 1983; Toyotama, 1981).

In this study, to elucidate the ionic process of thermoreception in *Paramecium*, we first examined whether the thermosensitive response at one end of the cell was different from that at the other end since it is known that mechanoreceptors produce different responses at each end (Naitoh & Eckert, 1969; Ogura & Machemer, 1980).

Key words: *Paramecium*, thermoreception, membrane potential, membrane permeability.

Application of thermal stimulation to discrete areas of *Paramecium* membrane is technically difficult, so the cell was transversely dissected with a glass micro-needle into anterior and posterior fragments, and the thermosensitive responses of each fragment were examined.

MATERIALS AND METHODS

Cells

Paramecium multimicronucleatum (supplied by Professor Dr K. Hiwatashi) was cultured in a hay infusion inoculated with *Klebsiella pneumoniae*. *Paramecium* cells at stationary phase were collected by low speed centrifugation and suspended in a solution of 2 mmol l^{-1} KCl, 0·25 mmol l^{-1} $CaCl_2$, 0·5 mmol l^{-1} $MgCl_2$ and 2 mmol l^{-1} Tris-HCl (pH 7·2). This solution was used as a standard solution in all experiments. For experiments investigating ionic dependence, potassium or calcium concentration of the standard solution was varied. The culture and suspension solution were maintained at 22 °C.

Dissection of cells

The cell was observed under an inverted microscope (80×), and then transversely dissected, approximately at the centre, with a glass micro-needle, into anterior and posterior fragments. Fragments which were actively swimming a few minutes after the dissection were used for experiments. Phase contrast images of a whole cell, and anterior and posterior fragments are shown in Fig. 1.

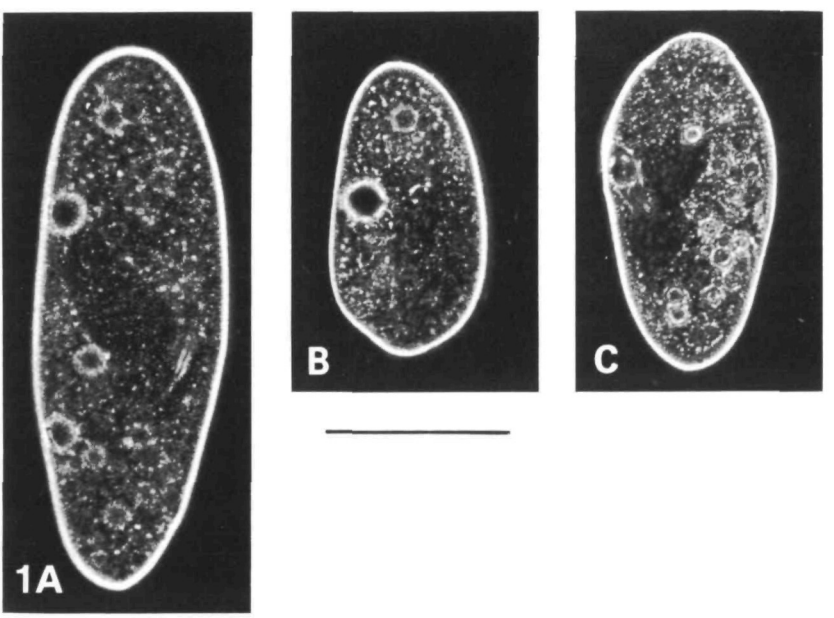

Fig. 1. Phase contrast images of a whole cell (A), and anterior (B) and posterior (C) fragments. The anterior end of the cell is at the top of the picture. Scale bar, 100 μm.

Observation of swimming behaviour

A whole cell or a cell fragment was placed in a glass vessel whose temperature was controlled by water flowing beneath it (Nakaoka & Oosawa, 1977). The temperature was monitored by a thermistor probe submerged in the vessel. Swimming behaviour was recorded with a camera mounted just above the vessel.

Intracellular recording

The method of recording from cells and cell fragments was similar to that described by Naitoh & Eckert (1972). The electrodes were filled with 1 mol l^{-1} KCl and their resistances were 50–80 MΩ. The cells were placed in a glass vessel mounted on an inverted microscope. The temperature was changed by switching water flow beneath the vessel, and was monitored with a thermistor probe submerged in the vessel.

