Biophys. Struct. Mechanism 5, 223–230 (1979)

Biophysics of Structure and Mechanism

The Effects of Mn^{2+} and Ca^{2+} on the Prolonged Depolarising After-Potential in Barnacle Photoreceptor*

C. Shaw, M. Hanani, and P. Hillman

Institute of Life Sciences, Neurobiology Unit, Hebrew University of Jerusalem, Jerusalem, Israel

Abstract. We have studied the effects on the PDA of modifying intracellular and extracellular concentrations of Ca^{2+} and Mn^{2+}. The effect of decreased Ca^{2+} concentration or addition of EGTA is mainly an increase in the PDA amplitude and length. Raising Ca^{2+} concentration using ruthenium red or high external Ca^{2+} has the opposite effect. The effect of Mn^{2+} is much more striking: In the presence of 50–100 mM Mn^{2+} the PDA is initially greatly depressed but can rise slowly for up to 20 or 30 s (in the dark) until it approaches its original amplitude and time course. Bridge measurements showed that the depression of the PDA corresponds to a depressed conductance and so is not due to an increase in K^+ conductance. The Mn^{2+} effect is potentiated by decreased Ca^{2+}. Appropriate stimulation suppresses the rising PDA as promptly as it does a normal PDA, suggesting that if lateral diffusion is the source of the slow rise, the PDA and PDA-depressing processes must be spatially linked. The action of the anti-PDA is apparently prolonged by both Ca^{2+} and Mn^{2+}.

Key words: Prolonged depolarising after-potential (PDA) – Visual pigment – Invertebrate photoreceptor – Manganese – Calcium.

Introduction

The ionic basis of the light response of the barnacle photoreceptor is now largely understood. Both the stimulus-coincident late receptor potential (LRP) and prolonged depolarising after-potential (PDA) arise from an increase in g_{Na} (Brown et al., 1970; Brown and Cornwall, 1975). In addition an early, transient, negative-going potential, the "dip", seems to be an increase in g_K (Hanani and Shaw, 1977). Calcium appears to reduce g_{Na} of the LRP and may mediate the increase in g_K (Brown et al., 1970; Hanani and Shaw, 1977).

* Based on material presented at the European Neurosciences Meeting, Florence, September 1978

0340-1057/79/0005/0223/$ 01.60

We have recently been investigating various aspects of the PDA and anti-PDA. The effects of Ca^{2+} seemed to be of particular interest, since Ca^{2+} may intermediate the anti-PDA and since it has a strong effect on the LRP as well as on facilitation and adaptation in the barnacle (Hanani and Hillman, 1976). A second divalent ion, Mn^{2+}, was also tested as a comparison, but turned out to have a more striking effect than the Ca^{2+}. We describe the effects of Ca^{2+} and Mn^{2+} on the PDA and on the anti-PDA and the effects of competition between the two ions.

Methods

The lateral ocelli of *Balanus amphitrite* were excised along with a short section of nerve and placed in a perfusion chamber. Details of this preparation have been described previously (Hochstein et al., 1973; Hanani and Hillman, 1976; Hanani and Shaw, 1977). The chamber was perfused with sea water or with barnacle Ringer (Brown et al., 1970) with Ca^{2+} and Mn^{2+} in various concentrations and to which ruthenium red (5–10 μM) could be added. Ruthenium red appears to increase Ca^{2+}_{in} by preventing uptake by internal stores (Moore, 1971). Calcium-sequestering agent EGTA was iontophorized intracellularly from electrodes with 1.5 mM EGTA concentrations. Intracellular recordings were made using 4 M potassium acetate electrodes. Light stimuli were presented via a fiber optic from a quartz-iodide source with unattenuated intensity of about 1×10^{16} photons $cm^{-2} \cdot s^{-1} \cdot nm^{-1}$ at 550 nm at the photoreceptor. "Blue" and "red" lights were obtained with Balzers broad band interference filters K3 and K5, passing totals of about 12×10^{16} and 50×10^{16} photons $cm^{-2} \cdot s^{-1}$ at the photoreceptor with peak wavelengths of about 490 and 600 nm respectively. Since, in the barnacle, the metarhodopsin absorption spectrum is blue-shifted with respect to the rhodopsin spectrum, a blue or white light puts most of the pigment into the rhodopsin state and red into the metarhodopsin. Thus a red stimulus after blue adaptation transfers most of the pigment from rhodopsin to metarhodopsin, inducing a PDA, while blue-after-red induces an anti-PDA, which either suppresses a PDA or impedes its induction. The amount of pigment transferred by a given red stimulus was determined by the 'half-PDA' method previously described by Hillman et al. (1976). The anti-PDA strength was measured as the percent decrease in the induced PDA amplitude at different intervals after the blue conditioning stimulus which produced the anti-PDA.

