

IONIC REQUIREMENTS FOR NON-SYNAPTIC ELECTROGENESIS IN THE MUSCLE FIBRES OF A LEPIDOPTEROUS INSECT

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SUMMARY

Study was made of the influence of external sodium, calcium and magnesium concentrations on the directly elicited response of the segmental muscle fibres of the larva of *Galleria mellonella*, as recorded with intracellular microelectrodes. The graded response caused by an outward current pulse was converted into an all-or-none spike in the presence of 10 mM tetraethylammonium. The amplitude of these spikes increased with increasing external Ca concentration but not with increasing Na or Mg concentration. The spikes were not influenced by tetrodotoxin (3×10^{-6} M) but were suppressed by Mn ions (20 mM). After replacement of TEA-containing saline with an isotonic CaCl₂ solution, spikes were still produced in the latter. It appears that only calcium ions act as charge carriers of inward current at the non-synaptic membrane.

INTRODUCTION

Crustacean muscle fibres do not produce action potentials under normal conditions, and a strong depolarizing current pulse elicits oscillatory and graded responses only. These responses can be converted into all-or-none spikes by treatment with tetraalkylammonium ions externally (Fatt & Ginsborg, 1958; Werman & Grundfest, 1961) or internally (Hagiwara, Naka & Chichibu, 1964), or by elimination of internal calcium ions with chelating agents such as EDTA and EGTA (Hagiwara & Naka, 1964). Whereas sodium ions play a major role in generating action potentials in many excitable membranes (Hodgkin & Huxley, 1952; Katz, 1966), these crustacean muscle fibres produce action potentials involving an inflow of calcium ions instead of sodium ions (Hagiwara, Hayashi & Takahashi, 1969). Barium and strontium ions are also capable of generating all-or-none spikes.

Many insect muscle fibres seem to have similar properties to those of crustacean fibres. Graded responses elicited by outward current pulses in normal saline solution can be converted into all-or-none spikes by treatment with tetraethylammonium (TEA) (Belton & Grundfest, 1961; Washio, 1972) or ryanodine (Usherwood, 1962). Such action potentials are independent of external sodium concentration in the

skeletal muscle fibres of a locust (Washio, 1972) and a fly (Patlak, 1976). Ba and Sr ions can also generate spikes. On the other hand, Na-dependent electrogenesis has been reported by many investigators in a variety of insect muscle fibres; such as the skeletal muscle fibres of a grasshopper (Werman, McCann & Grundfest, 1961), *Tenebrio* (Belton & Grundfest, 1962) and a moth (Piek, 1975), the proctodeal muscle fibres of a cockroach (Nagai, 1972), and the heart muscle fibres of Orthoptera and Mantodea (S-Rózsa & V-Szöke, 1972).

In this paper, study was made of the ionic requirements for the generation of directly elicited responses in the muscle fibres of *Galleria mellonella*. This lepidopterous insect has a haemolymph which is characterized by a low concentration of sodium ions and high concentrations of potassium and magnesium ions (Duchâteau, Florkin & Leclercq, 1953; Florkin & Jeuniaux, 1974).

MATERIALS AND METHODS

The segmental muscle fibres of the larva of the waxmoth *Galleria mellonella* were used. The electrophysiological techniques were similar to those described in a previous paper (Yamamoto & Fukami, 1976). The muscle fibre membrane was depolarised intracellularly by square current pulses. These currents were injected through glass microelectrodes filled with 2 M potassium citrate having a resistance of 1–10 M Ω when tested in normal saline. Membrane potentials were recorded intracellularly with a second microelectrode filled with 2 M potassium citrate. The resistance of the recording electrode ranged from 5 to 20 M Ω . The recording and current electrodes were inserted near one another into the same fibre. The normal saline (Belton, 1969) had the following composition (mM): NaCl, 35; KCl, 36; CaCl₂, 12; MgCl₂, 16; glucose, 269; Tris(hydroxymethyl)aminomethane, 5. The pH of the solution was adjusted to 7.4 with HCl. Test solutions were prepared by replacing glucose with an osmotically equivalent amount of test ions. To maintain the isosmolarity of the TEA-containing 100 mM Ca-saline, CaCl₂ replaced part of the NaCl also. Salines containing lower concentrations of Na, K, Ca and/or Mg ions were prepared by substitution of an osmotically equivalent amount of glucose for the ions.

