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CALCIUM-DEPENDENT ACTION POTENTIALS IN THE SECOND-ORDER NEURONES OF COCKROACH OCELLI

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SUMMARY

The ionic basis of the action potential in the large second-order neurones (L-neurones) of the ocellus of the cockroach, *Periplaneta americana*, was studied. L-neurones generated action potentials, usually once, at the off-set of hyperpolarizing light responses, or at the termination of hyperpolarizing current stimuli. The action potential was blocked by replacing saline Ca^{2+} with Mg^{2+} but maintained when Ba^{2+} was substituted. A block was produced by 2 mmol l^{-1} Cd^{2+} or 20 mmol l^{-1} Co^{2+}. The peak amplitude of the action potential increased by 26 mV for a 10-fold increase in external Ca^{2+} concentration, at concentrations below 1·8 mmol l^{-1}. The action potential was not affected by sodium-free saline or by 3×10^{-6} mol l^{-1} tetrodotoxin (TTX). These observations suggest that calcium ions are the major carrier for the inward current of the action potential. This finding supports the suggestion that the off-set responses of hyperpolarizing visual neurones of both vertebrates and invertebrates have a common ionic mechanism, including voltage-sensitive calcium currents.

INTRODUCTION

Most insects have two or three ocelli in addition to compound eyes. The ocellus contains a large number of photoreceptors that converge onto a few large second-order neurones (L-neurones). The L-neurones extend dendritic branches in the ocellus, and their axons project into the ocellar tract neuropile of the brain (Goodman, 1981). The ocellar L-neurone responds to illumination with a hyperpolarizing potential (Goodman, 1981; Milde, 1981; Mizunami, Yamashita & Tateda, 1982), as do the second-order cells of insects' compound eyes. At the repolarizing phase of the hyperpolarizing response, the cockroach L-neurones generate action potentials (Mizunami *et al.* 1982; Mizunami, Tateda & Naka, 1986). Spike-like events or transient depolarizations have also been observed in the second-order cells of insects' compound eyes (Laughlin, 1973; Zettler & Järvilehto, 1973).

Some visual neurones in both vertebrates and invertebrates generate transient depolarizations or spike-like potentials at the off-set of their hyperpolarizing light

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responses. Vertebrate photoreceptor cells generate transient depolarizations at the off-set of long light stimulation (Baylor & Hodgkin, 1974). These off-set responses become regenerative spikes in the presence of tetraethylammonium (TEA^+) ions, the amplitudes of which depend on external calcium concentrations (Fain, Quandt & Gerschenfeld, 1977). Cornwall & Gorman (1979) found that the off-set responses in scallop hyperpolarizing photoreceptors are similarly based on changes in Ca^{2+} permeability. They proposed that the (hyperpolarizing) photoreceptors of both vertebrates and invertebrates share common ionic mechanisms. This idea was given added support by the observation that the barnacle's second-order neurone generates a transient depolarization at the off-set of the hyperpolarizing light response and that the off-set response is associated with a change in Ca^{2+} permeability (Oertel & Stuart, 1981). However, the ionic mechanisms of the off-set responses of second-order visual neurones in insects have not been established. We have therefore examined the ionic basis of the action potential of cockroach ocellar L-neurones and report that Ca^{2+} is the major carrier of the inward current of the action potential.

MATERIALS AND METHODS

Preparation

All experiments were done on adult male cockroaches, *Periplaneta americana*, reared in the laboratory of Kyushu University. The insect was mounted, dorsal side up, on a Lucite stage and its head was fixed in a Lucite bath with beeswax. The dorsal surface of the head capsule was removed, and the brain was exposed by removing the jaw muscles. The oesophagus was excised and the brain was stabilized by inserting a glass rod into the oesophageal foramen. Care was taken not to disturb the tracheal system. Saline containing 1 % pronase (Sigma, Type IV) was applied to the brain for 2 min, to facilitate insertion of the electrode. The dorsal surface of the brain was desheathed, using a fine tungsten needle, to expose the underlying tissue to the bathing solution. The bath had a volume of about 0·7 ml and the solution was continuously perfused at 2 ml min^{-1}.

Recording

Intracellular recordings were made using glass capillary microelectrodes filled with 2 mol l^{-1} potassium acetate with d.c. resistances of 50–70 MΩ. Electrodes were inserted into the ocellar tract of the brain or the ocellar nerve. The electrodes were connected to a high-impedance, negative-capacity-compensated preamplifier (MEZ-8201, Nihon Kohden, Japan) which had provision for passing constant current through an active bridge circuit. A small piece of platinum in the bathing solution served as an indifferent electrode. The electrical signals were observed on an oscilloscope and also stored in a digital memory (DM-703, Iwatsu, Japan). The stored signals were recorded on a pen recorder and on film. Light stimuli were provided by the beam of a tungsten lamp, focused on the head of the animal. The light intensity was controlled by calibrated neutral-density filters. The recorded cells could be identified as L-neurones by their light responses: in particular (1) large

membrane potential fluctuations in the dark or during dim-light stimuli, (2) hyperpolarizing responses of more than 30 mV to bright light stimuli, (3) one or a few spikes at the off-set of the stimulus (Mizunami *et al.* 1982). In some preparations, the neurones were stained, by injecting cobalt ions through the recording electrode, and identified anatomically (e.g. Fig. 2A). The cobalt-filled preparations were intensified as wholemounts (Bacon & Altman, 1977). Stable recordings could be made for 1–2 h.

