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MEMBRANE POTENTIAL RESPONSES OF *PARAMECIUM CAUDATUM* TO BITTER SUBSTANCES: EXISTENCE OF MULTIPLE PATHWAYS FOR BITTER RESPONSES

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Summary

The membrane potential responses of *Paramecium caudatum* to the external application of bitter substances were examined by employing conventional electrophysiological techniques. Mutant cells defective in voltage-gated Ca^{2+} channels were used to record the potential responses in the absence of contamination by Ca^{2+} action potentials. The cells produced a transient depolarization followed by a transient hyperpolarization in response to a rapid whole-cell application of chloroquine, strychnine nitrate or brucine. Of these chemicals, chloroquine was the most potent. Cells produced a simple depolarization in response to a localized application of test chemicals to the anterior region, whereas they produced a transient hyperpolarization in response to an application to the posterior region. Membrane potential responses to an application of chloroquine declined with repeated application. The presence of chloroquine in the external bathing solution strongly inhibited the membrane potential responses to an application of brucine or strychnine. However, the presence of chloroquine did not affect the membrane potential responses to an application of quinine. It is suggested that chloroquine, strychnine and brucine share a common component of their transduction pathways, but that the transduction pathway for quinine is different.

Key words: *Paramecium caudatum*, receptor potential, bitter substance, chemoreception.

Introduction

Members of the ciliate genus *Paramecium* are known to show behavioural responses to various chemicals and to accumulate in, or disperse from, regions of solutions containing these chemicals (Jennings, 1906; Van Houten, 1992). According to these behavioural responses, chemicals are classified as attractants or repellents for *Paramecium*. In *Paramecium tetraurelia*, the behavioural responses and changes in membrane potential in response to attractants have been studied by Van Houten and her colleagues (reviewed by Van Houten, 1992). However, the responses of *Paramecium caudatum* to repellents are not fully understood. Quinine and its isomer quinidine are known to be potent repellents for *Paramecium* (Dryl, 1973; Van Houten, 1978, 1992; Oami, 1996*a*). The behavioural responses causing chemodispersal of *Paramecium* are controlled by quinine-induced membrane potential responses (Van Houten, 1978; Oami, 1996*a*). The membrane potential responses to a rapid application of quinine consist of a transient depolarization followed by a transient hyperpolarization and a sustained depolarization. The depolarizing receptor potential is produced by application of quinine to the anterior region of the specimen, while the hyperpolarizing receptor potential occurs in response to application to the posterior region of the cell (Oami, 1996*b*).

Quinine is a plant alkaloid known to taste bitter to man. To understand the mechanisms of chemosensory transduction in *Paramecium caudatum* in response to bitter substances, I examined the effects of some alkaloids on the membrane potential of *Paramecium caudatum*. I also examined whether the potential responses were produced through the same pathways as the quinine responses. The results indicated that *Paramecium caudatum* produced biphasic membrane potential responses following application of the bitter substances tested. As with quinine, depolarizing responses were produced by application of the chemicals to the anterior region of the specimen, while hyperpolarizing responses followed application to the posterior region. Although the characteristics of these responses are similar to those of quinine receptor potentials, the receptor systems for the substances examined in this study appear to be different from those for quinine. Some of these results have been presented verbally and in abstract form elsewhere (Oami, 1995).

Materials and methods

Behavioural mutant specimens of *Paramecium caudatum* (*cnrD* stock 18D504; Takahashi *et al.* 1985) defective in voltage-gated Ca^{2+} channels (CNR-mutants) were used throughout the experiments. Specimens were cultured in a hay infusion medium inoculated with *Klebsiella pneumoniae* as food and collected in an early stationary growth phase. They

were washed three times with the reference solution, consisting of 4 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$ and 1 mmol l^{-1} Tris–HCl buffer (adjusted to pH 7.4), and kept in that solution for more than 30 min before experimentation. Quinine hydrochloride, chloroquine, strychnine nitrate and brucine (Wako Pure Chemical Co., Tokyo) were dissolved in the reference solution.

The membrane potential was measured using the method described previously (Oami, 1996*a*). Test solutions containing chemicals were rapidly applied to the specimen through a pipette by increasing the hydrostatic pressure (30–50 Pa; 3–5 mmH_2O) inside the pipette (internal diameter of tip approximately 200 μm). The opening of the pipette was first placed 3–4 mm below the cell. It was then raised to the level of the cell and at a distance of approximately 200 μm from it. To apply the test solution to a localized region of the cell, ciliary beating was stopped with $NiCl_2$ (Kuznicki, 1963; Oami, 1996*b*). A single cell was impaled by a microelectrode in the reference solution, and then the external solution was replaced by a Ni^{2+}-containing solution (1 mmol l^{-1} $NiCl_2$ in reference solution) for 2–3 min until the cilia on the cell surface ceased beating. In this solution, the cell exhibited a membrane depolarization of 10–20 mV. The Ni^{2+}-containing solution was then replaced with the reference solution. Whereupon, the membrane repolarized to a level almost identical to the original value. The Ni^{2+} treatment did not affect the input resistance of the cell and suppressed ciliary beating for 20–30 min. The diameter of the pipette tip used for local application of the test solution was approximately 30 μm. The timing and duration of the application were followed microscopically and indicated on the recorder simultaneously with the membrane potential responses. During the experiment, the external solution was continuously perfused to minimize the accumulation of test solution around the specimen (Oami, 1996*b*). All the experiments were conducted at room temperature (20–24 °C).