Results

1. The Effects of Ca^{2+}

The upper traces in Figure 1 are normal PDAs from three cells. The lower trace of A shows that reducing external Ca^{2+} concentration broadens the early part of the PDA and prolongs it. High Ca^{2+} (not illustrated) does the opposite. Addition of ruthenium red to the medium (lower trace of B) depresses and shortens the PDA. Injection of EGTA (lower trace of C) broadens and prolongs the PDA. These results suggest

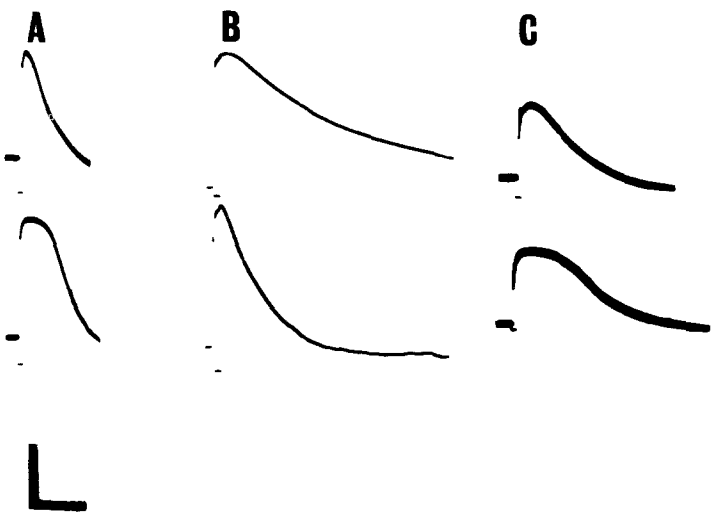

Fig. 1A–C. The effects of Ca^{2+} on the prolonged depolarising after-potential (PDA): All traces in all figures are intracellular recordings at about 22° C from excised lateral photoreceptors of *Balanus amphitrite*. The calibration bars represent 20 mV and 10 s. For all traces in all figures, the cell was adapted to blue light at least 3 min before the start of the trace. The stimulus indicated by the black bars under the response traces was in all cases in this figure *red*, inducing a PDA. The upper traces in this figure are normal PDAs in three cells. The lower traces show the effects of reducing or raising the external Ca^{2+} concentration: **A** low Ca^{2+} of 0.5 mM enhances the amplitude and duration of the PDA. **B** Addition of 10 μM ruthenium red to the medium shortens and lowers the PDA. This treatment presumably raises the internal Ca^{2+} concentration in the cell. **C** Injection of EGTA at 3 nA for 10 min mimics the effect of lowering external Ca^{2+} (**A**). All Ca^{2+} effects were largely reversible

that lowering internal Ca^{2+} concentrations broadens and prolongs the PDA. Similar effects have been seen on the LRP in *Limulus* (Lisman and Brown, 1972).

Small decreases in resting potential were noted (approximately 10 mV) as calcium concentration was increased with ruthenium red (Fig. 1B). In addition, increasing calcium concentration frequently diminished the post-stimulus hyperpolarisation arising from the electrogenic pump.