Solutions

The ionic mechanism of the action potential was studied by changing the ionic environment and also by observing the effects of tetraethylammonium (TEA^+) ions, tetrodotoxin (TTX), Cd^{2+} and Co^{2+}. The normal physiological saline was modified from that used by Yamasaki & Narahashi (1959), and contained (in mmol l^{-1}): NaCl, 214; KCl, 31; $CaCl_2$, 1·8; Tris-HCl, 10; pH 7·2. Variations in the Na^+ concentration were made by replacing Na^+ with $Tris^+$. High concentrations of Ca^{2+} were obtained by replacing Na^+ with Ca^{2+}. TEA^+, Cd^{2+}, Co^{2+} or TTX were simply added to the saline. Mg^{2+} or Ba^{2+} saline was made by replacing Ca^{2+} with Mg^{2+} or Ba^{2+}. All salines were made up from stock solutions on the day of experiments. Experiments were performed at a room temperature of 20–24°C.

RESULTS

The action potential of the L-neurone

The ocellus of the cockroach contains about 10 000 photoreceptors which have synapses onto four large second-order neurones, the L-neurones (Weber & Renner, 1976; Toh & Sagara, 1984). The L-neurones extend dendritic branches into the ocellus, and their axons project into the ocellar tract neuropile of the brain, through the ocellar nerve (see Fig. 2A). In the ocellar tract neuropile, L-neurones make synaptic contacts with the higher-order neurones (Toh & Hara, 1984; Mizunami & Tateda, 1986).

The L-neurones produced solitary action potentials at the off-set of the hyperpolarizing light response (Fig. 1A). They also produced action potentials, usually once, at the off-set of a hyperpolarizing current pulse (Fig. 1B). Depolarizing current pulses were not effective in eliciting action potentials in L-neurones (Fig. 1B).

The action potential of the L-neurone originates within the ocellar tract of the brain rather than in the ocellus or the ocellar nerve. The L-neurone has an axon 700–900 μm in length, measured from the point where it leaves the ocellus to the proximal end of the ocellar tract (Fig. 2A). Recordings were made from L-neurones at five points along the axons: (1) the point where the axon leaves the ocellus, (2) the medial part of the ocellar nerve, (3) the part where the axon enters the brain, (4) the medial part of the ocellar tract and (5) the proximal terminal of the ocellar tract, as shown in Fig. 2A. Action potentials induced at the off-set of hyperpolarizing current pulses of 8 nA, 500 ms (Fig. 2B) were largest (30–40 mV amplitude) and shortest

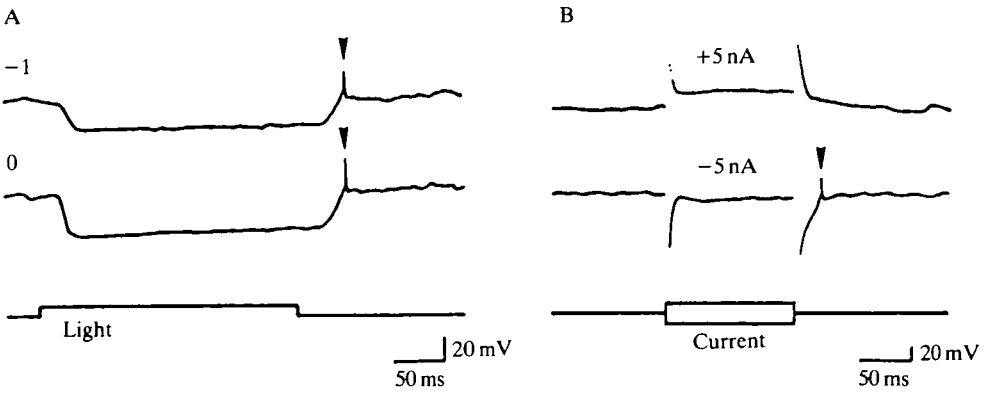
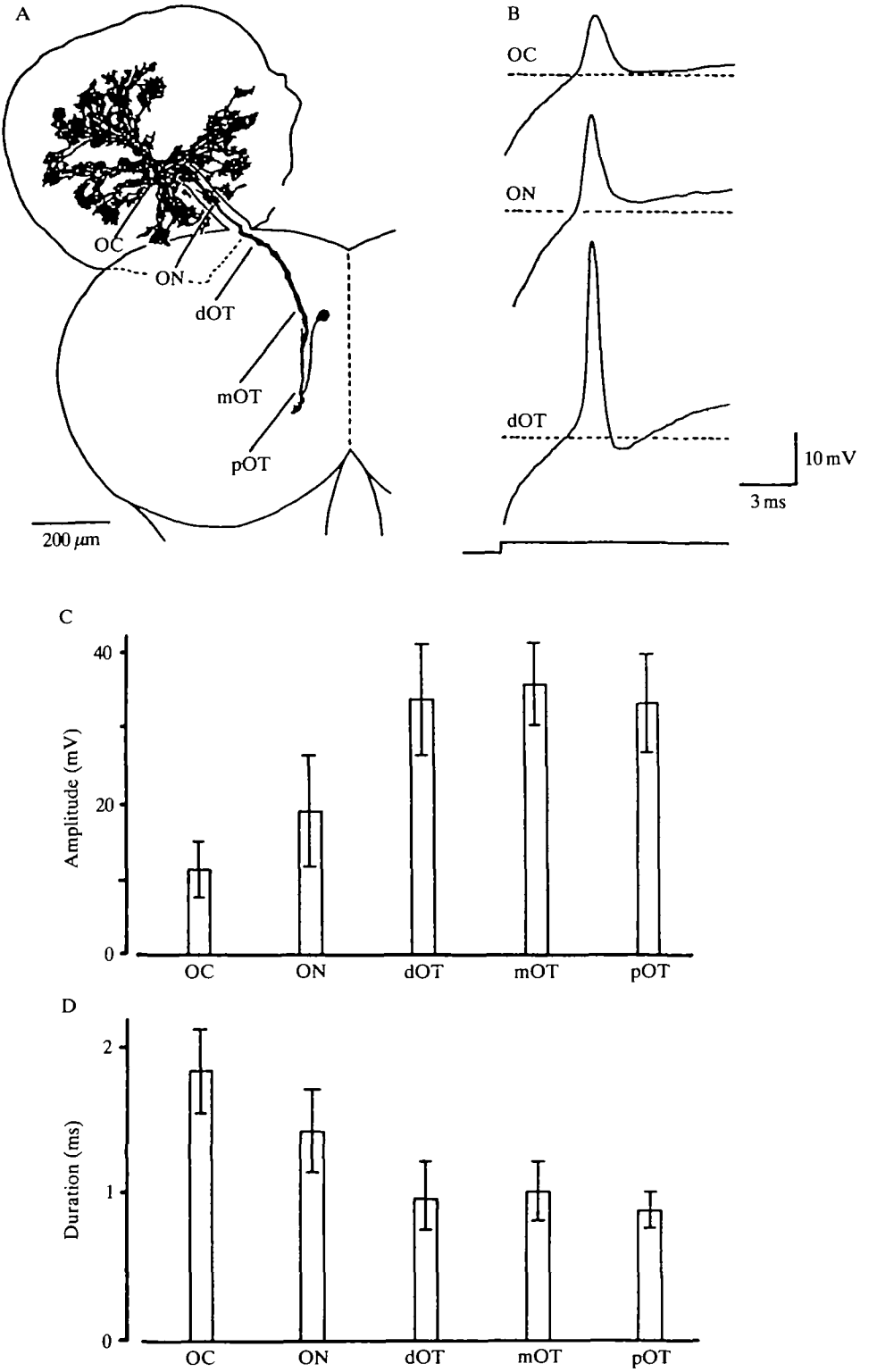


