Examination of the differences between control and CVF-treated animals at each 3-h-period demonstrates that the total leukocytic and neutrophilic response to CVF is dependent upon the time of day the factor is given. Total leukocytic and neutrophilic response is greater during the early morning (06.00–09.00 h) than during the afternoon (12.00–18.00 h). The lymphocytes on the other hand, showed little difference in variation and increase following CVF administration.

McCall et al.[4] reported alterations in neutrophil kinetics in the rabbit after CVF activation of the complement cascade. Following intravenous injection of 0.6 to 1.0 ml of purified cobra venom factor, a profound neutropenia occurred within 60–120 sec followed by a marked neutrophilia. In this same study, CVF was injected into C_6-deficient rabbits which again caused an initial neutropenia followed by a neutrophilia. To further characterize the active factor, fresh plasma was treated with CVF and filtered through a 20,000 MW filter. The filtrate was then injected into the rabbit ear vein and the same neutrophilic response obtained. These results suggested that the neutrophilic changes depended on the elaboration of a factor of low molecular weight ($<20{,}000$) which may be derived from either C_3 or C_5[4].

Because the present investigation was concerned with only a 45-min-response, a neutropenia was not observed. However, a 10fold increase between the 24-h-mean control and experimental neutrophil levels was evident, confirming the neutrophilia following CVF activation of the complement cascade[4].

It is not known whether complement regulates hourly variations in neutrophil kinetics. However, this study has demonstrated that the extent of the neutrophilia following CVF complement activation in the rat is a time-dependent phenomena which varies according to the time of day the factor is given and has a circadian rhythm.

Electrically excitable neurosecretory cell bodies in the periphery of the stick insect, Carausius morosus

I. Orchard[1] and L. H. Finlayson

Department of Zoology and Comparative Physiology, University of Birmingham, P. O. Box 363, Birmingham B15 2TT (England), 15 July 1976

Summary. Intracellular recordings have been made from the cell bodies of both neurosecretory and non-neurosecretory multipolar neurons in the periphery of Carausius morosus. The neurosecretory neurons have cell bodies which are electrically excitable and produce overshooting action potentials, whilst the cell bodies of the non-neurosecretory neurons are electrically inexcitable.

There is a sparsity of electrophysiological data about the basic membrane properties underlying neuroendocrine integration and regulation in insects. A suitable preparation for such a study is found in the stick insect, in which multipolar neurons showing the ultrastructural characteristics of being neurosecretory have been described[2,3]. These multipolar neurosecretory neurons, the link nerve neurons (LNNs), lie with their cell bodies on or near the link nerve and have processes passing superficially along a number of major peripheral nerves[2,3]. Each of the processes propagates action potentials towards their terminals where presumably release of neurosecretory material is triggered[3].

As a preliminary to investigating the control mechanisms of these cells, intracellular recordings were made to determine the properties of the membrane of the cell body. Previous intracellular recordings from nerve cell bodies of insects have been confined to monopolar neurons in the central nervous system. The majority of these cell bodies are electrically inexcitable[4], although a specific group of dorsally situated cell bodies are electrically excitable[5,6].

In this study we provide the first account of intracellular recordings from multipolar neurons in insects and present evidence for the presence of overshooting action potentials recorded from the cell bodies of LNNs.

Adult stick insects were dissected mid-dorsally, pinned out on 'Sylgard' (Dow Corning Corporation) and the gut removed. The preparation was flooded with a modified version of Wood's saline[7] (composition: KCl, 18 mM; $MgCl_2$, 50 mM; $CaCl_2$, 7.5 mM; NaH_2PO_4 6 mM; $NaHCO_3$, 9 mM; glucose 185 mM; made up to 1000 ml H_2O). A window was cut through the cuticle and tissue underlying the LNNs of an abdominal segment, and the preparation viewed under a compound microscope by interference contrast using transmitted light. In this way the insertion of microelectrodes was under visual control, providing no doubt as to the intracellular nature of the recordings and their origin in the soma. Glass micro electrodes of between 40 and 60 MΩ resistance filled with 3 M KCl were used for intracellular recording, and current injection was provided through the same electrode using a bridge circuit (Mentor N-950 Intracellular probe system).