Experiments were conducted at a room temperature of 25 °C.

RESULTS

Graded responses were converted into spikes by TEA in concentrations above 10 mM (Fig. 1). No further effect was observed up to 50 mM. This contrasts with the situation in *Romalea*, in which the conversion is achieved at 1×10^{-3} M, and a block is caused at higher concentrations (Belton & Grundfest, 1961).

The effects of Na, Ca and Mg ions on the action potential produced by an outward current pulse were examined in the presence of 10 mM TEA. Changing the external Na or Mg concentration had only negligible effects on the overshoot of the action potential (Fig. 2). In contrast, the overshoot of the action potential and the rate of rise of the spike were markedly increased as the external concentration of Ca ions was raised (Fig. 3). The relationship between the peak potential of the spike and the

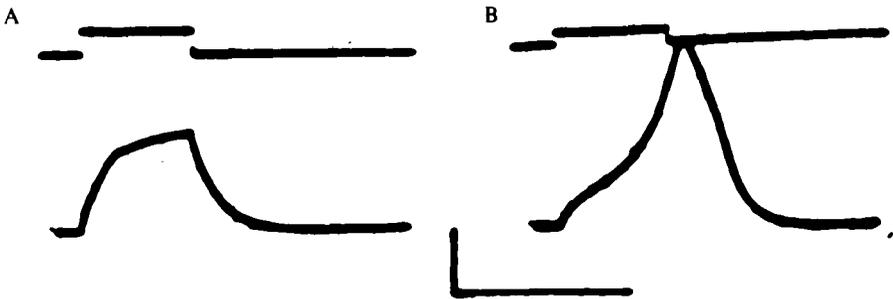


Fig. 1. Effect of 10 mM TEA on the membrane response (lower tracing) elicited by outward current (upper tracing) in a single muscle fibre. (A) Before and (B) 10 min after perfusion of TEA. Calibration: 100 ms, 20 mV, 1.5×10^{-8} A.

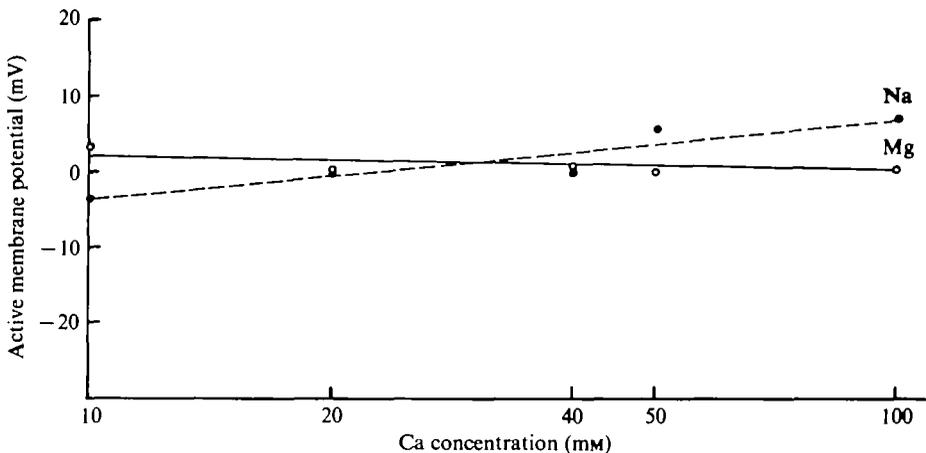


Fig. 2. Relationship between the overshoot of the action potential evoked by outward current and the external concentrations of Na and Mg in the presence of 10 mM TEA. Each symbol represents the mean of three fibres.

logarithm of the external calcium concentration was almost linear, with a slope of 30 mV for tenfold change in the concentration (Fig. 4). The threshold membrane potential was not significantly changed in the Ca concentration range between 10 and 100 mM (Fig. 4).

Fig. 5 illustrates the effects of TTX (3×10^{-5} M) and manganous ions (20 mM) on the action potentials elicited by outward current in the presence of TEA. TTX had no effect on the action potential (record B), whereas Mn ions markedly suppressed both the amplitude and the rate of rise of the action potential (record D).