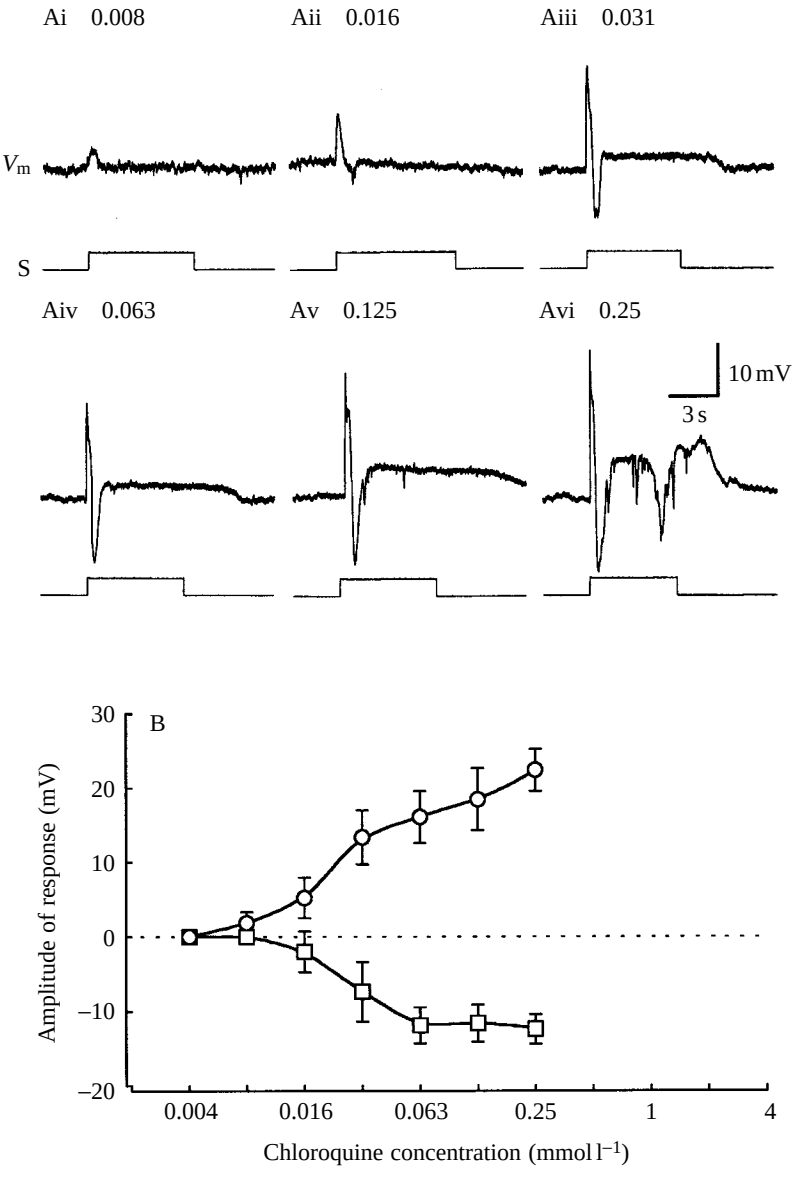


Fig. 1. (A) Membrane potential responses of CNR-mutant specimens of *Paramecium caudatum* to the external application of chloroquine: (i) 0.008 mmol l^{-1} chloroquine, (ii) 0.016 mmol l^{-1} chloroquine, (iii) 0.031 mmol l^{-1} chloroquine, (iv) 0.063 mmol l^{-1} chloroquine, (v) 0.125 mmol l^{-1} chloroquine, (vi) 0.25 mmol l^{-1} chloroquine. The upper trace in each pair of recordings shows the membrane potential responses (V_m), and the lower trace shows the timing and duration of the application of the test solution (S). (B) The amplitude of the chloroquine-induced transient depolarization (circles) and the transient hyperpolarization (squares) plotted against chloroquine concentration. Values are means ± S.E.M., N=5–9 measurements from different specimens.

Results

Membrane potential responses of Paramecium caudatum *to whole-cell application of bitter substances*

To examine membrane potential responses upon application of bitter substances, CNR-mutant specimens of *Paramecium caudatum* were impaled with a microelectrode, and test solutions containing bitter substances were applied through a pipette. The resting membrane potential of the specimens used in the present study ranged from −25 to −30 mV. Fig. 1 shows the membrane potential responses exhibited by CNR-mutant specimens following whole-cell application of chloroquine.

