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AN ANTICALMODULIN DRUG, W-7, INHIBITS THE VOLTAGE-DEPENDENT CALCIUM CURRENT IN *PARAMECIUM CAUDATUM*

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SUMMARY

The anticalmodulin drug, W-7 [*N*-(6 aminohexyl)-5-chloro-1-naphthalenesulphonamide] specifically inhibits the voltage-dependent Ca-current of *Paramecium* as well as the behavioural consequence of Ca^{2+} influx, backward swimming. The dechlorinated analogue, W-5, is four to five times less effective. Analysis of membrane currents under voltage clamp shows that W-7 not only reversibly inhibits the voltage-dependent Ca-current but also shifts the voltage sensitivity of this Ca-current towards less negative voltages in a concentration-dependent manner. We suggest that *Paramecium* can be used as a system to screen behaviourally for other Ca-channel blockers as well as to study the mechanism of action of these drugs.

INTRODUCTION

A *Paramecium* swims by beating the thousands of cilia which cover its body. Depolarizing stimuli can cause backward swimming by activating a voltage-dependent Ca^{2+} action current. This current increases intracellular Ca^{2+} level to cause reversal of the ciliary beat direction and subsequent backward swimming (for review see Kung & Saimi, 1982). The duration of stimulated backward swimming upon transferring the *Paramecium* into a high concentration of K^+ (20 mM) has been used as an estimate of the voltage-dependent Ca-channel activity (Haga, Forte, Saimi & Kung, 1982).

The calmodulin antagonist W-7 [*N*-(6 aminohexyl)-5-chloro-1-naphthalenesulphonamide] inhibits backward swimming in *Paramecium*. The concentration of drug which inhibits 50% of the bovine calmodulin-dependent phosphodiesterase activity, the ID_{50}, is about 30 μM for W-7 and about 240 μM for its dechlorinated analogue, W-5 (Nelson, Andrews & Karnovsky, 1983). W-7 reduces the backward swimming elicited by Ba^{2+} by 50% at a concentration of 40 μM, while W-5 is ineffective at this concentration (Otter, Satir & Satir, 1983). Although this correlation suggests a calmodulin involvement in the swimming behaviour of *Paramecium* it should be noted that W-7 also has effects on several calmodulin-independent functions (Hirata, Suematsu & Koga, 1982; Luthra, 1982; Tanaka, Ohmura & Hidaka, 1982; Wise *et al.* 1982; Schatzman, Raynor & Kuo, 1983).

Key words: Ca-channel, W-7, *Paramecium*.

Since no pharmacological agent has been previously described which can block t[redacted] *Paramecium* Ca-current (Eckert & Brehm, 1979), a genetic approach has been used to isolate the Ca-current (Oertel, Schein & Kung, 1977) and other membrane currents (Kung & Saimi, 1982). We now report that W-7 is a suitable pharmacological agent which can selectively and reversibly inhibit the voltage-dependent Ca-current of *Paramecium*.

MATERIALS AND METHODS

Stocks and cultures

Wild type *Paramecium caudatum*, stock G3, and a mutant which lacks a voltage-dependent calcium current, *cnrA* (16A712) (Takahashi & Naitoh, 1978), were cultured at 25 °C in Cerophyl medium supplemented with 0·03 % proteose peptone (Difco) and 5 mg l^{-1} stigmasterol (Sigma). These stocks were kindly provided by Drs M. Takahashi and Y. Naitoh. Cultures were innoculated with *Enterobacter aerogenes* 24 h before introducing the paramecia (Sonneborn, 1970).

Solutions

The standard recording solution (Ca-K solution) contained 4 mM-KCl, 1 mM-$CaCl_2$, 1 mM-MOPS (morpholinopropanesulphonic acid) and was buffered to pH 7·0 with Tris-base [Tris(hydroxymethyl)aminomethane]. To isolate the inward Ca-current, the K-currents were inhibited by adding 10 mM-TEA-Cl (tetraethylammonium chloride) to the Ca-K solution and using 2 M-CsCl in the microelectrodes. This procedure blocked 90–95 % of the voltage-dependent K-current (Eckert & Brehm, 1979; Hinrichsen & Saimi, 1984). The behavioural testing solution contained 20 mM-KCl in Dryl's solution as described by Haga *et al.* (1982). W-7 and W-5 (Rikaken Co., Ltd) were dissolved in DMSO (dimethylsulphoxide) or ethanol and added to the recording and behavioural testing solutions with a concentration of organic solvent not exceeding 1·5 % (v/v).

Recordings

The techniques for voltage clamp and current injection and methods of recording were similar to those described by Satow & Kung (1979). The 2 M-KCl or 2 M-CsCl microelectrodes used for voltage clamp and current injection experiments had resistances of 10–40 MΩ. The relatively high resistance electrodes were used to assure optimal cell condition during the multiple perfusion experiments. The perfusion rate was about 10 ml min^{-1} and recording was begun within 1 min after perfusion. The recording chamber volume was 1 ml. The membrane was held at −40 mV and the currents were recorded following step depolarizations and hyperpolarizations from this level. Current measurements presented in Figs 2, 4, 5 and 7 were background subtracted by determining the difference between the measured value and the leakage current, which was linearly extrapolated from the measured leakages near the holding level. The membrane resistance, R_m, was determined from the slope of the extrapolated leakage-current line.

RESULTS

Behavioural effects

W-7 inhibits the duration of backward swimming in a concentration-dependent manner in *Paramecium*. About 40–50 cells were added to 2 ml of the Ca-K solution and left at room temperature (22 ± 1 °C) for 15 min without drug. Individual cells were behaviourally tested by transferring them to 2 ml of 20 mM-KCl in Dryl's solution with drug and timing the duration of backward swimming as described by Haga *et al.* (1982). This solution depolarizes the cells due to the high external K^+ concentration. With no drug in the test solution the duration of backward swimming was 56·0 ± 8·0 s ($N = 8$). This was reduced by 50 % by addition of about 20 μM W-7 or 150 μM W-5 to the test solution (Fig. 1). When the test solution contained 150 μM

