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MEMBRANE CURRENTS OF PAWN MUTANTS OF THE *pwA* GROUP IN *PARAMECIUM TETRAURELIA*

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SUMMARY

Membrane currents were recorded from the wild type and two pawn mutants of the *pwA* complementation group in *Paramecium tetraurelia* under a voltage clamp. Most currents are not changed by the mutations. Transient inward currents of a leaky mutant, pwA_{132}, upon step depolarizations are less than those in the wild type. The inward transient is completely lacking in a non-leaky mutant, pwA_{500}. The time course of the residual inward currents in the leaky mutant is not significantly different from that of wild type. The voltage sensitivity of the Ca channels in the leaky mutant is also similar to that of wild type. The inward currents upon membrane hyperpolarizations in the mutants show normal characteristics in the presence or absence of external K^+. With sufficiently large, prolonged depolarization, outward currents progressively develop in the wild type but decay in the mutants.

The simplest conclusion we can draw is that the *pwA* mutations reduce the number of functional Ca channels but do not change the channel characteristics. From the conductance measurements, 45% of the Ca channels remain in the leaky mutant pwA_{132}, and none remain in the non-leaky mutant pwA_{500}.

By subtracting the outward currents of pwA_{500} from the slow and prolonged outward currents of the wild type, we have tentatively separated a Ca-induced K^+ current from the voltage-dependent K^+ current. The time courses of these two currents differ by two orders of magnitude.

INTRODUCTION

Ca action potentials have been reported in many excitable membranes (Fatt & Katz, 1953; Hagiwara, 1973). The Ca^{2+} entering during the action potential has a direct effect on a variety of cellular functions. In *Paramecium*, the Ca^{2+} reverses the direction of ciliary beat. Pawn mutants have a reduced Ca conductance and have a reduced tendency to swim backward when properly stimulated. The typical (non-leaky) pawn has no Ca conductance and does not swim backward at all.

In *Paramecium* the calcium current shows inactivation (Oertel, Schein & Kung, 1977; Brehm & Eckert, 1978) as in several other systems (e.g. Mounier & Vassort,

1975). Brehm & Eckert (1978) showed that the inactivation is induced by internal Ca^{2+}, not voltage as for the sodium channel. Internal calcium also opens potassium channels in several systems (Meech, 1976) and this has been demonstrated in *Paramecium* by injection of Ca or EGTA (Satow, 1978*a*; Brehm, Dunlap & Eckert, 1978). However, Oertel *et al.* (1977) have provided evidence that Ca-induced K conductance is not involved in excitation events occurring within tens of milliseconds after depolarization in *Paramecium*. The role of this conductance in excitation has also been questioned by Eckert & Brehm (1979).

In this paper we describe various currents observed in the *Paramecium* membrane under voltage clamp, including the calcium-induced K^+ current. We examined two mutants of the *pwA* complementation group (see Chang *et al.* 1974) – one a typical pawn, the other a leaky pawn – and compared them with the wild type to see which characteristics of the Ca channels are altered by the mutations. The pawn was found to have normal membrane characteristics except for its lack of Ca conductance, and has since been used as null control for a variety of studies (see Kung, 1979, for a review). An abstract of part of this work has been reported elsewhere (Satow, 1979).

MATERIAL AND METHODS

Stocks and culture

Cells of *Paramecium tetraurelia* were cultured at room temperature (22 ± 1 °C) in Cerophyl medium inoculated with *Enterobacter aerogenes* 20 h before use (Sonneborn, 1970). Only robust cells in log-phase growth were used. There was no systematic difference in the size of the paramecia used from different strains. The two mutant strains were allelic variants of the *pwA* complementation group. They were a typical pawn, stock d4–500 (abbreviated as pwA_{500} herein), and a leaky pawn, stock d4–132 (abbreviated as pwA_{132}) (Chang *et al.* 1974). The wild type used is stock 51s from which the mutants were derived.

Solutions

A bath solution containing 1 mM-Ca $(OH)_2$, 0·5 mM-$CaCl_2$ and 1 mM-citric acid adjusted to pH 7·2 with approximately 1·3 mM-Tris is termed the 'Ca solution' throughout this paper. The free Ca^{2+} concentration in this solution is calculated to be 0·91 mM. Addition of 4 mM-KCl to the Ca solution yielded the 'Ca-K solution'. This solution was used in the investigation of currents which may be carried by K^+. One, 4, 8 or 16 mM-KCl was added to the Ca solution for the experiments in Fig. 6.

Recordings

The methods used in intracellular recording were similar to those given by Naitoh & Eckert (1972). All experiments were performed using a voltage clamp, as described by Satow & Kung (1979). The membranes were first held at the resting levels and were then depolarized or hyperpolarized in steps. This procedure was followed to maximize the transient inward currents and to keep the membranes close to their normal states. Since the resting potentials of different strains were very similar in a given solution, the holding potentials were essentially the same for different strains (Table 1). All experiments were performed at room temperature (22 ± 1 °C).