The cell produced a transient membrane depolarization in response to an application of 0.008 mmol l^{-1} chloroquine (Fig. 1Ai). At higher chloroquine concentrations, the depolarization was followed by a transient hyperpolarization (Fig. 1Aii–vi). The amplitude of both the depolarizing and hyperpolarizing responses increased as the chloroquine concentration increased. A small sustained depolarization was often observed following the transient depolarization and hyperpolarization when the chloroquine concentration was high (Fig. 1Aiii–vi).

Fig. 1B shows the amplitudes of the chloroquine-induced transient depolarization and hyperpolarization plotted as a function of chloroquine concentration. The amplitude of both responses increased with increasing chloroquine concentration and tended to saturate at high concentrations.

Fig. 2A shows the membrane potential responses to the whole-cell application of strychnine nitrate. The cell produced a transient depolarization when strychnine concentration was low (Fig. 2Ai,ii). This was followed by a transient hyperpolarization when the strychnine concentration was raised (Fig. 2Aiii).

Fig. 2B shows the amplitude of the strychnine-induced depolarization and the hyperpolarization plotted as a function of strychnine concentration. The amplitude of both responses increased as the strychnine concentration increased. The depolarizing response persisted even when the strychnine concentration was reduced to 0.008 mmol l^{-1}.

Fig. 3A shows membrane potential responses to an application of brucine. The characteristics of the membrane potential responses were similar to those of the chloroquine and strychnine responses. The effective concentration range for inducing the depolarizing and hyperpolarizing potential responses was higher for brucine than for chloroquine and strychnine (Fig. 3B).

Membrane potential responses to localized applications of bitter substances

CNR-mutant specimens were treated with Ni^{2+}-containing solution and then with a locally applied bitter substance through a fine pipette either to the anterior or the posterior end.

Fig. 4 shows representative membrane potential responses. Localized application of 0.125 mmol l^{-1} chloroquine to the anterior end produced a simple transient depolarization

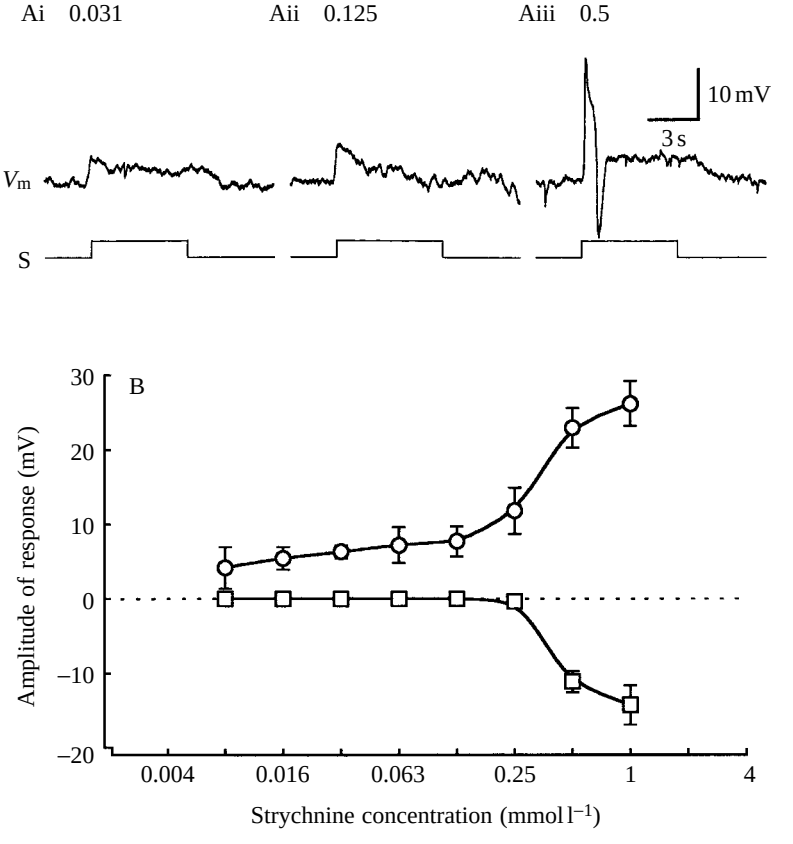


Fig. 2. (A) Membrane potential responses of CNR-mutant specimens of *Paramecium caudatum* to the external application of strychnine nitrate: (i) 0.031 mmol l^{-1} strychnine, (ii) 0.125 mmol l^{-1} strychnine, (iii) 0.5 mmol l^{-1} strychnine. Upper traces, membrane potential (V_m); lower traces, the timing and duration of the application (S). (B) The amplitude of the depolarizing (circles) and hyperpolarizing (squares) responses plotted against strychnine concentration. Values are means ± S.E.M., N=5–9 measurements from different specimens.