2. *The Effects of* Mn^{2+}

A previous report by Wulff and Mendez (1973) showed that Mn^{2+} increased the latency of the LRP in *Limulus* retinula cells. Further, Mn^{2+} depressed several components of the LRP. We found similar effects at low Mn^{2+} concentrations (20–30 mM), and, in addition, noted that the LRP continued to rise for several seconds during the stimulus. At higher Mn^{2+} concentrations (50–100 mM) this rise is extended, the latency of the LRP is increased by a factor of 2–3, and the LRP and PDA may be strongly depressed, especially their initial phases. Figure 2 shows these effects. A and B are recordings without and with 50 mM Mn^{2+}. The lower trace of B shows that the LRP is depressed and slowly-rising in Mn^{2+}. The upper trace of B shows that the PDA, starting with an initially depressed LRP, continued to rise in

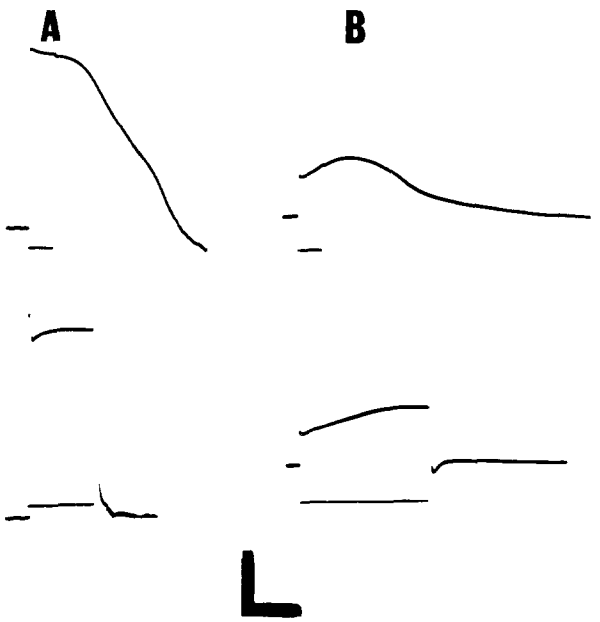

Fig. 2A and B. The effects of Mn^{2+} on the LRP and the PDA: **A** In 0.5 mM Ca^{2+}, a long blue stimulus, giving an LRP (lower trace), and a following red stimulus, giving a PDA (upper trace), are compared to responses to similar stimuli in **B** when 50 mM Mn^{2+} was added to the medium. Calibration: 40 mV/10 s

the dark for about 10 s. (In higher Mn^{2+} concentrations rises of 20–30 s were sometimes seen.) The PDA decline was not consistently affected by Mn^{2+}.

Injection of Mn^{2+} intracellularly through a Mn^{2+} electrode (2 M) for several minutes induced a similar change in the rising phase of the PDA. Mn^{2+} also lowered the cell's resting potential, and lowered, or abolished, the pump. Mn^{2+} also lowered criterion sensitivity by more than 2 log units. The Mn^{2+} effects were completely reversible after washing in normal sea water. We have occasionally seen the same phenomena described above occurring spontaneously, but have little idea of the conditions necessary for their appearance.

The dominant ionic basis of the LRP and the PDA is an increase in g_{Na} (Brown and Cornwall, 1975). We have confirmed with bridge measurements that the initial depression of the LRP and the PDA corresponds to a decreased conductance. Figure 3 shows a rising PDA and a rising LRP in 50 mM Mn^{2+}. As the PDA and LRP rise the resistance decreases (conductance increases) and returns to its original value as the PDA falls or as the LRP stimulus is switched off.

3. Competition between Ca^{2+} and Mn^{2+}

The action of Mn^{2+} is usually prevented by high Ca^{2+}_{out}. Figure 4 illustrates this effect. In high Ca^{2+}_{out} (20 mM), 100 mM Mn^{2+} does not affect the PDA rise time; while in low Ca^{2+}_{out} (0.5 mM) 50 mM Mn^{2+} causes a pronounced initial depression.