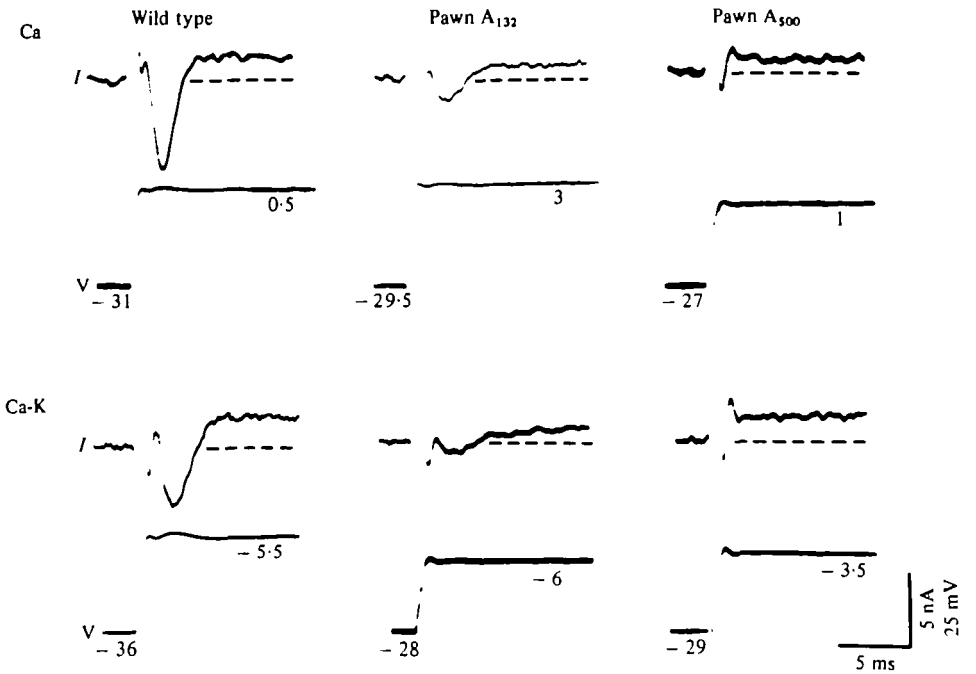

Fig. 1. Currents (*I*) in the wild type, pwA_{132} and in pwA_{500} upon step depolarizations, in the Ca solution (upper panels) and the Ca-K solution (lower panels). The lower traces (*V*) in each frame show the voltage steps from the holding potential, which is equal to the resting potential of the membrane in the given solution. The numbers on the *V* traces indicate the levels of the depolarization steps in mV. The transient inward currents in wild type and pwA_{132} are near the maxima. The transient inward current is not induced in pwA_{500} at all voltages.

RESULTS

Transient inward currents

When the wild-type membrane is first held at the resting potential level (−31 mV in the Ca solution and −36 mV in the Ca-K solution) and then subjected to a step depolarization, a transient inward current is observed as shown in Fig. 1 (left). The inward current reaches its peak within 3 ms and subsides rapidly afterward. An outward current follows the inward transient. For reasons not understood, the inward transient is smaller when K^+ is present in the solution (Fig. 1, lower left). Descriptions of the inward transients of wild-type *P. tetraurelia* have been given by Oertel *et al.* (1977) and by Satow & Kung (1979).

If the membrane of the leaky pawn, pwA_{132}, is subjected to step depolarizations, inward currents smaller than those of wild type can be observed when the step depolarization is from the holding level of about −30 mV to beyond −10 mV (Fig. 1, centre). The maximal peak inward current (I_{max}), measured directly from the zero current level without adjusting for leakage or rectifying currents (see below), is observed when the membrane potential is stepped up to +3 mV in cells bathed in the Ca solution. Still smaller inward current is seen when the cell is bathed in the Ca-K solution (Fig. 1, lower centre). The I_{max} of pwA_{132} is about 25% of that found in the wild type regardless of the bath solutions (Table 1).

Table 1. *Various characteristics of the Ca current of the wild type and the two pawnA mutants*

ion ...	Ca			Ca-K		
n ...	wild type	pwA_{132}	pwA_{500}	wild type	pwA_{132}	pwA
	$-6{\cdot}8 \pm 0{\cdot}7$	$-1{\cdot}6 \pm 0{\cdot}4$	$+1{\cdot}7 \pm 0{\cdot}7$	$-4{\cdot}1 \pm 1{\cdot}3$	$-1{\cdot}0 \pm 0{\cdot}3$	$+1{\cdot}7$
	$+1{\cdot}4 \pm 2{\cdot}3$	$+3{\cdot}7 \pm 4{\cdot}3$	—	$-0{\cdot}5 \pm 5{\cdot}7$	$+0{\cdot}6 \pm 4{\cdot}0$	—
	$2{\cdot}1 \pm 0{\cdot}3$	$2{\cdot}0 \pm 0{\cdot}2$	—	$2{\cdot}3 \pm 0{\cdot}1$	$2{\cdot}4 \pm 0{\cdot}3$	—
wild type (%)	—	24	0	—	24·4	0
a (%)	—	—	0	60·5	62·5	0
	$-32{\cdot}0 \pm 2{\cdot}7$	$-31{\cdot}0 \pm 3{\cdot}4$	$-33{\cdot}5 \pm 5{\cdot}6$	$-32{\cdot}6 \pm 4{\cdot}2$	$-33{\cdot}4 \pm 3{\cdot}8$	$-30{\cdot}4$
	7	8	7	8	5	5
A)*	−7·8	−3·5	0	−4·9	−2·2	0
	100	44·9	0	100	44·9	0
ho/cell)	70	32	0·0	41	19	0·0
)	100	45·7	0·0	100	46·3	0·0

d V_h are given as mean ± S.D.
peak inward current; pwA_{500} gave no inward current and the positive numbers stand for outward current at 2·2 ms at a
arable to T_{max} and V_{max} in wild type.
evel where I_{max} was seen.
e of I_{max}.
al.
of each strain.
used for calculation of G_{Ca}.
evel given by the current of pwA_{500}; all currents are measured at 2·2 ms for this calculation.

