0014-4754/96/080831-03$1.50 + 0.20 © Birkhäuser Verlag Basel, 1996

An increase in the influx of calcium ions into cilia induces thigmotaxis in *Paramecium caudatum*

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Received 20 June 1995; received after revision 18 October 1995; accepted 5 December 1995

Abstract. To understand the role of calcium ions in thigmotaxis in *Paramecium caudatum*, the effects of caffeine, ruthenium red and lanthanum ($LaCl_3$) on thigmotaxis were examined. Thigmotaxis in the CNR mutant, which lacks voltage-dependent Ca^{2+}-channels in the ciliary membrane, was also examined. Ruthenium red and $LaCl_3$ suppressed thigmotaxis in *P. caudatum*, while caffeine enhanced it. The CNR mutant showed hardly any thigmotaxis. It can be thought that an increase in Ca^{2+} influx and the intraciliary concentration of Ca^{2+} ions induces thigmotaxis in *Paramecium*.

Key words. *Paramecium caudatum*; thigmotaxis; Ja-value; CNR; calcium; ruthenium red; $LaCl_3$; caffeine.

The ciliate protozoan *Paramecium caudatum* may react to mechanical stimuli with a typical 'avoiding reaction' or it may slow down its progressive movement while remaining attached to the substrate. The latter response is called thigmotaxis[1–5]. Thigmotaxis is a very important behavioral response in *Paramecium* because it helps to keep the organism within regions of favorable conditions[6]. Since it was impossible to induce thigmotaxis in *P. caudatum* until recently, the possible mechanisms involved have not been well studied. Iwatsuki and Hirano[5] recently succeeded in inducing thigmotaxis by changing the ionic conditions of the surrounding solution. This breakthrough now permits studies of thigmotaxis in *P. caudatum*. In this report, we describe our initial efforts to understand the role of Ca^{2+} ions in thigmotaxis. We suggest that an increase in Ca^{2+} influx and the intraciliary concentration of Ca^{2+} ions induces thigmotaxis in *P. caudatum*.

Materials and methods

Paramecium caudatum (G3 mating type V, belonging to syngen 3) and the CNR mutant (16A1107, syngen 3, kindly provided by Dr. M. Takahashi, Tsukuba University, Japan) were cultured in a hay infusion at 21 °C under fixed illumination (1 W/m^2 from fluorescent lamps) as described previously[7]. All the experiments were carried out with wild type cells (G3) and mutant cells (CNR), which were collected from 2–3 h light-adapted cultures[8]. Cells of *Paramecium* from the logarithmic phase of growth were used for all experiments[4].

For studies of thigmotaxis, the cells of *Paramecium* were washed with various saline solutions prior to each measurement. Then 0.1 ml of a suspension (100 ± 10 cells/ml) of saline-equilibrated cells (20 min adaptation) was put on a glass slide. To make a water column (height: 0.45 mm, diameter: 15 mm), the suspension was covered by a cover slip with a spacer (0.45 mm thick).

Paramecium cells swam, crept, or remained motionless in the water column enclosed by the two parallel glass surfaces of the experimental chamber. We counted the number of creeping or motionless cells as an index of thigmotaxis in each solution[5,9,10], and each value is presented as a percentage of the total number of cells. All experiments were repeated 4 or 5 times and all the solutions were buffered with Tris-HCl (1 mM) at pH 7.2[11].

Results and discussion

The cells of *Paramecium* alternate between swimming (non-thigmotactic cell) and touching an object (thigmotaxis). The cells crept or stopped completely on the object. The behavior of the *Paramecium* cell touching the surface with the tip of its oral groove and raising its posterior end is defined as thigmotaxis by Iwatsuki and Hirano[5]. They found that the cilia touching the surface scarcely beat when *Paramecium* cells crept or were stopped on the object. The velocity of creeping is much less than that of normal forward swimming. In this report, we quantified the degree of thigmotaxis as described previously by Iwatsuki and Hirano[5].