Once treated with TEA *Galleria* muscle fibres were capable of producing action potentials in a saline solution containing 100 mM-CaCl₂, 195 mM glucose and 5 mM Tris-HCl, but devoid of Na, Mg, K and TEA ions. Fig. 6 illustrates an example, in which the spike was first recorded in TEA-containing 100 mM Ca-saline (see Materials and Methods) (record A), and then in a solution devoid of Na, K, Mg and TEA

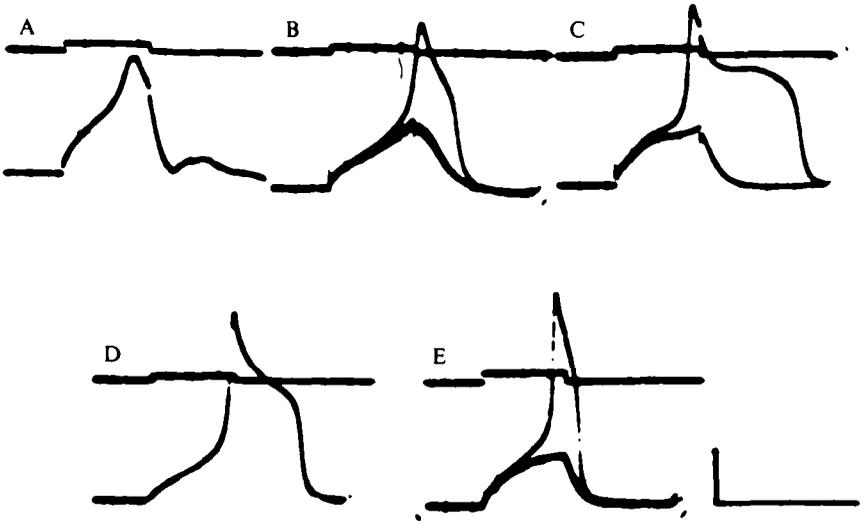


Fig. 3. Action potentials elicited by outward currents in a single muscle fibre at different Ca concentrations in the presence of 10 mM TEA. Ca concentrations are 10 mM (A), 20 mM (B), 40 mM (C), 50 mM (D) and 100 mM (E). Outward current pulses of an intensity just below or above the threshold were applied and potential changes for these pulses were superimposed in records B, C and E. Calibration: 100 ms, 20 mV, 8×10^{-4} A.

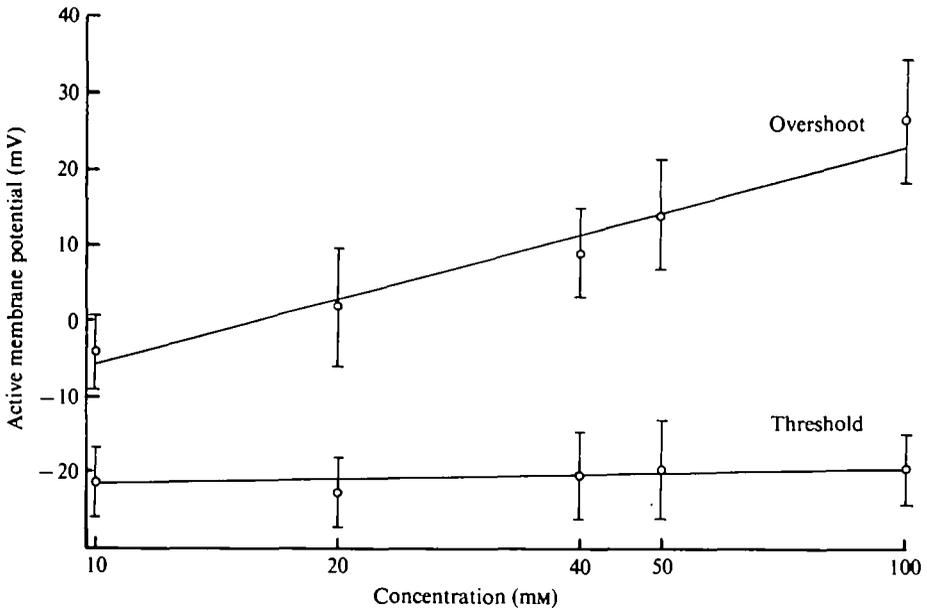


Fig. 4. Effects of external Ca concentration on the overshoot of spikes elicited by outward current (upper line) and the threshold potential for spiking (lower line) in the presence of 10 mM TEA. Each symbol represents the mean of six fibres and vertical bars represent the standard deviation of the mean.