Fig. 1. Action potentials of cockroach ocellar L-neurones. (A) The light responses of an L-neurone. Solitary action potentials are evoked at the off-set of hyperpolarizing responses. The light intensities are shown at the left of each traces as $\log_{10}$ attenuation (0 = 2 lx). (B) The responses of an L-neurone to current pulses. Currents were injected into the neurone from the recording electrode through a bridge circuit. An action potential is evoked at the off-set of a hyperpolarizing current pulse. Depolarizing currents are not effective at eliciting the action potential. A and B are from different L-neurones, recorded in the ocellar nerve.

(0·8–1·2 ms half-width) in the ocellar tract (Fig. 2C). They were smallest and longest in the ocellus (Fig. 2C). There were no significant differences between the different regions of the ocellar tract in either amplitude or duration of the action potentials. These observations suggest that the action potentials of cockroach L-neurones originate in the ocellar tract and spread passively towards the ocellus, as do the action potentials of locust L-neurones (Wilson, 1978).

The amplitude of the action potential changed when the duration or magnitude of the preceding hyperpolarizing current pulses were altered (Fig. 3A). In most cases, as shown in Figs 1 and 2B, solitary action potentials were induced at the off-set of hyperpolarization, but in other cases, as shown in Fig. 3A, an initial large action potential was followed by one or two action potentials with small amplitudes, especially when the preceding hyperpolarizing currents were strong. The amplitudes of the action potentials increased with increasing magnitude (Fig. 3B) or duration (Fig. 3C) of hyperpolarizing current pulses. Increasing the pulse length beyond 100 ms or the pulse magnitude above 6 nA had no further effect on spike amplitude.

Fig. 2. The origin of the action potential in the L-neurone. The action potentials were recorded at five points along the axon; the ocellus (OC), the ocellar nerve (ON), and distal (dOT), medial (mOT) and proximal (pOT) ocellar tract (A). The drawing was made dorsally from a cobalt-filled L-neurone. (B) Examples of action potentials of L-neurones. Dashed lines indicate the resting potential. The amplitudes (C) and the durations (D) of the action potentials recorded at five points along the axon. Averages from 5–8 L-neurones for each point are shown with the standard deviations. The amplitudes, measured from the resting potential, are largest and the durations, the half-widths, are shortest in the ocellar tract, showing that the action potentials originate in the ocellar tract. In all recordings, the action potentials were elicited at the off-set of hyperpolarizing pulses of 8 nA, 500 ms.


A
OC
ON
dOT
mOT
pOT
200 μm
B
OC
ON
dOT
10 mV
3 ms
C
Amplitude (mV)
40
20
0
OC
ON
dOT
mOT
pOT
D
Duration (ms)
2
1
0
OC
ON
dOT
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pOT