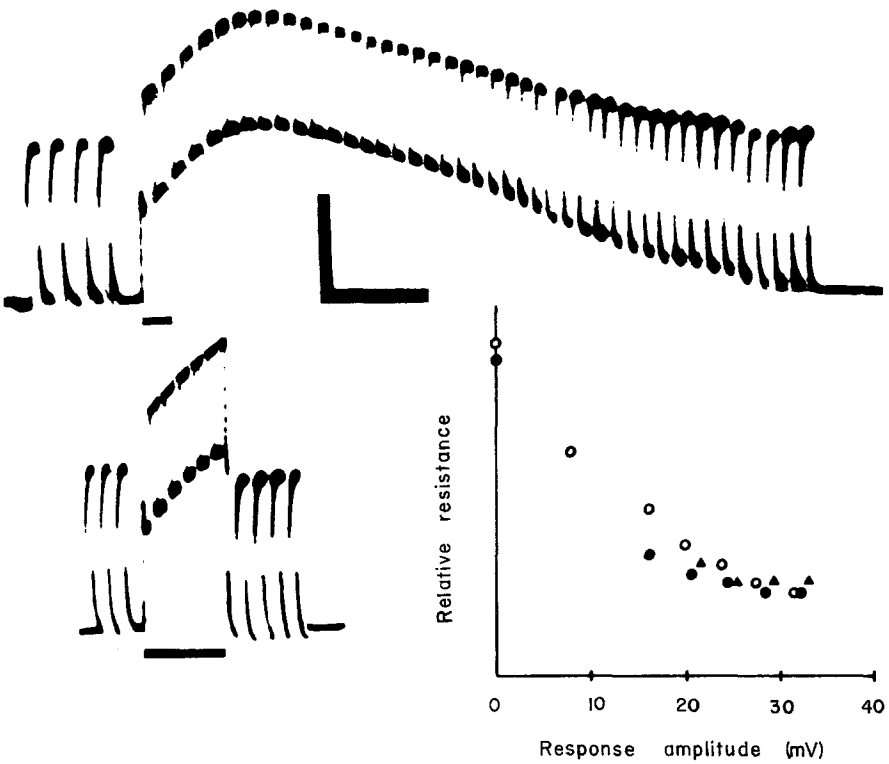

Fig. 3. Bridge measurement of the PDA and LRP in Mn^{2+}: Cell resistance during a PDA (A) and an LRP (B), was measured by passing 3 nA pulses about 0.5 s long through the recording electrode. The graph shows the amplitude of the current responses as a function of amplitude of the LRP (triangles) or of the PDA during its rising phase (filled circles) or falling phase (open circles). Conductance is monotonically correlated with amplitude both of the PDA and of the LRP, so that it is clear that the initial depression of the PDA and the LRP by Mn^{2+} is not primarily due to a K^+ conductance increase. Nevertheless, there is a suggestion of a small discrepancy between the points, suggesting that Mn^{2+} may change the ionic basis of the response a little. Calibration 20 mV/10 s

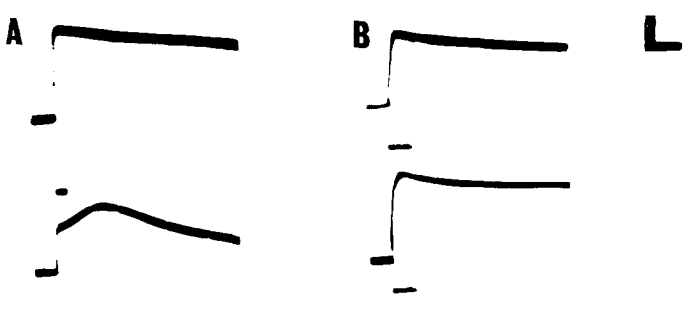

Fig. 4A and B. The competitive effects of Ca^{2+}: **A** The PDA response to red light in 0.5 mM Ca^{2+} sea water. In this cell addition of 50 mM $MnCl_2$ had a clear effect on the PDA (second trace). **B** Raising of the Ca^{2+} concentration to 20 mM without Mn^{2+} (first trace) does not alter the PDA as compared to the first trace of **A.** Raising Mn^{2+} to 100 mM has little or no effect in this Ca^{2+} concentration (second trace). Calibration: 20 mV/5 s

Several experiments suggest the existence of a hysteresis effect. In high Ca^{2+}_{out}, no effect of Mn^{2+} is apparent until Ca^{2+} concentration is lowered. However, if Mn^{2+} is added when Ca^{2+} is low, the effect of Mn^{2+} remains, even if Ca^{2+} is raised to the previously ineffective level.