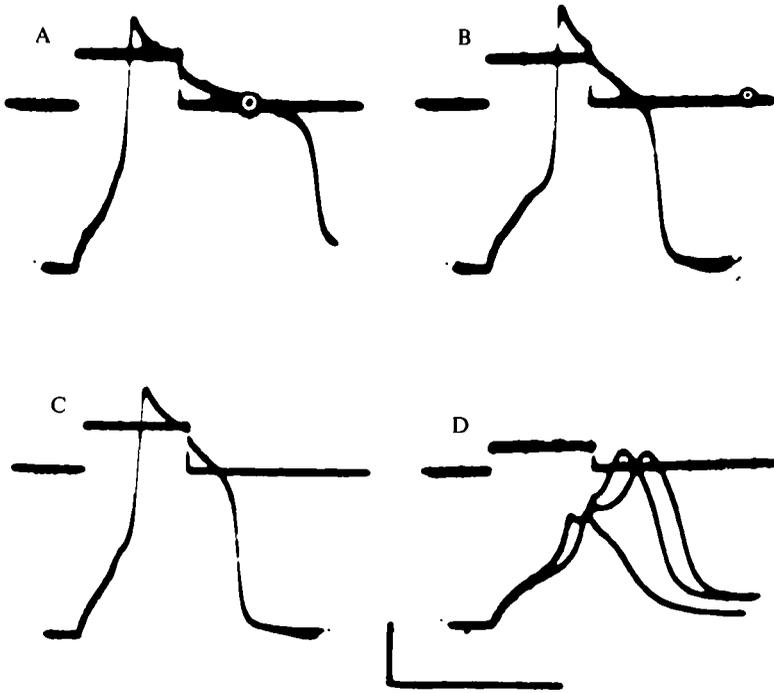


Fig. 5. Effects of 3×10^{-6} M TTX and 20 mM-Mn ions on the spikes of a single muscle fibre in a saline containing 50 mM-Ca and 10 mM TEA. (A) Before, (B) 60 min after perfusion with TTX, (C) 45 min after withdrawal of TTX, and (D) 25 min after perfusion of Mn ions. In record D, two abortive spikes in response to suprathreshold stimulations and one subthreshold electrotonic potential were superimposed. Calibration; 100 ms, 20 mV, 8×10^{-6} A.

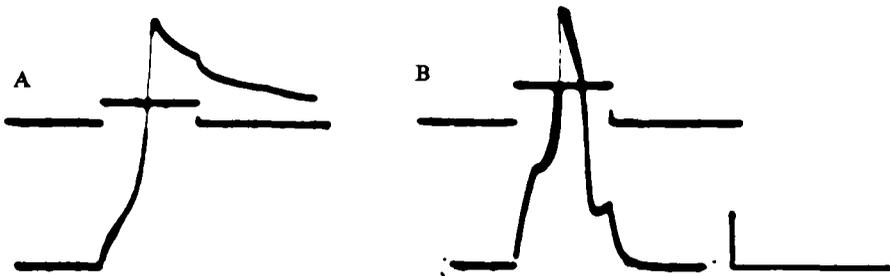


Fig. 6. The action potentials (lower tracings) elicited by outward currents (upper tracings) in a solution containing 27.5 mM-Na, 36 mM-K, 16 mM-Mg, 100 mM-Ca and 10 mM-TEA (A), and 60 min after application of a solution containing 100 mM-Ca but devoid of Na, K, Mg and TEA (B). Calibration: 100 ms, 20 mV, 7×10^{-6} A.

ions (record B). The spikes could be recorded even 1 h after the change of saline, although the critical firing level was shifted in the positive direction and the falling phase of the action potential became faster. It should be pointed out that withdrawal of Ca ions from the bathing medium effectively suppressed the action potential in the presence of a high concentration of sodium or magnesium ions.