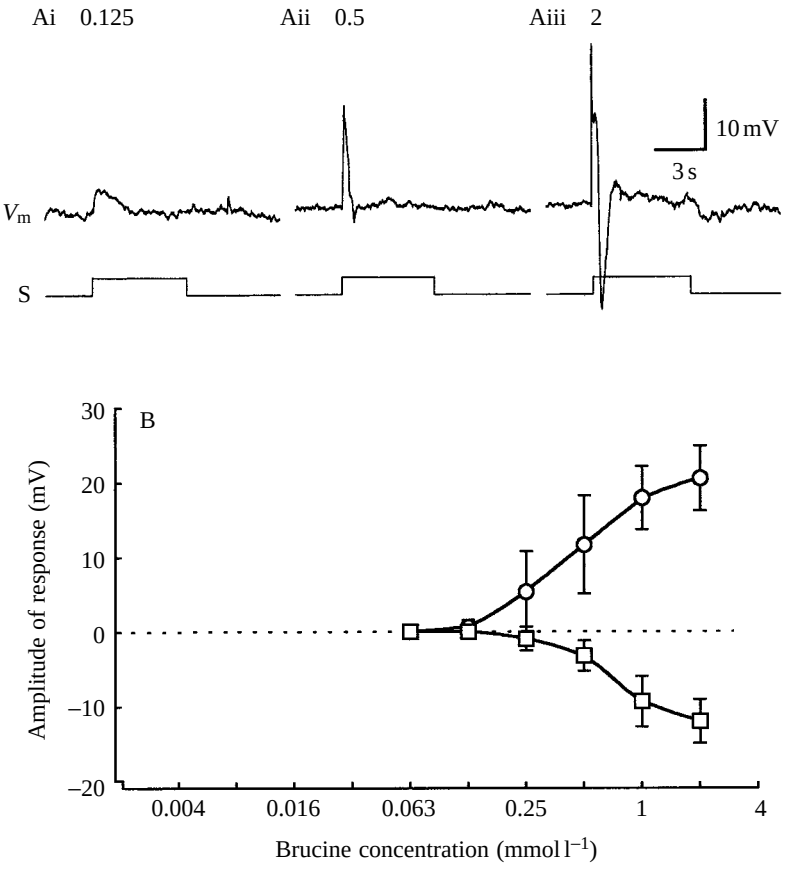


Fig. 3. (A) Membrane potential responses of CNR-mutant specimens of *Paramecium caudatum* to external application of brucine: (i) 0.125 mmol l^{-1} brucine, (ii) 0.5 mmol l^{-1} brucine, (iii) 2 mmol l^{-1} brucine. Upper traces, membrane potential (V_m); lower traces, timing and duration of the application (S). (B) The amplitude of the depolarizing (circles) and hyperpolarizing (squares) responses plotted against brucine concentration applied. Values are means ± S.E.M., N=5–9 measurements from different specimens.