As shown in figure 1a, the greater the concentration of ruthenium red in the solution, the greater the decrease in the number of cells showing thigmotaxis. Similarly, addition of $LaCl_3$ suppressed thigmotaxis, as shown in figure 1b. It is thought that $LaCl_3$ and ruthenium red do not pass through the cell membrane[12]. $LaCl_3$ and ruthenium red are both known to inhibit the influx of Ca^{2+} ions through cell membranes[13–19]. Therefore, it ap-

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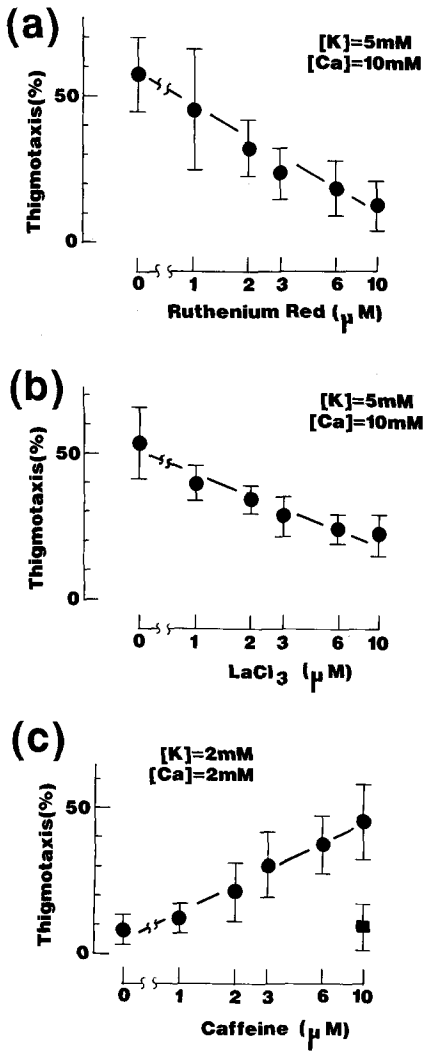

Figure 1. Thigmotaxis in *Paramecium caudatum*. The points and vertical bars represent the means ± SE of results from 55–65 organisms. (*a*) The effect of ruthenium red. (*b*) The effects of $LaCl_3$. (*c*) The effects of caffeine. Squares indicate the CNR mutant, circles the wild type cells.

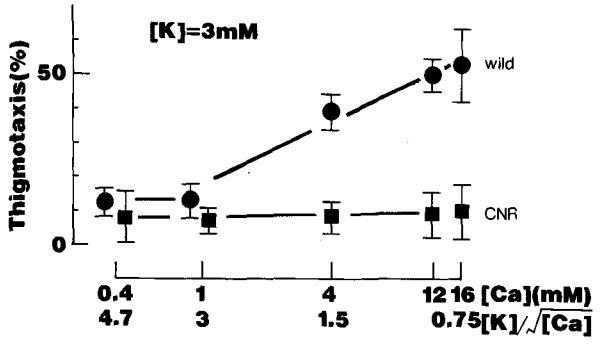

Figure 2. Thigmotaxis of the wilde type (circles) and the CNR mutant (squares) in various solutions. Error bars represent standard errors of the mean (N = 55–65). $[K^+]/[Ca^{2+}]^{1/2}$ means Ja-value (refer to Iwatsuki and Hirano[5]).

peared that an influx of Ca^{2+} ions was necessary for thigmotaxis in *Paramecium*. The greater the concentration of caffeine, the greater the number of organisms that showed thigmotaxis, as depicted in figure 1c. One of the effects of caffeine might be an increase in the influx of Ca^{2+} ions across the cell membrane[17–20]. Other possible effects of caffeine are increased Ca^{2+} release in the cytoplasm[21], and inhibition of phosphodiesterase[22]. In the latter case, the intracellular concentration of cAMP increases, while in the former the intracellular concentration of Ca^{2+} increases. The results using these three reagents lend support to the hypothesis that an increase in the intracellular Ca^{2+} ion concentration induces thigmotaxis in *P. caudatum*.

As shown in figure 2, the CNR mutant of *Paramecium*, which lacks voltage-dependent Ca^{2+}-channels[23–26] in the ciliary membrane, showed hardly any thigmotaxis at all. Addition of 10 μM caffeine to the surrounding medium did not affect thigmotaxis in the CNR mutant, as shown in figure 1c. These results suggest that the voltage-dependent Ca^{2+}-channels of the ciliary membrane are necessary for thigmotaxis. Since the depolarizing mechanoreceptor potential carried by Ca^{2+} is normal in the CNR mutant[23], it seems that the Ca^{2+}-channels in the non-ciliary membrane are not essential to thigmotaxis.

In accord with our previous data[5], our results here support the hypothesis that Ca^{2+} influx into the cilium is necessary for thigmotaxis. It is possible that an increase in the intracellular concentration of Ca^{2+} induces thigmotaxis in *P. caudatum*. If so, it remains to be determined how the cell modulates its response to an increase in intraciliary Ca^{2+} to produce thigmotaxis in some cases, but backward swimming in other cases[27–30]. We will discuss this in a future paper.

Acknowledgements. The authors express their gratitude to Dr. Hiroshi Onimaru (Showa University, Japan) for extremely useful discussions.

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