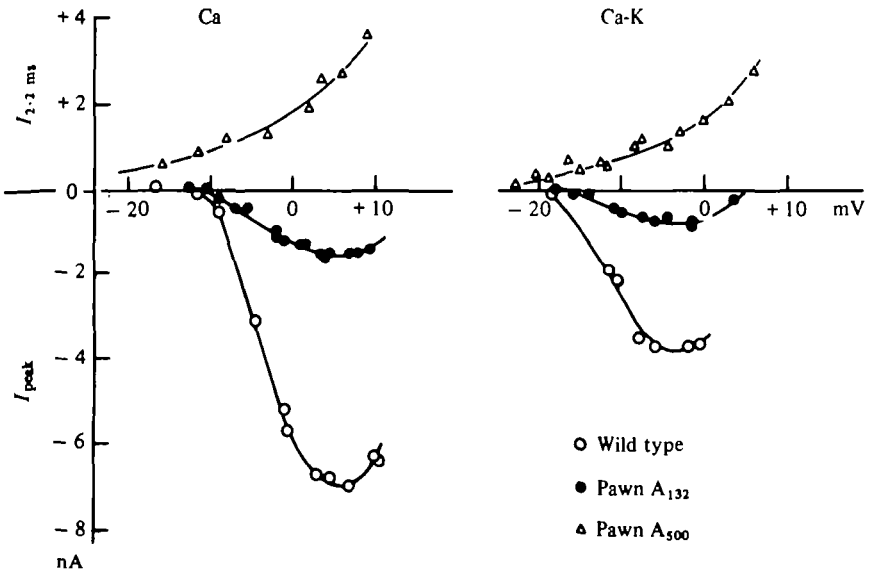

Fig. 2. V–I_{peak} relations of wild type (open circles) and pwA_{132} (filled circles) in the Ca- and the Ca-K solutions. There is no inward current in pwA_{500} at these voltages, but the outward currents at 2·2 ms after the voltage step changes in pwA_{500} are given (triangles). Note that the transient inward currents in pwA_{132} are smaller than those in the wild type, but the maximal peak inward currents occur at the same voltage, V_{max}.

As shown in Fig. 1 (right), no transient inward current is seen in the typical pawn, pwA_{500}, beyond an early downward oscillation associated with the step depolarizations. Outward currents are recorded from this membrane at a time (2·2 ms) when maximal inward current is seen in the wild type.

The time course of the transient inward current can be characterized by the time (T_{max}) when the inward current is maximal. The T_{max}'s of wild type and pwA_{132} are both about 2 ms in the Ca solution and about 2·3 ms in the Ca-K solution (Fig. 1). The mean and standard deviations of T_{max}'s are listed in Table 1. There is clearly no significant difference between the wild type and pwA_{132} in their T_{max}'s.

To compare the voltage sensitivity of the Ca conductance in pwA_{132} with that in the wild type, V–I_{peak} relations in the Ca and the Ca-K solutions are plotted in Fig. 2. As judged by the voltage at which the I_{max} is observed, V_{max}, the voltage sensitivity of the pwA_{132} membrane is similar to that of the wild type (Fig. 2; Table 1).

Assuming that pwA_{500} completely lacks the voltage-sensitive Ca conductance but is otherwise normal, the total Ca^{2+} current (I_{Ca}) of the wild type and pwA_{132} can be measured by subtracting the outward current in pwA_{500} from the inward currents of wild type and pwA_{132} at peak time (cf. Oertel *et al.* 1977). Averaged I_{Ca} of wild type and pwA_{132} in the Ca solution are shown in Fig. 3A. At +5 mV steps, I_{Ca} is 7·8 nA in the wild type and 3·5 nA in pwA_{132}. Chord conductance (G_{Ca}) calculated using an estimated E_{Ca} of +115 mV (from an estimated internal concentration of free Ca^{2+} of 10^{-7} M, see Eckert, Naitoh & Machemer, 1976), is 70 nmho/cell in the wild type and 32 nmho/cell in pwA_{132}. The conductance voltage plot is another estimate of the voltage sensitivity of the channels involved (Fig. 3B). The $V_{\frac{1}{2}G}$, the voltage

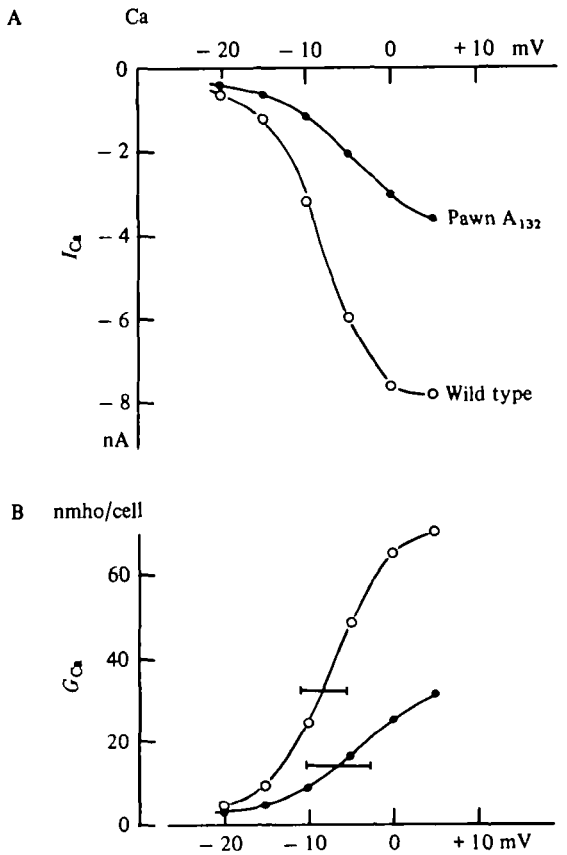

Fig. 3. Voltage relations of total inward current, I_{Ca} (A) and Ca conductance (B) in wild type (open circles) and pwA_{132} (filled circles) in the Ca solution (mean values from 7 to 8 cells each). I_{Ca} is derived from a background subtraction procedure given in the text. G_{Ca} is the chord conductance calculated using an estimated E_{Ca} of +115 mV. The horizontal bars of the G_{Ca} curves indicate the standard deviations of voltage at which the G_{Ca} is estimated to be half maximal, $V_{\frac{1}{2}G}$. Note that no significant difference in the $V_{\frac{1}{2}G}$ can be detected between the wild type and pwA_{132}.