The reversal potential of the thermoreceptor response was studied using a current clamp. The membrane potential was set at various levels by injection of constant current of the order of 10^{-10}A. One or two minutes after the potential had been set, and when it had become relatively stable, a temperature change was applied. Reversal potential was determined as the potential at which the electrical response changed its polarity.

RESULTS

Behavioural responses of cells and cell fragments

A temperature change away from the culture temperature produced an increase in the frequency of directional changes in swimming in whole cells, as reported previously (Nakaoka & Oosawa, 1977), and in cell fragments (Fig. 2). In the whole cell (Fig. 2A), both lowering and raising from the culture temperature (22°C) increased the frequency of directional changes, and returning the temperature towards 22°C decreased the frequency of directional changes. In anterior fragments (Fig. 2B), there was a transient increase in the frequency of directional changes upon cooling below 22°C, but warming above 22°C had little effect on directional changes. However, in posterior fragments (Fig. 2C) there was a transient increase in the frequency of directional changes upon warming above 22°C, whereas cooling induced little change in the swimming behaviour. When only the anterior quarter of the cell was removed, the remainder of the cell still did not show the avoiding response upon cooling, but showed it upon heating.

These results show that sensitivity to warming is concentrated in the posterior part of the cell, whereas sensitivity to cooling is apparently restricted to a small portion of the anterior.

Responses of the membrane potential

Both cooling and warming elicited a slow, transient depolarization in the whole cell, together with fast depolarizing spikes (Fig. 3A). During warming a sustained