DISCUSSION

The experiments indicated that calcium influx was responsible for the rising phase of the TEA-induced spikes in the non-synaptic membrane of muscle fibre of *Galleria*. TEA was shown not to be involved by the observation that the spikes persisted after its withdrawal (Fig. 6). A similar observation has been made for tetraalkylammonium salts in crustacean muscle fibres (Fatt & Ginsborg, 1958). Evidence for the role of calcium was as follows. Firstly, the overshoot of the spikes increased with external Ca concentration, but was unaffected by change in Na and Mg concentrations (Figs. 2-4). Secondly, in fibres pretreated with TEA, spikes were generated in a solution which contained only calcium as a cation (Fig. 6). Finally, in the presence of TEA, the spikes were not affected by high concentration of TTX (3×10^{-5} M) but were suppressed by 20 mM manganous ions (Fig. 5). In many excitable membranes, the Na channel is readily blocked by 10^{-7} to 10^{-8} M TTX (Narahashi, Moore & Scott, 1964; Narahashi, 1974), and the Ca channel is selectively suppressed by Mn ions (Hagiwara & Nakajima, 1965; 1966; Narahashi, 1974). Since it has been established that TTX and TEA act independently upon Na and K channels, respectively (Hille, 1967), the possibility that TEA prevents the TTX action appears unlikely.

Among insects, calcium-dependent action potentials have been reported in skeletal muscle of a locust (Washio, 1972), *Drosophila* (Ikeda, Ozawa & Hagiwara, 1976), *Sarcophaga* (Patlak, 1976) and *Ephesia* (Deitmer & Rathmayer, 1976); the heart muscle of *Hyalophora* (McCann, 1971); and a motoneurone cell-body (Pitman, 1976). Na-independent and probably Ca dependent action potentials have been reported in the myocardia of *Locusta migratoria*, *Phaneroptera nana* and *Mantis religiosa* (S-Rózsa & V-Szöke, 1971, cited by Miller, 1975), and the hind-gut muscle of *Leucophaea maderae* (Cook & Reinecke, 1973).

One observation in this study of *Galleria* muscle was not in keeping with previous results; there is no effect of Ca concentration on threshold potential (Fig. 4), whereas the stabilizing action of Ca ions has been clearly demonstrated in locust muscle fibres (Washio, 1972). This may be due, at least in part, to the relatively high concentrations of Mg ions in the bathing media (Hagiwara & Takahashi, 1967).

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REFERENCES

- BELTON, P. & GRUNDFEST, H. (1961). Comparative effects of drugs on graded responses of insect muscle fibres, *Fedn Proc.* **20**, 339 (abstract).
 BELTON, P. & GRUNDFEST, H. (1962). Potassium activation and K spikes in muscle fibres of mealworm larva (*Tenebrio molitor*). *Am. J. Physiol.* **203**, 588-594.
 BELTON, P. (1969). Innervation and neural excitation of ventral muscle fibres of the wax moth, *Galleria mellonella*, *J. Insect Physiol.* **15**, 731-741.

