

COMPARISON BETWEEN THERMORECEPTOR AND MECHANORECEPTOR CURRENTS IN *PARAMECIUM CAUDATUM*

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Summary

1. A voltage-clamped *Paramecium* produced an inward membrane current upon thermal or mechanical stimulation of its anterior region, whereas it produced an outward membrane current upon similar stimulation of its posterior region.
2. Anterior thermo- and mechanoreceptor currents decreased when the membrane potential was shifted in a positive direction, showing sign reversal at a positive membrane potential, whereas posterior thermo- and mechanoreceptor currents decreased when the membrane potential was shifted in a negative direction, showing sign reversal at a membrane potential more negative than the resting potential.
3. The reversal potential for both anterior receptor currents shifted in a positive direction when external $[Ca^{2+}]$ was increased, whereas those for both posterior receptor currents shifted in a positive direction when external $[K^+]$ was increased.
4. External $[Mg^{2+}]$, $[Mn^{2+}]$, $[Na^+]$, $[Rb^+]$ and $[TEA^+]$ had similar effects on the thermo- and mechanoreceptor currents.
5. Thermoreceptor currents decreased whereas mechanoreceptor currents increased as the ambient temperature was raised.
6. When a mechanical stimulus was applied to the membrane where a thermoreceptor current was being produced, an algebraic summation of these receptor currents occurred.
7. It is concluded that thermoreceptor currents are dependent on ion channels different from those responsible for the mechanoreceptor currents, although the ionic pores for the channels are similar to each other in various respects.
8. A possibility that a thermoreceptor mechanism exclusively shares a Ca^{2+} pore in the anterior membrane, or a K^+ pore in the posterior membrane, with a mechanoreceptor mechanism is discussed.

Introduction

In a previous paper (Tominaga and Naitoh, 1992a) we demonstrated that the ciliate protozoan *Paramecium caudatum* exhibited a depolarizing membrane potential response to thermal stimulation of the anterior region of the cell, whereas it exhibited a

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hyperpolarizing response to thermal stimulation of the posterior region. These findings suggest topographical differentiation of two different kinds of thermoreceptor channels on the surface membrane of *Paramecium*.

Differences in the topographical distribution of two kinds of mechanoreceptor potentials on the cell surface of *Paramecium* were first demonstrated by Naitoh and Eckert (1969b). *Paramecium* showed a depolarizing membrane potential response when a mechanical stimulus was applied to its anterior region, whereas it showed a hyperpolarizing membrane potential response when a similar stimulus was applied to the posterior region. Similar mechanoreceptor potential distribution patterns on the cell surface have been reported in some other ciliate protozoans, such as *Euplotes* (Naitoh and Eckert, 1969a), *Stylonychia* (De Peyer and Machemer, 1978) and *Tetrahymena* (Takahashi *et al.* 1980) (see review articles by Naitoh, 1982, 1984; Machemer, 1988; Deitmer, 1992).

In *Paramecium*, the similarity in the topographical distribution of thermoreceptor potentials and mechanoreceptor potentials suggests that they may share a common underlying mechanism. Receptors that respond to both thermal and mechanical stimulation have been reported in mammals (Hensel, 1973, 1974a; Burgess and Perl, 1973), birds (Gottschaldt, 1985; Gentle, 1989), fish (Hensel, 1974b), crustaceans (Burkhardt, 1959) and insects (Altner and Loftus, 1985). These facts strongly suggest that both receptor systems have a common developmental and evolutionary origin.

The primary objectives of the research described in this paper were to examine and compare the electrophysiological characteristics and mechanisms of the thermoreceptor and mechanoreceptor currents in voltage-clamped specimens of *P. caudatum*. Some of these results have been presented verbally elsewhere (Tominaga and Naitoh, 1992b).

Materials and methods

Specimens of *Paramecium caudatum* (strain G3, mating type V) were cultured in a bacterized (*Klebsiella pneumoniae*) lettuce infusion at $25 \pm 2^\circ\text{C}$. After their final feeding, the culture was kept immersed in a constant-temperature water bath ($25 \pm 0.1^\circ\text{C}$) for more than a day. The temperature of the water bath is regarded as the 'culture temperature'. Prior to experimentation, the specimens were washed with a standard saline solution [4 mmol l^{-1} KCl, 1 mmol l^{-1} CaCl_2 , 1 mmol l^{-1} Mops-KOH or Tris-HCl buffer (final concentration); pH 7.2], and then maintained in this solution in the water bath for more than 30 min.

Conventional electrophysiological techniques were employed for examining membrane electrical responses to a thermal and/or a mechanical stimulus (Naitoh and Eckert, 1972a). The tips of two glass microcapillary electrodes ($1 \mu\text{m}$ inner diameter) filled with 1 mol l^{-1} KCl were inserted into the central portion of a specimen of *Paramecium* to measure membrane potential and membrane current. When the anterior mechanoreceptor response was examined, microelectrodes filled with 1 mol l^{-1} CsCl were used (Hinrichsen and Saimi, 1984). The tip of a similar microcapillary electrode filled with 3 mol l^{-1} KCl was placed in the external solution close to the cell surface of the impaled specimen and used as the reference electrode. The electrical resistance of these

microcapillary electrodes ranged from 4 to 10 M Ω . The overall time constant of the voltage-clamp system was about 900 μ s, sufficiently fast to follow the receptor currents.