Fig. 2

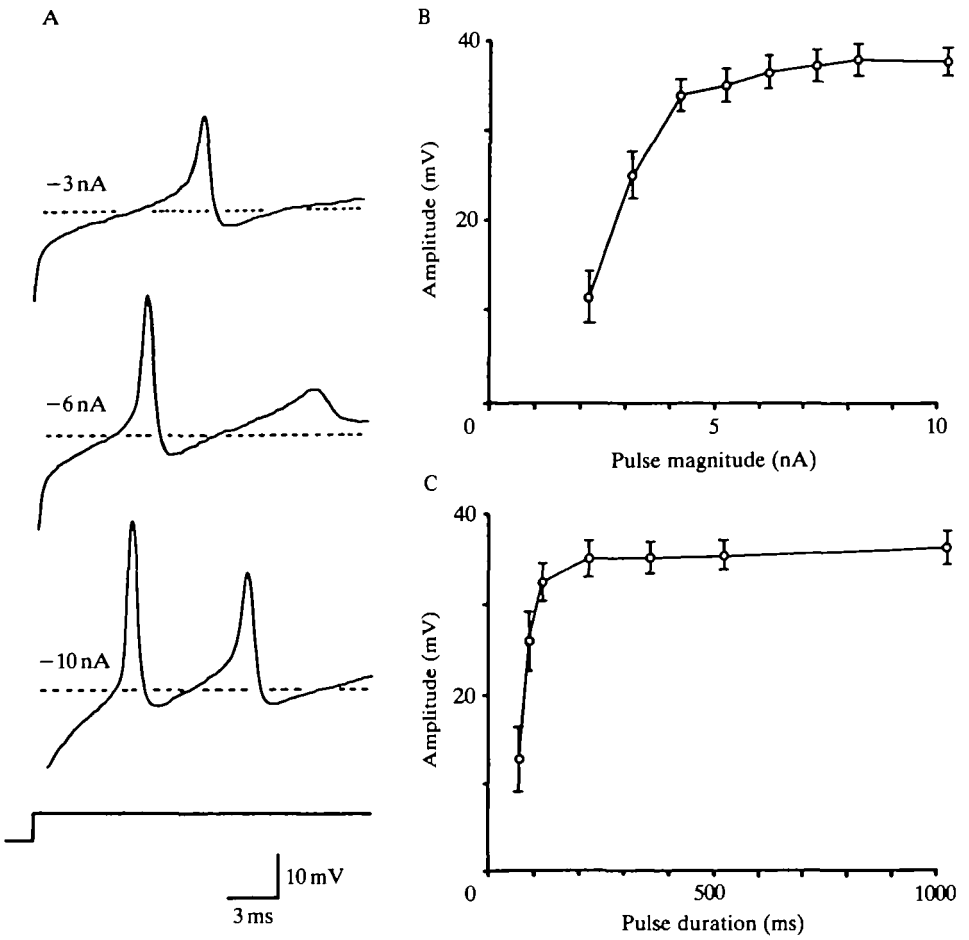

Fig. 3. (A) Action potentials of an L-neurone produced after hyperpolarizing current pulses of varying magnitudes. Dashed lines indicate the resting potentials (about −45 mV). (B,C) The amplitudes of the action potentials, measured from the resting level, are plotted against the magnitudes (B) and the durations (C) of hyperpolarizing current pulses. An average from five L-neurones is shown with the standard deviations. Recordings were made in the ocellar tract of the brain. In A and B, the pulse durations are constant at 500 ms. In C, the pulse magnitudes are constant at 6 nA.

The maximum spike amplitude was 35 mV above the resting potential. The resting potentials of the L-neurones were 46 ± 5 mV ($N = 23$), and overshooting action potentials (peak potential exceeding the zero-level) were not observed. In the locust L-neurone, Simmons (1982) also found that the spike amplitude changed when the magnitude or the duration of preceding hyperpolarization was altered, and that the peak potentials never exceeded the zero-level.

In the following experiments we inserted electrodes into the L-neurones in the ocellar tract and examined the ionic mechanism of their action potentials. The action potentials were induced at the off-set of hyperpolarizing current pulses of 7–10 nA, 500 ms.

Effects of tetraethylammonium

TEA^+ is known to block the voltage-sensitive K^+ current in most excitable membranes (Narahashi, 1974). When 25 mmol l^{-1} TEA^+ was added to the saline, the duration of the action potential was prolonged by 0·3–1·0 ms and the peak amplitude was increased by 2–8 mV (Fig. 4). This shows that, in addition to the inward current, an outward K^+ current is present. The increase in the peak amplitude can be explained as follows: membrane permeability to K^+ was increased before the action potential reached the peak potential, and the outward K^+ current was superimposed on the inward current at the peak of the action potential. The peak potential did not exceed the zero-level even in the presence of TEA^+, probably because the inward current at the rising phase of the action potential is weak and/or TEA^+ cannot completely suppress the outward K^+ current.

Effects of changes in external calcium concentration

The effects of changes in calcium concentration were studied in the presence of 25 mmol l^{-1} TEA^+. When normal saline (containing 25 mmol l^{-1} TEA^+) was replaced by a calcium-free saline, the amplitude of the action potential declined and the action potential was reversibly abolished within 20 min (Fig. 5). The experiments were repeated on six occasions.

It has been shown that Mg^{2+} cannot substitute for Ca^{2+} in calcium action potentials (Hagiwara & Byerly, 1981). The action potential was reversibly abolished within 20 min in calcium-free saline containing 1·8 mmol l^{-1} Mg^{2+}. This was repeated on three occasions.

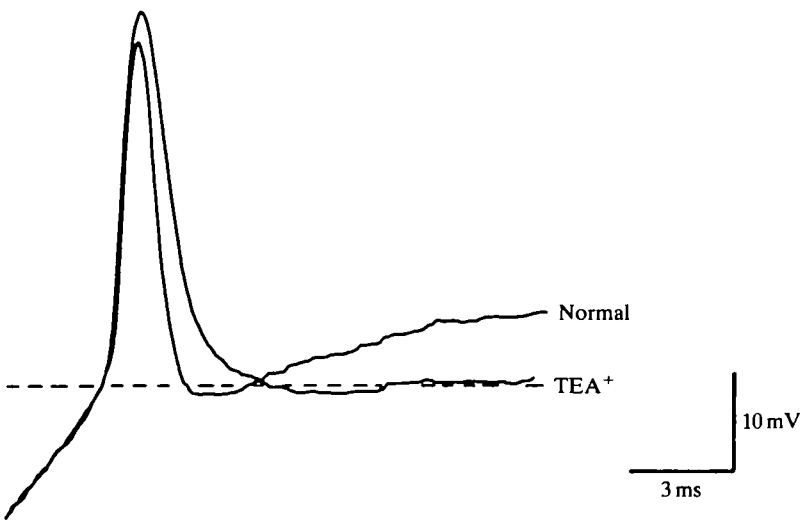

Fig. 4. Effects of TEA^+ on the action potential of an L-neurone. The action potential in normal saline and that after 16 min in saline containing 25 mmol l^{-1} TEA^+ are shown. Dashed line indicates the resting membrane potential. The recording was made in the ocellar tract and the action potentials were elicited at the off-set of hyperpolarizing current pulses of 9 nA, 500 ms.