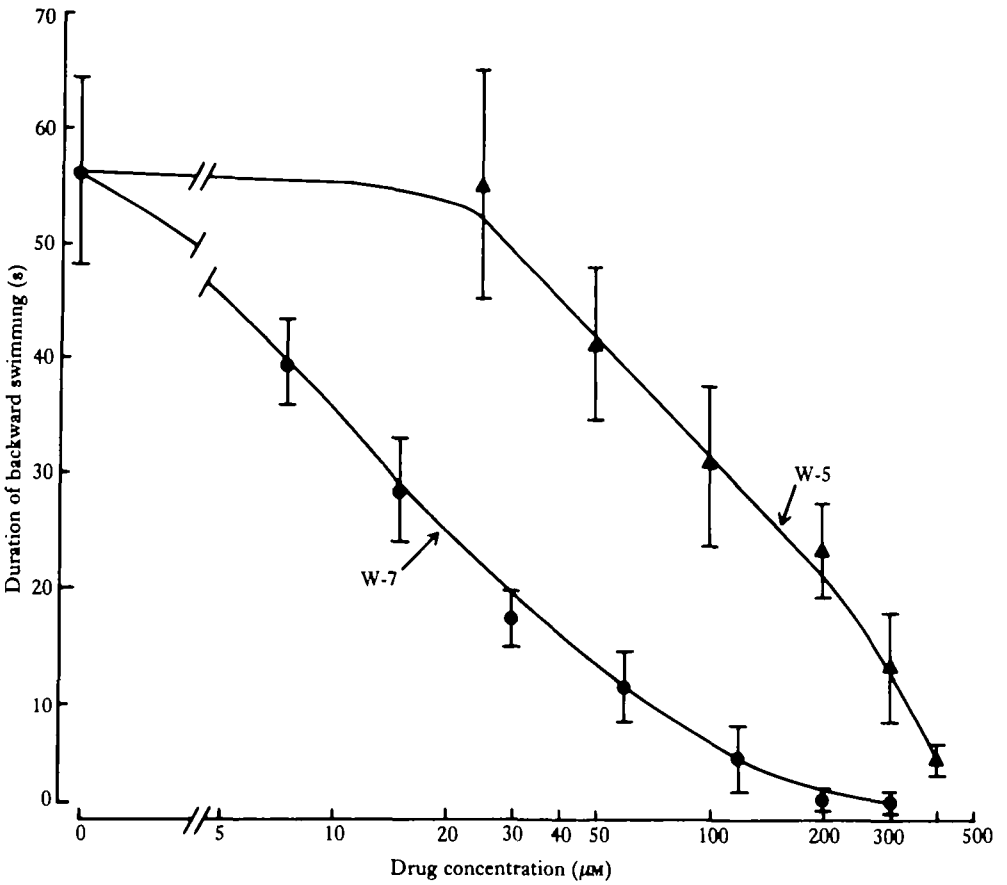

Fig. 1. The behavioural response of *Paramecium caudatum* to stimulation by 20 mM-K^+ in the presence of W-7 and W-5 is concentration dependent. Cells were tested for duration of backward swimming in 20 mM-KCl in Dryl's solution with drug present at various concentrations. W-5 (▲——▲) and W-7 (●——●) both inhibit backward swimming but higher concentrations of W-5 were necessary for the same effects. The estimated drug concentrations which inhibits backward swimming by 50 %, shown by the arrows, are about 20 μM for W-7 and about 150 μM for W-5. Each point shows the mean ± S.D., $N = 4$–8.

Table 1. *Duration of backward swimming of cells with or without exposure to 20 μM W-7 tested with and without 20 μM W-7*

	Duration of backward swimming (s)	
Time in 20 μM W-7 (min*)	Tested with 20 μM W-7†	Tested without drug†
0	22·8 ± 3·3	48·5 ± 7·5
5	20·5 ± 0·7	53·5 ± 0·7
5 min in 20 μM W-7 then 5 min in Ca-K	21·0 ± 2·0	55·8 ± 8·7

* Cells were incubated in the Ca-K solution (see Methods) for 15 min and then transferred to the same solution with or without 20 μM W-7 for the times indicated before being challenged with the test solution.
† Test solution contained 20 μM-KCl in Dryl's solution.
All numbers are mean ± S.D. ($N = 4$).

W-7, the cells showed about 1 s backward swimming followed by forward swimming. Since W-7 was dissolved in ethanol the ethanol concentration was as high as 0·8 % in the final test solution. Addition of up to 1·5 % ethanol to the test solution without drug did not affect the duration of backward swimming. Note that at the concentration at which W-7 is 50 % effective (20 μM), W-5 was ineffective (Fig. 1).

The behavioural effects of W-7 were rapidly reversible and were not time dependent. After a 5 min incubation of cells in the Ca-K solution with 20 μM W-7, the cells were either tested or transferred to the Ca-K solution without drug. The suppression of backward swimming was dependent only upon the presence of the drug in the test solution, showing that the drug was rapidly effective and almost immediately reversible (Table 1). Exposure to 20 μM W-7 for up to 1 h did not change the responsiveness of the cells to 20 μM W-7 or its reversibility (data not shown).

Effects of W-7 on membrane currents

The only voltage-dependent membrane electrophysiological property which was observed to change following perfusion with 20 μM W-7 was the transient inward current. The experimental protocol was as follows: (1) a cell was voltage clamped at −40 mV in the Ca-K solution and membrane currents recorded following various hyperpolarizing and depolarizing voltage steps, (2) the recording chamber was perfused with 20 μM W-7 in the same solution and the voltage steps repeated, and (3) the cell was returned by perfusion to the Ca-K solution without drug and the recordings repeated. Recording began as soon as the perfusions were completed. At 20 μM, W-7 had no effect on the voltage-dependent K-current or the hyperpolarization-dependent currents (both measured at 15 ms) but the transient depolarization-dependent inward current (largely the Ca-current, see below) was decreased by about 40 % (Fig. 2). 20 μM W-5 had no effect on any of these currents.

The effects on the transient inward current were largely reversible. Perfusion of a cell with the Ca-K solution following recording in 20 μM W-7 caused the maximal inward current (I_{max}) to return to near the original level (Fig. 2, Table 2). A possible shift in the voltage at which the I_{max} was seen (V_{max}) was suggested in the presence of 20 μM W-7 (Fig. 2, Table 2). This was studied more directly with the isolated Ca-current