4. The Anti-PDA and Depression of the PDA

We have investigated the effects of both Ca^{2+} and Mn^{2+} on the anti-PDA (not illustrated). (For details about the anti-PDA see Methods, and Hochstein et al., 1973.) Ruthenium red-induced high internal Ca^{2+} concentration increases the apparent duration of the anti-PDA by a factor of three or four. Similar results were obtained by altering external Ca^{2+} concentration. In principle, however, it is difficult to distinguish between the depressing effects of Ca^{2+} on the PDA and a possible extension of the anti-PDA. This difficulty is less serious with Mn^{2+}. Mn^{2+} clearly depresses the PDA and delays its rise time but appears not to have a drastic effect on its duration. An extension of the apparent duration of the anti-PDA is nevertheless seen, which, though relatively small, may suggest that Mn^{2+} extends the anti-PDA.

The delayed rise of the PDA in Mn^{2+} suggested the possibility of a delayed onset of the depressing effect of the anti-PDA. Figure 5A illustrates the depression of a rising PDA in Mn^{2+}. As in the normal PDA, depression is virtually instantaneous, and there is no further rise in the PDA after depression.

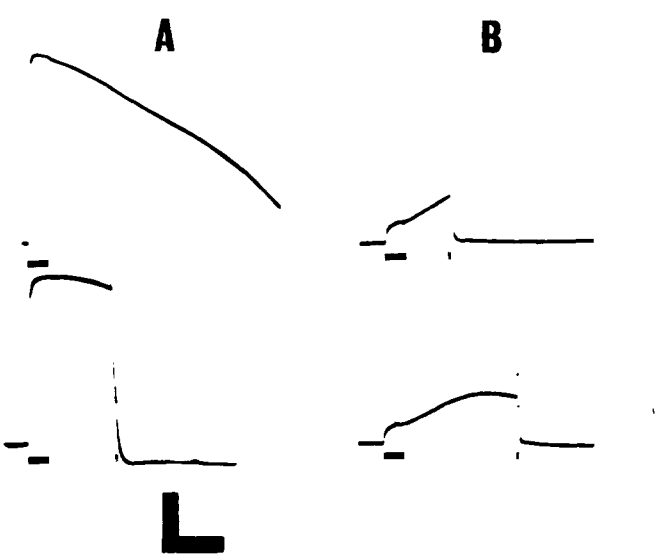

Fig. 5A and B. The anti-PDA and depression of the PDA in Mn^{2+}: **A** In a normal medium, a PDA (first trace) and a PDA depressed by blue anti-PDA-inducing 100 ms light (second trace). **B** in 50 mM Mn^{2+}, the blue light causes an immediate and maintained repolarization to baseline (depression) when presented during the PDA rise (first trace) or at the peak (second trace). Calibration: 20 mV/10 s

Discussion

Several conclusions can be drawn from the current results. Both ions alter, to differing degrees, cell sensitivity, resting potential and pump. The effects of both are largely reversible. They differ, however, in several significant ways. Ca^{2+} has been shown previously to lower g_{Na} of the LRP in barnacle and *Limulus* photoreceptors (Brown et al., 1970; Millechia and Mauro, 1969). Our results show a decrease in PDA amplitude and duration in high Ca^{2+}. Mn^{2+} in other preparations may block slow Na^+ and Ca^{2+} channels (Zipes and Mendez, 1973; Rougier et al., 1968). In *Limulus* photoreceptors Mn^{2+} depresses the LRP and delays its onset and time to peak (Wulff and Mendez, 1973). Mn^{2+} in barnacle acts similarly on the LRP and PDA, but acts further to prolong greatly the rising phases of the LRP during stimulation and the PDA in the dark. It does not drastically affect PDA duration. We conclude that while in many ways similar, the mechanisms of action of the two ions differ substantially. The sites of action, however, must be the same, as indicated by their competitive effects. This is an important point as Ca^{2+} may play a major role in transduction in invertebrate photoreceptors. By understanding where and how Mn^{2+} works, we may better understand the site and action of Ca^{2+} in transduction.