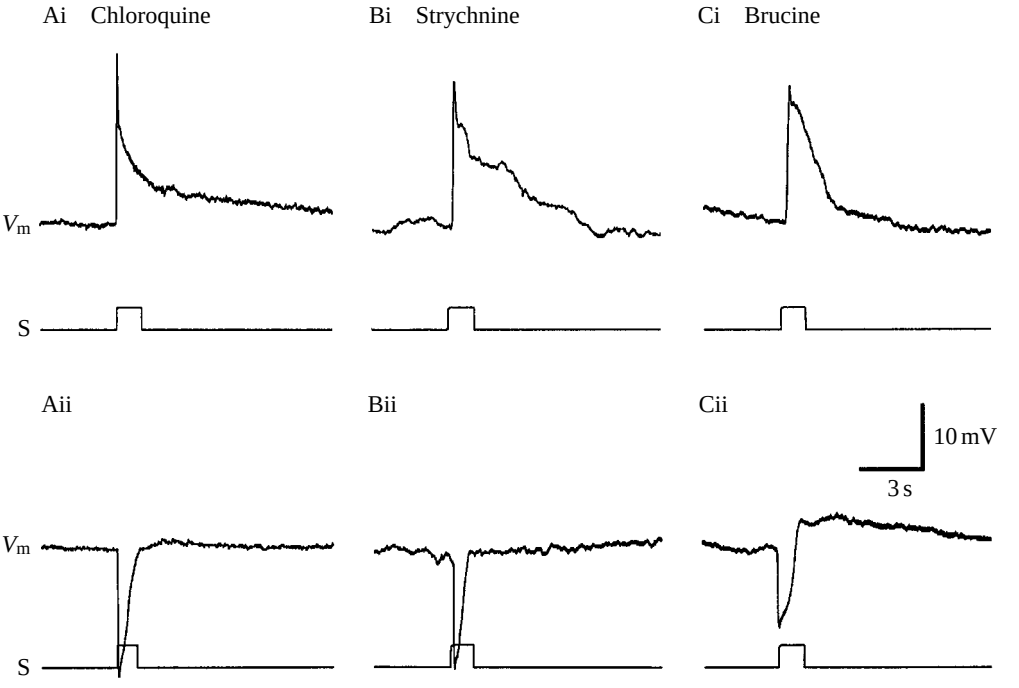


Fig. 4. Membrane potential responses of $NiCl_2$-treated CNR-mutant specimens of *Paramecium caudatum* to the localized application of 0.125 mmol l^{-1} chloroquine (Ai,ii), 1 mmol l^{-1} strychnine (Bi,ii) and 2 mmol l^{-1} brucine (Ci,ii). Ai, Bi and Ci are responses to an anterior stimulation, while Aii, Bii and Cii are responses to a posterior stimulation. V_m, membrane potential; S, stimulus application.

(Fig. 4Ai). In contrast, the cell produced a transient hyperpolarization in response to an application to the posterior end (Fig. 4Aii).

Similarly, the cell produced a transient depolarization in response to an application of 1 mmol l^{-1} strychnine or 2 mmol l^{-1} brucine to the anterior end (Fig. 4Bi,Ci), while it

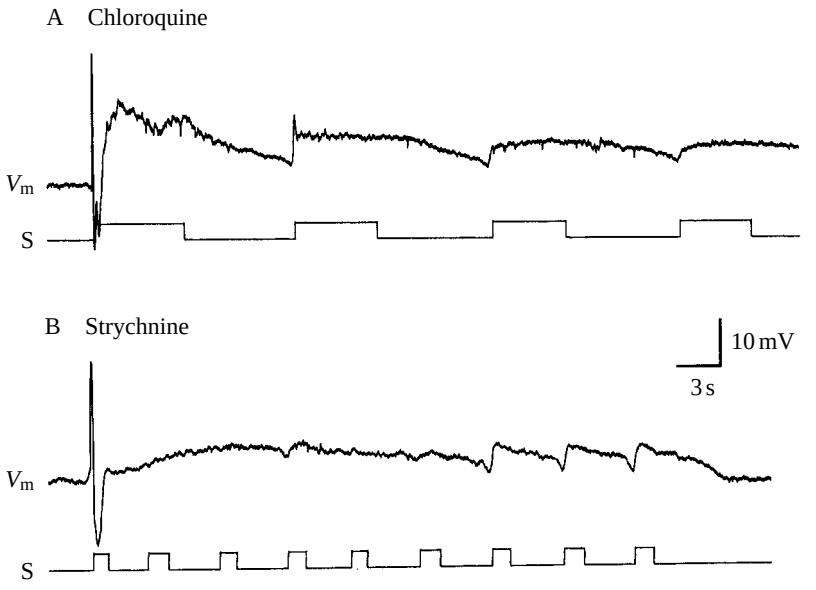


Fig. 5. Effect of repeated applications of the test substances on the membrane potential responses of *Paramecium caudatum*. (A) Responses to 0.125 mmol l^{-1} chloroquine. (B) Responses to 0.5 mmol l^{-1} strychnine. V_m, membrane potential; S, stimulus application.

produced a transient hyperpolarization in response to an application to the posterior end (Fig. 4Bii,Cii).

Effects of repeated application of bitter substances on the membrane potential responses

The effects of repeated applications of bitter substances on the membrane potential responses were examined. The transient depolarizing and hyperpolarizing responses induced by an application of 0.125 mmol l^{-1} chloroquine became smaller as the application was repeated and entirely disappeared within three repetitions (duration of application, approximately 5 s; interval, 5 s; Fig. 5A). Membrane potential responses to the application of 0.5 mmol l^{-1} strychnine or 1 mmol l^{-1} brucine also disappeared with repeated applications, but a shorter interval between stimulations was necessary for complete suppression of the responses to these chemicals (duration, 2 s; interval, 3 s; Fig. 5B).

Recovery of depolarizing and hyperpolarizing chloroquine responses after an application of chloroquine

The time courses of recovery of the depolarizing and the hyperpolarizing chloroquine responses after an application of chloroquine were examined by paired application experiments. Chloroquine (0.125 mmol l^{-1}) was applied twice, and the responses to the second application were examined by changing the interval between applications. A second response was absent when the interval between applications was 1 s (Fig. 6Ai). The depolarizing and hyperpolarizing responses recovered as the interval between stimulations increased (Fig. 6Aii–v).

The time course of recovery of the depolarizing response is shown in Fig. 6B and that for the hyperpolarizing response in Fig. 6C. The relative amplitude of the second response with respect to that of the first response was plotted against the interval between applications. Both the depolarizing and the hyperpolarizing responses recovered more or less exponentially. The responses resumed their original amplitude within 5 s of the first application.

