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A TIME-DEPENDENT EXCITABILITY CHANGE IN THE SOMA OF AN IDENTIFIED INSECT MOTONEURONE

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Summary

Long-term, current-clamp recordings were made from the cell body of the fast coxal depressor motoneurone (D_f) of the third thoracic ganglion of the cockroach *Periplaneta americana*. In freshly dissected preparations the response to short-duration, suprathreshold, depolarising current pulses was a graded series of damped membrane oscillations similar to those reported previously in this neurone. The response to current injection changed, however, with increasing time after setting up the preparation: cells developed the ability to exhibit all-or-none action potentials. Their amplitude, however, was usually insufficient to overshoot 0 mV. Our observations suggest that the enhancement in excitability is dependent on time following dissection rather than on time following impalement.

Recordings taken from neurone somata mechanically divided from their processes indicated that the time-dependent changes in excitability were not attributable to changes in synaptic input to the neurone and, moreover, that the cell body was involved in action potential genesis. The action potentials were resistant to treatment with the sodium channel blocker tetrodotoxin (up to 10^{-5} mol l^{-1}), but were reversibly abolished when preparations were bathed in saline containing cadmium ions (1 mmol l^{-1}) or manganese ions (20 or 40 mmol l^{-1}) and, therefore, the inward current underlying these events was largely, if not entirely, carried by calcium ions.

These time-dependent action potentials can co-exist with plateau potentials. In neurones giving both plateau potentials and time-dependent action potentials, plateau potentials can drive action potentials in bursts.

Introduction

Insect motoneurones are unipolar and the soma is not on the direct route of electrical information flow. The cell bodies of insect motoneurones have been

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generally accepted as inexcitable and, therefore, unable to generate action potentials (Pitman *et al.* 1972; Hoyle and Burrows, 1973; Gwilliam and Burrows, 1980; Pitman, 1979). In this respect, they have been considered to be similar to those of leech and some crustacean motoneurones and to contrast with central neurones of gastropod molluscs, virtually all of which can produce overshooting all-or-none action potentials.

The excitability of at least some insect motoneurones, however, can be enhanced by surgical or pharmacological interventions. The somata of both the cockroach fast coxal depressor motoneurone, D_f, and the locust fast extensor tibiae, FETi, produce action potentials, which are predominantly Na^+-dependent, some days after the neurones have been axotomized or their axons exposed to colchicine (Pitman *et al.* 1972; Pitman, 1975; Goodman and Heitler, 1979). Anoxia also causes a similar delayed development of Na^+-dependent action potentials in the cockroach motoneurone D_f (Pitman, 1988). Therefore, sodium channels capable of supporting action potentials can be induced in the soma membrane of some insect neurones, although, apparently, they are normally absent or not expressed.

Insect motoneurones can also be rendered excitable by manipulating the transmembrane calcium gradient or by suppressing the potassium conductance. For example, the cell body of the cockroach motoneurone D_f can generate Ca^{2+}-dependent spikes after intracellular injection of calcium chelating agents or after extracellular application of the potassium channel blocker tetraethylammonium (TEA^+; Pitman, 1979). It seems that such motoneurones possess the channels necessary for the generation of Ca^{2+}-dependent action potentials, although the inward currents these channels carry may be masked by a high potassium conductance in the 'normal' state.

Although insect motoneurone somata had been thought to play no active role in generating electrical activity except under the experimental conditions described above, we have recently shown that motoneurone D_f can, in fact, actively participate in producing plateau potentials (Hancox and Pitman, 1991). Plateau potentials in this preparation, however, are normally only produced by depolarizing events of relatively long duration (lasting tens or hundreds of milliseconds) and, therefore, exhibit relatively slow activation. In the present study, we report that, during the course of experiments lasting several hours, the cell body of the cockroach motoneurone D_f becomes able to generate Ca^{2+}-dependent action potentials in response to relatively brief excitation; this occurs in the absence of any pharmacological intervention.