Insertion of a microelectrode into a neurosecretory cell body revealed negative resting potentials of 30–62 mV (mean 46 mV, $n = 50$). The total membrane resistance of the cells was found by applying hyperpolarizing current pulses and varied from 40 to 80 MΩ (mean 58 MΩ, $n = 10$). Total time constant was found to be 30–50 ms (mean 43 ms, $n = 10$). Assuming the cell to be a sphere of 40 μm diameter and the time constant to be the time taken for the membrane to reach 63% of its final value[8] (10 ms), the specific membrane resistance can be calcu-

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lated as 3000 $\Omega \cdot cm^2$ and the specific membrane capacity as 3.3 $\mu F \cdot cm^{-2}$. These should be considered as rough approximations; they do however lie within the range for other neurons.

Current-voltage relationships indicate that the membrane of the soma follows an essentially ohmic response up to a critical membrane potential level (figure 1). Above this level the cell bodies of the LNNs are electrically excitable in that they generate overshooting action potentials at the threshold membrane potential in response to depolarizing pulses (figure 1). Delayed rectification is evident when the steady amplitude reached by the membrane following local responses or action potentials is plotted against the current (figure 1).

A constant feature of these cells was the generation of only a single action potential in response to a depolarising pulse of long duration (figure 1), thereby resembling certain molluscan neurosecretory cells[9]. Spikes were also evoked after the termination of a long hyperpolarizing pulse, illustrating accomodation.

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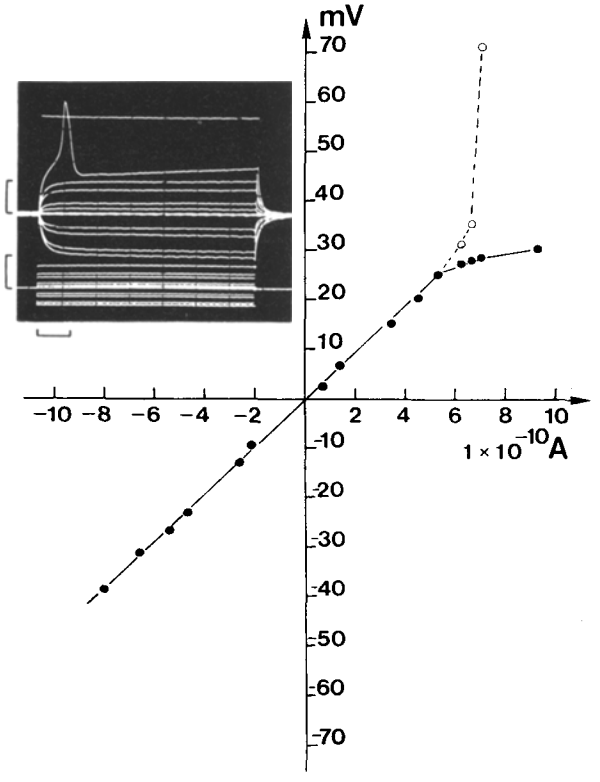

Fig. 1. Current-voltage relationship from the cell body of a link nerve neurosecretory neuron of Carausius. Inset shows response of a cell (upper traces) to depolarizing and hyperpolarizing currents of varying intensity (lower traces). Note that above the threshold membrane potential one action potential with an overshoot of 10 mV is generated. Zero potential is shown by the single upper line. Graph illustrates current vs voltage based on the experiment shown in part in the inset. Below a critical membrane potential the response is in accordance with Ohm's law. Above this level, however, the resistance or capacitance of the membrane changes during local responses and action potentials. Solid circles indicate the measurement at the final steady amplitude, and illustrate delayed rectification above a critical membrane potential; hollow circles indicate peak amplitude and illustrate the electrical excitability of the membrane. Scale bars: 20 mV, 1 namp, 50 ms.

