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Light Activated Electrogenic Na^+-Ca^{2+}-Exchange in Fly Photoreceptors: Modulation by Na^+/K^+-Pump Activity*

E. Armon and B. Minke

Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

Abstract. We found recently that illumination of white-eyed *Musca* photoreceptors following application of ruthenium red (RR), (a known inhibitor of Ca^{2+}-uptake into intracellular organelles) induced a transient post-illumination-afterdepolarization (TA). We found that the TA depended strongly on Na^+- and Ca^{2+}-gradients across the cell membrane suggesting that it arose from activation of electrogenic Na^+-Ca^{2+}-exchange. Since the magnitude of the TA was dependent on the Na^+-gradient, in this work we studied the effects of the Na^+/K^+-pump, which controls the Na^+-gradient, on the TA. Increasing extracellular K^+-concentration which largely increased pump activity also increased the TA. Application of 100 μM ouabain, for a short time, depressed the TA considerably without causing any noticeable effect on the resting or the receptor potential. Only after longer exposure to ouabain, when the TA was totally depressed, were the resting and receptor potentials abolished by only a few strong light flashes. Trains of strong light pulses either facilitated or inhibited the TA depending on the degree of increase in internal Ca^{2+}-ions by light and on the activity of the Na^+/K^+-pump.

Key words: *Musca* photoreceptors – Ruthenium red – Ouabain – Na^+- and Ca^{2+}-gradients

Introduction

A great deal is known today about the existence and characteristics of Na^+-Ca^{2+}-exchange mechanisms in a variety of tissues (for a detailed review, see Requena and Mullins 1979).

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The main characteristics of the Na^+-Ca^{2+}-exchange, as seen in the squid axon are as follows: under physiological conditions the Na^+-gradient across the cell membrane is used to extrude Ca^{2+} from the cell. Since 3–5 Na^+-ions are exchanged for one Ca^{2+}-ion, this mechanism is electrogenic and therefore depends on the membrane potential, however, this predicted electrogenicity has not been directly demonstrated. Studies of Ca^{2+}-fluxes in squid axon indicated that the factors which determine the direction of net flux of Ca^{2+} either into or out of the axon are the Na^+- and Ca^{2+}-gradients across the membrane and the membrane potential (Requena and Mullins, 1979). If the energy available in the Na^+-gradient across the membrane is to energize the efflux of Ca^{2+}, then:

$$rzF\ (E_{\mathrm{Na}}-E_m) \geqslant 2F(E_{\mathrm{Ca}}-E_m) \tag{1}$$

when r is the coupling ratio Na^+/Ca^{2+}, E_{Na}, and E_{Ca} are the Na^+- and Ca^{2+}-equilibrium potentials, E_m is the membrane potential, z is the ion valency and F is the Faraday constant (Mullins 1977). If $r = 4$, Eq. (1) can be rewritten in the form:

$$[Ca^{2+}]_0\ [Ca^{2+}]_i^{-1} = [Na^+]_0^4\ [Na^+]_i^{-4} \exp\ \{-2\ E_mF/RT\} \tag{2}$$

where $[Ca^{2+}]_0$, $[Na^+]_0$, $[Ca^{2+}]_i$, $[Na^+]_i$ are the extracellular and intracellular Ca^{2+}- and Na^+-concentrations respectively, R is the gas constant and T is the absolute temperature. Equation (2) shows that when $[Ca^{2+}]_0$ and $[Na^+]_0$ are constant (under physiological conditions), $[Ca^{2+}]_i$ depends strongly (to the 4th power) on $[Na^+]_i$. Experiments conducted by Lisman and Brown (1972) in the *Limulus* ventral photoreceptors were consistent with Eq. (2). Injection of Na^+-ions into *Limulus* ventral photoreceptor greatly increased the intracellular Ca^{2+}-level as evidenced by the large reduction in sensitivity to light. However, at very low $[Ca^{2+}]_0$ injection of Na^+ did not increase $[Ca^{2+}]_i$, and reduction in sensitivity to light was not observed.

In photoreceptors, illumination is known to increase intracellular $[Ca^{2+}]$ (Brown and Blinks 1974). During anoxia the increase in internal $[Ca^{2+}]$ is very large (Lo et al. 1980).