Materials and methods

All experiments were performed on the metathoracic 'fast' coxal depressor motoneurone, D_f (Pearson and Iles, 1970), of adult male cockroaches (*Periplaneta americana*). Animals were decapitated, the ventral nerve cord was dissected out in such a way that the second and third thoracic ganglia remained connected and the

metathoracic ganglion was prepared for electrophysiological recording as described previously (Pitman, 1975). Experiments were performed in recirculating, oxygenated normal physiological solution containing: NaCl 214 mmol l^{-1}; KCl 3.1 mmol l^{-1}; $CaCl_2$ 9.0 mmol l^{-1}; and Tes buffer (pH 7.2) 10 mmol l^{-1}. Stock solutions of cadmium chloride ($CdCl_2$), manganese (II) chloride ($MnCl_2$) and tetrodotoxin (TTX) were made up by addition to normal saline without osmotic compensation. $CdCl_2$, $MnCl_2$ and TTX were obtained from Sigma Chemical Co. Experiments were typically carried out at temperatures between 20 and 23 °C, but temperature was not monitored throughout the experiments.

In experiments where the cell body of D_f was surgically isolated from its axon and synaptic inputs, a sharpened tungsten needle was used to cut around and behind the soma of this neurone. The neurone was not physically removed from the ganglion. Routine injection of the fluorescent dye Lucifer Yellow into the cell at the end of experiments confirmed the success of this 'undercutting' procedure: in cells where isolation had been successful the dye remained restricted to the soma.

Cells were penetrated with a pair of borosilicate glass thin-walled, fibre-filled microelectrodes (Clark Electromedical, Pangbourne) containing 2 mol l^{-1} potassium acetate as electrolyte (electrode resistance 15–20 MΩ). One electrode was used to pass current into the neurone, the second to record the resulting changes in membrane potential. Current was measured using a laboratory-built 'virtual earth' current monitoring device. Data were recorded on tape using an FM tape recorder (Racal 7DS) and displayed on a Gould 1604 oscilloscope. Hardcopy data were down-loaded from tape using a Gould Colorwriter 6120 plotter.

Results

The resting potential of D_f measured in oxygenated, recirculating saline was in the region of −70 mV (−70.8±1.0 mV N=25; mean±S.E.). The effective membrane resistance [5.9±0.4 MΩ (S.E., N=25)] was measured by determining the current necessary to hyperpolarize the cell to a steady-state potential 10 mV more negative than the cell resting potential. Membrane time constant (9.0±0.7 ms; S.E., N=9) was determined by measuring the membrane potential relaxation following 10–20 mV hyperpolarizing square pulses applied to the neurone.

The response of D_f to short, 50 ms depolarising pulses of small magnitude was essentially passive (Fig. 1Ai). In freshly dissected, recently impaled preparations the response to increased levels of current injection was a series of damped membrane oscillations (Pitman, 1979). The magnitude of these oscillations increased with the size of the current pulse injected (Fig. 1Aii, 1Aiii). Preparations demonstrating such responses were left in normal saline and the membrane response to current injection was monitored over long periods. With increasing time into experiments (between 1–4 h, depending on the preparation), the response to current injection changed from graded membrane oscillations to an all-or-none action potential (Fig. 1B). (Henceforth, we term these 'time-

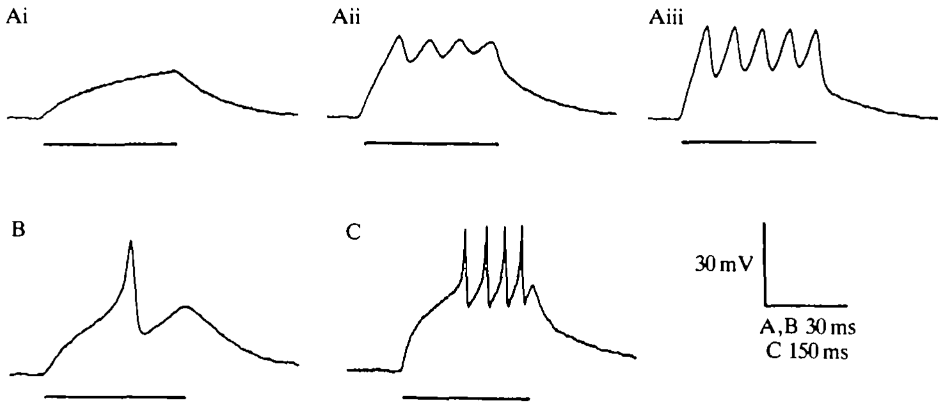

Fig. 1. Development of all-or-none action potentials in the soma of D_f. (Ai) Passive response to a subthreshold depolarising pulse (4 nA) applied to a freshly dissected recently penetrated neurone; (Aii,iii) damped membrane oscillations produced in response to 10 nA and 16 nA depolarising pulses, respectively. (B) All-or-none action potential evoked in the same cell by an 8 nA depolarising pulse 2 h after impalement. (C) Train of action potentials evoked by a 5 nA depolarising pulse applied to a different neurone 1.5 h after impalement. Horizontal bars represent the duration of injected current pulses. Resting potentials of both cells were −70 mV.