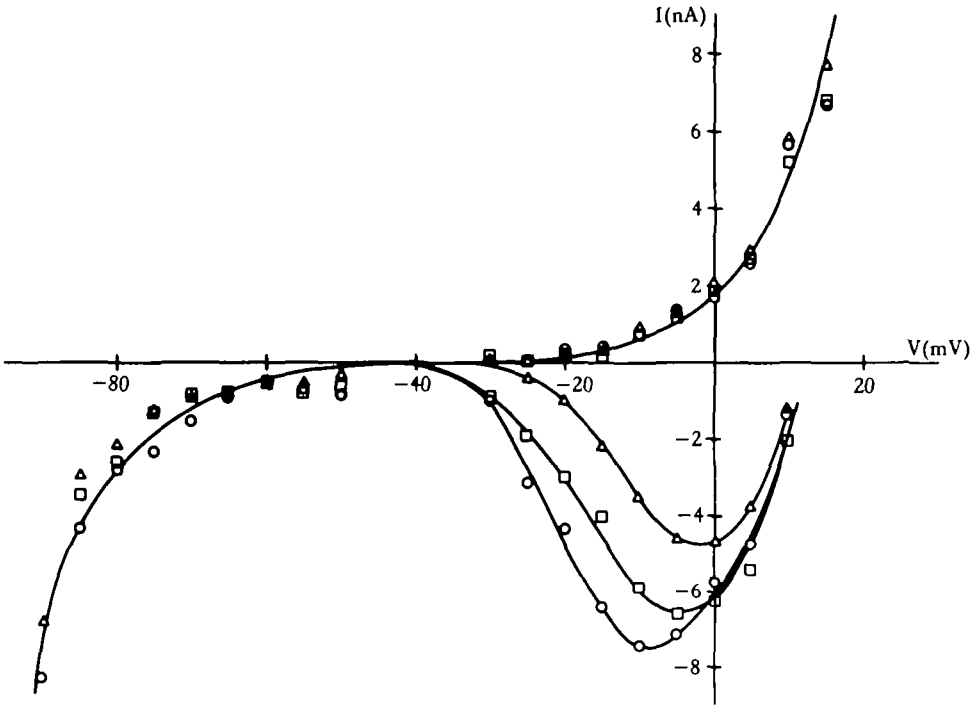

Fig. 2. Electrophysiological effects of 20 μM W-7. At the I_{50} for behavioural effects of W-7, 20 μM, membrane currents were measured under voltage clamp following hyperpolarizing and depolarizing steps from the holding level of −40 mV. The transient inward current, which peaked within 5 ms, seen in the Ca-K solution (○——○) was decreased by perfusion with 20 μM W-7 (△——△). The peak inward current returned to near the original level following perfusion again with the Ca-K solution (□——□). There was no change in either the early outward currents or the hyperpolarization-induced inward currents (both measured at 15 ms) under any of these conditions. The effects of 20 μM W-7 are therefore specific for the transient inward current. The estimated background leakage, which did not change in the presence of 20 μM W-7, was subtracted in all cases. Each point represents the mean of three to eight cells. Some of the data points shown here also appear in Table 2.

Table 2. *The effects of 20 μM W-7 are specific and reversible*

	I_{max} (nA)	V_{max} (mV)	T_{max} (ms)	$I^{In}_{15\,ms}$ (nA)	$I^{Out}_{15\,ms}$ (nA)	R_m (MΩ)
Ca-K	−7·4 ± 1·0	−2·6 ± 3·4	3·4 ± 0·6	−4·3 ± 0·9	2·6 ± 1·9	18·9 ± 3·1
20 μM W-7	−4·7 ± 0·9	3·6 ± 4·6	3·8 ± 0·6	−3·1 ± 1·3	2·9 ± 1·9	25·0 ± 7·6
Return to Ca-K	−6·6 ± 1·1	1·4 ± 2·3	3·6 ± 0·4	−3·4 ± 0·6	2·7 ± 1·5	24·6 ± 6·4

I_{max}, the maximal peak inward current; V_{max}, voltage where I_{max} is seen; T_{max}, peak time of I_{max}; $I^{In}_{15\,ms}$, inward current measured at 15 ms with a −45 mV step; $I^{Out}_{15\,ms}$, outward current measured at 15 ms with a +45 mV step; R_m, membrane resistance estimated from the extrapolated background leakage current; all numbers are mean ± S.D. $N = 8$ cells. This data was taken from the same cells as those represented in Fig. 2.

Recordings from cells with free running membrane potentials showed that there was also no change in the resting membrane potential, membrane resistance, or the height of the action potential triggered by injected current when cells were bathed in 20 μM W-7. The membrane potential was unaffected by concentrations of W-7 as high as 150 μM (data not shown).

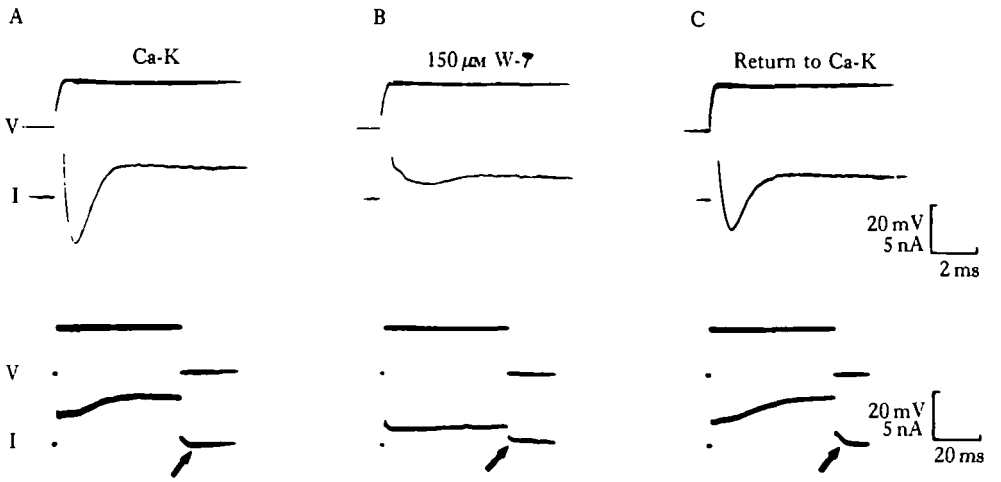

Fig. 3. Effects of 150 μM W-7 on the transient inward current and the Ca^{2+}-dependent K-current. (A) Both the rapid transient inward current (top traces) and the slow Ca^{2+}-dependent K-current (lower traces) were seen in the Ca-K solution in response to a +35 mV step. (B) Perfusion with the same solution containing 150 μM W-7 caused a decrease in both the transient inward current and the Ca^{2+}-dependent K-current. (C) Perfusion once again with the Ca-K solution caused a partial return of both currents. Note that the tail current (arrows) of the Ca^{2+}-dependent K-current is clearly seen in A, is decreased in B, and returns in C. The decrease in the Ca^{2+}-dependent K-current and its tail current is consistent with the decreased Ca^{2+} influx in the presence of 150 μM W-7.