A typical recording of the effects of changes in Ca^{2+} concentration on the amplitude of the action potential is shown in Fig. 6A. When the saline was replaced with that containing a higher concentration of Ca^{2+}, the amplitude of the action potential increased within 5 min, and reached a steady level within 20 min. In

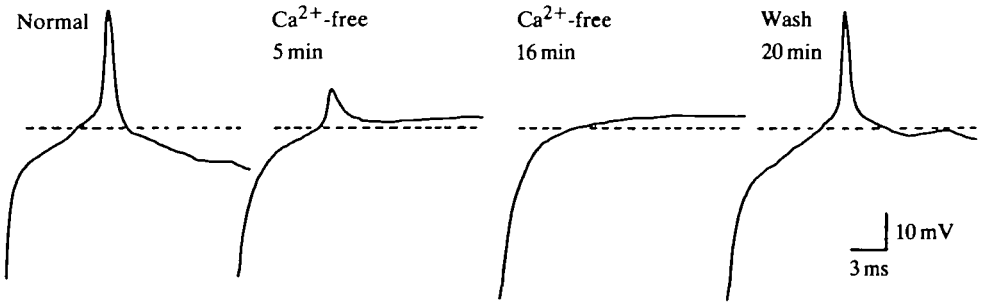

Fig. 5. Effects of perfusion with calcium-free saline on the action potential of an L-neurone. The action potential was abolished within 16 min when normal saline containing 25 mmol l^{-1} TEA^+ was replaced with Ca^{2+}-free saline. The effects were reversible. Dashed lines indicate resting potentials. The recording was made in the ocellar tract and the action potentials were evoked at the off-set of hyperpolarizing pulses of 8 nA, 500 ms.

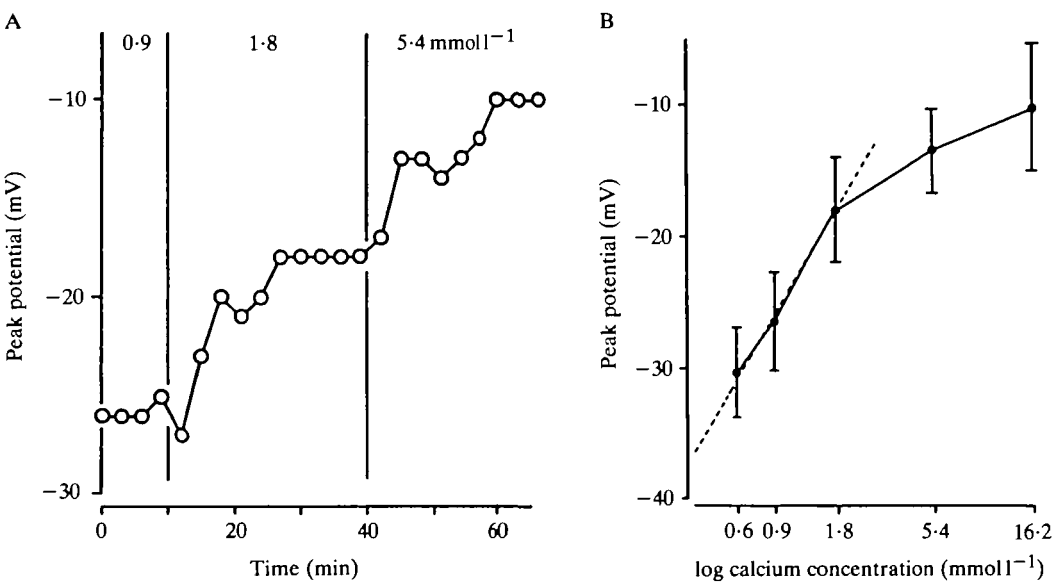

Fig. 6. Effects of external calcium concentration on the action potential. (A) A typical example of the effects of Ca^{2+} concentration change on the peak potential of the action potential. The peak potential reaches a steady level about 20 min after the solution change. The saline contained 25 mmol l^{-1} TEA^+. In B the peak potential is plotted against the external calcium concentration, in the presence of 25 mmol l^{-1} TEA^+. Averaged data from six L-neurones are shown with standard deviations. At low Ca^{2+} concentrations, the slope of the plot is 26 mV/10-fold change (dashed line). Recordings were made in the ocellar tract and the action potentials were evoked at the off-set of hyperpolarizing current pulses of 7 nA, 500 ms.

averaged data from six L-neurones, a plot of the maximum amplitude against log calcium concentration showed a slope of about 26 mV/10-fold change at concentrations of less than 1·8 mmol l^{-1} (Fig. 6B). This is in good agreement with the theoretical slope for a membrane acting as a calcium electrode (29 mV/10-fold change), and suggests that calcium is the major carrier for the inward currents of the action potential. At a higher concentration, the peak potentials of the action potential were smaller than that predicted from the theoretical slope. A similar effect has been documented for Ca^{2+} currents of some other excitable membranes (Hagiwara, 1973; Hagiwara & Byerly, 1981).

Effects of Cd^{2+} and Co^{2+}

Cadmium and cobalt ions have been shown to block calcium action potentials in a number of excitable cells (Hagiwara & Takahashi, 1967). Saline containing 2 mmol l^{-1} cadmium ions blocked the action potential within 10 min in a reversible manner (Fig. 7A). This experiment was repeated three times. Replacement of the normal saline with saline containing 20 mmol l^{-1} cobalt ions also reversibly blocked the action potential (Fig. 7B). This was repeated seven times. These observations support the hypothesis that calcium is a major charge carrier of the inward current of the action potential.

Effects of replacing Ca^{2+} with Ba^{2+}

Barium ions have been shown to substitute for calcium ions in calcium action potentials (Hagiwara & Byerly, 1981). The action potential could be maintained when Ca^{2+} in normal saline was replaced with Ba^{2+} (Fig. 8). The experiment was repeated three times. The action potential seemed to be slightly prolonged (see Fig. 8), which may indicate the presence of a calcium-activated outward current.