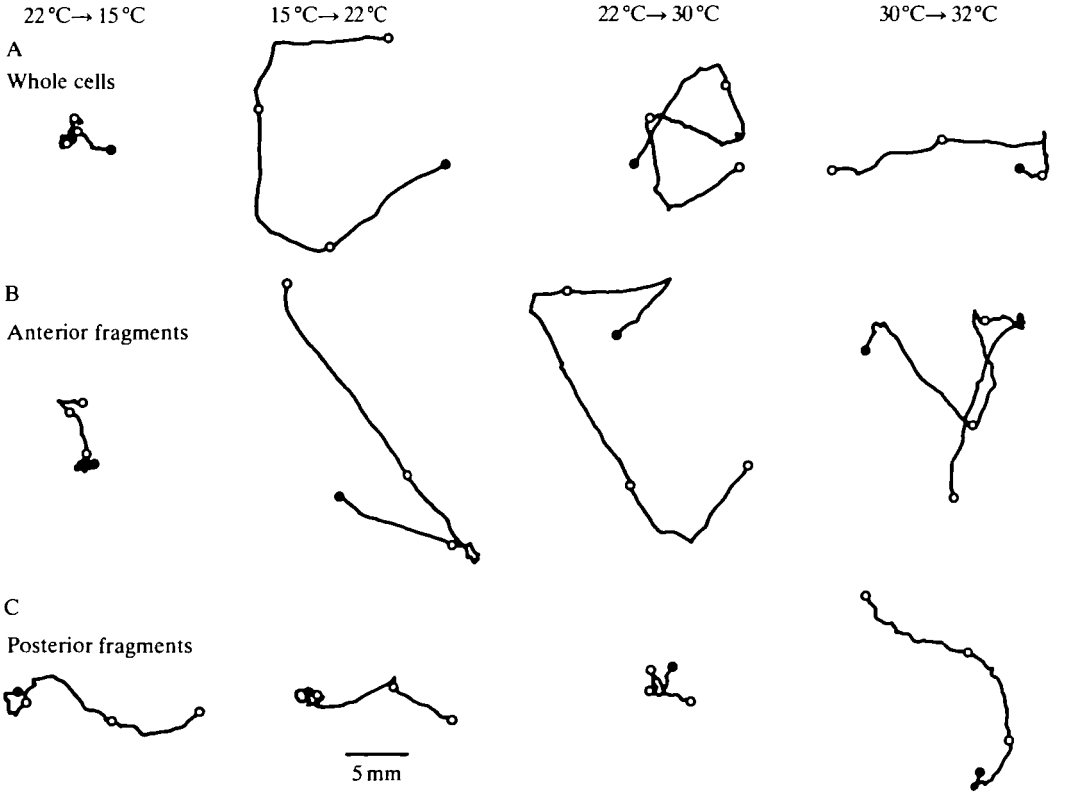

Fig. 2. Swimming tracks of whole cells (A), and anterior (B) and posterior (C) fragments. Initial and final temperatures of thermal stimuli are indicated above. After the specimen had been placed in the vessel containing standard solution, the temperature was held for 3 min, then changed towards the final temperature. The rate of temperature change at the initial phase was about $0{\cdot}16\,^{\circ}C\,s^{-1}$. The start of the tracks is indicated by the filled circles. The open circles are 10 s apart.

depolarization was sometimes observed. Similar changes in membrane potential have been reported previously (Hennessey *et al.* 1983; Toyotama, 1981). In anterior fragments (Fig. 3B), a transient depolarization was induced upon cooling and a small transient hyperpolarization was induced upon warming. However, in posterior fragments (Fig. 3C), a fast depolarization was induced upon warming and only a small, steady hyperpolarization upon cooling. The changes in the membrane potential of the whole cell can be approximated by the sum of the changes in the two fragments.

In the absence of any thermal stimulus, anterior fragments showed spontaneous depolarizations, posterior fragments showed spontaneous hyperpolarizations, and whole cells showed potential changes in both directions.

Reversal potential

The polarity and amplitude of the potential response to a thermal stimulus could be changed by the injection of a constant current (Fig. 4). To allow repetitive

stimulation during each recording, the thermal stimulus was removed after the initial phase of the response (about 1 min) and the potential was then restored. In the anterior fragment (Fig. 4A), the depolarization that was induced by cooling became larger when the membrane potential was made more positive than the resting potential. When the membrane potential was made more negative, the response to