at which G_{Ca} is half maximal, has been obtained from the conductance-voltage plot for each specimen using the I_{Ca} values obtained by subtracting the same base line derived from the mean values of outward currents of pwA_{500}. The mean $V_{\frac{1}{2}G}$ is −8·0 mV (S.D. 3·1, n = 5) for the wild type and −7·2 mV (S.D. 3·9, n = 7) for pwA_{132}. These figures are not significantly different.

Inward currents upon hyperpolarization

Anomalous rectification has been observed in *Paramecium* (Naitoh & Eckert, 1968*a*; Satow & Kung, 1976*a*; Schein, Bennet & Katz, 1976; Oertel, Schein & Kung, 1978). The current is carried by K^+ (Oertel *et al.* 1978) and so was measured in the Ca-K solution (4 mM-K). The sustained inward currents induced by step hyperpolarizations in the wild type are very similar to those in pwA_{500} (Fig. 4, upper panel). In this study, the earlier portion of the inward currents is not analysed. The steady-state inward

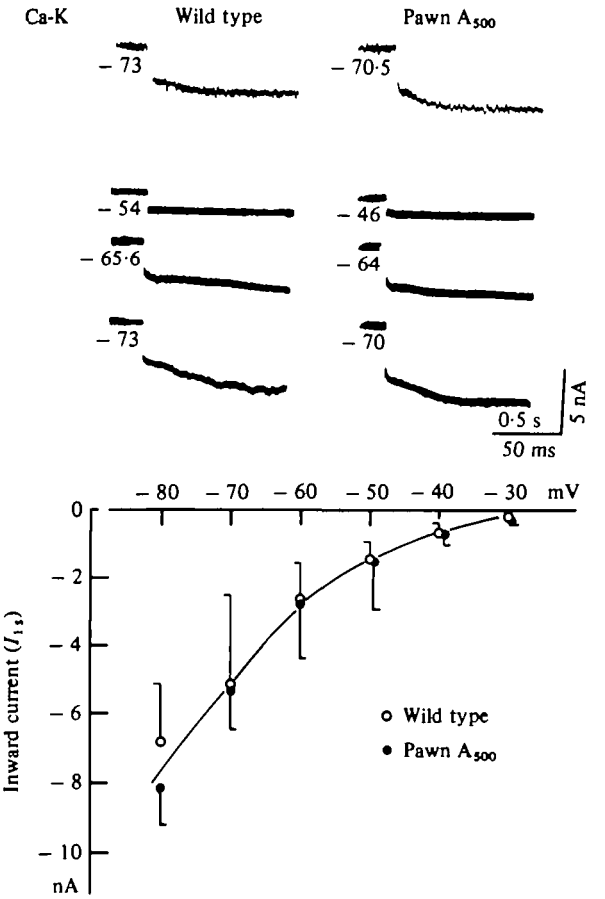

Fig. 4. Inward currents induced by membrane hyperpolarization in the Ca-K solution. Upper panels show the inward currents associated with steps at different amounts as indicated in each frame (in mV) or the wild type and pwA_{500}. The two uppermost panels are of higher sweep speed to show the anomalous rectification in the first 50 ms. Lower graph shows the relation between hyperpolarization level and the inward current at 1 s after the hyperpolarization in wild type (n = 6, open circles) and pwA_{500} (n = 5, filled circles). The differences between the two strains are statistically insignificant.

currents at 1 s after the beginning of hyperpolarizing steps (Fig. 4, middle panels with slow sweep speed) are plotted against the step voltages (Fig. 4, lower). The voltage dependencies of the anomalous rectification channels of the wild type and pwA_{500} are indistinguishable. There is no statistically significant intergroup difference between the inward currents recorded from the two strains at any of the hyperpolarizing steps.

We compared the channel properties in the wild type and pwA_{500} as revealed by a technique similar to that of Oertel *et al.* (1978). We first hyperpolarized the membrane by a step to activate the anomalous rectifying channels, held the membrane at this level for 720 ms, and then analysed the tail currents observed when the potential was stepped to other levels. The reversal potentials of the tail currents in the Ca-K solution were $-54{\cdot}8$ mV (S.D. 5·6, n = 8) in the wild type and $-55{\cdot}8$ mV (S.D. 6·3, n = 10) in