The Ca^{2+}-dependent K-current (Satow & Kung, 1980*b*) was also decreased by W-7 (Fig. 3). This is expected since this K-current is induced by internal Ca^{2+} delivered by the Ca-current.

Isolation of the voltage-dependent Ca-current

The *cnrA* mutant, which is defective in its voltage-dependent Ca-channel (Takahashi & Naitoh, 1978), was used to determine the effectiveness of 2 M-CsCl electrodes and external TEA-Cl in blocking the voltage-dependent outward K-current. 150 μM W-7 was added to ensure that the outward K-current (measured at 15 ms) was not contaminated with any Ca^{2+} currents. This early outward K-current seen in the Ca-K solution (using 2 M-KCl electrodes) was unaffected by 150 μM W-7 but was eliminated by using 2 M-CsCl electrodes and 10 mM external TEA-Cl, revealing a small sustained inward current similar to that described by Eckert & Brehm (1979). Addition of 150 μM W-7, along with 2 M-CsCl electrodes and 10 mM external TEA-Cl, eliminated the sustained inward current as well (Fig. 4). Under these conditions it was determined that the voltage-dependent outward K-current was inhibited by more than 95 % at all voltages tested.

As with *cnrA*, wild type showed no outward current (other than the background leakage current) in the presence of 2 M-CsCl electrodes and 10 mM external TEA-Cl. The unmasked sustained inward current had an I_{max} of $1{\cdot}4 \pm 0{\cdot}7$ nA ($N = 5$) with a V_{max} of about 10·0 mV. Addition of either 150 μM W-7 or 400 μM W-5 eliminated this sustained inward current as well (data not shown).

With the outward K-current blocked by 10 mM external TEA-Cl and 2 M-C

electrodes the isolated voltage-dependent Ca-current shown in Fig. 5 was very similar to the inward current seen with 2 M-KCl electrodes in the Ca-K solution (Fig. 2).

Concentration effects of W-7 and W-5

Both the I_{max} and the V_{max} of the isolated voltage-dependent Ca^{2+} inward current changed in a concentration-dependent manner following perfusion with W-7. Addition of 150 μM W-7 decreased the I_{max} to about 10 % and shifted the V_{max} of the

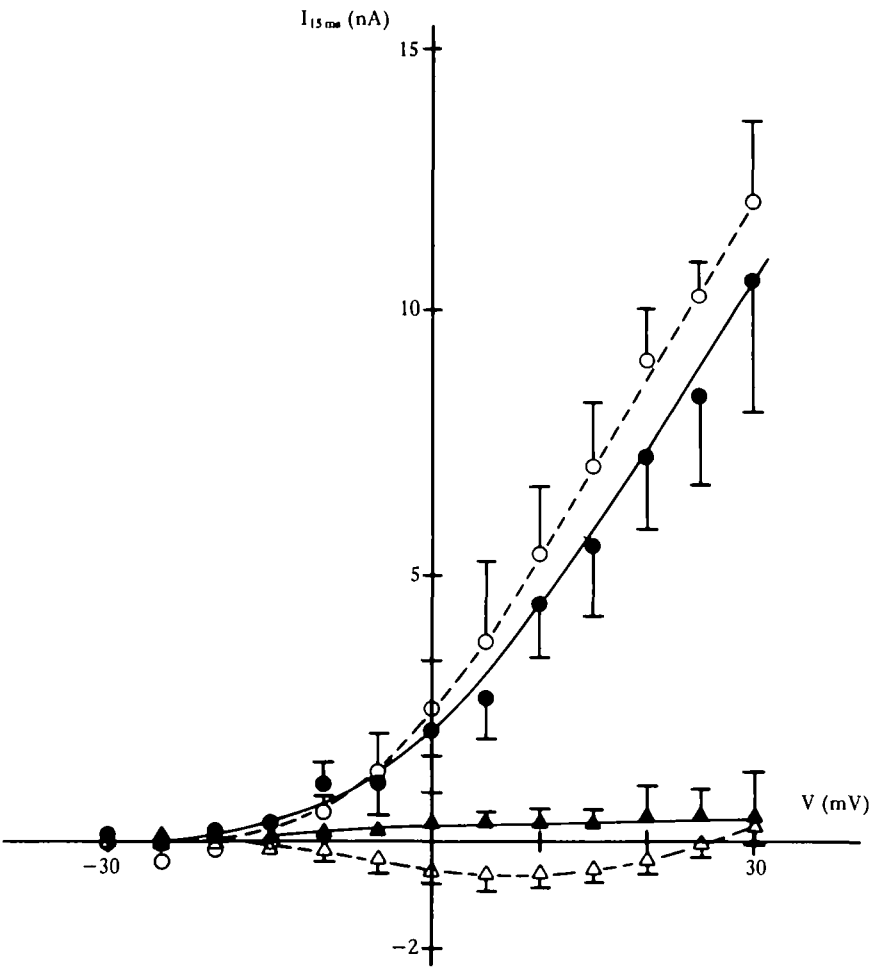

Fig. 4. The voltage-dependent K-current is eliminated by internal CsCl and external TEA-Cl. The membrane currents of the *cnrA* mutant were measured at 15 ms by step depolarizations from the holding level of −40 mV with 2 M-KCl electrodes in the Ca-K solution (○---○) and following perfusion with 150 μM W-7 (●——●). No transient inward current was seen and only the outward currents were present. The use of 2 M-CsCl electrodes and 10 mM external TEA-Cl eliminated this outward K-current and revealed a small sustained inward current (△---△) which was suppressed by addition of 150 μM W-7 (▲——▲). Thus, with 150 μM W-7, 2 M-CsCl electrodes and 10 mM external TEA-Cl there were virtually no measurable depolarization-dependent currents. The estimated background leakage was subtracted from each measurement and all measurements were made at 15 ms. Each point represents the mean ± S.D., $N = 3$–5.