The 'experimental temperature' of the vessel was kept constant (± 0.2 °C) with the aid of an electronically controlled Peltier module placed beneath the vessel. A specimen impaled by microelectrodes was equilibrated at an experimental temperature for 2 min then subjected to a stimulus. One end of a specimen impaled by microelectrodes was subjected to a localized thermal stimulus, which was given by a microheater placed 25 μ m from the end, as described in our previous paper (Tominaga and Naitoh, 1992a). Stimulus strength was given by the square of the current (A^2) applied to the microheater, since the amount of heat produced by the heater was proportional to A^2 . The duration of thermal stimulation was kept constant at 50 ms throughout the experiments. The end of the specimen was also subjected to a mechanical stimulus, which was given by hitting it with the tip of a microneedle (5 μ m tip diameter), as described by Naitoh and Eckert (1969a). Stimulus strength was given by the voltage of a square electric pulse applied to the piezoelectric element that drove the microneedle against the specimen. The excursion of the microneedle was proportional to the voltage in the range employed (Katoh and Naitoh, 1992). The duration of mechanical stimulation was kept constant at 5 ms throughout the experiments. Movement of the tip of the microneedle has been examined in detail by De Peyer and Machemer (1978).

Values given in the text are means \pm S.E.M. unless stated otherwise.

Results

Membrane electrical responses to localized thermal or mechanical stimulation

A specimen of *P. caudatum* exhibited a depolarizing membrane potential response upon thermal stimulation of its anterior end, whereas it exhibited a hyperpolarizing response upon thermal stimulation of its posterior end. When the membrane potential was clamped at its resting level, an inward membrane current was evoked by anterior stimulation, whereas an outward membrane current was evoked by posterior stimulation. Representative traces of the membrane electrical responses to thermal stimulation are shown in Fig. 1A.

As for thermal stimulation, a specimen exhibited a depolarizing membrane potential response upon mechanical stimulation of its anterior end, whereas it exhibited a hyperpolarizing membrane potential response upon mechanical stimulation of its posterior end. When the membrane potential was clamped at the resting level, an inward membrane current was evoked by anterior stimulation, whereas an outward membrane current was evoked by posterior stimulation. Representative traces of the membrane electrical responses to mechanical stimulation are shown in Fig. 1B. Hereafter, these receptor currents will be abbreviated to the following: ATC, anterior thermoreceptor current; PTC, posterior thermoreceptor current; AMC, anterior mechanoreceptor current; PMC, posterior mechanoreceptor current.

The intensity of each receptor current increased with increasing stimulus strength. The relationships between the current intensity and the stimulus strength at two different experimental temperatures, 15 °C and 25 °C, are shown in Fig. 2. The thresholds for the

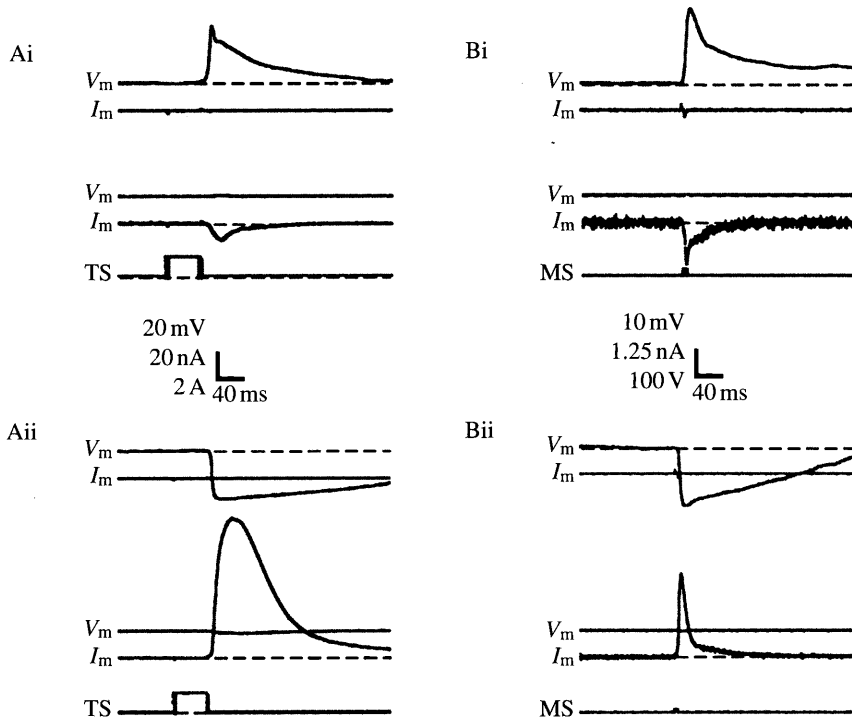


Fig. 1. Membrane potential and current responses to thermal (A) or mechanical (B) stimulation of *Paramecium caudatum*. (Ai, Bi) Responses to stimulation of the anterior region of the cell; (Aii, Bii) responses to stimulation of the posterior region of the cell. The lower V_m and I_m traces in each pair represent responses obtained under voltage-clamp. V_m , membrane potential; I_m , membrane current; TS, current pulse applied to the microheater for thermal stimulation; MS, voltage pulse applied to a piezoelectric phonocardiograph to drive a microneedle for mechanical stimulation. Stimulus strength was 2 A^2 for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation. The experimental temperature was 15°C in thermal stimulation experiments and 25°C in mechanical stimulation experiments.

thermoreceptor currents were lower at 15°C than at 25°C ($0.9 \pm 0.08 \text{ A}^2$ at 15°C and $1.7 \pm 0.09 \text{ A}^2$ at 25°C for ATC; $1.0 \pm 0.07 \text{ A}^2$ at 15°C and $2.6 \pm 0.19 \text{ A}^2$ at 25°C for PTC; $N=4-5$ different specimens). In contrast, the thresholds for mechanoreceptor currents were higher at 15°C than at 25°C ($24.5 \pm 1.79 \text{ V}$ at 15°C and $16.0 \pm 2.40 \text{ V}$ at 25°C for AMC; $5.3 \pm 0.55 \text{ V}$ at 15°C and $1.4 \pm 0.15 \text{ V}$ at 25°C for PMC).