We have found recently (Minke and Armon – manuscript submitted for publication) that a very pronounced electrical response can be induced in intact fly receptors by an increase in intracellular $[Ca^{2+}]$ produced by illumination following anoxia. The electrical response, which was manifested as a transient post illumination afterdepolarization (TA), could also be induced by light following treatment with ruthenium red (RR), a known inhibitor of Ca^{2+}-uptake by intracellular organelles (Alnaes and Rahamimoff 1975). The strong dependence of the TA on Na^+- and Ca^{2+}-gradients across the cell membrane, which was much stronger than the dependence of the receptor potential on the above parameters, suggested that the TA arose from an electrogenic Na^+-Ca^{2+}-exchange. The TA seemed to be activated by a large increase in internal $[Ca^{2+}]$ during illumination which could not be removed fast enough due to anoxia or RR.

Equation (2) shows that the Na^+-Ca^{2+}-exchange depends strongly on the Na^+-gradient and hence on the Na^+/K^+-pump activity which controls the internal $[Na^+]$. The main aim of this work is to examine the effect of Na^+/K^+-pump activity on the TA in order to provide further evidence for the hypothesis that the TA arises from activation of electrogenic Na^+-Ca^{2+}-exchange.

Methods

Isolated eyes of white-eyed *Musca* were superfused with oxygenated fly Ringer's solution during intracellular recordings from R1-6 photoreceptors. The Ringer's solution contained in mM: 2 or 10 KCl, 0.8 $CaCl_2$, 20 trehalose; 120 NaCl, 2 $MgCl_2$, 5 glucose, 148 sucrose, and 5 HEPES buffer adjusted to pH 7 with NaOH. The details of this technique are described elsewhere (Minke 1982).

Diffuse illumination was provided by a Xenon light source (XBO) combined with a 550 nm narrow-band interference filter and neutral density filters. The unattenuated 550 nm light intensity at the level of the preparation was 1.2×10^{16} photons/cm^2/s.

Results

Induction of the TA by Light After Application of RR

In order to induce the TA it appears to be necessary to increase $[Ca^{2+}]_i$ appreciably without reducing the Na^+-gradient. Figure 1 shows the effect of adding 15 μM RR for 30 s to the perfusate. The upper trace was recorded shortly (1 min) after RR was added and while a normal receptor potential was still observed. The lower trace was recorded 5 min later in response to the same light stimulus. The receptor potential in this record shows a large increase in transient to steady state ratio – indicative of an increase in $[Ca^{2+}]_i$ in the fly (Muijser 1979). The TA is clearly observed in the lower trace as a slow transient post illumination after depolarization.

Although the RR was applied for only 30 s, its effect was not reversible and persisted for hours. The decaying phase of the TA shows typically relatively slow and then faster decaying phases followed, in many cases, by a hyperpolarization. We interpret the faster decaying phase as a manifestation of Na^+/K^+-pump activity which is activated by the increase in $[Na^+]_i$ due to Na^+-Ca^{2+}-exchange. The hyperpolarizing phase is typical of electrogenic Na^+/K^+-pump activity (Koike et al. 1971; Brown and Lisman 1972).

Figure 2A shows the dependence of the TA on light intensity. Here and in the rest of the figures, a pulse of 3 μM RR for 30 s had previously been applied.

The figure shows that the TA has a threshold – it appears only above a certain light intensity and it shows a large facilitation (third and fourth lines).

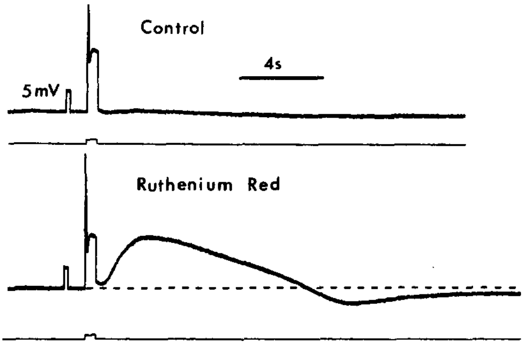

Fig. 1. The post illumination transient afterdepolarization (TA) can be induced by an increase in intracellular [Ca^{2+}] produced by ruthenium red (RR). The upper trace shows the receptor potential in response to a light stimulus of maximal intensity attenuated by 1.0 log unit. One minute before the trace was recorded 15 μM RR was applied for 30 s. The lower trace shows the response to the same stimulus in the same cell recorded five minutes later. In this trace, the effects of the RR were: (*a*) an increase in the transient to steady state ratio of the receptor potential and (*b*) induction of a pronounced TA. This TA presumably arises from an electrogenic Na^+-Ca^{2+}-exchange mechanism (Minke and Armon manuscript submitted for publication)

The figure also shows that when a weak short light stimulus did not induce a TA, repeating the same stimulus resulted in a TA which showed pronounced facilitation (third row). Thus the TA amplitude and duration depend on the amount and history of illumination. Figure 2B shows in the RR treated eye that the dependence of the TA on the amount of light is not a simple one: the upper trace (Fig. 2B) shows that 3 s of light stimulus did not induce a TA while separation of the long stimulus into three shorter stimuli with dark intervals between them did induce a TA.