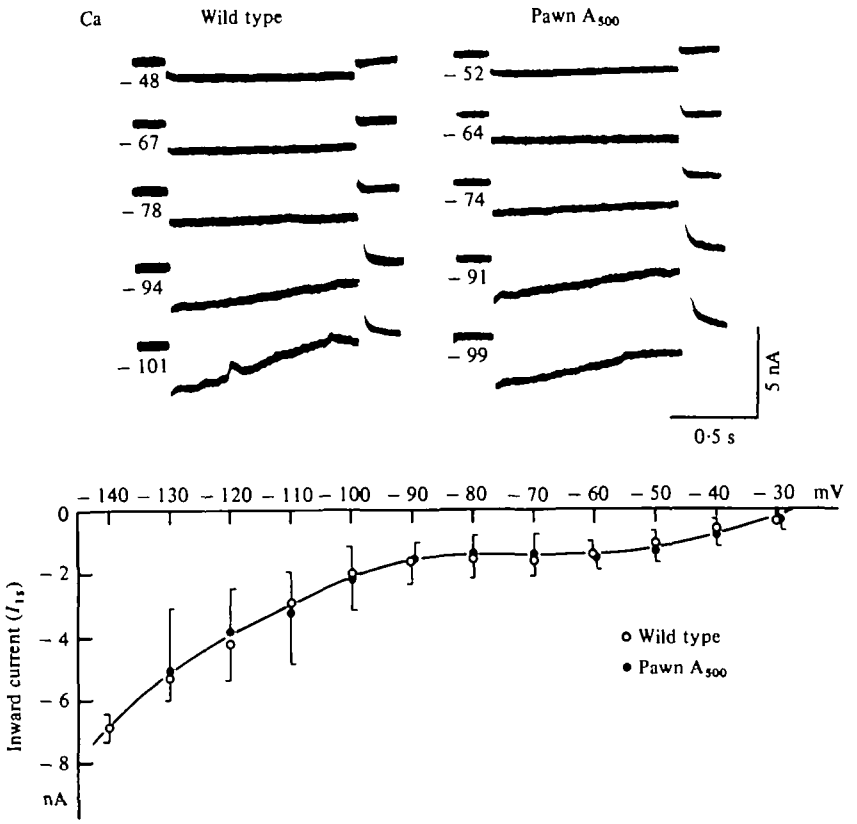

Fig. 5. Inward currents induced by membrane hyperpolarization in the Ca solution. Inward currents are shown in the upper panels. The extent of the hyperpolarization is given in mV in each frame. Lower graph shows the relation between hyperpolarization level and the inward current at 1 s after the hyperpolarization in wild type (n = 7, open circles) and *pwA*$_{500}$ (n = 6, filled circles). The differences between the two strains are not statistically significant.

pwA$_{500}$. Thus, *pwA*$_{500}$ appears to have normal hyperpolarization-activated channels (see Discussion on E_K).

As expected, the anomalous rectification is different in the K^+-free solution (the Ca solution) (Fig. 5). In the range from -30 to -50 mV, the response is ohmic. This is best attributed to the voltage-independent leakage conductance. From -50 to -90 mV, further hyperpolarization does not increase the inward current. This is most likely due to the efflux of K^+ through the anomalous-rectification channel resulting in outward currents that compensate for the inward leakage current. These findings are also consistent with those derived from previous current clamp experiments (Satow & Kung, 1977). When the step hyperpolarization is beyond -90 mV, the response is again ohmic, but with a large conductance. The mechanism of this inward rectification is not known. As in the Ca-K solution, there was no difference between the inward current in the wild type and that in *pwA*$_{500}$. This observation reinforces the notion that the mutational effect on the Ca channels does not extend to the channels responsible for the leakage current and the anomalous rectifying current.

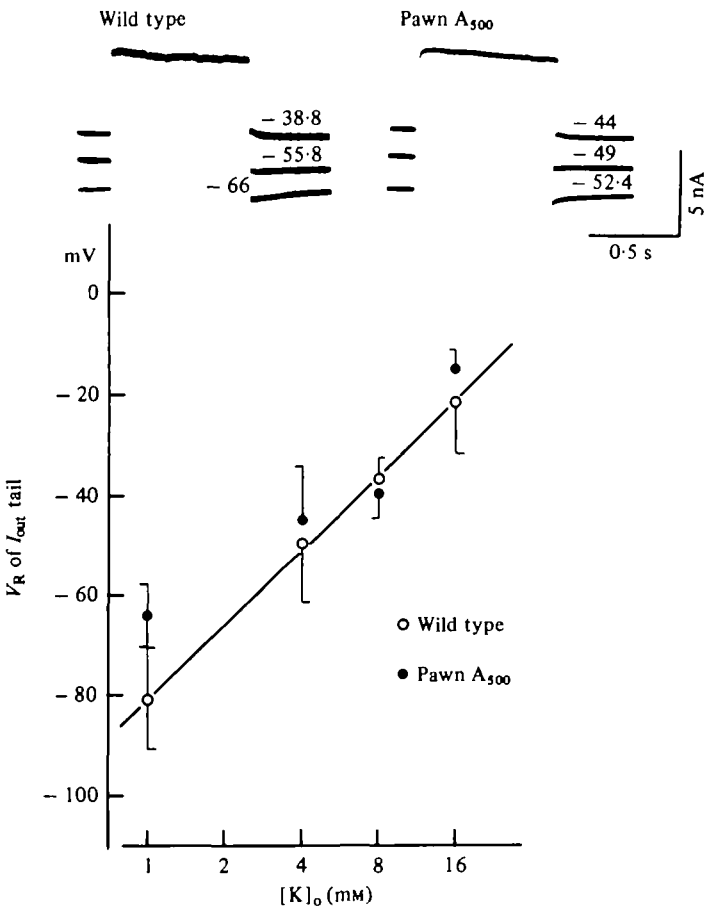

Fig. 6. Analysis of conductances corresponding to the slow outward currents induced by long depolarizations in the wild type and *pwA*$_{500}$ in the Ca-K solution (4 mM-K). The membranes are first held at their resting level (−36 mV in the wild type, −30 mV in *pwA*$_{500}$); the potentials are then stepped up to fixed levels (+6·6 mV or wild type and +11 mV for *pwA*$_{500}$) for 720 ms and then stepped down to various levels indicated by the numbers given (in mV). Three traces are shown in each case. The traces are not superimposed so as to show the 'tail' currents clearly. The reversal potentials, V_R, can be estimated from these 'tail' currents. The V_R of the 'tail' currents are plotted against the concentration of K^+ in the bath, $[K]_o$. Means ±S.D. of V_R's from wild type (n = 5 or 6, open circles) and *pwA*$_{500}$ (n = 4 or 5, closed circles) are shown. The straight line drawn by eye has a slope of 50 mV for a tenfold change in $[K]_o$.