ATC tended to saturate (at approximately -10 nA per cell) when stimulus strength was as high as approximately 2 A^2 at 15°C . The receptor current, however, showed a further increase when the stimulus intensity was higher than 3 A^2 . Saturation was not seen at 25°C in the range of stimulus strengths employed. PTC tended to saturate (at approximately 40 nA per cell) when stimulus strength was as high as approximately 2 A^2 at 15°C . The receptor current, however, increased further when the stimulus intensity was further increased. PTC was scarcely detectable at 25°C .

AMC did not saturate in the range of stimulus strengths employed, whereas PMC

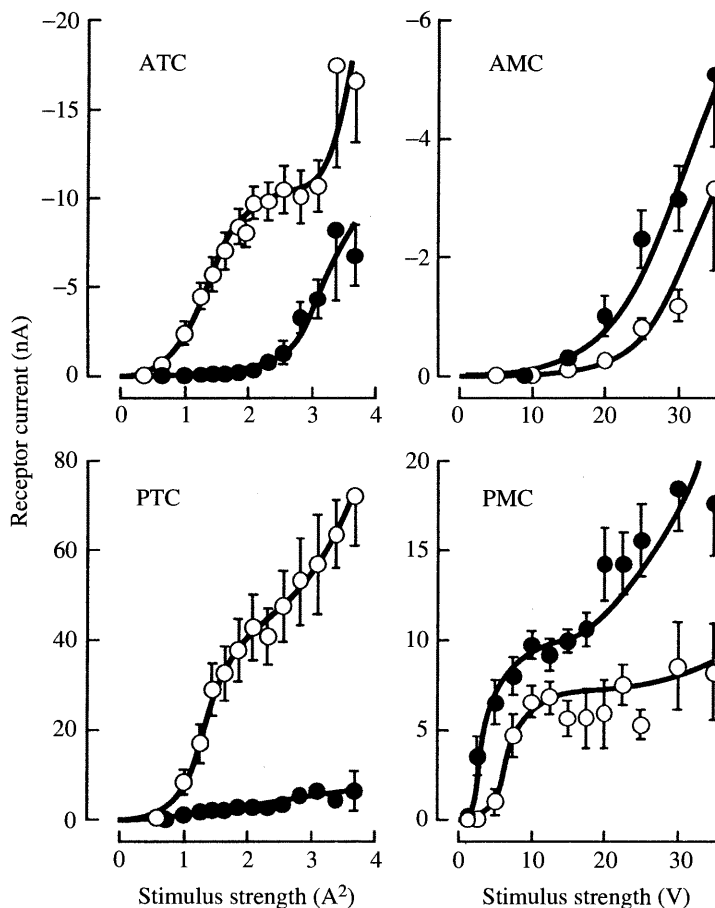


Fig. 2. The relationship between the receptor current intensity and stimulus strength at two different experimental temperatures in *Paramecium caudatum*. A negative current value corresponds to an inward current, a positive value to an outward current. ATC, anterior thermoreceptor current; AMC, anterior mechanoreceptor current; PTC, posterior thermoreceptor current; PMC, posterior mechanoreceptor current. Open circles, values obtained at the experimental temperature of 15 °C; filled circles, responses obtained at 25 °C. Each symbol is the mean (\pm S.E.M.) of 3–6 measurements with different specimens. The membrane potential was held at the resting level. The lines of best fit were drawn by hand.

tended to show saturation when the stimulus strength was as high as 10 V. The quasi-saturated receptor current was larger at 25 °C (approximately 10 nA per cell) than at 15 °C (approximately 6 nA per cell). When the stimulus strength was higher than 20 V, PMC tended to increase again. This tendency was more noticeable at 25 °C than at 15 °C.

Effect of experimental temperature on the receptor currents

As described in the previous section, the experimental temperature affected the receptor currents. To examine more precisely the effects of experimental temperature on

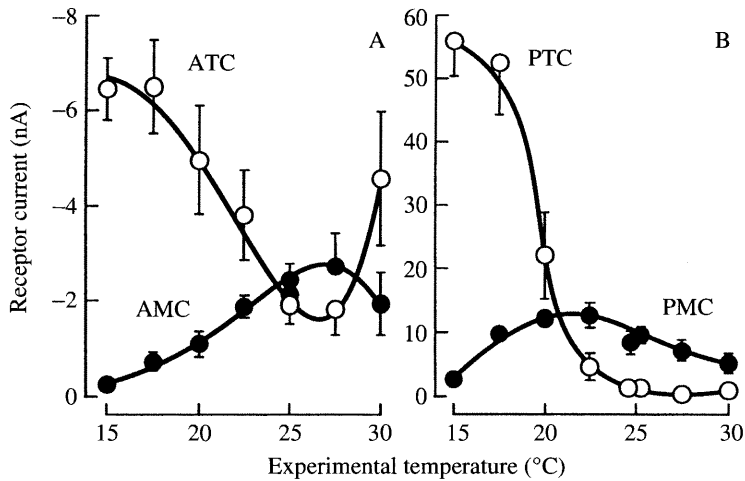


Fig. 3. The relationship between the receptor current intensity and the experimental temperature in *Paramecium caudatum*. (A) Anterior receptor currents; (B) posterior receptor currents. Open circles, thermoreceptor currents; filled circles, mechanoreceptor currents. Each symbol is the mean (\pm S.E.M.) of 5–16 measurements with different specimens. The lines of best fit were drawn by hand. ATC, AMC, PTC and PMC are defined in Fig. 2.