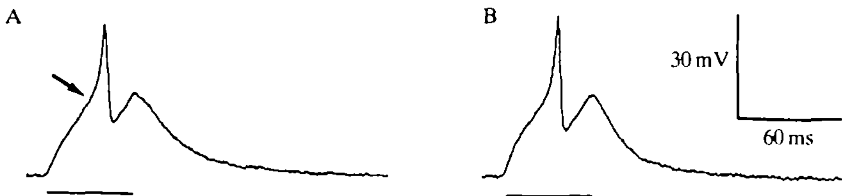

Fig. 2. Action potentials recorded from the soma of a spiking neurone 1.5 (A) and 2.5 h (B) after the first recordings had been made. The amplitude of the action potential in B is clearly larger than that in A (26 mV as opposed to 20 mV). Horizontal bars represent the duration of depolarising current pulses (6 nA). The arrow in A marks the inflection on the rising phase of the response. Resting potential −70 mV.

dependent action potentials'.) When long-duration pulses were applied to the neurone, a train of action potentials could be elicited, each with a similar amplitude and followed by a distinct after-hyperpolarization (Fig. 1C).

Action potentials recorded in this way had a mean amplitude of 18.6 mV (S.E. $\pm$0.7 mV, N=18) when measured from the inflection on their rising phase. (Inflection marked in Fig. 2A.) Measurements of action potential amplitude were taken in this way to avoid problems that could result from measuring amplitude from the resting potential, V_m (where changes in V_m could introduce false changes in amplitude), or the peak-to-trough of the action potential (where changes to the

after-hyperpolarization could similarly produce apparent changes in spike amplitude). In many cases the amplitude of spikes increased with time (Fig. 2) so attempts were made to standardise measurements: values for mean amplitude were taken from action potentials recorded immediately after the spiking response had been observed. Although spikes could show a progressive increase in amplitude during the course of experiments, only a small minority of preparations generated somatic action potentials that overshot zero potential.

The duration of action potentials at their half-height was measured as 3.5 ± 0.1 ms (S.E., $N=18$). Action potential duration was monitored throughout experiments; but, whilst spike amplitude could increase with time, spike duration appeared to remain unchanged.

The time-dependent increase in excitability did not appear to result from changes in the properties of D_f consequent upon damage associated with impaling the neurone with microelectrodes. Some preparations were left for several hours prior to impalement; all-or-none action potentials could be recorded from these immediately following dual impalement. This indicates that it is time after dissection that is the important variable, as opposed to time after impalement.

Changes in effective membrane resistance, time constant and resting potential

Measurements of effective membrane resistance and time constant were taken at increasing intervals during experiments. Changes in these variables did not appear to correlate with changes in the excitability of the neurone; in some cells, for example, the conversion to spiking occurred without any change in input resistance, whilst in others this did increase. Similar observations were made with respect to membrane time constant. Both membrane properties have previously been reported to increase with pharmacologically induced excitability (Pitman, 1979); the lack of consistent changes in the present work, however, indicates that these variables are not principal determinants of the time-dependent change in excitability observed in these experiments.

During the course of development of time-dependent action potentials, there was no consistent alteration in the resting potential of neurones that could account for the change in excitability.

Topographical location of the spiking phenomenon

Experiments were performed to determine the site of origin of the time-dependent action potentials. Long-term recordings were made from surgically isolated somata to establish whether the action potentials recorded in the cell body result from changes in somatic membrane properties or whether they reflect changes to some other part of the neurone.