The anti-PDA in the barnacle photoreceptor is not well understood. We know that it is the result of an $M \rightarrow R$ phototransition. Further, it does not directly affect membrane conductance but rather decreases the conductance rise associated with the PDA. The anti-PDA can be studied only by its effect on the PDA. For this reason, changes in the PDA, particularly the delayed-rise PDA in Mn^{2+}, can be useful in further understanding the anti-PDA. Both Ca^{2+} and Mn^{2+} may potentiate the anti-PDA. Mn^{2+}, which delays the LRP onset and slows the LRP and PDA rise times, does not delay the onset of the anti-PDA. Depression of the PDA occurs instantly and completely, and the PDA does not restart in the dark. These results tell us several things. The failure of the rising PDA to restart after a full depression may indicate that the anti-PDA acts at an early stage of the transduction process and not on its expression as a conductance increase. If the anti-PDA acts on some stage that can diffuse in the cell (or membrane), then the anti-PDA must be spatially linked to it since no delay in depression occurs. This would be in apparent conflict with the Hillman et al. (1976) observation of a non-local PDA-anti-PDA interaction, and would tend to support Hamdorf and Razmjoo's (1978) model of the PDA against that of Hochstein et al. (1973); but in fact a non-local range of a few hundred angstroms would satisfy the Hillman et al. result and yet presumably constitute sufficient spatial linking.

The present results suggest several new conclusions about the actions of Ca^{2+} and Mn^{2+} in transduction and help to clarify various aspects of the action of the PDA and anti-PDA.

Acknowledgements. Discussion with Dr. S. Hochstein helped us greatly. Miss Roni Laiwand provided technical help. This work was supported by a grant from the U.S.-Israel Binational Science Foundation (BSF), Jerusalem, Israel.

References

Brown, H. M., Hagiwara, S., Koike, H., Meech, R. M.: Membrane properties of a barnacle photoreceptor examined by the voltage clamp technique. J. Physiol. (Lond.) **208,** 385–413 (1970)

Brown, H. M., Cornwall, M. C.: Ionic mechanism of a quasi-stable depolarization in barnacle photoreceptor following red light. J. Physiol. (Lond.) **248,** 579–593 (1975)

Hamdorf, K., Razmjoo, S.: Photoconvertible pigment states and excitation in *Calliphora*. The induction and properties of the prolonged depolarising afterpotential. Biophys. Struct. Mech. **5,** 137–161 (1979)

Hanani, M., Hillman, P.: Adaptation and facilitation in the barnacle photoreceptor. J. Gen. Physiol. **67,** 235–249 (1976)

Hanani, M., Shaw, C.: A potassium contribution to the response of the barnacle photoreceptor. J. Physiol. (Lond.) **270,** 151–163 (1977)

Hillman, P., Hochstein, S., Minke, B.: Non-local interactions in the photoreceptor transduction process. J. Gen. Physiol. **68,** 227–245 (1976)

Hochstein, S., Minke, B., Hillman, P.: Antagonistic components of the late receptor potential arising from different stages of the pigment process. J. Gen. Physiol. **63,** 105–128 (1973)

Lisman, J. E., Brown, J. E.: The effects of intracellular iontophoretic injection of calcium and sodium ions on the light responses of *Limulus* ventral photoreceptors. J. Gen. Physiol. **59,** 701–719 (1972)

Millecchia, R., Mauro, A.: The ventral photoreceptor cells of *Limulus:* II. A voltage-clamp study. J. Gen. Physiol. **54,** 331–351 (1969)

Moore, C. L.: Specific inhibition of mitochondrial Ca transport by ruthenium red. Biochem. Biophys. Res. Comm. **42,** 248 (1971)

Rougier, O., Vassort, G., Garnier, D., Gargouil, Y-M., Corboeuf, E.: Données nouvelles concernant le rôle des ions Na^+ et Ca^{2+} sur les propriétés electrophysiologiques des membranes cardiaques: existence d'un canal lent. C. R. Acad. Sci. [D] (Paris) **266,** 802–805 (1968)

Wulff, V. J., Mendez, C.: The effect of manganous chloride and tetrodotoxin on *Limulus* lateral eye retinular cells. Vision Res. **13,** 2327–2334 (1973)

Zipes, D. P., Mendez, C.: The action of manganese ions and of tetrodotoxin on atrioventricular nodal transmembrane potentials in isolated rabbit hearts. Circ. Res. **32,** 447–454 (1973)

Received September 25, 1978/Accepted October 5, 1978