the receptor currents, the intensity of each receptor current evoked by a stimulus at a particular strength was determined at experimental temperatures ranging from 15 to 30°C. The stimulus strengths were 2.0 A² for thermal stimulation, 25.0 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation.

As shown in Fig. 3, ATC decreased to a minimum and AMC increased to a maximum when the experimental temperature was raised from 15 to 27°C. In contrast, PTC decreased to 0 nA and PMC increased to an approximately steady value as the experimental temperature was raised from 15 to 25°C.

Effects of membrane potential on the receptor currents

To determine the effects of membrane potential on the receptor currents, each current evoked by a particular stimulus intensity (2 A² for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation) was measured while the membrane potential was held at various levels in the range -100 to +70 mV. A stimulus was applied to the specimen 300 ms (for thermal stimulation) or 800 ms (for mechanical stimulation) after the membrane potential had been clamped at each potential. The experimental temperature was 15°C for thermal stimulation and 25°C for mechanical stimulation.

Fig. 4 illustrates a representative series of traces for each receptor current, together with corresponding graphs of the peak values of the receptor current, plotted against the membrane potential (the *I-V* relationships). The peak value was determined as the difference between the value for the membrane current measured immediately before stimulation (the steady membrane current) and the peak value for the membrane current during subsequent stimulation.

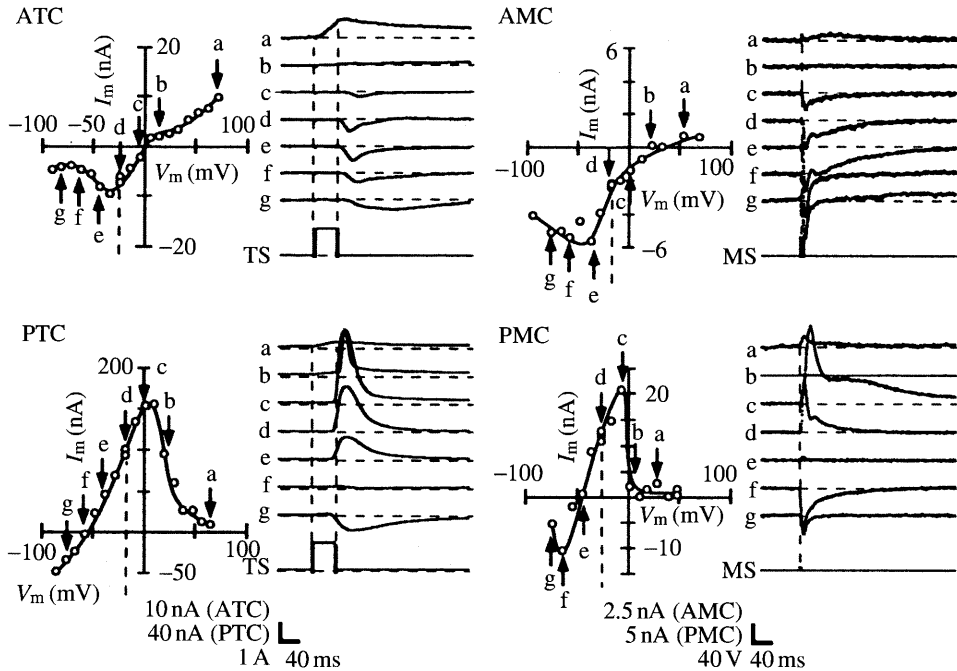


Fig. 4. The relationships between the peak values for the receptor currents and the membrane potential level (the $I-V$ relationship) in *Paramecium caudatum*. I_m , receptor current; V_m , membrane potential. Each $I-V$ relationship is accompanied by the corresponding series of receptor current traces. The membrane potential at which each current trace was obtained is indicated by the arrows. The potential indicated by the broken line corresponds to the resting membrane potential. ATC, PTC, AMC and PMC are defined in Fig. 2 and TS and MS in Fig. 1.

The (inward) ATC decreased as the membrane potential was made more positive than the resting potential (-26.3 ± 1.3 mV, $N=15$). The sign of the receptor current reversed (i.e. it reached the reversal potential) at a membrane potential of -3.7 ± 3.8 mV ($N=7$). This (outward) current increased as the membrane potential was made more positive than the reversal potential. In contrast, the inward ATC increased as the membrane potential was made more negative than the resting potential. However, it tended to decrease when the membrane potential was more negative than approximately -40 mV.

The (outward) PTC decreased as the membrane potential was made more negative than the resting potential. The reversal potential was -52.1 ± 5.9 mV ($N=5$). This inward current increased as the membrane potential was made more negative than the reversal potential. In contrast, the outward PTC increased as the membrane potential was made more positive than the resting potential. However, it decreased abruptly when the membrane potential was made more positive than approximately 10 mV, and only a very small outward current was observed at a membrane potential of 70 mV.