Shortly after nerve cords had been placed in the recording apparatus, some cell bodies were divided from their branches by the 'undercutting' procedure described in the Materials and methods. Such functionally isolated somata generated all-or-none action potentials in a similar time-dependent manner to that seen in intact neurones. Fig. 3 shows the response of a functionally isolated cell body to current

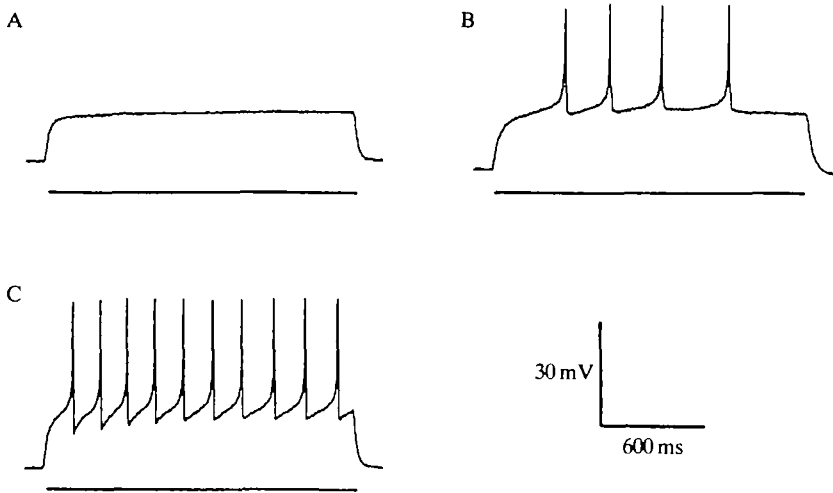

Fig. 3. Recordings from a cell body mechanically isolated *in situ*. (A) Response to a relatively long-duration, subthreshold (1.7 nA) depolarising current pulse applied 1.5 h after the first recordings had been made. (B) A larger pulse (1.8 nA) elicited a small train of action potentials. (C) A still larger depolarisation (2 nA), applied 10 min further into the experiment, significantly increased the spike frequency. Horizontal bars represent the duration of depolarising current pulses. Resting potential −75 mV.

injection 1.5 h after impalement. Injection of a relatively long-duration current pulse of low magnitude produced a passive membrane depolarisation; larger levels of current were sufficient to elicit a train of action potentials. The inferences from these observations on functionally isolated somata are twofold: first, the changes in excitability are not associated with time-dependent changes in synaptic modulation of the cell (the cell body is separated from its synaptic inputs, which occur in the neuropile). Second, the soma can participate in action potential genesis. The above observations do not, however, exclude the possibility that other regions of the neurone, such as dendrites, also participate in generating time-dependent action potentials.

Ionic dependence of time-dependent action potentials

To determine the ionic basis of the time-dependent action potentials, depolarizing pulses of sufficient magnitude to evoke a single action potential were delivered regularly to the soma throughout experiments (at a frequency of 0.6 Hz–2.0 Hz, depending upon the preparation). After sufficient time had elapsed to ensure that the response of the neurone was stable, the preparation was superfused with saline containing tetrodotoxin, manganese (II) chloride or cadmium chloride. After the effects of any of these agents had been observed, the preparation was washed with normal saline to reverse their action, where possible.