The Effect of Modulating Na^+/K^+-Pump Activity on the TA

It is well known that an increase in extracellular K^+-concentration ($[K^+]_0$) enhances the activity of the Na^+/K^+-pump (Lindenmayer et al. 1974; see also Koike et al. 1971; Brown and Lisman 1972). In Fig. 3, we show the effect of elevating the $[K^+]_0$-level in the RR treated eye from 2 mM to 10 mM. Although the resting and peak receptor potential were not affected in this case, the amplitude of the TA increased approximately three fold, two minutes after $[K^+]_0$ was elevated. The increase in the amplitude of the hyperpolarizing phase after the TA of Fig. 3, at 10 mM $[K^+]_0$ is a direct indication of higher activity of the Na^+/K^+-pump than at 2 mM $[K^+]_0$. The increase in the TA amplitude when $[K^+]_0$ was raised was not reversible. The amplitude of the TA remained high even after the $[K^+]_0$-level was reduced to 2 mM. We have no simple explanation for this reproducible observation. The enhancement of the TA by elevating $[K^+]_0$ was observed even in those experiments when elevation of $[K^+]_0$ resulted in depolarization and reduction in the amplitude of the receptor potential.

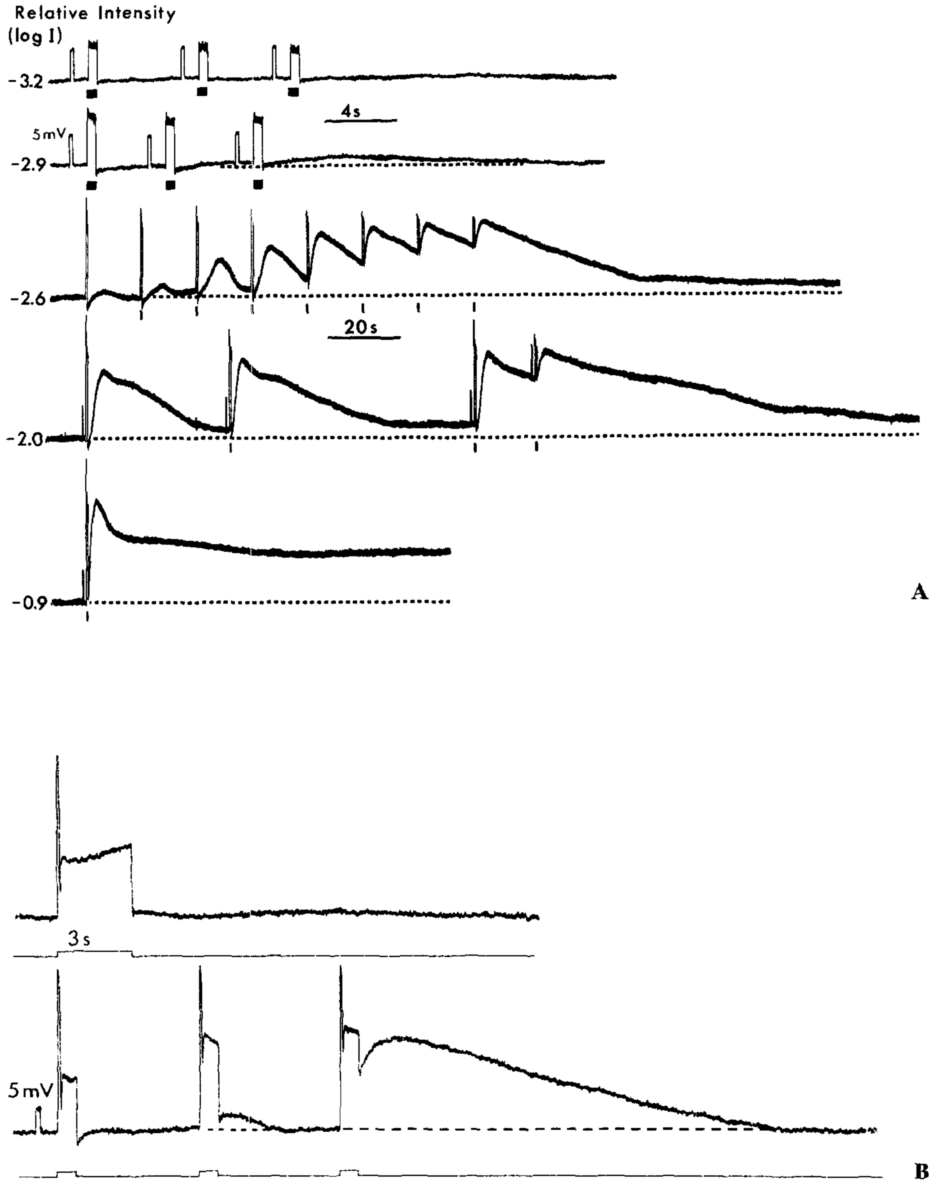