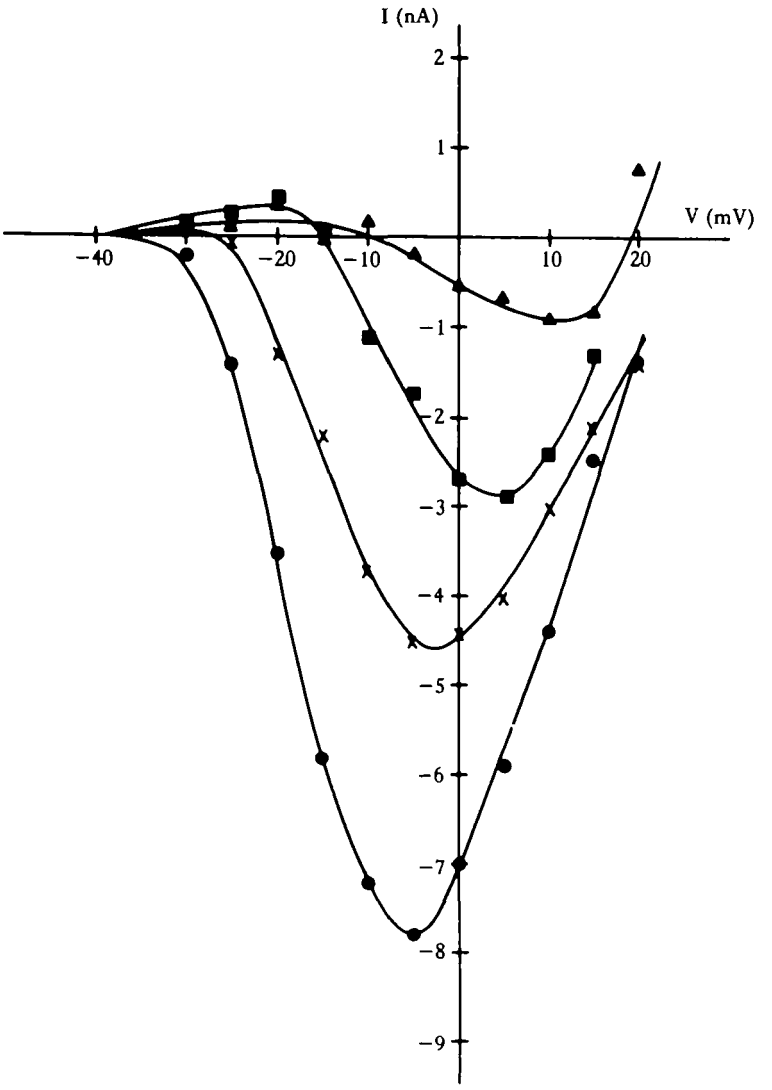

Fig. 5. W-7 affects the isolated voltage-dependent Ca-current in a concentration-dependent manner. The maximal peak inward current (I_{max}) decreased and the voltage where I_{max} is seen (V_{max}) shifted towards a less negative voltage as the concentration of W-7 increased from 0 (●——●) to 40 μM (x——x), 100 μM (■——■) and 150 μM (▲——▲). At each concentration the isolated Ca-current was first measured in the Ca-K solution with 10 mM-TEA-Cl and 2 M-CsCl electrodes and then the measurements repeated following perfusion with W-7 in the same solution. The zero concentration (●——●) is the mean of all of the zero drug controls. The estimated background leakage was subtracted from each measurement. Each point represents the mean of two to eight cells.

remaining current by 10–15 mV less negative. Smaller changes were seen at 40 and 100 μM W-7 (Fig. 5). Ethanol alone, at the same concentration as was present with 150 μM W-7, did not produce such changes.

W-5 had the same electrophysiological effects as W-7 but a higher concentration was required. W-5 was ineffective at 40 μM, the concentration at which W-7 inhibited 40 % of the Ca-current (Fig. 6A, B). At 150 μM W-5 inhibited 40 % of the current ar

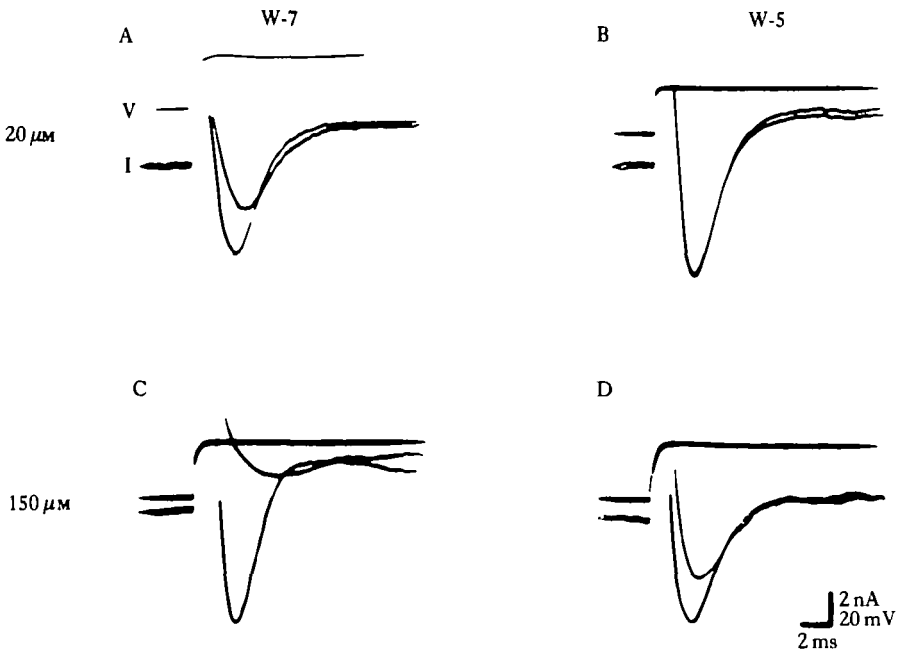

Fig. 6. Membrane currents of cells in two concentrations of W-7 and W-5. Cells were stepped to −5 mV from the holding level of −40 mV; first in the Ca-K solution with 10 mM-TEA-Cl and 2 M-CsCl electrodes and then in the same solution containing (A) 20 μM W-7; (B) 20 μM W-5; (C) 150 μM W-7; (D) 150 μM W-5. At 20 μM, W-7 reduces the Ca^{2+}-current by 40 % but W-5 is ineffective. W-5 is only effective near a concentration at which W-7 reduces the current to less than 10 % (150 μM). The remaining outward current in each trace is due to the background leakage. In each frame, the upper trace is the voltage (V) and the bottom traces the currents (I) before (larger) and after (smaller) the perfusion of drugs. Some of the downstrokes of the currents were retouched to clarify.

W-7 inhibited >90 % of that current (Fig. 6C, D). 150 μM W-5 also shifted the V_{max} 5–10 mV less negative (data not shown).

The estimated concentration for 50 % inhibition of the isolated voltage-dependent Ca^{2+} inward current was about 50 μM for W-7 and about 200 μM for W-5 (see Fig. 7). The W-7 effects were about 90 % reversible up to 40 μM but at higher concentrations reversibility was not as reliable. In one case 80 % of the inward current recovered after treatment with 150 μM W-7 while in most cases the recovery was only 30 % of the original I_{max} when 150 μM W-7 was used.