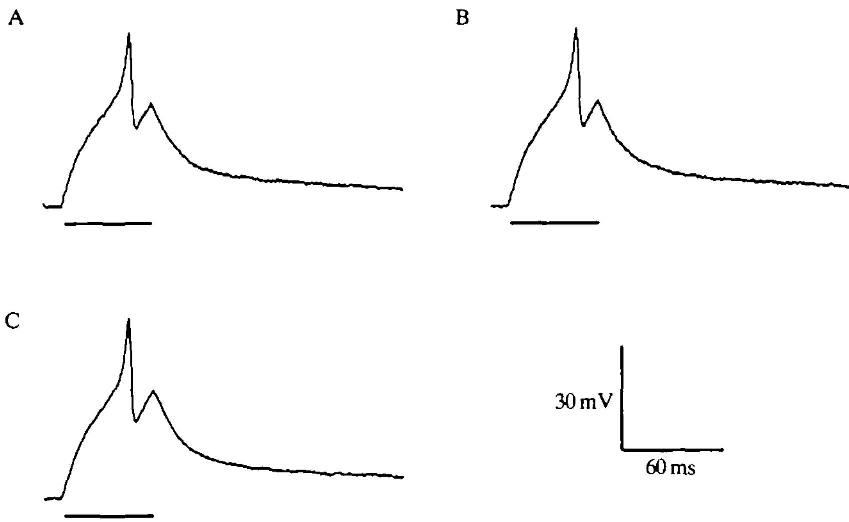

Fig. 4. Effect of tetrodotoxin on time-dependent action potentials. (A) Action potential recorded in normal saline. (B) Action potential recorded following 6 min of exposure to 10^{-6} mol l^{-1} tetrodotoxin (TTX). (C) Action potential recorded after a further 10 min of exposure to 10^{-5} mol l^{-1} TTX (total exposure 16 min). Horizontal bars represent the duration of continuously applied current pulses (7 nA, 0.8 Hz). Resting potential −75 mV.

Tetrodotoxin

Tetrodotoxin was applied to preparations producing all-or-none action potentials (N=4). Fig. 4A,B shows that the amplitude of action potentials was not reduced by exposure to 10^{-6} mol l^{-1} TTX for 6 min; at this point the concentration of this drug was increased tenfold. Even at this relatively high concentration, TTX failed to reduce the amplitude of time-dependent action potentials (Fig. 4C). In some experiments, such as that illustrated in Fig. 4, there was an increase in spike amplitude with time similar to that observed in cells not exposed to this agent.

The potency of the tetrodotoxin used in this study was tested on dorsal unpaired medial (DUM) neurones, which exhibit largely Na^+-dependent spikes (Jégo *et al.* 1970). TTX (10^{-5} mol l^{-1}) completely abolished action potentials in these neurones within 2 min.

Manganese(II) chloride and cadmium chloride

Bathing preparations in saline containing either Mn^{2+} (20 or 40 mmol l^{-1}) (N=4) or Cd^{2+} (1 mmol l^{-1}) (N=7) abolished the time-dependent action potentials within 5 min (Fig. 5); action potentials underwent a progressive decline before complete block. An increase in depolarising current injection (twofold or more) did not evoke action potentials in the presence of these ions, indicating that their effect was most certainly that of blockade, rather than a simple increase in action potential threshold. However, if the strength of the applied current was