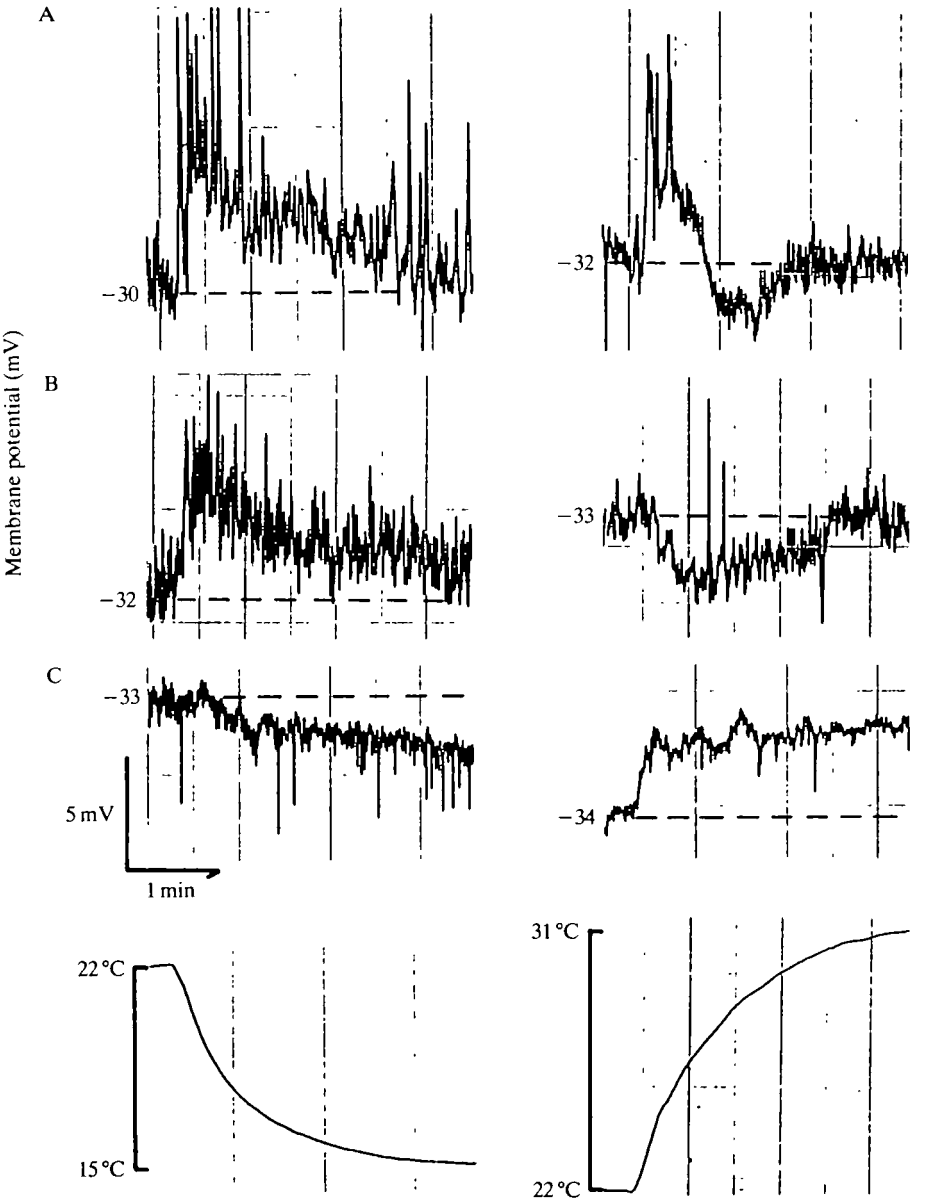

Fig. 3. Potential changes of a whole cell (A), and anterior (B) and posterior (C) fragments following thermal stimuli. After inserting the electrodes, the temperature was kept at 22 °C for a few minutes, then changed to 15 °C or 30 °C as indicated below. Dashed lines indicate the resting potential. Fragments were from different cells.

cooling became smaller until a potential was reached at which the response changed polarity. In the posterior fragment (Fig. 4B), the hyperpolarizing response to cooling became larger as the membrane was hyperpolarized, and became smaller and reversed in polarity at more positive values than the resting potential.