Outward currents

The outward currents induced by step depolarizations, commonly known as delayed rectifying currents, were compared between the three strains to test whether the *pwA* mutations affect the channels responsible for these currents. The kinetics of these currents within the first hundred ms after the depolarization steps are complex and are dependent strongly on the amount of depolarization. As shown below, these early outward currents are not significantly different in the three strains, although variations within strains are large, as was observed by Oertel *et al.* (1977). However, the late currents are different.

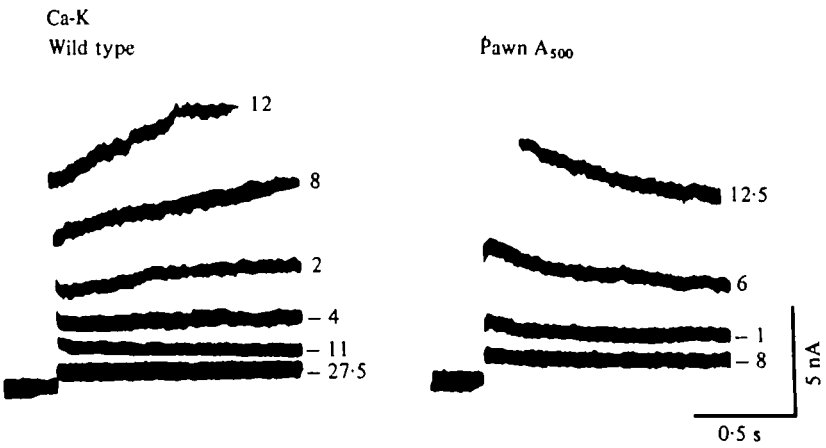

Fig. 7. The slow outward currents associated with long step depolarizations, in the Ca-K solution. The currents in wild type and pwA_{500} are shown. The membranes are first held at the resting potential (−33 mV in the wild type, −31 mV in pwA_{500}) and then depolarized to the levels indicated by the numbers (in mV). Note that currents activated with depolarizations to above −10 mV show a slow rise in the wild type and a fall in pwA_{500}.

The outward current induced by membrane depolarizations is considered to be carried largely by K^+ in *Paramecium* (Naitoh & Eckert, 1974; Satow & Kung, 1976*a*) as in other excitable systems. To investigate the K^+-dependence of the outward currents, the membrane was subjected to a prolonged depolarization (720 ms), and the potential was then stepped to various lower levels (Fig. 6) at a range of K^+ concentrations. Tail currents may be inward or outward depending on the level of the second step. The reversal potentials (V_R) of the tail currents induced in this manner in the Ca-K solution (4 mM-K) are $-49{\cdot}5 \pm 12{\cdot}1$ mV in the wild type and $-42{\cdot}5 \pm 6{\cdot}4$ mV in pwA_{500} (see Discussion). A plot of the V_R versus the K^+ concentration shows a slope of 50 mV for a tenfold change of concentration in both strains (Fig. 6). Thus, the outward current after a 720 ms depolarization is mostly carried by K^+. Similar examination of the tail currents after 30 ms of depolarization at the voltage of 0 mV shows that the V_R is about −20 mV in the Ca-K solution (4 mM-K) and the V_R is dependent on the amount of depolarization in both the wild type and pwA_{500}. Thus, the early outward current is not entirely dependent on K^+.

The outward current associated with very long and constant depolarization (seconds) are steady with depolarizations up to about the −10 mV level (Fig. 7). Larger depolarizations, however, produce currents which do not stay steady. In the wild-type membrane, the currents develop towards plateaus with half times measured in hundreds of ms (Fig. 7, left). These added currents can be as large as 4–5 nA in some cases. In the membrane of pwA_{500}, the currents decline rather than rise, before assuming a steady value (Fig. 7, right).

Fig. 8 is a plot of these plateau levels at 10 s of the depolarizations. Although the mean values of the plateau currents of the wild type are consistently higher than those of pwA_{500} in all cases, it is only with depolarizations to levels above 0 mV, that the wild-type current is significantly larger than that of pwA_{500} ($P < 0{\cdot}05$ at 0 mV; $P < 0{\cdot}01$ at +5 and +10 mV). We propose that this reduction of slow outward