DISCUSSION

W-7 inhibits both the duration of backward swimming in response to 20 mM-K^+ and the voltage-dependent inward Ca-current in a concentration-dependent and reversible manner in *Paramecium*. W-5 has the same effects as W-7 but higher concentrations of W-5 are needed. W-7 and W-5 are about 90 % effective at concentrations of about 150 μM and 400 μM respectively, but at 20 μM, the concentration where W-7 is 50 % effective at inhibiting backward swimming and about 30 % effective at

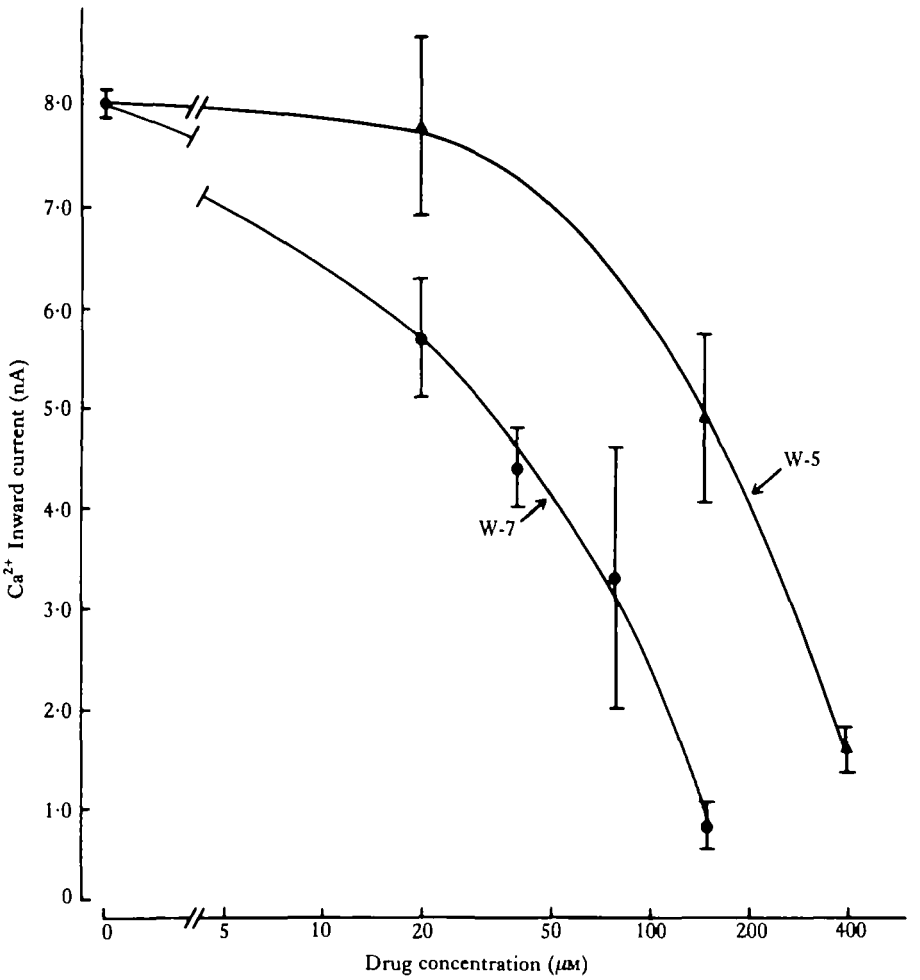

Fig. 7. The decrease in the isolated Ca-current in the presence of W-7 and W-5 is concentration dependent. The I_{max} was measured in the Ca-K solution with 10 mM-TEA-Cl and 2 M-CsCl electrodes (zero drug concentration point) and then measured in the same solution following perfusion with either W-7 (●——●) or W-5 (▲——▲) at various concentrations. The estimated background leakage was subtracted from each measurement. The 50 % effective concentrations are shown by the arrows to be about 50 μM for W-7 and 200 μM for W-5. Each point represents the mean ± S.D., $N = 2$–8.

inhibiting the voltage-dependent Ca-current, W-5 is ineffective. Thus W-5 can serve as a control for non-specific effects of W-7 at 20 μM.

The electrophysiological effects of W-7 appear to be specific for the voltage-dependent Ca-current. Both the hyperpolarization-induced currents and the voltage-dependent K-current are unaffected by up to 150 μM W-7. At the effective concentration of W-7, 20 μM, there was no effect on either the time to peak or the relaxation of the transient inward Ca-current. The membrane potential, membrane resistance and height of the action potential also did not change in 20 μM W-7. Secondary effects on other Ca^{2+}-dependent functions, such as the Ca^{2+}-dependent K-current, are expecte

sequences of the reduced Ca^{2+} influx in the presence of W-7. This Ca^{2+}-dependent K-current of *Paramecium* is also reduced in mutants with decreased inward Ca-current (Satow & Kung, 1980*a*), by EGTA iontophoresis (Saimi, Hinrichsen, Forte & Kung, 1983), and by the addition of an antibody which inhibits the inward Ca-current (Ramanathan *et al.* 1983). The reduced Ca^{2+}-dependent K-current does not affect the voltage-dependent K-current since the two currents are temporally distinct (Satow & Kung, 1980*b*). We cannot rule out direct effects of W-7 on the Ca^{2+}-dependent K-current, however, since it has been suggested that calmodulin antagonists can directly inhibit this current (Lackington & Orrego, 1981).

The voltage-dependent Ca-current was isolated for study by blocking the outward voltage-dependent K-current with internal CsCl (delivered by 2 M-CsCl electrodes) and external 10 mM-TEA-Cl. This procedure was greater than 95 % effective in blocking the outward currents without greatly affecting the inward Ca-current. The sustained inward current which was revealed under these conditions has been ascribed to the remainder of the Ca-current after the major inactivation and presumably uses the same channel as the transient inward Ca-current (Eckert & Brehm, 1979). Therefore the decrease in both the transient and sustained inward currents in the presence of W-7 may be due to inhibition of Ca-channel activity.

The behavioural and electrophysiological reversibility is seen at low concentrations of W-7 (see Tables 1 and 2) but the effects of higher concentrations are not as readily reversible. As seen in Fig. 3, the effects of 100 μM W-7 are not completely reversible. This may be due either to some irreversible damage at high drug concentration or to accumulation of intracellular W-7 (Kanamori, Naka, Asano & Hidaka, 1981). The anticalmodulin effects of W-7 could affect viability. W-7 binds to calmodulin (Hidaka *et al.* 1980) which is present in *Paramecium* (Walter & Schultz, 1981; Maihle *et al.* 1981; Rauh & Nelson, 1981) and serves important functions (Satir, Garofalo, Gilligan & Maihle, 1980; Garofalo, Gilligan & Satir, 1983; Rauh, Levin & Nelson, 1980). Although cells are viable in 20 μM W-7 for hours, 150 μM W-7 kills cells in 5–10 min.