Effects of sodium-free saline

There were no significant differences in the amplitude and the duration between the action potentials observed after treatment with sodium-free saline for 30 min and those in normal saline (Fig. 9) in nine preparations. As a control, electrodes were inserted into six neurones in close proximity to the recording site of the L-neurones (the estimated distance was less than 100 μm), and the effects of Na^+-free saline were examined. These neurones exhibited spike discharges in response to depolarizing current pulses in normal saline, and the spikes were abolished in Na^+-free saline within 20 min. These results indicate that sodium ions have little role in carrying charges for the inward current during the action potential of L-neurones.

Effects of tetrodotoxin

TTX blocks sodium action potentials (Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965). Calcium action potentials are not blocked by TTX at a concentration of less than 10^{-6} mol l^{-1} (Hagiwara & Byerly, 1981). TTX applied to 15 of our preparations at a concentration of 3×10^{-6} mol l^{-1} had no measurable effects of the action potential (Fig. 10A). As a control, we impaled four

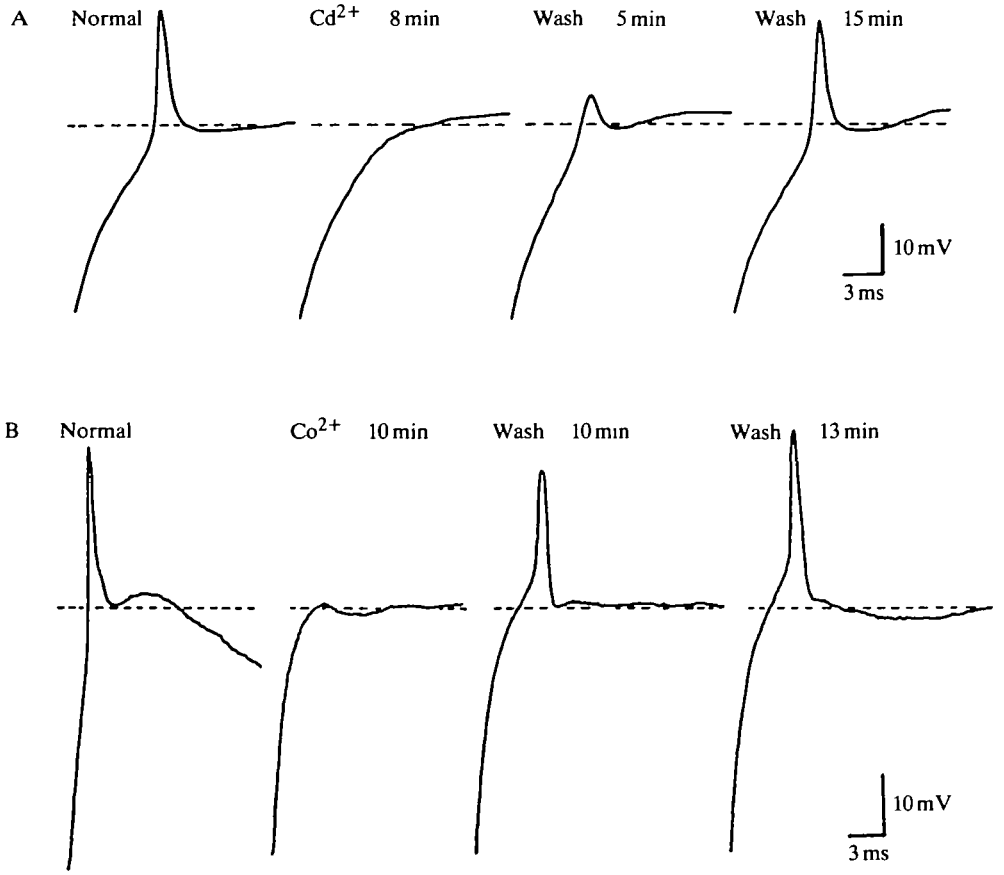

Fig. 7. Effects of Cd^{2+} and Co^{2+} on the action potential. The action potential was abolished within 10 min when normal saline was replaced with that containing 2 mmol l^{-1} Cd^{2+} (A). The effects were reversible. The action potential was also reversibly blocked by 20 mmol l^{-1} Co^{2+} (B). Dashed lines indicate the resting potentials. The recordings were made in the ocellar nerve and the action potentials were elicited at the off-set of hyperpolarizing pulses of 8 nA, 500 ms.

neurones in close proximity to the recording site of the L-neurone (the estimated distance was less than 100 μm) and the effects of TTX were observed. The action potentials, induced by the depolarizing currents, diminished in the presence of TTX within 10 min.

These observations support the finding that sodium ions do not have any major role in carrying the inward current during the action potential. 3×10^{-5} mol l^{-1} TTX, however, did block the action potential within 15 min (Fig. 10B). The TTX concentration for 50 % inhibition was about $1{\cdot}0\times10^{-5}$ mol l^{-1} (Fig. 10C).

DISCUSSION

The evidence presented here suggests that calcium ions are the major charge carriers for the inward current during the action potential of the cockroach

L-neurone. The action potential diminished in Ca^{2+}-free saline, and the slope of peak action potential *versus* log Ca^{2+} concentration was 26 mV/10-fold change, at a Ca^{2+} concentration of less than 1·8 mmol l^{-1}. The observed value is similar to that predicted for a calcium electrode (29 mV/10-fold change).

The results also suggest that under normal conditions sodium ions have little role in generating the action potential since treatment with sodium-free saline did not affect the size or shape of the action potential. It may be argued, however, that there is either a barrier between the haemolymph and the cell membrane or a store of sodium ions outside the membrane which maintain the action potential in sodium-free saline. However, these possibilities can probably be ruled out, because we impaled some neurones in close proximity to the recording site of L-neurones (the estimated distance was less than 100 μm), and found that the spikes produced by these neurones diminished in Na^{+}-free saline.