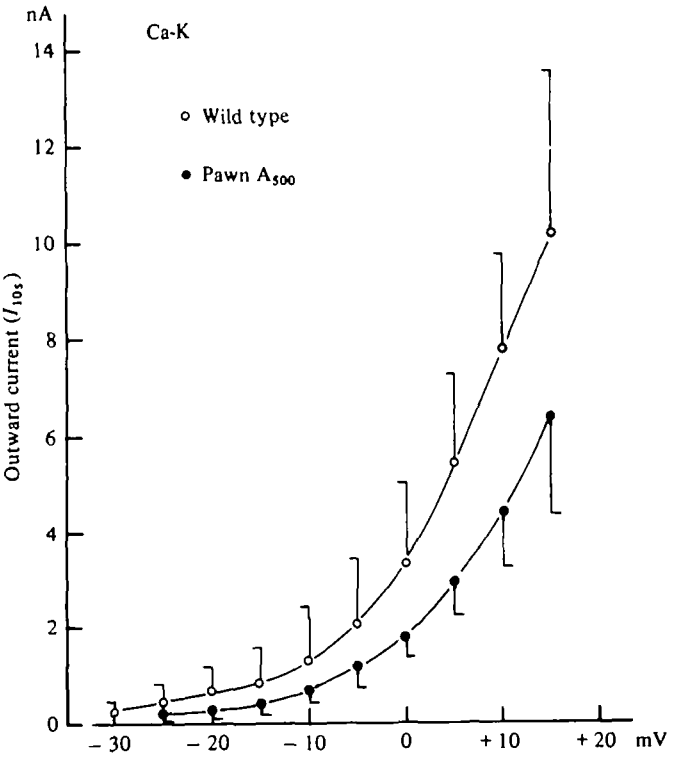

Fig. 8. The steady-state slow outward currents at different levels of sustained depolarizations. The membranes are first held at the resting levels and then stepped to various depolarized levels indicated on the abscissa. Means ±S.D. of currents of wild type (n = 10) and *pwA*$_{500}$ (n = 9) as 10 s after potential changes are shown. The differences between currents from the two strains at depolarizations above 0 mV are statistically significant.

current in *pwA*$_{500}$ is due to the lack of a Ca-induced K^+ current (see Discussion). Assuming that *pwA*$_{500}$ suffers a complete loss of this current, the kinetics of the Ca-induced K current in the wild type can be estimated by a subtraction of the trajectory of the outward current of *pwA*$_{500}$ from that of the wild type, as shown in Fig. 9. In this example, the proposed Ca-induced K current rises slowly with a half-time of approximately 0·25 s. This rise is two orders of magnitude slower than that of the voltage-sensitive K^+ current responsible for the major portion of the delayed rectification.

DISCUSSION

The effect of pwA mutations

The *pwA* gene product may regulate the production of Ca channels, or it may code for the channel structures or define the immediate environment of the channels. Thus, the reduction of the Ca^{2+} inward transient in *pwA*$_{132}$ and the total lack of this current in *pwA*$_{500}$ may be due to a reduction in the number of normal Ca channels, or due to changes in the channel properties. Based largely on the broad range of leakiness and the lack of effects on the anomalous rectification channels of the *pwA*

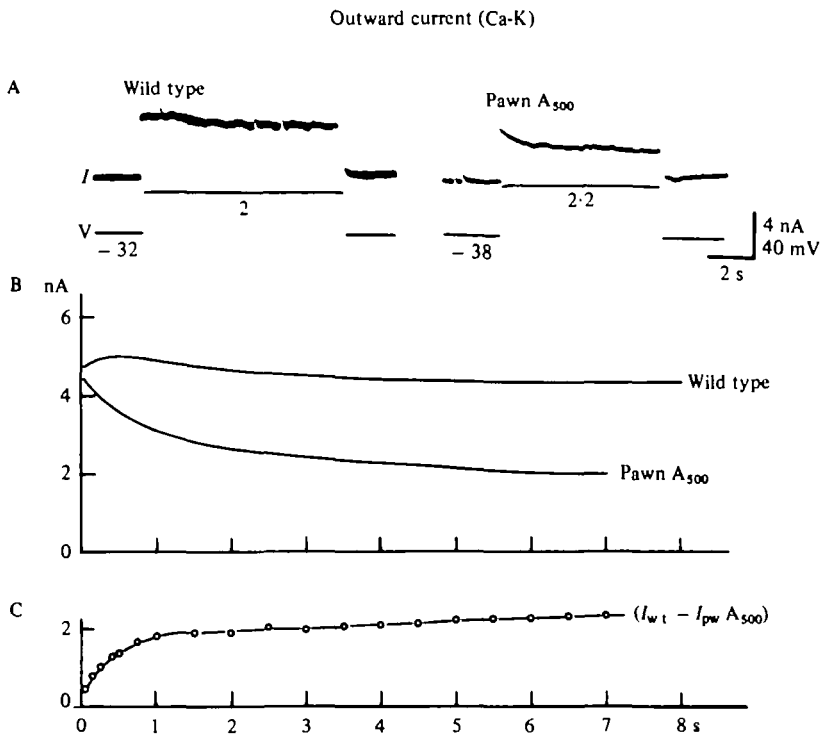

Fig. 9. (A) the slow outward currents, I, in wild type and pwA_{500}. These currents are induced by depolarizations from the holding potentials of −32 mV in the wild type and −38 mV in pwA_{500} to +2 mV in wild type and +2·2 mV in pwA_{500}, as shown on the V traces. These currents are traced in (B). The difference between the two curves (C) is considered the Ca-induced K current in the wild-type membrane. See text for details.

mutations, Schein *et al.* (1976) speculated that these mutations affect the voltage sensitivity of the Ca channels.