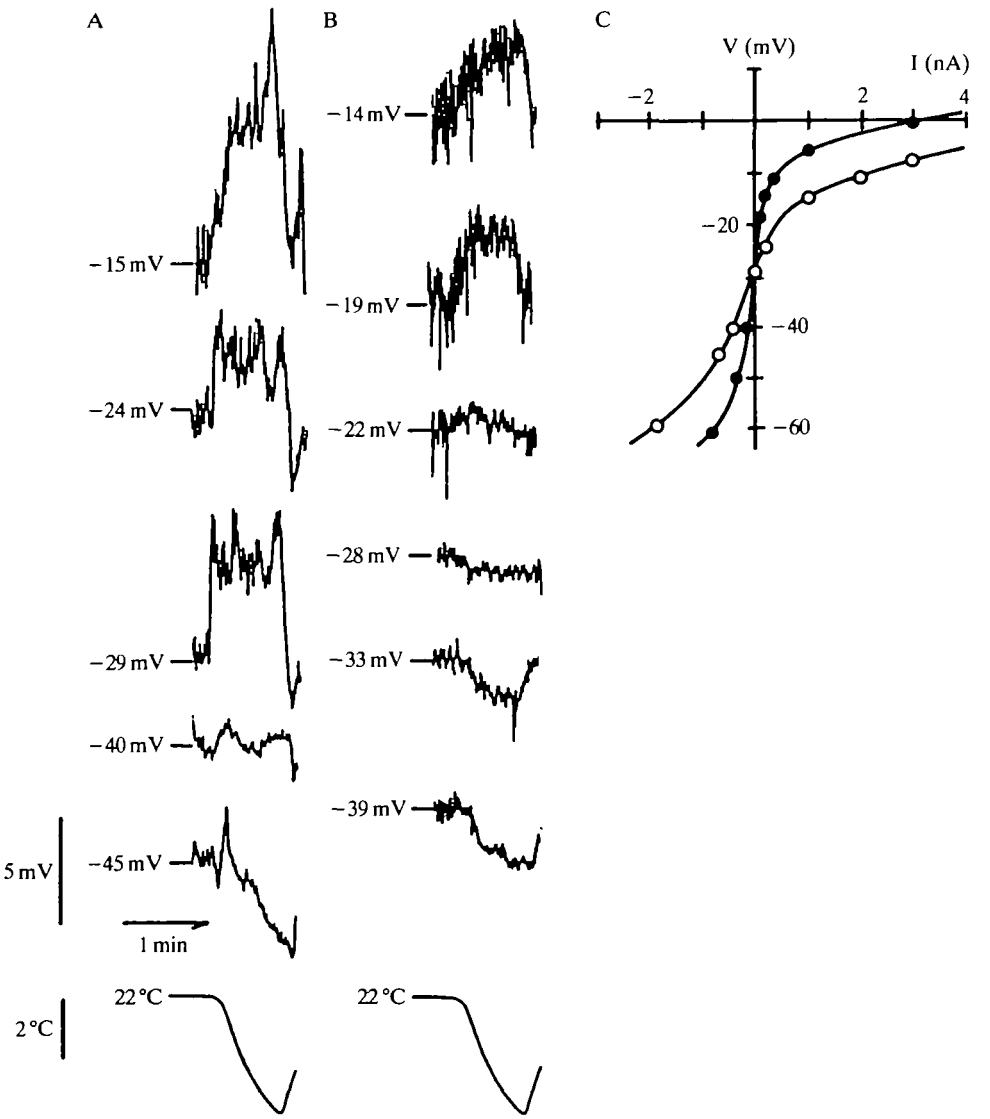

Fig. 4. Initial phase of potential response at various potential levels. Membrane potentials of anterior (A) and posterior (B) fragments were set at various levels by injection of a constant current through an impaling electrode, then the temperature was dropped as shown in the bottom row. To repeat many stimuli on the same fragment, only the initial phase of potential response (1 min) after stimulus was recorded, and the potential and temperature were then restored. Before current injection, resting potentials of anterior and posterior fragments were −29 mV and −28 mV, respectively. (C) I/V relationships of anterior (○) and posterior (●) fragments.