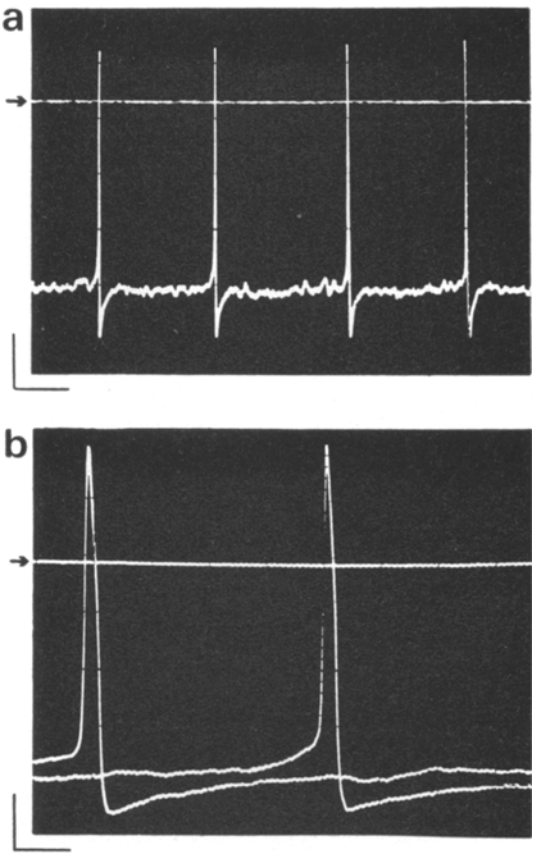

Fig. 2. Spontaneous activity recorded intracellularly from a link nerve neurosecretory cell body of Carausius. *a* Example of low frequency spike activity; *b* 2 action potentials on a faster time base to show impulse shape with long duration, positive overshoot and prominent undershoot. Arrows indicate zero potential. Scale bars: *a* 10 mV, 2s; *b* 10 mV, 200 ms.

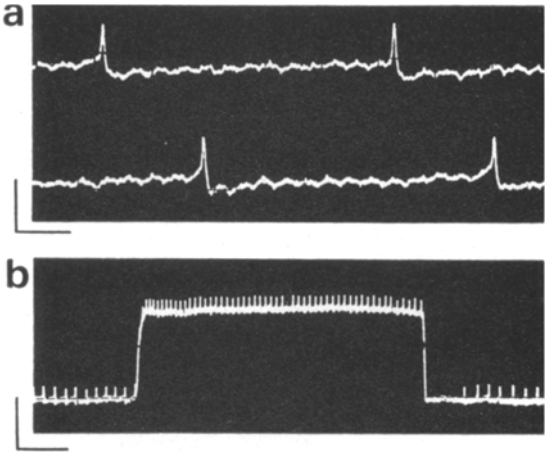

Fig. 3. Intracellular recordings from the cell body of a peripheral nerve stretch receptor of Carausius. *a* Spontaneous activity of small amplitude and short duration, 2 traces are continuous; *b* the effects of electrical stimulation on frequency of action potentials from these non-neurosecretory cells. Note the increase in spike discharge caused by depolarisation of the cell body, and also the post-excitation inhibitory period on return to resting potential. The current strength was 8×10^{-10} A. Scale bars: *a* 2 mV, 50 ms; *b* 5 mV, 2s.

'Spontaneous' action potentials were occasionally observed, although the general lack of 'spontaneous' discharge may reflect an inactivity of these cells during daylight hours, a fact which has been observed from extracellular recordings (unpublished data). The 'spontaneous' action potentials reached 70 mV in height, with a positive overshoot of 10–20 mV and an undershoot of about 8 mV (figure 2). Spike duration is very long even for insect neurosecretory cells (7–20 ms at 50% spike height) and in this respect they again resemble molluscan neurosecretory cells[10]. The smallness of the overshoot as well as the long duration of the spike may be due in part to the unusual ionic content of the haemolymph found in these phytophagous insects[7] and this is at present under investigation.