Effects of bath application of chloroquine on the membrane potential responses to a rapid application of chloroquine

To examine the effects of a continuous application of chloroquine on the chloroquine responses, 0.125 mmol l^{-1} chloroquine was introduced into the external medium and then 0.5 mmol l^{-1} chloroquine was applied to the cell through a pipette. Fig. 7A shows a control experiment in which 0.5 mmol l^{-1} chloroquine was applied to a cell immersed in reference solution. The response consisted of a conspicuous transient depolarization followed by a transient hyperpolarization. Fig. 7B shows the membrane potential response to 0.5 mmol l^{-1} chloroquine when the specimen was immersed in a solution containing 0.125 mmol l^{-1} chloroquine. Again, the cell produced a transient depolarization followed by a transient hyperpolarization, but the amplitudes of these responses were much smaller and their duration longer than those obtained in the reference solution. Fig. 7C shows the recovery of the initial response when the external medium was replaced with reference solution.

Effects of a bath application of chloroquine on the membrane potential responses induced by the application of strychnine, brucine or quinine

The effects of bath application of 0.125 mmol l^{-1} chloroquine on the membrane potential responses induced by a rapid whole-cell application of strychnine, brucine and quinine were examined.

Fig. 8A shows representative membrane potential responses to an application of strychnine. Application of 1 mmol l^{-1} strychnine caused a transient depolarization followed by a transient hyperpolarization in the reference solution (Fig. 8Ai). However, these responses were abolished when 0.125 mmol l^{-1} chloroquine was present in the external solution (Fig. 8Aii).

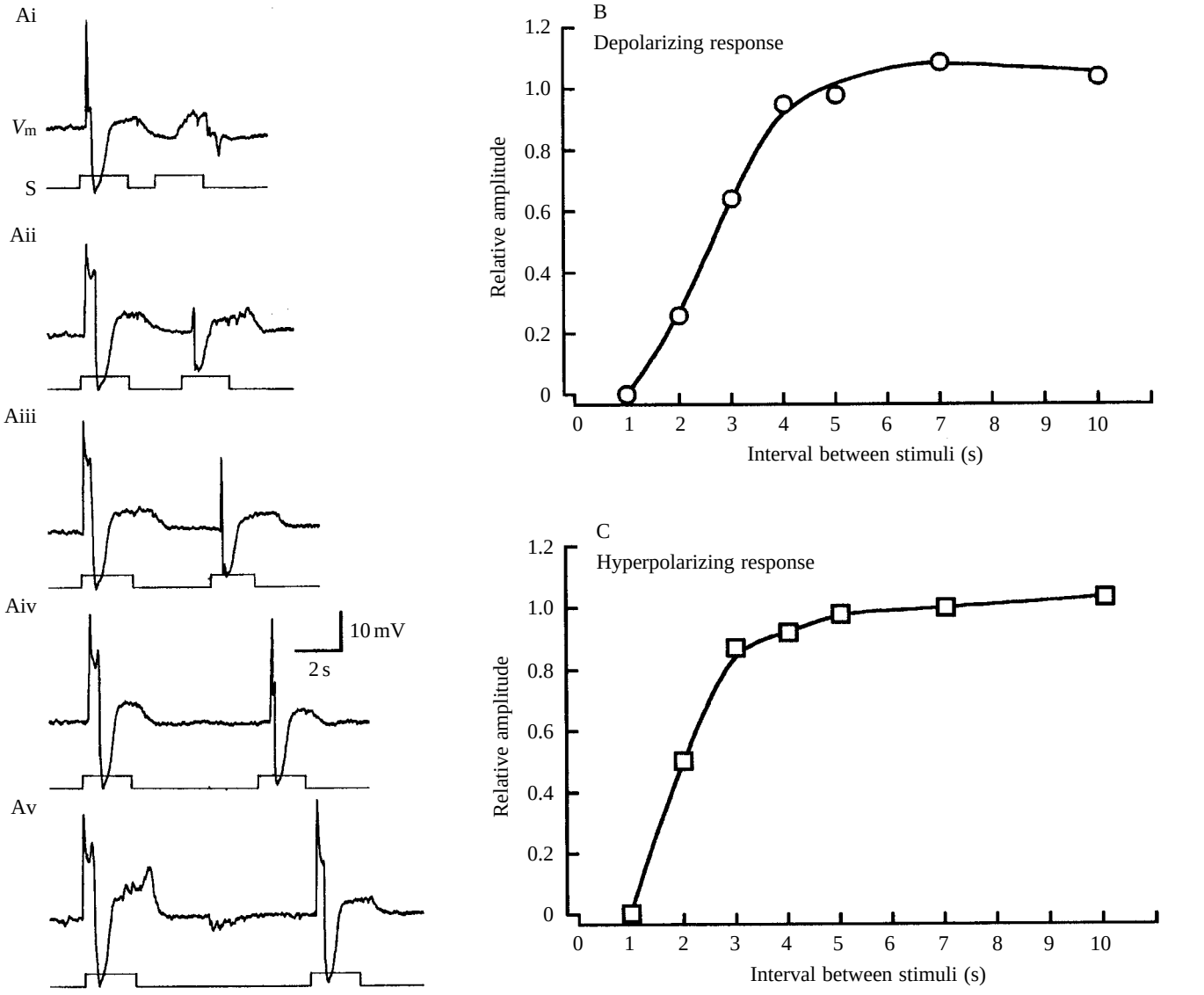
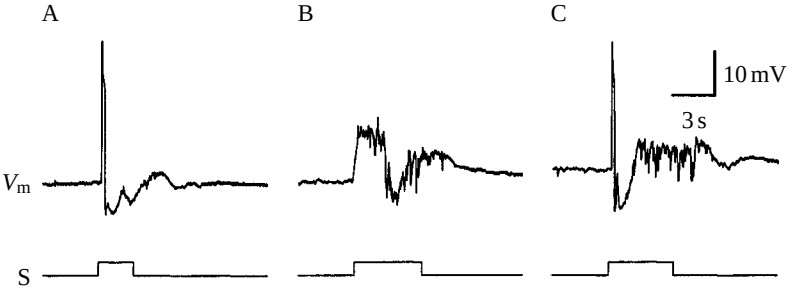