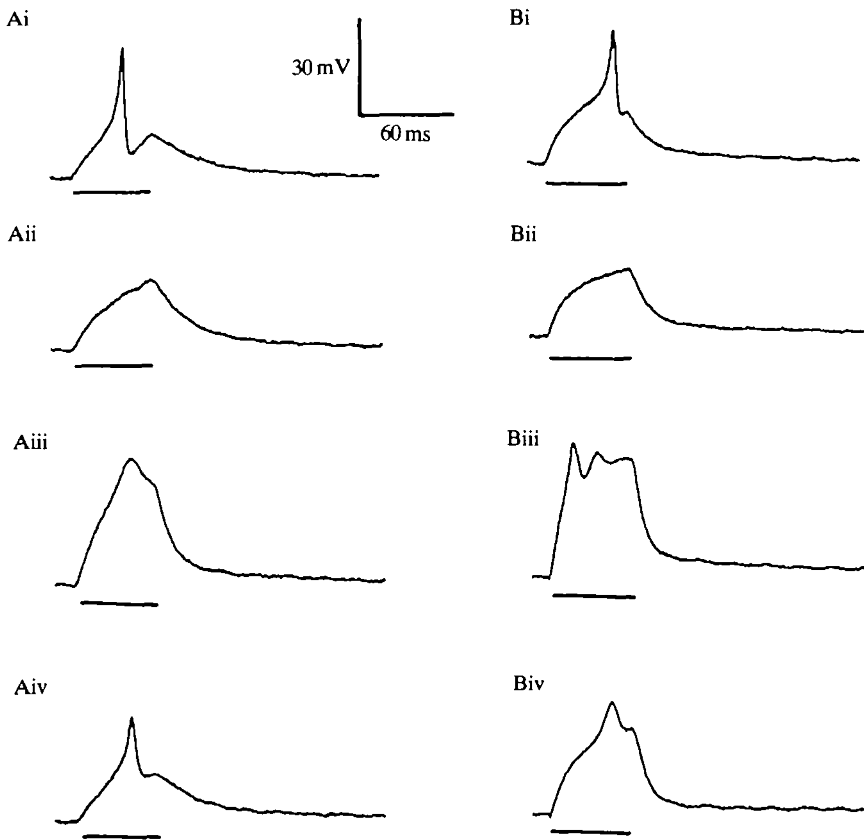

Fig. 5. (A) Effect of manganese (II) ions (40 mmol l^{-1}) on time-dependent spikes. (Ai) Action potential recorded in normal saline. (Aii) Blockade of action potential within 30 s of manganese addition. (Aiii) A twofold increase in current injection failed to evoke an action potential 2 min after Mn^{2+} addition, although some graded response could be observed. (Aiv) Action potential recorded after 10 min washout in normal saline. Magnitude of applied depolarising pulses: Ai, Aii, Aiv, 3 nA; Aiii, 6 nA. Resting potential: Ai, Aii, −67 mV; iii, −65 mV; iv, −67 mV. (B) Effect of cadmium ions (1 mmol l^{-1}) on time-dependent spikes (different preparation from A). (Bi) Action potential recorded in normal saline (pulse amplitude 4 nA). (Bii,iii) Responses to 4 nA and 11 nA, respectively, recorded 1 min after addition of Cd^{2+}, demonstrating blockade of the action potential; some graded oscillatory response could be evoked by increased levels of current injection (Biii). (Biv) Limited recovery of response after 10 min washout in normal saline (pulse amplitude 7 nA). Resting potential: i, −68 mV; ii,iii, −64 mV; iv, −65 mV. In both A and B horizontal bars represent the duration of depolarising current pulses (stimulus frequency 1 Hz).

increased sufficiently, the neurone could give graded oscillations (Fig. 5Biii), but these had characteristics that differed fundamentally from those of all-or-none action potentials. Block of spikes by Mn^{2+} or Cd^{2+} was at least partially reversible on washing the preparation with normal saline solution. Reversal was normally

more easily attained in experiments on isolated rather than non-isolated cell bodies.

The above findings indicate that a large proportion, if not all, of the inward current of time-dependent spikes is carried by calcium ions. Often, the blockade of action potentials was accompanied by a steady membrane depolarisation that was reversible on washing the preparation in normal saline. The magnitude of this depolarization (generally less than 10 mV), however, was insufficient to cause spike block by inactivation of inward currents.

Co-existence of time-dependent action potentials and plateau potentials

We have recently reported (Hancox and Pitman, 1991) that motoneurone D_f is capable of generating plateau potentials. In most instances, recordings of plateau potentials have been made shortly after nerve cords had been dissected from animals and set up for recording. In such recordings, plateau potentials appear as prolonged depolarizing events that may outlast the stimulus that initiated them. In many cases, plateau potentials drive bursts of axonal spikes which can be observed as attenuated depolarizations on the crest of each plateau potential (Fig. 6A). The