- BOOK, B. J. & REINECKE, J. P. (1973). Visceral muscles and myogenic activity in the hind-gut of the cockroach, *Leucophaea maderae*. *J. comp. Physiol.* **84**, 95-118.
- DEITMER, J. W. & RATHMAYER, W. (1976). Calcium action potentials in larval muscle fibres of the moth *Ephestia kuehniella* Z. (Lepidoptera). *J. comp. Physiol.* **112**, 123-132.
- DUCHÂTEAU, G., FLORKIN, M. & LECLERCQ, J. (1953). Concentrations des bases fixes et types de composition de la base totale de l'hémolymphe des insectes. *Archs int. Physiol.* **61**, 518-546.
- FATT, P. & GINSBORG, B. L. (1958). The ionic requirements for production of action potentials in crustacean muscle fibres, *J. Physiol., Lond.* **142**, 516-543.
- FLORKIN, M. & JEUNIEUX, C. (1974). Haemolymph: composition. In *The Physiology of Insecta*, 2nd ed., vol. 5 (ed. M. Rockstein), pp. 256-307. New York: Academic Press.
- HAGIWARA, S. & NAKA, K. (1964). The initiation of spike potential in barnacle muscle fibres in low intracellular Ca^{++} . *J. gen. Physiol.* **48**, 141-162.
- HAGIWARA, S., CHICHIBU, S. & NAKA, K. (1964). The effects of various ions on resting and spike potentials of barnacle muscle fibres, *J. gen. Physiol.* **48**, 163-179.
- HAGIWARA, S. & NAKAJIMA, S. (1965). Tetrodotoxin and manganese ions: Effect on action potential of the frog heart, *Science, Wash.* **149**, 1254-1255.
- HAGIWARA, S. & NAKAJIMA, S. (1966). Differences in Na and Ca spikes in the barnacle muscle fibre membrane. *J. gen. Physiol.* **50**, 583-601.
- HAGIWARA, S. & TAKAHASHI, K. (1967). Surface density of calcium ions and calcium spikes in the barnacle muscle fibre membrane, *J. gen. Physiol.* **50**, 583-601.
- HAGIWARA, S., HAYASHI, H. & TAKAHASHI, K. (1969). Calcium and potassium currents of the membrane of a barnacle muscle fibre in relation to the calcium spike. *J. Physiol., Lond.* **205**, 115-129.
- HILLE, B. (1967). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.* **50**, 1287-1302.
- HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve, *J. Physiol., Lond.* **117**, 500-544.
- IKEDA, K., OZAWA, S. & HAGIWARA, S. (1976). Synaptic transmission reversibly conditioned by single-gene mutation in *Drosophila melanogaster*. *Nature, Lond.* **259**, 489-491.
- KATZ, B. (1966). *Nerve, Muscle and Synapse*. New York: McGraw Hill.
- MCCANN, F. V. (1971). Calcium action potentials in insect myocardial fibres. *Comp. Biochem. Physiol.* **40A**, 353-357.
- MILLER, T. A. (1975). Insect visceral muscle. In *Insect Muscle* (ed. P. N. R. Usherwood), pp. 545-606. New York: Academic Press.
- NAGAI, T. (1972). Insect visceral muscle: Ionic dependence of electrical potentials in the proctodeal muscle fibres. *J. Insect Physiol.* **18**, 2299-2318.
- NARAHASHI, T., MOORE, J. W. & SCOTT, W. R. (1964). Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. *J. gen. Physiol.* **47**, 965-974.
- NARAHASHI, T. (1974). Chemicals as tools in the study of excitable membrane. *Physiol. Rev.* **54**, 813-889.
- PATLAK, J. B. (1976). The ionic basis for the action potential in the flight muscle of the fly, *Sarcophaga bullata*. *J. comp. Physiol.* **107**, 1-11.
- PIEK, T. (1975). Ionic and electrical properties. In *Insect Muscle* (ed. by P. N. R. Usherwood), pp. 218-336. New York: Academic Press.
- PITMAN, R. M. (1976). Calcium dependent action potentials in a cell body of an insect motoneurone. *J. Physiol., Lond.* **251**, 62-63P.
- S-ROZSA, K. & V-SZÖKE, I. (1972). Ion mechanisms of the resting and action potentials in the heart of some insect species. *Comp. Biochem. Physiol.* **41A**, 495-506.
- USHERWOOD, P. N. R. (1962). The action of alkaloid ryanodine on insect skeletal muscle. *Comp. Biochem. Physiol.* **6**, 181-199.
- WASHIO, H. (1972). The ionic requirements for the initiation of action potentials in insect muscle fibres. *J. gen. Physiol.* **59**, 121-134.
- WERMANN, R., MCCANN, F. V. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. I. The effects of alkali-earth cations on the neuromuscular system of *Romalea microptera*. *J. gen. Physiol.* **44**, 997-1027.
- WERMANN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibres. *J. gen. Physiol.* **44**, 997-1027.
- YAMAMOTO, D. & FUKAMI, J. (1976). Effect of chlordimeform on nerve-muscle system of the larva of the waxmoth, *Galleria mellonella*. *J. Insect Physiol.* **22**, 1511-1516.