W-7 has many properties which could be involved in the mechanism of action of its Ca-current inhibition: (1) W-7 is a potent anticalmodulin drug (Hidaka, Naka & Yamaki, 1979; Hidaka, Yamaki, Totsuka & Asano, 1979; Hidaka *et al.* 1978, 1980, 1981*b*; Hidaka Asano & Tanaka, 1981*a*; Kanamori *et al.* 1981; Niki, Niki & Hidaka, 1981; Tanaka *et al.* 1982) and calmodulin may be involved in membrane excitation (Carp, Aronstam, Witkop & Albuquerque, 1983; Takahashi, Ogura & Maruyama, 1983). (2) W-7 inhibits calmodulin-independent enzymes such as phospholipid-sensitive protein kinase (Tanaka *et al.* 1982; Schatzman *et al.* 1983) and Na^+,K^+-ATPase and Mg^{2+}-ATPase (Luthra, 1982). Such enzymes could be involved either directly or indirectly in Ca^{2+} channel regulation. (3) W-7 inhibits Ca^{2+} uptake by mitochondria (Hirata *et al.* 1982) and Ca^{2+} binding to membranes (Tanaka *et al.* 1982). This could affect internal Ca^{2+} concentration which could change the driving force for Ca^{2+} influx. Since EGTA injections did not change the W-7 effect in *Paramecium* (unpublished observation) we feel that these effects alone cannot account for the decreased Ca^{2+} inward current. (4) W-7 can partition into membrane lipids and possibly disrupt the physical properties of the excitable membrane. (5) 7 is positively charged at neutral pH, a property which may affect membrane

surface charge and surface potential (see Satow & Kung, 1981). (6) W-7 has an affinity for hydrophobic areas of proteins (Tanaka *et al.* 1982; Schatzman *et al.* 1983). Since the Ca-channel must have hydrophobic areas, the drug may cause an allosteric inhibition. W-7 could also block the Ca-channel in a manner similar to the action of D600 and verapamil in other systems (Lee & Tsien, 1983). It is also possible that the Ca-channel of *Paramecium* is simply very sensitive to general membrane changes (Ramanathan *et al.* 1983). Thus the mechanism of action of W-7 remains an area for future study.

The inhibition of a voltage-dependent Ca-current has also been shown with another anticalmodulin drug, TI233, in pheochromocytoma cells (Takahashi *et al.* 1983). TI233 inhibited high K^+-stimulated norepinephrine release due to a decreased Ca^{2+} influx. This is analogous to the decreased high K^+-stimulated backward swimming and decreased Ca^{2+} current by W-7 in *Paramecium*. Although a calmodulin involvement has been suggested for TI233 action (Takahashi *et al.* 1983), Hidaka *et al.* (1981*a*) have suggested that the blockage of norepinephrine release in the thoracic aorta by W-7 is independent of calmodulin. A clear warning from this work and our work with W-7 is that if anticalmodulin drugs are used to study some cell function it must be remembered that anticalmodulin drugs might inhibit Ca^{2+} influx, a property which could affect Ca^{2+}-dependent cellular processes.

We suggest that *Paramecium* can be used as a behavioural screen for other drugs which may inhibit Ca^{2+} inward current as well as providing a system for analysing the mechanism of action of such drugs.

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REFERENCES

CARP, J. S., ARONSTAM, R. S., WITKOP, B. & ALBUQUERQUE, E. X. (1983). Electrophysiological and biochemical studies on enhancement of desensitization by phenothiazine neuroleptics. *Proc. natn. Acad. Sci. U.S.A.* **80**, 310–314.

ECKERT, R. & BREHM, P. (1979). Ionic mechanisms of excitation in *Paramecium*. In *Annual Review of Biophysics and Bioengineering*, Vol. 8, (eds L. J. Mullins, W. A. Hagins & C. M. Newton), pp. 353–383. Palo Alto: Annual Reviews.

GAROFALO, R. S., GILLIGAN, D. M. & SATIR, B. H. (1983). Calmodulin antagonists inhibit secretion in *Paramecium*. *J. Cell Biol.* **96**, 1072–1080.

HAGA, N., FORTE, M., SAIMI, Y. & KUNG, C. (1982). Microinjection of cytoplasm as a test of complementation in *Paramecium*. *J. Cell. Biol.* **97**, 559–564.

HIDAKA, H., ASANO, M., IWADARE, S., MATSUMOTO, I., TOTSUKA, T. & AOKI, N. (1978). A novel vascular relaxing agent, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, which affects vascular smooth muscle actomysin. *J. Pharmac. exp. Ther.* **207**, 8–15.

HIDAKA, H., ASANO, M. & TANAKA, T. (1981*a*). Activity-structure relationship of calmodulin antagonist: naphthalenesulfonamide derivatives. *Mol. Pharmac.* **20**, 571–578.

HIDAKA, H., NAKA, M. & YAMAKI, T. (1979). Effect of novel specific myosin light chain kinase inhibitors on Ca^{2+}-activated Mg^{2+}-ATPase of chicken gizzard actomyosin. *Biochem. biophys. Res. Commun.* **90**, 694–699.

HIDAKA, H. Y., SASAKI, T., TANAKA, T., ENDO, S., OHNO, Y., FUJII, Y. & NAGATA, T. (1981*b*). *N*-(6-Aminohexyl)-5-chloro-1-naphthalene sulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4354–4357.

HIDAKA, H., YAMAKI, T., NAKA, M., TANAKA, T., HAYASHI, H. & KOBAYASHI, R. (1980). Calcium-regulated modulator protein interacting agents inhibit smooth muscle calcium-stimulated protein kinase and ATPase. *Mol. Pharmac.* **17**, 66–72.

HIDAKA, H., YAMAKI, T., TOTSUKA, T. & ASANO, M. (1979). Selective inhibitors of Ca^{2+}-binding modulator of phosphodiesterase produce vascular relaxation and inhibit actin-myosin interaction. *Mol. Pharmac.* **15**, 49–59.

HINRICHSEN, R. D. & SAIMI, Y. (1984). A mutation that alters properties of the calcium channel in *Paramecium tetraurelia*. *J. Physiol, Lond.* (in press).