The (inward) AMC decreased as the membrane potential was made more positive than the resting potential (-4.4 ± 4.0 mV; $N=9$). The reversal potential was 9.9 ± 11.5 mV

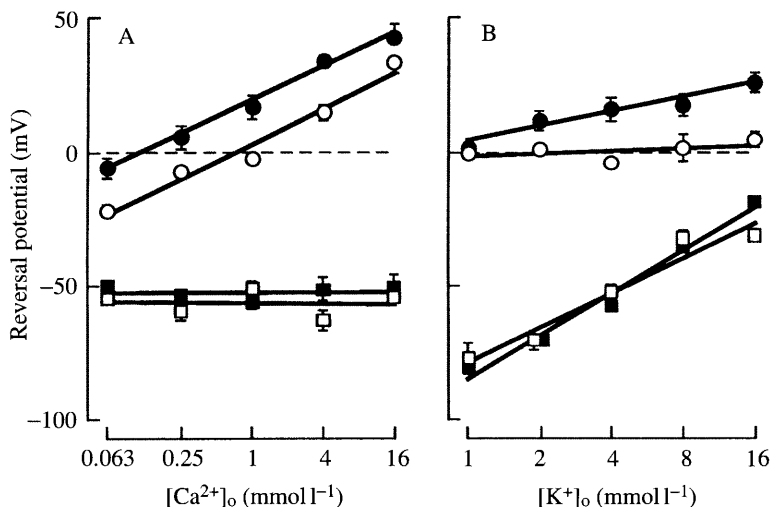


Fig. 5. Effect of the external concentration of Ca²⁺ ([Ca²⁺]_o) and of K⁺ ([K⁺]_o) on the reversal potentials for the receptor currents in *Paramecium caudatum*. (A) [Ca²⁺]_o was changed while [K⁺]_o was kept constant at 4 mmol l⁻¹; (B) [K⁺]_o was changed while [Ca²⁺]_o was kept constant at 1 mmol l⁻¹. Open circles, ATC; open squares, PTC; filled circles, AMC; filled squares, PMC. Each symbol is the mean (± S.E.M.) of 3–5 measurements with different specimens. The lines represent regressions of reversal potential on ion concentration. Note the logarithmic scale of the abscissa. ATC, PTC, AMC and PMC are defined in Fig. 2.

($N=5$). This (outward) current increased as the membrane potential was made more positive than the reversal potential. The inward AMC increased as the membrane potential was made more negative than the resting potential. However, it tended to decrease as the membrane potential was made more negative than -40 mV.

The (outward) PMC decreased as the membrane potential was made more negative than the resting potential. The reversal potential was -58.1 ± 5.1 mV ($N=5$). This (inward) current increased as the membrane potential was made more negative than the reversal potential. However, it tended to decrease as the membrane potential was made more negative than approximately -60 mV. The outward PMC increased as the membrane potential was made more positive than the resting potential, but it abruptly disappeared when the membrane potential was more positive than approximately -5 mV.

Effects of the concentrations of some cations on the receptor currents

To identify species of ions which carry the receptor currents, we examined the effects of the external concentration of Na⁺, K⁺, Rb⁺, Mg²⁺, Ca²⁺ and Mn²⁺ on the reversal potential for each receptor current. Stimulus strength was 2 A² for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation. The experimental temperature was 15 °C for thermal stimulation and 25 °C for mechanical stimulation.

As shown in Fig. 5A, an increase in the external Ca²⁺ concentration, [Ca²⁺]_o, at a constant [K⁺]_o (4 mmol l⁻¹), brought about a marked positive shift in the reversal

Table 1. Shift in the reversal potential (mV) per tenfold change in the external concentration of some cations in thermoreceptor and mechanoreceptor currents in *Paramecium caudatum*

Ion species	Thermoreceptor		Mechanoreceptor	
	Anterior	Posterior	Anterior	Posterior
Ca ²⁺	22.1±2.5	-0.4±2.9	20.7±1.2	0.7±1.8
K ⁺	3.4±3.7	39.0±6.0	17.7±2.9	53.0±4.3
Mg ²⁺	13.8±0.8	5.1±0.6	7.9±0.8	8.7±2.2
Mn ²⁺	15.7±2.4	3.7±0.0	8.6±0.1	5.9±1.5
Rb ⁺	13.7±0.5	37.9±3.4	6.3±4.4	35.5±2.5
Na ⁺	0.2±7.9	7.7±5.5	25.7±2.2	-13.0±6.4

Each value is the mean ± s.d. obtained from 3–5 measurements with different specimens.

potential in both ATC and AMC (Table 1). In contrast, the reversal potential scarcely shifted as [Ca²⁺]_o increased in both PTC and PMC (Table 1).

As shown in Fig. 5B, an increase in the external K⁺ concentration, [K⁺]_o, at a constant [Ca²⁺]_o (1 mmol l⁻¹), brought about a marked positive shift in the reversal potential in both PTC and PMC, but it brought about only a slight shift in ATC and AMC (Table 1).

When 8 mmol l⁻¹ Mg²⁺ or Mn²⁺ was added to the external standard saline, the reversal potential shifted in the positive direction for both ATC and AMC (Table 1). The reversal potential for both PTC and PMC also shifted towards the positive direction with increasing [Mg²⁺]_o or [Mn²⁺]_o, but to a lesser degree than for ATC and AMC (Table 1).