The action potential of the cockroach L-neurone is insensitive to 3×10^{-6} mol l^{-1} TTX, confirming that sodium ions have little role in generating the action potential. In dragonfly L-neurones, Chappell & Dowling (1972) found that the off-set depolarization is also insensitive to TTX (6×10^{-7} mol l^{-1}). The action potential of cockroach L-neurones is, however, blocked by 3×10^{-5} mol l^{-1} TTX. It seems unlikely that the inward current passes through the Na^{+} channel, because (1) perfusion with sodium-free saline has little effect on the action potential and (2) the TTX concentration required to suppress the action potential by 50% (10^{-5} mol l^{-1}) is three orders of magnitude higher than that required for 50% suppression of the sodium current in cockroach giant axons (10^{-8} mol l^{-1}; Sattelle, Pelhate & Hue, 1979). Therefore, it may be concluded that the inward calcium current is slightly sensitive to TTX.

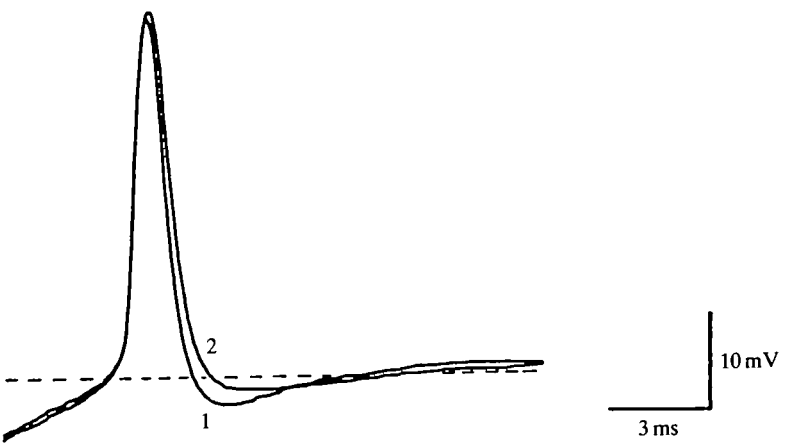

Fig. 8. Effects of replacing Ca^{2+} with Ba^{2+}. Record 1 shows the action potential in normal saline (containing 1·8 mmol l^{-1} Ca^{2+}). Record 2 shows the action potential after 25 min in normal saline containing 1·8 mmol l^{-1} Ba^{2+} and 0 mmol l^{-1} Ca^{2+}. Dashed line indicates the resting potential. The recording was made in the ocellar tract and the action potentials were elicited at the off-set of hyperpolarizing pulses of 7 nA, 500 ms.

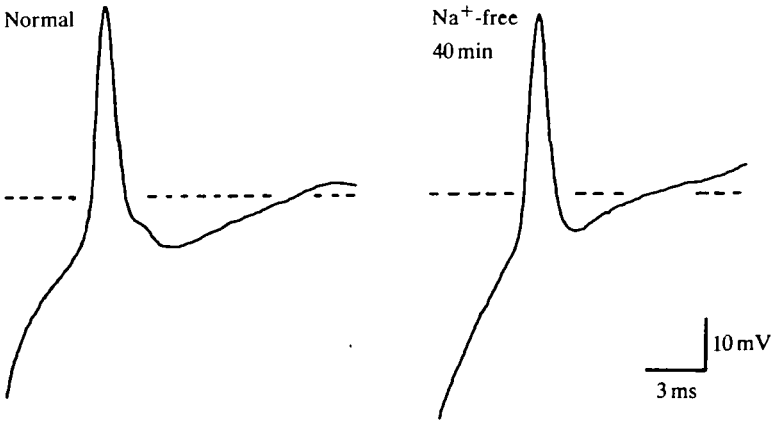

Fig. 9. Effects of Na^+-free saline on the action potential. No detectable effects on the duration and amplitude of the action potential were observed. Dashed lines indicate the resting potential. The recording was made in the ocellar tract and the action potentials were elicited at the off-set of hyperpolarizing pulses of 8 nA, 500 ms.

The action potential of the cockroach L-neurone originates in the ocellar tract of the brain. In the locust, Wilson (1978) and Simmons (1982) found that the action potential of the L-neurone also originates in the brain. L-neurones make output synapses onto a number of higher-order neurones along the full length of the axon in the ocellar tract (Toh & Hara, 1984). Fain, Gerschenfeld & Quandt (1977) suggested that the Ca^{2+} channels, which are responsible for generating off-responses in vertebrate photoreceptors, may be associated with the presynaptic sites of the axon terminals. In the cockroach L-neurone, therefore, the Ca^{2+} channels responsible for generating action potentials may be associated with the presynaptic sites of their axons in the ocellar tract.

The peak potentials at high Ca^{2+} concentrations are smaller than those predicted from a theoretical slope. One of the probable reasons for this effect is that the outward K^+ current would be superimposed on the inward current at the peak of the action potential if the 25 mmol l^{-1} TEA^+ could not completely suppress the outward K^+ current. Another possible reason for this effect is that the concentration of Ca^{2+} at the outer surface of the membrane would not increase as much as it does in the external solution if high concentrations of Ca^{2+} reduced the magnitude of the membrane surface potential. Saturation of calcium currents at high calcium concentrations has been noted in some excitable membranes (Hagiwara, 1973; Hagiwara & Byerly, 1981; Cota & Stefani, 1984).