Fig. 6. (A) Membrane potential responses of a CNR-mutant specimen of *Paramecium caudatum* to paired applications of 0.125 mmol l^{-1} chloroquine with varying intervals between applications. (B,C) Time course of recovery of the chloroquine responses in the paired application experiments. Relative amplitudes of the depolarizing response (B) and the hyperpolarizing response (C) produced in response to the second application of chloroquine plotted against the interval between stimulations. The amplitude is expressed relative to the value of the initial response in each pair of applications. V_m, membrane potential; S, stimulus application.

Fig. 7. Effects of bath application of 0.125 mmol l^{-1} chloroquine on the membrane potential responses induced by 0.5 mmol l^{-1} chloroquine. Membrane potential responses of CNR-mutant specimens to a rapid application of 0.5 mmol l^{-1} chloroquine were compared before (A) and during (B) the bath application of 0.125 mmol l^{-1} chloroquine. (C) Recovery after the bathing solution had been replaced with reference solution. V_m, membrane potential; S, stimulus application.



Membrane potential responses to strychnine recovered when the external solution was exchanged for the reference solution (Fig. 8Aiii).

Fig. 8B shows the effects of bath application of chloroquine on the membrane potential responses induced by brucine. As with the strychnine responses, the membrane potential

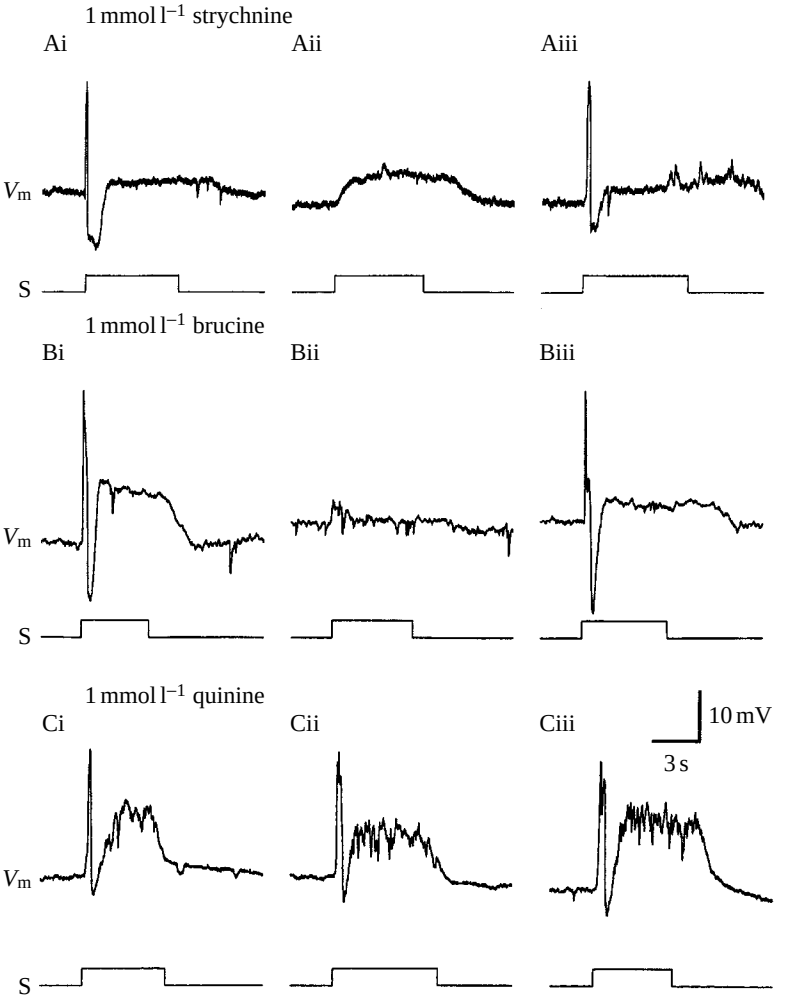


Fig. 8. Effects of bath application of 0.125 mmol l^{-1} chloroquine on the strychnine-induced (A), brucine-induced (B) and quinine-induced (C) membrane potential responses of CNR-mutants. Membrane potential responses to the substances were compared before (Ai,Bi,Ci) and during (Aii,Bii,Cii) the bath application of chloroquine. When chloroquine was applied to the external medium, each test solution contained chloroquine at a concentration identical to that in the external solution. Aiii, Biii and Ciii show responses after the bathing solution had been replaced with reference solution. V_m, membrane potential; S, stimulus application.

responses induced by 1 mmol l^{-1} brucine were abolished by an application of 0.125 mmol l^{-1} chloroquine in the external solution, but the effect was reversible (Fig. 8Bi–iii).

Fig. 8C shows the effects of bath application of chloroquine on the membrane potential responses induced by quinine. A specimen immersed in the reference solution exhibited a transient depolarization followed by a transient hyperpolarization and a sustained depolarization in response to an application of 1 mmol l^{-1} quinine (Fig. 8Ci). These quinine-induced membrane potential responses were scarcely affected by the presence of 0.125 mmol l^{-1} chloroquine in the external medium (Fig. 8Cii) and remained almost unchanged after washing (Fig. 8Ciii).

Discussion