When the external rubidium concentration, [Rb⁺]_o, was increased while keeping [Ca²⁺]_o constant at 1 mmol l⁻¹, the reversal potential for both PTC and PMC shifted in the positive direction by an amount similar to that caused by an increase in [K⁺]_o (Table 1). The reversal potential for both ATC and AMC also shifted in the positive direction with increasing [Rb⁺]_o, but to a lesser degree than for PTC and PMC (Table 1).

An increase in the external concentration of Na⁺, [Na⁺]_o, at a constant [Ca²⁺]_o (1 mmol l⁻¹) had little effect on the reversal potentials for both ATC and PTC (Table 1). The reversal potential for AMC shifted in the positive direction, whereas that for PMC shifted in the negative direction with increasing [Na⁺]_o (Table 1).

Effects of TEA⁺ on the posterior receptor currents

The presence of tetraethylammonium (TEA⁺) in the external solution brought about a reduction of both PTC and PMC, but did not affect ATC and AMC. Concentration-dependent effects of TEA⁺ on the receptor currents are shown in Fig. 6. In this figure, the receptor current intensity in the presence of TEA⁺ is expressed as a value (P) relative to the current intensity in the absence of TEA⁺ and plotted against the logarithm of external TEA⁺ concentration, [TEA⁺]_o. A small difference in the plots is found between PTC and PMC. The Hill plot for the relationship between [TEA⁺]_o and P is shown as an inset of Fig. 6 and will be considered in the Discussion. The receptor currents resumed their respective original values when TEA⁺ was removed from the external solution.

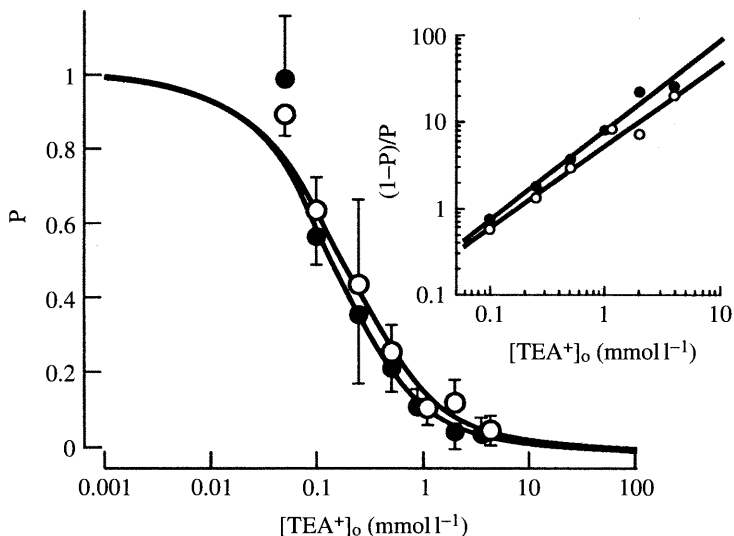


Fig. 6. Effects of the external concentration of TEA^+ ($[\text{TEA}^+]_o$) on the intensity of the posterior receptor currents in *Paramecium caudatum*. P , the intensity of receptor current in the presence of TEA^+ relative to that in the absence of TEA^+ . Open circles, PTC; filled circles, PMC. The inset is the Hill plot of the relationship between $[\text{TEA}^+]_o$ and P . Each symbol is the mean (\pm S.E.M.) of at least five measurements with different specimens. The smooth curved lines were drawn according to corresponding regression lines drawn in the inset. See the text for more details, and the legend of Fig. 2 for definitions of PTC and PMC.

Successive application of thermal and mechanical stimulation

In this series of experiments, thermal and mechanical stimuli were applied to the cell so that both thermoreceptor and mechanoreceptor currents reached their respective peak levels simultaneously, allowing us to examine their summation. The experimental temperature was 25°C for anterior stimulation experiments and 20°C for posterior stimulation experiments, because the thermo- and mechanoreceptor current intensities were almost the same at these temperatures. Examination of the summation was therefore reliable (Fig. 3). The strengths of the stimuli applied to the anterior end of the specimen were 2.6 A^2 for thermal stimulation and 25 V for mechanical stimulation, and for posterior stimulation, 2.3 A^2 for thermal stimulation and 15 V for mechanical stimulation.

Two representative series of traces for the anterior receptor currents and for the posterior receptor currents are shown in Fig. 7. Each series consists of a trace for a current evoked by a thermal stimulus (T), by a mechanical stimulus (M), by both thermal and mechanical stimuli (T+M), and for the difference between T and T+M, $[(\text{T}+\text{M})-\text{T}]$. As is clear from the T+M traces, the mechanoreceptor current was superimposed on the thermoreceptor current evoked by a preceding thermal stimulus. Each $(\text{T}+\text{M})-\text{T}$ trace was almost identical with each corresponding M trace.