HIRATA, M., SUEMATSU, E. & KOGA, T. (1982). Calmodulin antagonists inhibit Ca^{2+} uptake of mitochondria of guinea pig peritoneal macrophages. *Biochem. biophys. Res. Commun.* **105**, 1176–1181.

KANAMORI, M., NAKA, M., ASANO, M. & HIDAKA, H. (1981). Effects of *N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide and other calmodulin antagonists (calmodulin interacting agents) on calcium-induced contraction of rabbit aortic strips. *J. Pharmac. exp. Ther.* **217**, 494–499.

KUNG, C. & SAIMI, Y. (1982). The physiological basis of taxes in *Paramecium*. In *Annual Review of Physiology*, Vol. 44, (eds I. S. Edelman & R. M. Berne), pp. 519–534. Palo Alto: Annual Reviews.

LACKINGTON, I. & ORREGO, F. (1981). Inhibition of calcium-activated potassium conductance of human erythrocytes by calmodulin inhibitory drugs. *FEBS Letters* **133**, 103–106.

LEE, K. S. & TSIEN, R. W. (1983). Mechanism of calcium channel blockade by verapamil, D600 diltiazem and nitrendipine in single dialysed heart cells. *Nature, Lond.* **302**, 790–794.

LUTHRA, M. G. (1982). Trifluoperazine inhibition of calmodulin-sensitive Ca^{2+}-ATPase and calmodulin-insensitive (Na^{+}-K^{+}) and Mg^{2+}-ATPase activities of human and rat red blood cells. *Biochim. biophys. Acta.* **692**, 271–277.

MAIHLE, N. J., DEDMAN, J. R., MEANS, A. R., CHAFOULEOUS, J. G. & SATIR, B. H. (1981). Presence and indirect immunofluorescent localization of calmodulin in *Paramecium tetraurelia*. *J. Cell Biol.* **89**, 695–699.

NELSON, G. A., ANDREWS, M. L. & KARNOVSKY, M. J. (1983). Control of erythrocyte shape by calmodulin. *J. Cell Biol.* **96**, 730–735.

NIKI, H., NIKI, A. & HIDAKA, H. (1981). Effect of a new calmodulin interacting agent (W-7) on insulin secretion. *Biomed. Res.* **2**, 413–417.

OERTEL, D., SCHEIN, S. J. & KUNG, C. (1977). Separation of membrane currents using a *Paramecium* mutant. *Nature, Lond.* **268**, 120–124.

OTTER, T., SATIR, P. & SATIR, B. H. (1983). Possible mechanisms by which calmodulin antagonists alter swimming in *Paramecium*. *Biophys. J.* **41**, 89a (abstr.).

RAMANATHAN, R., SAIMI, Y., PETERSON, J. B., NELSON, D. L. & KUNG, C. (1983). Antibodies to the ciliary membrane of *Paramecium tetraurelia* alter membrane excitability. *J. Cell Biol.* **97**, 1421–1428

RAUH, J., LEVIN, A. E. & NELSON, D. L. (1980). Evidence that calmodulin mediates calcium-dependent ciliary reversal in *Paramecium*. In *Calcium Binding Proteins: Structure and Function*, (eds F. L. Seigel, E. Carafoli, R. H. Kretsinger, D. H. MacLennan & R. H. Wasserman), pp. 231–232. New York: Elsevier North Holland.

RAUH, J. J. & NELSON, D. L. (1981). Calmodulin is a major component of extruded trichocysts from *Paramecium tetraurelia*. *J. Cell Biol.* **91**, 860–865.

SAIMI, Y., HINRICHSEN, R. D., FORTE, M. & KUNG, C. (1983). Mutant analysis shows that the Ca^{2+}-induced K^{+} current shuts off one type of excitation in *Paramecium*. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5112–5116.

SATIR, B. H., GAROFALO, R. S., GILLIGAN, D. M. & MAIHLE, N. J. (1980). Possible functions of calmodulin in protozoa. *Ann. N.Y. Acad. Sci.* **356**, 83–91.

SATOW, Y. & KUNG, C. (1979). Voltage-sensitive Ca-channels and the transient inward current in *Paramecium tetraurelia*. *J. exp. Biol.* **78**, 149–161.

SATOW, Y. & KUNG, C. (1980*a*). Membrane currents of pawn mutants of the pwA group in *Paramecium tetraurelia*. *J. exp. Biol.* **84**, 57–71.

SATOW, Y. & KUNG, C. (1980*b*). Ca^{2+}-induced K^{+}-outward current in *Paramecium tetraurelia*. *J. exp. Biol.* **88**, 293–303.

SATOW, Y. & KUNG, C. (1981). Possible reduction of surface charge by a mutation in *Paramecium tetraurelia*. *J. Membr. Biol.* **59**, 179–190.

SCHATZMAN, R. C., RAYNOR, R. L. & KUO, J. F. (1983). *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), a calmodulin antagonist, also inhibits phospholipid-sensitive calcium-dependent protein kinase. *Biochim. biophys. Acta* **755**, 144–147.

SONNEBORN, T. M. (1970). Methods in *Paramecium* research. In *Methods in Cell Physiology*, Vol. 4, (ed. D. Prescott), pp. 241–339. New York: Academic Press.

TAKAHASHI, M. & NAITOH, Y. (1978). Behavioural mutants of *Paramecium caudatum* with the defective membrane electrogenesis. *Nature, Lond.* **271**, 656–658.

TAKAHASHI, M., OGURA, A. & MARUYAMA, M. (1983). Inhibition of transmitter release by TI233, a calmodulin antagonist, from clonal neural cells and a presumed site of action. *Biochem. Pharmac.* **32**, 249–252.

TANAKA, T., OHMURA, T. & HIDAKA, H. (1982). Hydrophobic interactions of the Ca^{2+}-calmodulin complex with calmodulin antagonists: naphthalenesulfonamide derivatives. *Mol. Pharmac.* **22**, 403–407.

WALTER, M. F. & SCHULTZ, J. E. (1981). Calcium receptor protein calmodulin isolated from cilia and cells of *Paramecium tetraurelia*. *Eur. J. Cell Biol.* **24**, 97–100.

WISE, B. C., GLASS, D. B., JEN CHOU, C.-H., RAYNOR, R. L., KATOH, N., SCHATZMAN, R. C., TURNER, R. S., KIBLER, R. F. & KUO, J. F. (1982). Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. I. Substrate specificity and inhibition by various agents. *J. biol. Chem.* **257**, 8489–8495.