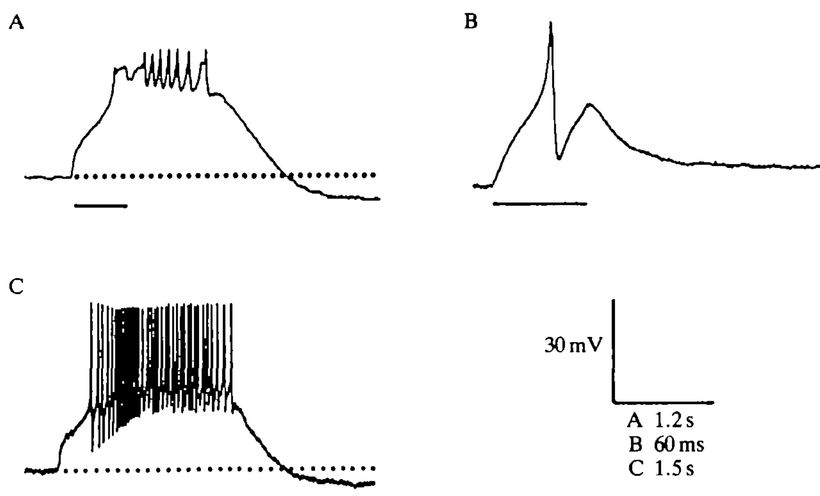

Fig. 6. Plateau potentials and action potentials in D_f. (A) Plateau potential recorded from a neurone that was not producing somatic spikes. The crest of the plateau potential is surmounted by a train of attenuated axonal action potentials. The plateau potential was driven by a 2 nA intracellular current pulse (horizontal bar). (B,C) Responses of a neurone to short (B) and relatively long-duration (C) suprathreshold depolarising pulses applied 2 h after the neurone has been impaled with microelectrodes (current pulse magnitude 6 nA and 2 nA, respectively). Whilst a relatively short (<60 ms) pulse elicits a single action potential, a longer-duration pulse (1.2 s) produces a plateau potential outlasting the pulse and driving a burst of action potentials. The dotted lines in A and C represent the resting potentials of the cells: the plateau is followed by an after-hyperpolarization. Horizontal bars represent durations of depolarising current pulses. Resting potential −70 mV.

interaction between plateau potentials and time-dependent action potentials was investigated during recordings made from neurones some time after preparations had been set up. In such recordings, brief depolarizing pulses could drive one or more all-or-none action potentials without a plateau potential (Fig. 6B). Longer depolarizing pulses, in contrast, could evoke a plateau potential, upon which was superimposed a burst of somatic action potentials (Fig. 6C). This observation demonstrates not only that plateau potentials and action potentials can co-exist in the same neurone, but also that plateau potentials can actually drive bursts of somatic all-or-none action potentials in motoneurone D_f.

Discussion

The work presented here is the first report of all-or-none action potentials recorded from the cell body of an identified cockroach motoneurone without pharmacological or other experimental manipulation. These action potentials are Ca^{2+}-dependent and appear to involve different channels from those responsible for generating plateau potentials, which are also predominantly Ca^{2+}-dependent (Hancox and Pitman, 1991), since these events can co-exist (Fig. 6). The observation that these action potentials appear in a time-dependent way raises a number of questions.

First, can time-dependent action potentials be ascribed to changes occurring in a healthy neurone, or are these changes consequent upon cell damage and, therefore, related to the demise of the neurone? It is unlikely that the observed changes in excitability are related to damage caused by impalement (or recovery from impalement), for action potentials could be recorded immediately following penetration, if this took place some time after the nerve cord had been isolated and placed in the recording chamber. It appears, therefore, that time elapsed after dissection, rather than time following impalement, is the important variable determining the degree of excitability. The evidence that the appearance of spikes was not a consequence of progressive deterioration in the membrane properties of the neurone is that enhancement in excitability did not appear to correlate with a fall in resting membrane potential or in effective membrane resistance, which might be expected during pathological changes in the neurone. Indeed, the values for both membrane resistance and membrane time constant obtained in the current study are somewhat higher than those reported in an earlier examination of D_f (Pitman, 1979). Furthermore, all-or-none action potentials could be recorded from the soma of D_f for long periods (several hours) after their initial appearance. Thus, the available criteria provide no indication that the time-dependent appearance in D_f of Ca^{2+}-dependent action potentials is a consequence of pathological processes leading to alterations in the passive membrane properties of the neurone.

A second question relates to the mechanisms underlying the time-dependent changes in excitability observed in this series of experiments. Ca^{2+}-dependent

action potentials have been recorded from the soma of D_f following extracellular application of TEA^+ or intracellular injection of calcium chelators (Pitman, 1979) and these have been associated with changes in a tonically active outward conductance that is dependent on intracellular calcium ion concentration (Pitman, 1979). A calcium-dependent potassium current (I_C) has been shown to make a major contribution to outward currents recorded from motoneurone D_f, from the basalar/coxal depressor motoneurone (cell 3) and from DUM neurones of the cockroach (Thomas, 1984; Nightingale and Pitman, 1989). It was proposed that changes to this conductance resulting from manipulations of intracellular calcium concentration could enable normally inexcitable somata to generate action potentials, altering the balance between inward and outward currents in favour of inward currents. Normally, when the neurone was depolarized, the K^+ conductance became large enough to shunt inward Ca^{2+} currents and so prevent an action potential from occurring. Reduction in $[Ca^{2+}]_i$ would lower I_C, reduce the shunt on Ca^{2+} currents and so enable the neurone to generate action potentials (Pitman, 1979). It is difficult, however, to account for the changes in excitability reported here in terms of changes to an outward conductance that is active at the normal resting potential of the cell since, unlike spikes induced by injection of Ca^{2+} chelators (Pitman, 1979), the appearance of time-dependent spikes takes place without an increase in effective membrane resistance (determined using hyperpolarizing pulses from the resting potential). This difference, however, may be attributable, at least in part, to the more negative mean resting potential obtained in the present study (-70.8 ± 1.0 mV) compared to that recorded by Pitman (1979) (-61.2 ± 1.6 mV; $\pm$s.e. $N=5$). The resting potentials obtained in the earlier study may have been sufficiently positive to activate an outward current that is absent at the resting potentials recorded here.