Fig. 2. A The TA has a threshold and shows a pronounced facilitation. The figure shows the dependence of the TA on light intensity as indicated on the left by a log scale. When the stimulus was too weak to induce a TA, repeating the same stimulus resulted in a very pronounced TA. Thus the TA depends on both the amount and the history of illumination. Note a change in time scale in the lower three traces. At the beginning of the experiments of this and the following figures a 30 s pulse of 3 μM RR was applied allowing TA induction for hours; **B** The TA does not depend on the amount of light in a simple manner. The upper trace shows a receptor potential in response to a 3 s light pulse of maximal intensity attenuated by 1.0 log unit. The same light intensity was used to induce the responses of the lower row of the figure, and the responses presented in the rest of the figures. In the lower row, the light stimulus was divided into three shorter pulses with dark intervals between them. In this paradigm of stimulation a clear TA was induced

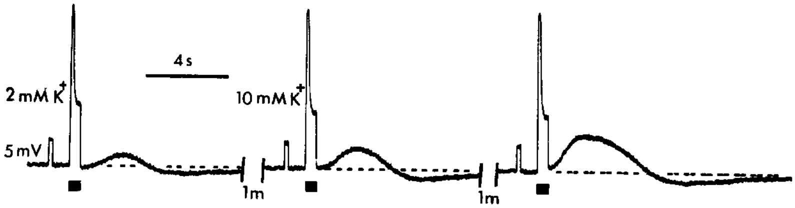

Fig. 3. An increase in extracellular [K$^+$]-level which increases Na$^+$/K$^+$-pump activity also increases the amplitude of the TA. All responses are from the same cell. This figure shows that when [K$^+$]$_0$-level is elevated from 2 to 10 mM the amplitude of the TA increases while the receptor potential does not change. The increase in TA amplitude at elevated [K$^+$]$_0$ was not reversible