Some hyperpolarizing neurones in the visual systems of both vertebrates and invertebrates generate, in some cases, transient depolarizations or spike-like potentials at the recovery phase of the hyperpolarization. Such is the case in the photoreceptors of vertebrates (Baylor & Hodgkin, 1974) and scallops (McReynolds & Gorman, 1970) and the second-order neurones of insect compound eyes (Zettlar & Järvilehto, 1973; Laughlin, 1973), insect ocelli (Chappell & Dowling, 1972; Goodman, 1981), barnacle ocelli (Stuart & Oertel, 1978) and vertebrate retinas

(centre-hyperpolarizing bipolar cells: Schwartz, 1974). Studies on the ionic mechanisms of these off-set responses led to the conclusion that they are associated with changes in voltage-dependent calcium permeability. Oertel & Stuart (1981) examined the ionic mechanism of the off-set depolarizing response of barnacle second-order ocellar cells, I-cells. They found that the graded off-response of I-cells becomes an action potential when an outward K^+ current was blocked by TEA^+, and that the peak amplitude and rate of rise depended on the concentration of external

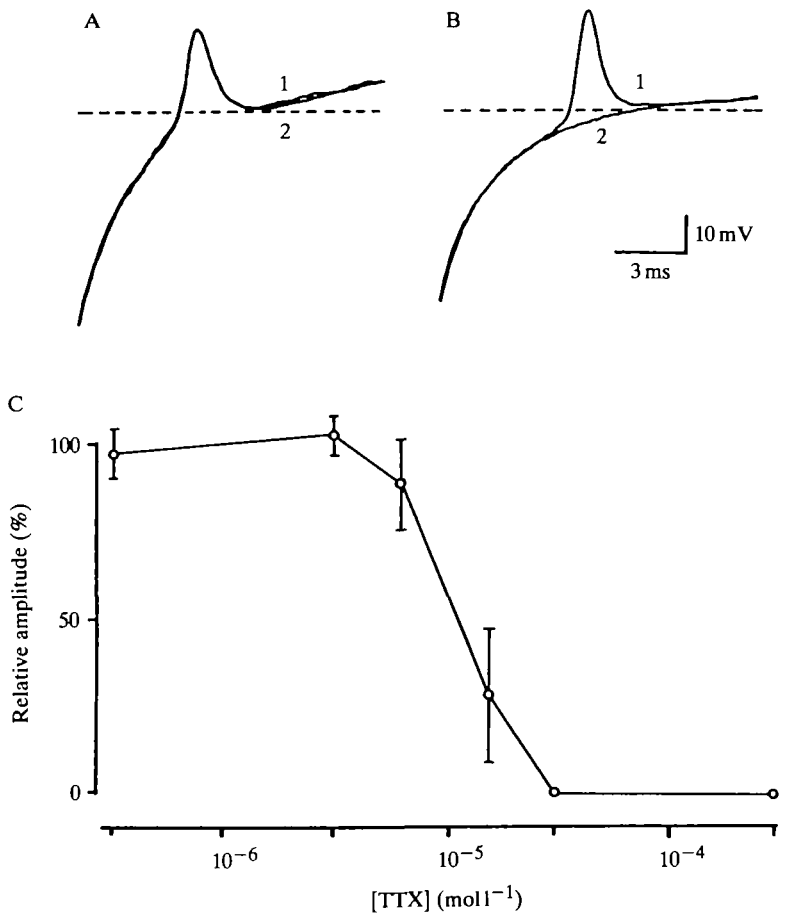

Fig. 10. Effects of tetrodotoxin (TTX) on the action potential. (A) The effects of 3×10^{-6} mol l^{-1} TTX on the action potential. The action potential in normal saline (record 1) and that after 30 min in TTX-containing saline (record 2) are shown. No detectable effects are observed. (B) The effects of 3×10^{-5} mol l^{-1} TTX. Record 1: an action potential in normal saline. Record 2: a response after 15 min in the presence of TTX. Dashed lines indicate resting potentials. (C) The amplitude of the action potential plotted against the TTX concentration in the saline. The amplitude is normalized to the extent of that observed in normal saline. An average of recordings from 5–15 L-neurones for each TTX concentration is shown with the standard deviation. All recordings were made in the ocellar tract and the action potentials were elicited at the off-set of hyperpolarizing pulses of 8–9 nA, 500 ms.

Ca^{2+}. Similar observations were made in vertebrate rods (Fain *et al.* 1977, 1980) and cones (Piccolino & Gerschenfeld, 1978, 1980; Gerschenfeld & Piccolino, 1980) and scallop photoreceptors (Cornwall & Gorman, 1979). Vertebrate horizontal cells, one of the (hyperpolarizing) second-order neurones of the retina, can also generate calcium action potentials when isolated (Tachibana, 1981).

In the second-order neurones of insect's compound eyes, the off-set responses appear to be transient depolarizations or spike-like potentials (Laughlin, 1973; Zettler & Järvilehto, 1973). In these neurones, voltage-dependent conductance changes at the off-set of the responses may be weaker than those in the cockroach ocellar L-neurones and, therefore, all-or-none action potentials would not be generated, at least in a normal ionic environment.

Off-set responses in second-order neurones of other ocelli and compound eyes in insects are also likely to depend upon voltage-sensitive calcium currents, since they have similar properties to those in the cockroach L-neurones (Goodman, 1981; Mizunami *et al.* 1982). Recently, Ammermüller & Zettler (1986) have performed voltage-clamp experiments on locust L-neurones. They considered that the fast inward current, which reflects the action potential, may be a sodium current because (1) its voltage characteristics, at a voltage range from -70 to -30 mV, were similar to those of a sodium current and (2) it could be blocked by 10^{-5} mol l^{-1} TTX. However, these observations do not exclude the possibility that the inward current is a calcium current because (1) the voltage characteristics of a calcium current in that voltage range are very similar to those of a sodium current (Hagiwara, 1973) and (2) the TTX concentration required to suppress the inward current is one or two orders of magnitude higher than that expected for a sodium current (Hagiwara, 1973; Sattelle *et al.* 1979). Therefore, further study remains to be done to clarify if the action potential of locust L-neurones is sodium- or calcium-dependent: for example, can it be maintained in sodium- or calcium-free saline, etc.

In conclusion, our results on cockroach ocellar L-neurones provide evidence to suggest that the off-set responses of hyperpolarizing visual neurones of both vertebrates and invertebrates have a common ionic mechanism involving a voltage-dependent change in calcium permeability. This is an extension of the original suggestion of Cornwall & Gorman (1979) for hyperpolarizing photoreceptors.

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