The reasons for the differences in resting potential and excitability of motoneurone D_f observed in this and the earlier studies are not entirely clear. Although in early experiments the delay between setting up preparations and impaling neurones was normally short (less than half an hour), experiments could easily last longer than 2 h. This should have been sufficient for the appearance of time-dependent action potentials. The only clearly defined alteration that has been made since those early experiments on excitability is that the experimental chamber has been redesigned so that recirculation of the saline solution over the preparation is considerably more efficient.

Switching from non-spiking to spiking modes is most likely to result from alterations in calcium or potassium currents. For two reasons, any modulation of calcium currents that might occur, however, appears not to involve transformation of the relatively slow currents that carry the inward current of plateau potentials to faster currents that underlie the rising phase of spikes. First, plateau potentials did not exhibit the same time-dependency as spikes: plateaux could be recorded from preparations throughout the course of experiments; spikes could usually only be recorded after a delay. Second, whilst some preparations exhibited either one or other of these events, in many instances spikes and plateau potentials could co-

exist. It should be remembered, of course, that the effects of Ca^{2+} current modulation may be complex because of the many potential roles of Ca^{2+} as a charge carrier, second messenger or regulator of other ionic conductances (such as I_C). Potassium current modulation offers a particularly attractive mechanism by which excitability could be controlled, since experiments with TEA^+ have already demonstrated that suppression of K^+ currents can enhance excitability in this preparation (Pitman, 1979).

Although time-dependent changes in excitability could result from a change in ionic currents resulting directly from a change in the transmembrane gradient of an ion, they are more likely to result from modulation of ion channel function. This could be brought about either by changes in intracellular conditions or through some extracellular signal. An example of the first type of mechanism is the appearance of Ca^{2+}-dependent action potentials under conditions of anoxia or lowered intracellular pH in crustacean muscles that are normally electrically inexcitable. This enhanced excitability results from blockade of delayed K^+ currents by intracellular protons (Moody, 1978, 1980). Time-dependent action potentials in motoneurone D_f are unlikely to be produced by a similar mechanism, since previous experiments have shown that lowering intracellular pH by acid injection or external application of ammonium chloride reduced rather than enhanced the excitability of this neurone (Pitman, 1988). It is, therefore, unlikely that the increase in excitability in this preparation results from a gradually developing metabolic acidosis in the neurone. However, since a fall in intracellular pH reduces excitability of motoneurone D_f, it is possible that neurones undergo a fall in intracellular pH during dissection and isolation of the nerve cord and that the increase in excitability reflects a long-term recovery to normal pH. Obviously, electrical properties could be influenced by other progressive intracellular metabolic changes that are a consequence of the experimental conditions.

There is considerable precedence for modulation of ion channel function by signalling molecules (see reviews by Levitan and Kaczmarek 1987; Levitan, 1988; Koketsu, 1984). In this instance, modulation would have to be exerted by neuromodulators which are released widely rather than from discrete synapses, since action potentials appear in a time-dependent way even in neurone somata that have been functionally isolated from any synaptic input. Thus, any modulatory effect must result from substances released generally into the ganglionic environment or even into the perfusing solution that recirculates over the preparation.

The final question that should be addressed concerns the implications of the current observations. Until recently, insect motoneurone somata have been viewed as electrically inexcitable in the normal state (Pitman *et al.* 1972; Hoyle and Burrows, 1973; Gwilliam and Burrows, 1980). More recently, Laurent (1990) has suggested that non-linear rectification can reduce synaptic transfer in locust interneurones. Conversely, plateau potentials in motoneurone D_f provide a means for synaptic amplification (Hancox and Pitman, 1991). Although, at present, the role of time-dependent action potentials in this neurone is unknown, they provide

the soma with a second non-linear property that may potentially influence the electrical output of the neurone.

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