Discussion

Our localized thermal stimulation of *P. caudatum* with a microheater (Tominaga and

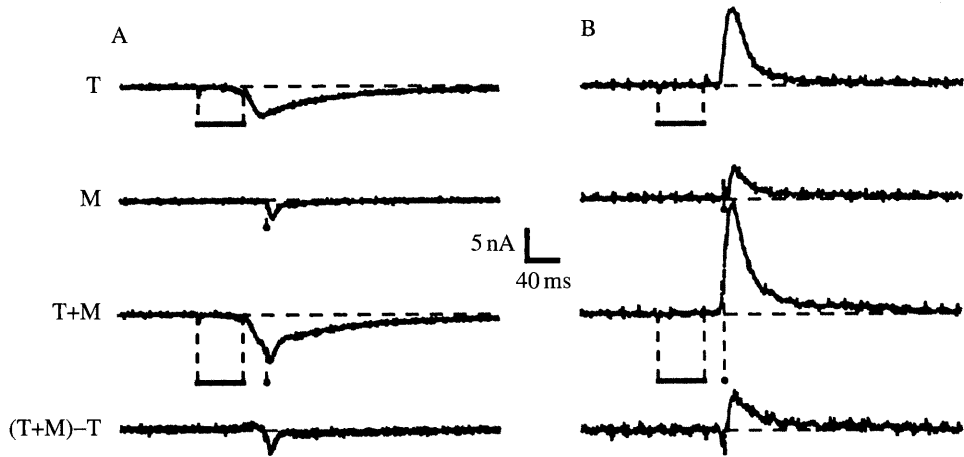


Fig. 7. Traces for the anterior (A) and the posterior (B) receptor currents in one specimen of *Paramecium caudatum*. T, receptor currents evoked by thermal stimulation; M, receptor currents evoked by mechanical stimulation; T+M, receptor currents evoked by mechanical stimulation applied immediately after thermal stimulation; (T+M)-T, electrical subtraction of the T trace from the T+M trace. Each horizontal bar indicates the timing and duration of thermal stimulation. Each black dot indicates the time when mechanical stimulation was applied to the cell. See the text for more details.

Table 2. The fractional conductance (T_X) of the anterior or the posterior membrane of *Paramecium caudatum* to cation X during thermal or mechanical stimulation

Ion species (X)	Thermal		Mechanical	
	Anterior	Posterior	Anterior	Posterior
Ca ²⁺	0.77±0.09	-0.01±0.10	0.70±0.04	0.02±0.06
K ⁺	0.06±0.06	0.68±0.10	0.30±0.05	0.90±0.07
Mg ²⁺	0.48±0.03	0.18±0.02	0.27±0.03	0.29±0.07
Mn ²⁺	0.55±0.08	0.13±0.00	0.29±0.00	0.20±0.05
Rb ⁺	0.24±0.01	0.66±0.06	0.11±0.07	0.60±0.04
Na ⁺	0.00±0.14	0.13±0.10	0.43±0.04	-0.22±0.11

Each value is the mean (\pm S.D.) obtained from 3–5 measurements with different specimens and is estimated from the corresponding rates of shift shown in Table 1.

The experimental temperature was 15°C for thermal stimulation and 25°C for mechanical stimulation.

Naitoh, 1992a) revealed that ion channels responsible for the inward thermoreceptor current are present predominantly in the anterior region of the cell, whereas those responsible for the outward thermoreceptor current are present predominantly in the posterior region of the cell (Fig. 1). The distribution of these thermoreceptor channels resembles that of the mechanoreceptor channels: i.e. ion channels responsible for the inward mechanoreceptor current are present predominantly in the anterior region,

whereas those underlying the outward mechanoreceptor current are present predominantly in the posterior region of the cell (Fig. 1) (Ogura and Machemer, 1980).

The receptor currents increased with increasing stimulus strength and tended to saturate, but they then increased further with further increases in the stimulus strength (Fig. 2). This unorthodox shape of the stimulus–response curve is probably attributable to the combined effects of an increase in the stimulus strength and the concomitant spread of the stimulated area along the membrane. The absence of saturation in AMC is attributable to the lower mechanical sensitivity of the anterior region of the cell (Naitoh and Eckert, 1969*a*). The magnitudes of the thermoreceptor currents were several times larger than those of the mechanoreceptor currents. This large difference in magnitude may have been caused by a difference in the membrane area affected by the thermal and mechanical stimuli.

It can be assumed that the receptor currents described in this paper are carried by Ca^{2+} and/or K^+ in the mixtures of KCl and CaCl_2 employed, because membrane conductance to anions is negligible in *Paramecium* (Kamada, 1934; Tominaga and Naitoh, 1992*a*). Therefore, the reversal potentials for the receptor currents are determined by membrane conductances to both Ca^{2+} and K^+ and by the concentrations of these cations.

According to Hodgkin and Horowicz (1959), the ratio of the membrane conductance to a cation X^{z+} (where z is the valency of the ion) to the total membrane conductance, T_X can be formulated as:

$$T_X = \frac{z\alpha_X}{RT/F}, \quad (1)$$

where α_X is the constant of proportionality between the reversal potential and $\log[X^{z+}]_o$, R is the gas constant, T is the absolute temperature and F is Faraday's constant.

The fractional conductances to Ca^{2+} (T_{Ca}) and to K^+ (T_{K}) during thermal or mechanical stimulation were estimated by introducing the value for the rate of shift in the reversal potential per tenfold change in $[\text{Ca}^{2+}]_o$ or $[\text{K}^+]_o$ (Table 1), respectively, into equation 1 (see Table 2).

The higher T_{Ca} value (and therefore lower T_{K} value) during anterior stimulation than during posterior stimulation supports the idea that thermal or mechanical stimulation of the anterior region of the cell predominantly activates Ca^{2+} channels. In contrast, the higher T_{K} value (and therefore lower T_{Ca} value) during posterior stimulation than during anterior stimulation supports the idea that thermal or mechanical stimulation of the posterior region of the cell activates predominantly K^+ channels.