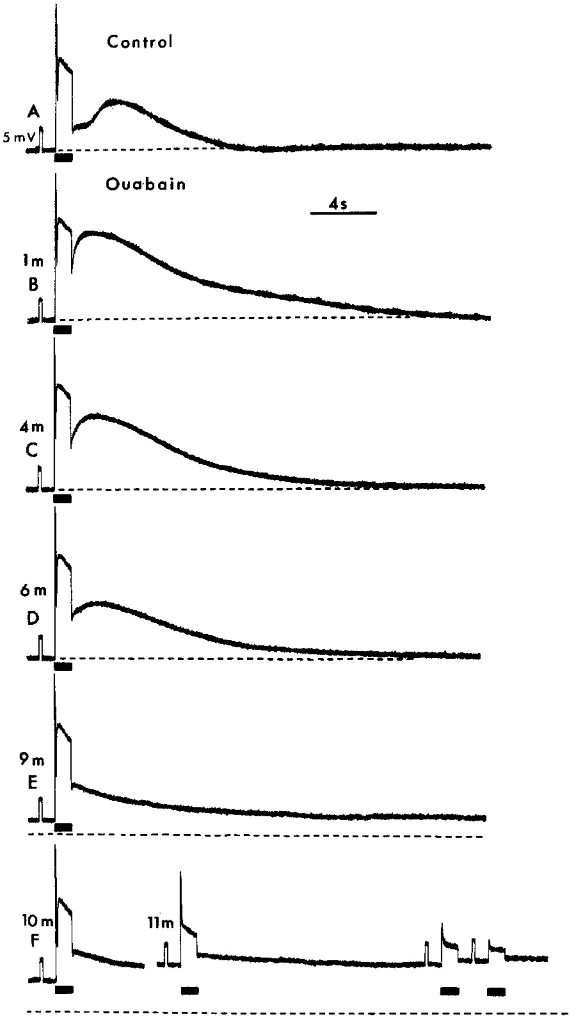

Fig. 4. The TA is very sensitive to changes in the activity of the Na$^+$/K$^+$-pump following ouabain application. Trace A shows the control before ouabain was added. One min after 100 μM ouabain was added to the perfusate the TA was much enhanced. The peak amplitude then slowly declined with time until after 9 min no peaked TA followed the immediate response. The modulation in the TA amplitude (traces B–D) was not accompanied by changes in the resting or the receptor potential, indicating a higher sensitivity of the TA than of the receptor potential to changes in Na$^+$-gradient. The afterpotential in traces E–F is not a TA, but rather a depolarization produced, presumably, by an increase in [K$^+$]$_0$-level. When the resting potential (dotted line) started to decline, a few light pulses were sufficient to suppress the receptor potential

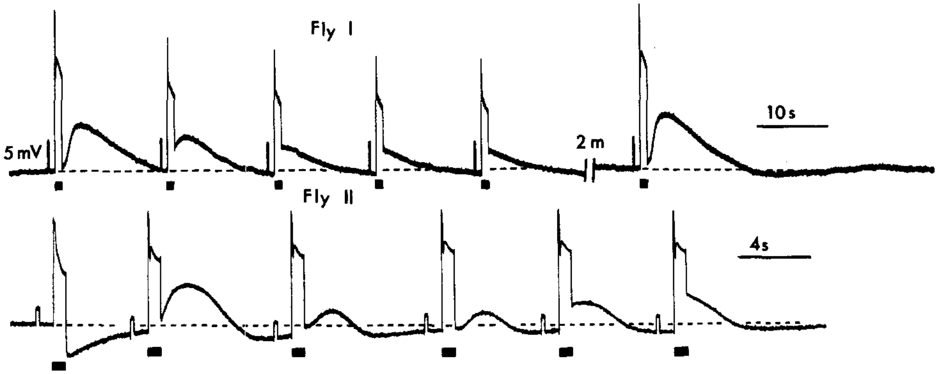

Fig. 5. The TA can be reversibly suppressed by a train of strong light pulses. A train of light pulses depressed the TA, possibly due to inhibition of the Na$^+$/K$^+$-pump by elevated [Ca^{2+}]$_i$ in a cell with initially a "weak" Na$^+$/K$^+$-pump. In the lower row an initial facilitation was changed into inhibition of the TA in another fly. The lack of TA facilitation in cells with weak pump activity suggests that the facilitation of the TA is related to Na$^+$/K$^+$-pump activity

In Fig. 4 the effects of 100 μM ouabain are examined. Figure 4A is a control, before application of ouabain. Shortly after adding the ouabain to the perfusate the TA amplitude was increased (Fig. 4B). This TA enhancement is consistent with the phenomenon of ouabain enhancement of pump activity in muscles at low concentrations (Brown 1966). Only after longer exposure to ouabain, when the concentration of ouabain in the vicinity of the cell had presumably increased, did the TA amplitude start to decline, but without any noticeable reduction in either the resting or the receptor potential (Fig. 4C and D). This fact clearly demonstrated the different dependence of the receptor potential and the TA on the reduction in Na$^+$-gradient. After longer exposure to ouabain a reduction in resting and receptor potential amplitudes was observed (Fig. 4E and F). At this stage, when the Na$^+$/K$^+$-pump was presumably completely inhibited, additional light pulses of the same intensity reduced the resting potential and the receptor potential in a staircase fashion, suggesting that the Na$^+$-gradient across the cell was almost completely abolished (Fig. 4F). Figure 4F thus convincingly demonstrates that when the Na$^+$/K$^+$-pump is completely inhibited, a few strong light pulses are sufficient to abolish the Na$^+$-gradient across the cell.

In Fig. 4E and F, an afterpotential with only a monotonically decaying phase is observed. This decaying afterpotential does not seem to be a TA since it was not as sensitive as the TA to reduction in [Na$^+$]$_0$. We consistently observed this phase in deteriorated cells just before they lost their light response. Therefore it seems possible that this phase arises from transient accumulation of K$^+$-ions in the extracellular space following illumination in the presence of ouabain. The appearance of this phase then suggested partial pump inhibition.