A contrast to these properties of LNNs was obtained with intracellular recordings from non-neurosecretory multipolar neurons which occur on peripheral nerves in the stick insect[3,4]. These 'peripheral nerve stretch receptors'[3] are 'spontaneously' active and when penetrated the cell bodies revealed resting potentials of about 40 mV, with small action potentials of between 1 and 10 mV in height occurring with a frequency of 2–3 sec^{-1} (figure 3). The action potentials never show overshoot, and are of 2–3 ms in duration (at 50% spike height). We consider that they are remnants of action potentials initiated in the axon which have electrotonically invaded the cell body. The cell bodies of these multipolar neurons are therefore electrically inexcitable, in contrast to LNNs. Long depolarizing pulses cause an increase in the frequency of these action potentials (figure 3b), which increases proportionally with higher depolarizing pulses. Hyperpolarizing pulses lower or inhibit the firing of these cells. These multipolar neurons then act as typical stretch receptor neurons with the dc levels of the cell body affecting the frequency of initiation of the action potentials at some point distant from the cell body.

The majority of cell bodies of central neurons in insects are inexcitable[4]. Exceptions to this have been shown for protocerebral neurosecretory cells[11] and a group of dorsally situated cell bodies in the ventral ganglia[5,6]. One cell of the group of dorsal cells in the thoracic ganglia has been demonstrated to be neurosecretory[5]. The LNN is an example of a clearly defined insect neurosecretory cell which has an electrically excitable cell body. The close relationship between the metabolic state of a nerve cell and its electrical properties has been previously demonstrated[12]. Neurosecretory cells are clearly metabolically different from 'ordinary' nerve cells and this seems to be reflected in the excitable nature of the cell body. The advantages conveyed to the LNNs in having an electrically excitable cell body are presumably ones of coordination of release. The LNNs are multipolar neurons with each process capable of propagating centrifugal action potentials[4]. An electrically excitable cell body would be an efficient means of ensuring that the spike initiation site caused a concomitant action potential in each of the processes, thus causing simultaneous release of neurosecretory material from the terminals.

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Häutungen von Insekten ohne Häutungsdrüse: Befunde mit Larven von Periplaneta americana

Moulting of insects without moulting gland: Results with larvae of Periplaneta americana

M. Gersch[1,2]

Sektion Biologie der Friedrich-Schiller-Universität, Erbertstrasse 1, DDR-69 Jena (Deutsche Demokratische Republik), 19 Juli 1976

Summary. The course of moulting of Periplaneta americana larvae of which the prothoracic glands were exstirpated, was investigated in 2 succeeding series. It could be proved that all animals underwent 1 or 2 moulting processes inspite of the absence of the moulting gland. These results demonstrate that the generally accepted classical schema of the endocrine control of the insect moulting must be renewed.

Es besteht weitgehend unangefochten die Auffassung, dass die Prothoracaldrüse der Insekten der Produktionsort des Häutungshormons und damit eine für die Häutungsprozesse unbedingt erforderliche Voraussetzung darstellt. Diese Auffassung wurde auch durch einige andersartige Feststellungen an Galleria mellonella[3] sowie an Periplaneta americana[4–6] kaum ernsthaft berührt, geschweige erschüttert. Zweifel an der Allgemeingültigkeit der konventionellen Vorstellungen musste allerdings auch der Nachweis der Synthese von Ecdyson und Ecdysteron ohne Beteiligung der Prothoracaldrüsen hervorrufen, wie das einerseits in den Abdomina der Saateule Mamestra brassicae nach Applikation von ^{3}H-Cholesterin[7] und andererseits bei Bombyx mori[8] sowie neuerdings in den isolierten Abdomina des Kartoffelkäfers gefunden wurden[9].

Aus In-vitro-Experimenten mit Beinregeneraten von Blabera craniifer wurde ebenfalls geschlossen, dass das Häutungshormon in Geweben des Metathorax und des Abdomens gebildet wird und dass das klassische Schema der Funktion der Prothoracaldrüse mit diesen Ergebnissen nicht im Einklang steht. In unmittelbarem Gegensatz dazu konnte allerdings bei verschiedenen Insekten nach-

1 Für die wertvolle technische Unterstützung mit der sorgfältigen Präparation der Prothoracaldrüsen sowie der gewissenhaften Kontrolle und Betreuung der Versuchsserien bin ich Frau R. Winkler zu grossem Dank verpflichtet.
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