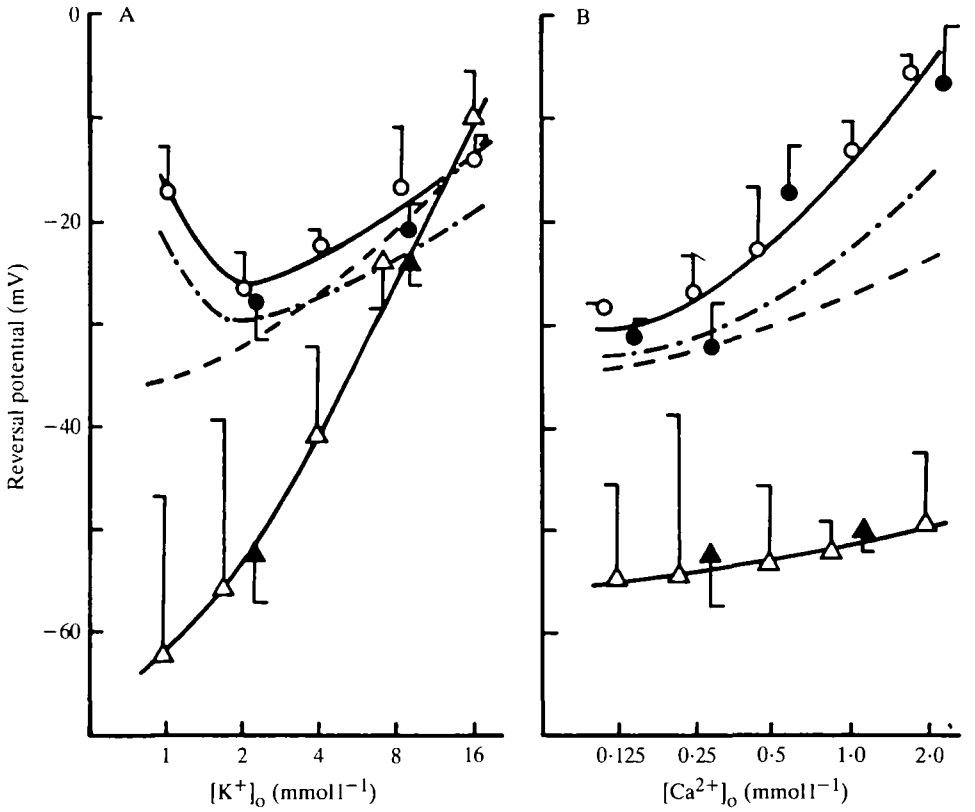

Fig. 5. Dependence of reversal potential on $[K^+]$ (A) and $[Ca^{2+}]$ (B). In A, Ca^{2+} was held at 0·25 mmol l^{-1} and in B, K^+ was held at 2 mmol l^{-1}. In all measurements, the external medium contained 0·5 mmol l^{-1} $MgCl_2$ and 2 mmol l^{-1} Tris-HCl (pH 7·2). Upon cooling from 22 °C, the reversal potentials of anterior (△) and posterior (○) fragments were measured. Upon warming from 22 °C, the reversal potentials of anterior (▲) and posterior (●) fragments were measured. Vertical bars show standard deviation of 5–8 measurements on as many different specimens. Dashed lines and interrupted lines indicate the mean levels of the resting potentials of anterior and posterior fragments (5–8 specimens), respectively.

The reversal potentials obtained upon cooling were almost the same as those obtained upon heating, in both anterior and posterior fragments.

Dependence on K^+ and Ca^{2+}

The membrane potential of *Paramecium* is mainly determined by membrane permeabilities for potassium and calcium ions (Naitoh & Eckert, 1974). The roles of these ions in the thermal response were therefore examined by measuring the reversal potential at various concentrations of K^+ and Ca^{2+}. The reversal potential of the anterior fragment showed a slope of 52 mV for a 10-fold change in the concentration of K^+ (Fig. 5A) and was slightly dependent on Ca^{2+} (5 mV/log$[Ca^{2+}]_o$) (Fig. 5B). The reversal potential of the posterior fragment showed a slope of 28 mV for a 10-fold change in the concentration of Ca^{2+} (Fig. 5B) and was almost independent of K^+ (Fig. 5A).

DISCUSSION

The present results show that the thermal response of *Paramecium* to cooling can be ascribed to receptors on the anterior membrane, while the response to warming is mediated by receptors on the posterior part: the anterior membrane is responsible for transient depolarization upon cooling, whereas the posterior membrane is responsible for depolarization upon warming (Fig. 3). These depolarizations trigger the spike-like depolarizations which cause directional changes in swimming.

Depolarization of the anterior membrane upon cooling can be explained by a decrease in K^+ permeability, since the reversal potential was dependent on $[K^+]_o$ and more negative than the resting potential. Depolarization of the posterior fragment upon warming can be explained by an increase in Ca^{2+} permeability since the reversal potential was dependent on $[Ca^{2+}]_o$ and was more positive than the resting potential.

The behavioural response to thermal stimulation is given after 2–3 h adaptation to a new temperature (Nakaoka *et al.* 1982). The present results suggest that this adaptation requires a modulation of membrane permeability to K^+ and Ca^{2+}. This may be related to the change in lipid composition that occurs in *Paramecium* following a change in temperature (Hennessey & Nelson, 1983).

The sites of the K^+ and Ca^{2+} channels involved in thermoreception are opposite to those observed for the channels involved in mechanoreception, for which Ca^{2+} permeability is located on the anterior membrane and K^+ permeability is mainly on the posterior part (Naitoh & Eckert, 1969; Ogura & Machemer, 1980). Such opposite sites suggest that the channels for thermoreception are different from those for mechanoreception.

Although the ionic mechanism of thermoreception in higher organisms is unknown, it has been reported that in afferent fibres of the rat the removal of extracellular K^+ results in an increased frequency of cold receptor discharge (Pierau, Torrey & Carpenter, 1975) and that in the cat an increase in extracellular $[Ca^{2+}]$ causes a marked increase in warm receptor discharge (Hensel & Schäfer, 1974). These features of afferent fibres agree well with the present results obtained on *Paramecium*, because such changes in $[K^+]$ and $[Ca^{2+}]$ increase the electromotive forces for the respective ions, which tend to increase the amplitudes of depolarizations induced by cold and warm stimuli. The ionic mechanism of thermoreception in *Paramecium* may thus be similar to that in higher organisms.

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