Figure 5 shows the effect of trains of strong light pulses on the TA in two different flies. The upper row shows that, in contrast to Fig. 2, the TA did not show facilitation but rather an inhibition by the successive light pulses. Also the monotonically decaying afterpotential appeared in the last responses. The difference between Figs. 2 and 5 may arise from differences in the efficiency of the Na^+/K^+-pump which is known to be inhibited by Ca^{2+}-ions. Fig. 5 bottom shows that facilitation and inhibition of the TA can sometimes be observed in the same cell. The effects of repetitive stimulation were completely reversible after short (1 min) dark time.

Discussion

In a well oxygenated perfusate the TA was not observed unless we added RR. The reason for this might be that usually the increase in $[Ca^{2+}]_i$ following illumination was quickly reduced by metabolically dependent mechanisms. Our results are consistent with the hypothesis that when RR was added to the medium, and these mechanisms were inhibited, $[Ca^{2+}]_i$ increased to a high enough level to activate Na^+-Ca^{2+}-exchange resulting in the TA. Our results can be conveniently explained by equation (2): In physiological conditions the Na^+/K^+-pump determines the Na^+-gradient which in turn imposes a very low concentration of $[Ca^{2+}]_i$ through the Na^+-Ca^{2+}-exchange and other mechanisms. An increase in $[Ca^{2+}]_i$ during illumination, if the Na^+-gradient is high, should result in Ca^{2+}-extrusion and Na^+-uptake until Eq. (2) is satisfied. However, illumination not only increases $[Ca^{2+}]_i$, it also increases $[Na^+]_i$, and to a large degree (see Fig. 4F). This is probably why the TA shows a rising phase at the cessation of the light. The increase in $[Na^+]_i$ during illumination initially inhibits the Na^+-Ca^{2+}-exchange. However, the increase in $[Na^+]_i$ activates the Na^+/K^+-pump, and only after sufficient Na^+-gradient is created, is the Na^+-Ca^{2+}-exchange activated and the TA produced. Figure 2B supports this interpretation: The long light pulse (upper trace) increased both $[Na^+]_i$ and $[Ca^{2+}]_i$, so that the Na^+-Ca^{2+}-exchange was inhibited and no TA was observed. However, when the long stimulus was separated into three shorter pulses the $[Ca^{2+}]_i$-level did not change much during the dark intervals, so that the $[Ca^{2+}]_i$-level was similar at the end of the long stimulus and after the third short stimulus. However, the $[Na^+]_i$-level was reduced considerably during the dark interval in the lower trace due to activation of the Na^+/K^+-pump which was also active during the dark intervals. This fact is manifested by the increase in the steady state phase of the second and third receptor potentials. The TA declining phase can be explained by a reduction in $[Ca^{2+}]_i$ on the one hand and by an increase in $[Na^+]_i$ during the exchange on the other hand. This increase in $[Na^+]_i$ activates the Na^+/K^+-pump, speeding up the repolarization.

Our present study together with a previous study (Minke and Armon, submitted) shows that the TA is induced only under conditions when the cell is loaded with Ca^{2+} due to illumination in the presence of RR. However, we found several phenomena which cannot be explained by an increase in $[Ca^{2+}]_i$ alone. A modulation of $[Na^+]_i$ by the Na^+/K^+-pump was also needed to explain the above phenomena; Thus, (*a*) the induction of the TA was possible only when sufficient Na^+-gradient was produced by the activity of the Na^+/K^+-pump; (*b*) the observed facilitation of the TA (Fig. 2) could only be observed when the Na^+/K^+-pump was not inhibited (Fig. 5); and (*c*) the peculiar shape of the TA, mainly its slow time course, also requires the involvement of Na^+/K^+-pump activity in order to be explained.

Thus it appears that a very delicate balance between an increase in $[Ca^{2+}]_i$ and changes in $[Na^+]_i$ induced by light and by the Na^+/K^+-pump determines the TA waveform and characteristics. This is fully in accord with the known characteristics of Na^+-Ca^{2+}-exchange.

In conclusion, in spite of the lack of direct measurements of Ca^{2+}-ions, the observed complicated phenomenology of the TA and the possibility to account for it, at least qualitatively, by a single known mechanism strongly support our suggestion that the TA arises from activation of an electrogenic Na^+-Ca^{2+}exchange mechanism.

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