A variety of alkaloids have been identified as bitter substances. However, their effects on the membrane potential responses in *Paramecium* have not been studied except in the case of quinine and its isomer quinidine (Van Houten, 1978; Oami, 1996*a*). The present experiments revealed that *Paramecium caudatum* showed membrane potential responses to chloroquine, strychnine and brucine (Figs 1–3). The responses consisted of an initial depolarization followed by a transient hyperpolarization. Because the cells used in the present experiments were defective in voltage-gated Ca^{2+} channels (CNR-mutant; Takahashi *et al.* 1985), Ca^{2+} action potentials were not involved in the membrane potential responses even when the membrane was depolarized and, therefore, the membrane potential responses recorded are receptor potentials to the substances examined. The amplitude of the responses increased as the concentration of applied alkaloid increased, but tended to saturate at high concentrations (Figs 1–3). These results suggest that *Paramecium caudatum* possesses receptors to chloroquine, strychnine and brucine.

The cells produced a membrane depolarization in response to a localized application of a bitter substance to the anterior end and a hyperpolarization in response to an application of the substance to the posterior end (Fig. 4), indicating that the receptors responsible for the depolarizing responses are present mainly around the anterior region of the cell, while those responsible for the hyperpolarizing responses are mainly around the posterior region of the cell. This distribution is similar to that found for quinine receptors (Oami, 1996*b*). Similar anterior depolarizing and posterior hyperpolarizing responses are produced by mechanical (Naitoh and Eckert,

1969) and thermal (Tominaga and Naitoh, 1992) stimulation in *Paramecium caudatum*.

A loss of responsiveness of the specimen to the chemicals during repeated applications (Fig. 5) indicates the existence of a desensitization or adaptation mechanism in the receptor systems. The responses to a second application of chloroquine were absent when the interval between paired applications was short (<1 s) and recovered more or less exponentially as the interval increased (Fig. 6).

It is assumed that desensitization or adaptation of the chloroquine responses continued during the bath application of chloroquine since this treatment partially suppressed the responses to a rapid application of 0.5 mmol l^{-1} chloroquine (Fig. 7). In this condition, the membrane potential responses to strychnine or brucine were abolished (Fig. 8A,B). If the transduction pathways for strychnine and brucine were different from those for chloroquine, the suppression of the chloroquine receptor system should have no effect on the other pathways. Therefore, it is suggested that these three chemicals share the same transduction pathways, such as receptors and/or ion channels.

In contrast to the responses to strychnine and brucine, the responses of *Paramecium caudatum* to quinine were not affected by bath application of chloroquine (Fig. 8C). This strongly suggests that the transduction pathways for quinine are different from those for chloroquine, strychnine or brucine.

The present experiments indicate the existence of multiple receptor systems for bitter substances in *Paramecium caudatum*. It has been reported that the chemoreceptor potential to an attractant, folate or acetate, is produced by activation of electrogenic pump activity in *Paramecium tetraurelia* (Preston and Van Houten, 1987). The transduction pathways for quinine and other bitter substances should be further examined to elucidate the mechanisms of chemoreception in *Paramecium caudatum*.

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