The fractional conductances of thermally or mechanically stimulated membranes to the cations Mg^{2+} , Mn^{2+} , Rb^+ and Na^+ were estimated in a similar way (Table 2). T_{Mg} and T_{Mn} for the thermally or mechanically stimulated anterior region of the cell were higher than those for the posterior region. This suggests that the stimulus-activated Ca^{2+} channels are also permeable to Mg^{2+} and to Mn^{2+} . These cations, however, are less permeant than Ca^{2+} , as shown by their smaller values of T .

Naitoh and Eckert (1972*b*) reported that Mn^{2+} did not reduce the amplitude of the depolarizing membrane potential response caused by mechanical stimulation of the anterior end of *P. caudatum*. De Peyer and Deitmer (1980) reported that the mechanosensitive Ca^{2+} channels in *Stylonychia*, a relative of *Paramecium*, were as

permeable to Mg^{2+} as they were to Ca^{2+} , but that they were blocked to some extent by Mn^{2+} .

The fractional conductance of the posterior membrane to Rb^+ (T_{Rb}) during thermal or mechanical stimulation was always higher than that of the anterior membrane. This indicates that thermally or mechanically activated K^+ channels are also permeable to Rb^+ .

In contrast to Rb^+ , the fractional conductance of the membrane to Na^+ , T_{Na} , was very low in most cases. This indicates that thermally or mechanically activated receptor channels are almost impermeable to Na^+ . The exceptionally high value for T_{Na} during anterior mechanical stimulation is presently unexplained. Naitoh and Eckert (1973) reported that $[Na^+]_o$ did not affect the membrane potential responses to mechanical stimulation in *P. caudatum*.

Externally applied TEA^+ blocked the posterior receptor currents, PTC and PMC (Fig. 6). The inset of Fig. 6 shows the ratio of the number of blocked channels to the number of non-blocked channels plotted against $[TEA^+]_o$ on a logarithmic scale (i.e. a Hill plot). From this, we can estimate the number of TEA^+ binding sites on the ion channel (corresponding to the slope of the plot) and the binding constant (K_d) of the binding site for TEA^+ (calculated from the intersection of the plot with the x -axis, which corresponds to $\log K_d^{-1}$). No significant ($P < 0.05$) difference in the number of binding sites was found between the posterior thermoreceptor channel and the posterior mechanoreceptor channel (0.95 ± 0.10 for the thermoreceptor channel; 1.02 ± 0.07 for the mechanoreceptor channel, mean \pm s.d., $N=5$). However, the binding constant (K_d) was slightly, but significantly ($P < 0.05$), different for these two kinds of receptor channels (0.19 mmol l^{-1} for thermoreceptor channel, 0.12 mmol l^{-1} for the mechanoreceptor channel).

The effects of the membrane potential on the thermoreceptor currents were essentially identical to those on the mechanoreceptor currents (Fig. 4). The similarities in their dependence on membrane potential of ATC and AMC, and of PTC and PMC, imply that the charge properties of the ionic pores responsible for the receptor currents are similar for thermoreceptor channels and mechanoreceptor channels.

In spite of many similarities in the characteristics of the currents, the effects of experimental temperature on thermoreceptor currents were different from those on mechanoreceptor currents (Figs 2, 3). This suggests that the thermoreceptor currents are dependent on different ion channels from those responsible for the mechanoreceptor currents.

It should be noted that, when a mechanical stimulus was applied to a membrane in which a thermoreceptor current was being produced by a preceding thermal stimulus, a mechanoreceptor current was superimposed on the thermoreceptor current (T+M traces in Fig. 7). The intensity of ATC evoked by only a thermal stimulus (trace T) was approximately 50 % of its saturated value (approximately 10 nA, Fig. 2). It is therefore presumed that the intensity of AMC evoked by a subsequent mechanical stimulus should be 50 % of the intensity of AMC evoked by only a mechanical stimulus (trace M), if AMC is dependent on the same ion channels responsible for ATC. Subtraction of trace T from trace T+M gives an AMC evoked by a subsequent mechanical stimulus. The time course and peak value of the AMC were almost identical with those for an AMC evoked by a

mechanical stimulus alone. Similarly, a PMC evoked by a mechanical stimulus during a PTC was almost identical with that evoked by a mechanical stimulus alone [compare (T+M)–M with M in Fig. 7]. This strongly supports the idea that the thermoreceptor currents are dependent on ion channels different from those responsible for the mechanoreceptor currents.

It should be noted that the thermoreceptor current decreased, whereas the mechanoreceptor current increased, with increasing experimental temperature (Fig. 3). This, together with similarities between the effects of external cations and the membrane potential on the thermoreceptor currents and on the mechanoreceptor currents, suggests that the thermoreceptor mechanism exclusively shares an ionic pore (Ca^{2+} pore or K^{+} pore) with a mechanoreceptor mechanism. Lowering the experimental temperature makes the thermoreceptor mechanism dominant, while raising the experimental temperature makes the mechanoreceptor mechanism dominant.

It is interesting to note that a single-gene mutant of *P. caudatum*, *tsb* (temperature-sensitive behavior; Takahashi, 1979), shows vigorous avoidance responses to mechanical agitation as well as a long-lasting backward swimming response to increased temperature. These behavioural responses imply that the mutant cannot produce a hyperpolarizing receptor potential in response to mechanical or thermal stimuli. A membrane hyperpolarization is known to inhibit avoidance responses and backward swimming and to accelerate forward movement (Eckert and Naitoh, 1972; Naitoh, 1974). The abnormal behaviour exhibited by the mutant, therefore, can be understood if we assume that the mutant has a malfunction in the K^{+} -selective pores that the thermoreceptor and mechanoreceptor mechanisms share.

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