We have presented here a thorough study of the Ca-channel characteristics using the voltage clamp. The peak time, T_{max}, is an indicator of the kinetics of activation of available channels. As shown in Fig. 1 and Table 1, the T_{max} of pwA_{132} is not significantly different from that of the wild type in Ca- or Ca-K solutions. Moreover, the T_{max}'s of the two strains are also the same in a Ca-Ba solution (Y. Satow & C. Kung, unpublished observations), although Ba^{2+} slows the activation significantly (Satow, 1978*b*). The voltage sensitivity of the Ca channels can be estimated by the V_{max} on the V–I_{peak} curves or by the $V_{\frac{1}{2}G}$ on the G_{Ca}–V plots. Data presented in Table 1 and Figs. 2 and 3 show that V_{max} and $V_{\frac{1}{2}G}$ of wild type and pwA_{132} are not significantly different. Within the resolution of our present experimental techniques, we found no significant change in either the activation kinetics or the voltage sensitivity of the Ca channels remaining in the leaky mutant pwA_{132} although the Ca conductance is clearly reduced to less than half. While one can contrive more complicated alternatives, the simplest explanation would be a reduction in the number of functional Ca channels by the pwA_{132} mutation and a complete lack of them in pwA_{500}. This could mean that the *pwA* gene product is required for the production, assembly or maintenance of

normal Ca channels but does not affect the functions of assembled channels. This role of the *pwA* gene product fits well the observation that the phenotypic changes after abrupt temperature changes of the leaky mutant require a growth period (Satow, Chang & Kung, 1974). Berger (1976) showed that a wild-type cytoplasmic factor confers excitability to pawn A. This factor may be unassembled channels or a diffusible cofactor coded by the *pwA* locus required for Ca-channel function.

We have further investigated the possibility that the *pwA* product may affect other channels besides the voltage-sensitive Ca channel. The resting permeabilities of the mutants are normal since the resting membrane potential and the resting membrane resistance of pwA_{132} and pwA_{500} are the same as those of the wild type (Satow & Kung, 1976*b*). The inward currents through the anomalous-rectification channels are also similar in the wild type and the mutants (Figs. 4–6). Thus, there is no evidence for the *pwA* mutations affecting any conductances within the time scale of the transient inward current other than the Ca conductance. Significant differences in the slow outward current developing in seconds after the onset of depolarizations (Figs. 8, 9) will be discussed below.

The *pwA* mutant series is important in our future research since it provides the proper null control for experiments concerning excitation and excitation-related effects. pwA_{500} will be especially useful since it is a complete pawn mutant and has no detectable effects on other channel functions. *pwB* mutations, on the other hand, appear to be complicated since they affect the anomalous-rectification channel (Schein *et al.*, 1976) and non-K leakage current (Satow, 1979), they have an additional phenotype of being K^+ resistant (C. L. Shusterman & C. Kung, unpublished observations; Shusterman, Thiede & Kung, 1978) and show phenotypic reversion (Eckert & Brehm, 1979; T. Hennessey & C. Kung, unpublished observations), although they also have reduced or deleted Ca-channel activities.

Ca-induced K^+ current

It has been reported in molluscan neurones that the delayed outward current consists of two types of K^+ current; one voltage dependent and the other Ca dependent (Thompson, Aldrich & Getting, 1978). As described in the Introduction, there is evidence for both the voltage-dependent K conductance and the Ca-dependent K conductance in *Paramecium* (Satow, 1978*a*; Brehm *et al.* 1978). The latter is supposed to be induced by a high concentration of internal Ca^{2+} which enters during excitation. Therefore, it would be reasonable to expect that the Ca-induced K^+ current is small or missing in the pawn mutants. Since pwA_{500} shows no transient inward current upon membrane depolarizations (Fig. 1, Table 1), we attribute the outward currents of pwA_{500} to the voltage-sensitive channel and the leakage channel. While these currents rise to levels comparable to or slightly lower than those of the wild type within some 100 ms upon moderate to strong depolarizations, they then decay rather than stay steady or increase, as in the wild type (Figs. 7, 9). This decay describes the inactivation of the voltage-sensitive K conductance, following the above argument. It is probably a secondary effect of the mutational loss of Ca channels and not an independent effect of the mutation on the delayed rectification channel. The difference between the outward currents in the wild type and in pwA_{500} in response to the same depolarizations, as shown in Fig. 9C, is now assigned to the Ca-induced outward cur-

rents. These currents are probably carried largely by K^+ as deduced from the experiments shown in Fig. 6 where the 'tails' trailing the outward currents have a reversal potential close to E_K, although the Ca-induced currents are only parts of the total currents. (The tail currents from pwA_{500} similarly treated also have a reversal potential close to E_K, even though we believe that this mutant is lacking the Ca-induced outward current. This is probably because the currents remaining in this mutant are K^+ currents, although they are voltage-dependent instead of Ca-induced.) The reversal potentials of tail currents in 4 mM-K solution after 25 ms hyperpolarization from a holding potential of -20 to -25 mV were demonstrated by Oertel *et al.* (1978) to be close to E_K in *P. tetraurelia*. The reversal potentials of the tail currents after 720 ms depolarization from the holding potential of -30 to -35 mV (Fig. 6), as well as those associated with anomalous rectification (Fig. 4) in our studies, are more negative than the E_K established by Oertel *et al.* (1978). The reason for this departure is not fully understood although it may be related to the fact that the resting potential of the paramecia used in this study is consistently higher (-30 to -35 mV in the Ca-K solution, Table 1) than that observed by Oertel *et al.* (1977, 1978) (-20 to -25 mV in a similar solution).

The Ca-induced K^+ current, isolated from the voltage-induced K^+ current and the leakage current, has kinetics of hundreds of milliseconds to seconds (Fig. 9, see Y. Satow & C. Kung, in preparation, for details). Such slow kinetics are in contrast with those of the voltage-induced events in time scales of milliseconds or tens of milliseconds. This large difference in time scales explains why the Ca-induced K^+ current is largely not involved in early events in excitation. The slowly rising and falling Ca-induced K^+ current may be related to electrical events and behaviour of similar time scale such as the prolonged plateau depolarizations (Naitoh & Eckert, 1968*b*; Satow & Kung, 1974) and the continuous ciliary reversal (Jennings, 1906; Dryl, 1973) in *Paramecium*.

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